CLAY AND THE ORIGIN OF LIFE

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Abstract. Research concerning the possible role of clay in chemical evolution is reviewed. The probable importance of clays in the origin of life is assessed.

1. Introduction

The abiotic evolution of life, postulated independently by Oparin (1924) and Haldane (1928), has been the subject of continuing investigation during the last three decades. The theory and empirical studies of chemical evolution have cosmic implications, for they suggest that the origin of life may be common in the universe, and that, if life began according to universal physical and chemical laws, it is not unique to the planet Earth. The postulated stages of chemical evolution leading to the first life include the formation of organic molecules and gaseous constituents and their accumulation in the primordial seas, the increased survival of aggregated monomer molecules due to higher thermodynamic stability, followed by the evolution of a function for the grouped molecules eventually leading to replicative ability.

Today, there is a large amount of experimental data available concerning the abiotic synthesis of biomonomers and biopolymers. (Lemmon, 1970; Stephen-Sherwood and Oró, 1973; Ponnamperuma, 1978). The Oparin-Haldane hypothesis has been further substantiated by the analysis of organic compounds in extra-terrestrial materials (Ponnamperuma, 1971; Ponnamperuma and Buhl, 1976; Kvenvolden *et al.*, 1971; and Kotra *et al.*, 1979).

Although much knowledge has been gained in recent years in the study of the abiotic origin of life, many questions remain. Most of these questions center on the mechanism whereby small biological molecules, such as amino acids and nucleic acid bases, became organized into a system of polymers which evolved into catalysts, templates, and self-replicating systems. As yet, the processes involved in the first peptide and polynucleotide formation have not been clearly elucidated. Thus, it is interesting to consider the contribution of solid surfaces to the 'self-organization' of biomonomers into the complex, surviving, replicating systems which we call life.

Because of their wide distribution in geological time and space and their strong affinity for organic compounds, clays and clay minerals are the most likely candidates among solid materials to have contributed to chemical reactions producing the poly-

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meric substances from which life emerged. The existence of clay minerals on the prebiotic Earth is most likely. Some evidence for their presence in Archean sediments has been proferred (Jaffe, 1975). Clay minerals are formed by igneous activity as an alteration product of silicate minerals and also during diagenesis of sediments. As soon as liquid water appeared in the surface of the primitive Earth, clay minerals probably accumulated on the surface and also became suspended in the primitive ocean.

The importance of clay minerals in chemical evolution was suggested by Bernal in 1951. He proposed that clays near the hydrosphere-lithosphere interface might have adsorbed organic micromolecules, thereby providing high local concentrations of reactants needed to form certain biologically important macromolecules and also protection for those molecules from destructive high energy radiation. Furthermore, he argued that clays could act as catalysts in polymerization reactions, giving rise to polymers which would themselves eventually become catalysts. We can extend Bernal's hypothesis of the role of clay minerals during the stages of chemical evolution on the primitive Earth in the following sequence:

1. Clay minerals catalyzed the reactions of biomonomer synthesis from gaseous constituents of the primordial atmosphere.
2. Clay minerals adsorbed biomonomers on their surfaces, providing a highly concentrated system in which the monomers had a specific orientation.
3. Clay minerals facilitated condensation reactions between adsorbed monomers in which biopolymers were formed. In addition, the surfaces of clay minerals might have served as templates for the specific adsorption and replication of organic molecules. The role of clays as information-carrying crystals has been envisioned by Cairns-Smith (1966), who proposed that after the evolution of a crystalline primitive gene, a 'genetic metamorphosis' would occur, with organic molecules taking control of information transfer and replication. Anderson and Banin (1975) and Rao *et al.* (1980) have recently reviewed the work on clays and chemical evolution.

The following discussion will be based upon the postulated roles of clay minerals in chemical evolution and will include recent work involving clay-organic complexes.

2. Possible Roles of Clay Minerals

2.1. Biomonomer synthesis

Prebiotic synthesis experiments performed with gas compositions ranging from extremely reducing to slightly oxidizing and with various energy sources have successfully produced organic monomers such as amino acids and/or nucleic acid bases, which are essential to life (Miller, 1953; Abelson, 1956; Oró and Kimball, 1961 and 1962; Palm and Calvin, 1962; Miller, 1963; Oró, 1963; Ponnamperuma *et al.*, 1963; Grossenbacher and Knight, 1965; Ponnamperuma, 1971; Ring *et al.*, 1972). Similar experiments have been performed in the presence of clays. In some experiments, a

small quantity of water was adsorbed on the clay mineral surface, while in others, the clay was suspended in water, a condition simulating the primordial ocean. The dry experiment, in which only a small quantity of water is present, maximizes surface acidity of the clays, due to dissociation of adsorbed water molecules. This increased acidity is considered to be important in catalysis of chemical reactions taking place at or near the clay surfaces (Fripiat *et al.*, 1965; Mortland, 1968).

2.1.1. *Amino Acids*

Simulated synthesis experiments using clay minerals have been reported by Yoshino *et al.* (1971), Fripiat *et al.* (1972), and Poncelet *et al.* (1975). The materials, methods, and results are listed in Table I for comparison. Similar gas mixtures were used. Although water was not purposely added, it is clear that the montmorillonite used by Yoshino *et al.* (1971) contained a substantial amount of adsorbed water. Fripiat *et al.* mentioned that a small amount of water was also present in their experiment. Although the reaction temperatures of the gas mixtures in the experiments were quite different, the experiments yielded similar kinds of amino acids. Interestingly, a significant amount of the larger acidic amino acids (aspartic and glutamic) and the basic ones (lysine and arginine) were synthesized. Yoshino *et al.*, ruled out laboratory contamination as a source of these amino acids in a following report, using a deuterated gas mixture (Hayatsu *et al.*, 1971). Using ^{14}CO, Poncelet *et al.* (1975), also showed

TABLE I

Abiotic synthesis of amino acids from a primordial gas mixture in the presence of a clay mineral

	Yoshino *et al.* (1971)	Fripiat *et al.* (1972)	Poncelet *et al.* (1975)
Clay mineral	Montmorillonite	Zeolites	Zeolites
Gas mixture	H_2, CO, NH_3	CO, NH_3	CO, NH_3
Energy	Heat	Heat	Heat
Temperature	700°C	250°C, 325°C	275°, 285°, 290°
Time	5 hr	1–8 days	120 hr
Amino acids detected			
Aspartic Acid	15 nanomoles		5–12
Threonine		+	tr–6
Serine	28 nm	+	18–38
Glutamic Acid	27 nm		tr–15
Glycine	242 nm		101–136
Aspartic Acid and/or Glycine		+	
Alanine	149 nm	+	10–14
Leucine	12 nm		trace
Valine			21–30
Phenylalanine and/or Leucine		+	
Lysine	12 nm	+	
Arginine	26 nm		

+ Indicates that the amino acid was detected.

that amino acids detected in the reaction mixture were not contaminants. Unfortunately, none of the non-protein amino acids were detected in these experiments. The effect of the montmorillonite and zeolite is not clear, since no comparative experiments without the clay minerals was reported.

Shimoyama *et al.* (1978) performed synthesis experiments using an electric discharge with a gas mixture of CH_4 and N_2 in the presence of Na-montmorillonite suspended in H_2O. For comparison, an experiment without clay mineral was also performed under the same conditions. Amino acids detected with an amino acid analyzer were serine, sarcosine, glutamic acid, glycine, alanine, α-aminobutyric acid, valine, isoleucine, β-alanine, and α-, γ-diaminobutyric acid. Alanine and α-aminobutyric acid were found to be racemic mixtures as shown by gas chromatographic analysis (Figure 1). The four most abundant amino acids, glycine, alanine, α-aminobutyric acid, and sarcosine, were also confirmed by gas-liquid chromatography combined with mass spectrometry. These four amino acids, which have the simplest structures, are the most probable amino acids to be synthesized. Their yields are shown in Figure 2. The proposed role of the clay mineral in this experiment is to promote the synthesis reaction. It is clear from Figure 1 that there are no qualitative differences in the types of amino acids produced in the presence and in the absence of the clay mineral. The difference between the two experiments is that glycine predominates in the experiment without the clay, and alanine is the most abundant amino acid in the presence of Na-

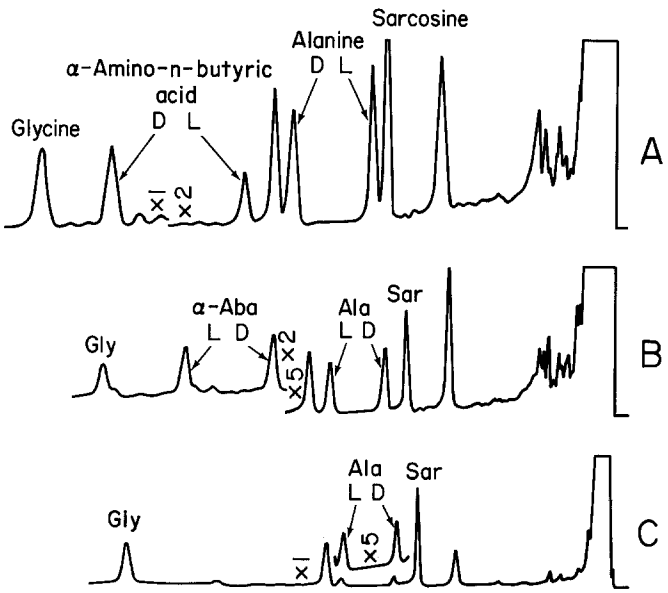

Fig. 1. (a) Gas chromatogram of amino acid enantiomers produced in the presence of Na-montmorillonite and examined by the phase D column. (b) As above by the phase L column. (c) Gas chromatograms of amino acid enantiomers produced in the absence of the clay and examined by the phase L column. From Shimoyama *et al.* (1978).

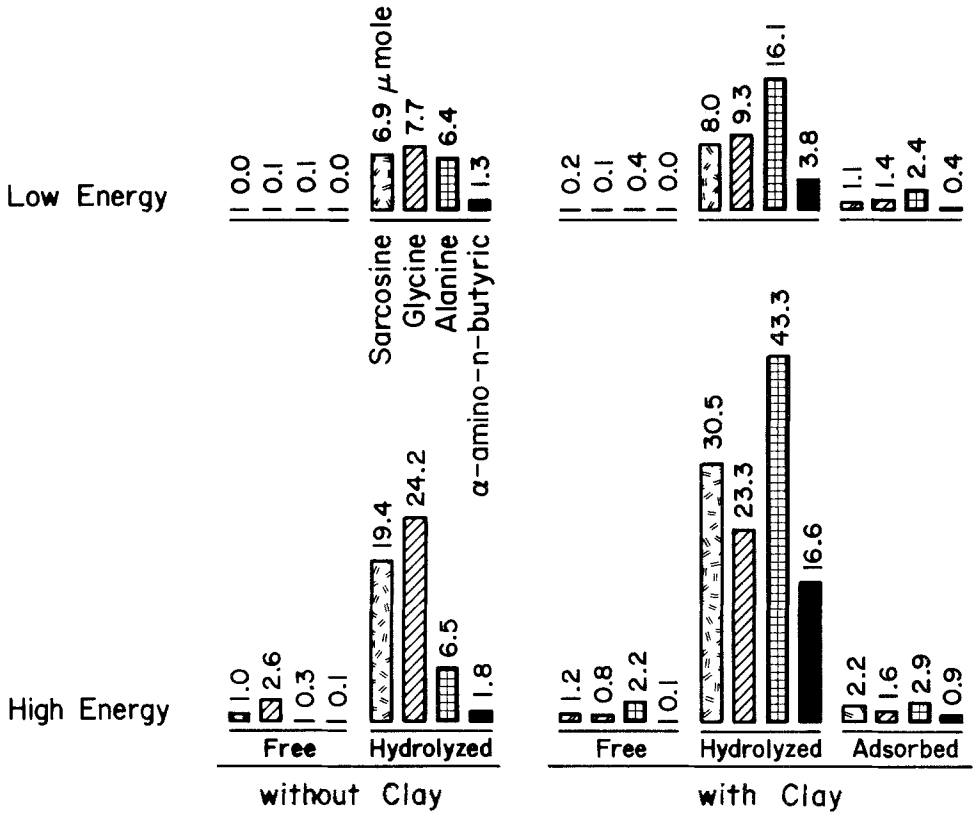

Fig. 2. Yields of the four most abundant amino acids produced from CH_4 and H_2O by electric discharge in the presence and absence of Na-montmorillonite – From Shimoyama *et al.* (1978).

montmorillonite. In this experiment the clay mineral promotes methylation of the intermediate product. This hypothesis is consistent with the increased yield of α-aminobutyric acid, α-alanine, and sarcosine in the presence of the clay mineral. Apparently, the amino group of the intermediate is already attached to the α-carbon before further methylation occurs, as indicated by the appreciable amount of sarcosine and the small amount of β-alanine produced.

Hubbard *et al.* (1973) observed the formation of formic acid and other organic compounds on montmorillonite and other silicaceous substrates when mixtures of CO, CO_2, and N_2 and H_2O were irradiated with UV light at 250 nm.

Several researchers have examined the interaction of HCN and HCN oligomers, the possible precursors of biological molecules, with clays. Cruz *et al.* (1974) studied the adsorption of HCN on Ca- and Cu-montmorillonites. Dry conditions in which there was no residual water allowed maximum adsorption of HCN. Heating the clay mixtures to 90°C under anhydrous conditions produced changes in IR spectra, suggesting the formation of carbonyl or carboxyl bonds, but the reaction products were not well characterized. Ferris *et al.* (1979) found that montmorillonites inhibit the

oligomerization of HCN. Further experiments showed that clays catalyze the decomposition of diaminomaleonitrile in aqueous solution, with two equivalents of HCN being formed per mole of DAMN. These workers suggest that HCN oligomers and the biomolecules derived from them were not formed in large quantities on the primitive Earth in the presence of clays.

2.1.2. *Purines, Pyrimidines, and Carbohydrates*

Two other important classes of biomonomers are the nucleic acid bases and sugars. It has been demonstrated that the bases can be synthesized from the constituents of the primitive atmosphere without solid catalysts (Oró and Kimball, 1961 and 1962; Ponnamperuma *et al.*, 1963) and also in the presence of iron meteorite powder (Hayatsu *et al.*, 1968).

The synthesis of cytosine, uracil and cyanuric acid from CO_2, NH_3, and H_2O in the presence of kaolinite has been reported by Harvey *et al.* (1971). After the mixture was heated at 80°C for 14 days, the liquid phase was chromatographed and tested for optical absorbance in the UV region. However, since the experiment was not performed without clay, the contribution of kaolinite to this reaction cannot be deduced. Chittenden and Schwartz (1976) observed that expandable lattice clays, such as montmorillonite, greatly increased the conversion of 5,6-dihydrouracil to uracil by photodehydrogenation.

The formation of sugars from formaldehyde refluxing together with kaolinite has been reported (Gabel and Ponnamperuma, 1967). Trioses, tetroses, pentoses, and hexoses were produced in the presence of alumina or kaolinite. It was proposed that the alumina octrahedral lattice of kaolinite provides basic sites which remove protons from formaldehyde, providing the necessary intermediate reactant molecules for the formation of sugars. Harvey *et al.* (1972) obtained polysaccharides by heating paraformaldehyde, water, and kaolinite to 80°C for 160 days. No saccharide synthesis was observed in controls treated in the same manner. They proposed that the Al coordination sites on the clay could catalyze aldol condensation.

Little work has been done with reactions in which nucleotides are formed in the presence of clay minerals. Steinman *et al.* (1965) reported the production of adenosine triphosphate (ATP) from ADP, H_3PO_4, and dicyandiamide at room temperature in the presence of kaolinite. The yield of ATP was enhanced from 0.03 % to 0.5 % by the use of kaolinite.

In summary, the most probable role of clay minerals (especially those with high adsorption capacity) in the synthesis of biomonomers is to promote the reaction, but not to change the direction of the reaction path. Therefore, clay minerals could have increased the rate of the production of a variety of biomonomers on the primitive Earth, decreasing the time span required for significant quantities of these molecules to accumulate.

2.2. Adsorption

Bernal (1951) postulated that clays can concentrate by adsorption the organic compounds in the 'primordial soup' proposed by Haldane (1928). Clay mineral adsorption of biologically important organic molecules (amino acids, peptides, proteins, nucleic acid bases, nucleosides, nucleotides, polynucleotides and sugars) has been studied extensively by many investigators. Mortland (1970), Theng (1974), Weiss (1969), and Lahav and Chang (1976) have reviewed in detail the adsorption and retention of organic compounds by clay minerals. To avoid repeating the work of these two reviews, we will discuss only those problems relevant to chemical evolution.

2.2.1. *Amino Acids*

Opinion differs concerning the possible upper limit of amino acids which can be adsorbed on the clay minerals. McLaren *et al.* (1958) reported that montmorillonite can adsorb alanine in excess of its exchange capacity, while Sieskind (1960) found that the exchange capacity of the clay mineral could not be saturated under similar conditions. The discrepancy in these results appears to be due to two different methods used for determining the quantity of amino acid adsorbed. McLaren *et al.* (1958), obtained their data indirectly, measuring the difference in the initial and the recovered concentrations of amino acids in solution at equilibrium. Sieskind estimated the quantity adsorbed directly, measuring the amount retained in the clay portion after washing with water. Cloos *et al.* (1966) performed both types of measurements, analyzing quantities of amino acid in the equilibrium solution (indirect) and amino acids on washed clay residues (direct). They found that the indirect method gave a higher estimate of amino acids adsorbed. The difference between these two methods can be attributed in part to weakly adsorbed molecules lost during the wash of the clay in the method used by Cloos *et al.* (1966). It is also likely that the difference is partially due to experimental error involved in the indirect method. Generally, in an

TABLE II

Adsorption of an equimolar mixture of α- and β-alanine on Na-montmorillonite*

	Amino acids	Initial (μM)	Amounts found in (μm)			Amounts adsorbed (meq/100 g)	
			Supernt.	Clay	Total	Indirect	Direct
pH 3.0	α-Ala	200	165	19.3	184 (92.0%)	8.8	4.8
	β-Ala	200	138	46.2	184 (92.0%)	15.5	11.6
pH 7.0	α-Ala	200	178	3.67	182 (91.0%)	5.5	0.9
	β-Ala	200	194	4.33	198 (99.0%)	1.5	1.1
pH 10.0	α-Ala	200	181	1.94	183 (91.5%)	4.8	0.5
	β-Ala	200	192	2.64	195 (97.5%)	2.0	0.7

* Na-montmorillonite is from the Source Clay Repository (Univ. of Missouri, U.S.A.) 400 mg of the clay (less than 2 μm particle size) was suspended in 40 ml of water and was used for adsorption experiments at each pH.

adsorption experiment, the amount of amino acids remaining in solution is at least one order of magnitude greater than that adsorbed on the clay surface. Therefore, any error in estimating the amount adsorbed is amplified by the indirect method. Furthermore, incomplete recovery of the molecules may occur, causing an overestimation of the quantity adsorbed if the indirect method of measurement is used. An experiment in our laboratory illustrates the difference in results obtained by the two methods. Table II shows the data from an experiment in which equimolar quantities of α- and β-alanine were mixed together with Na-montmorillonite at three hydrogen ion concentrations. After adsorption had occurred, two fractions were recovered: (1) the 'indirect' fraction – the supernatant after centrifugation, combined with a pH adjusted wash of the clay fraction, and (2) the 'direct' fraction, the HCl extraction of the amino acids from the clay. The quantity of the two amino acids recovered in both

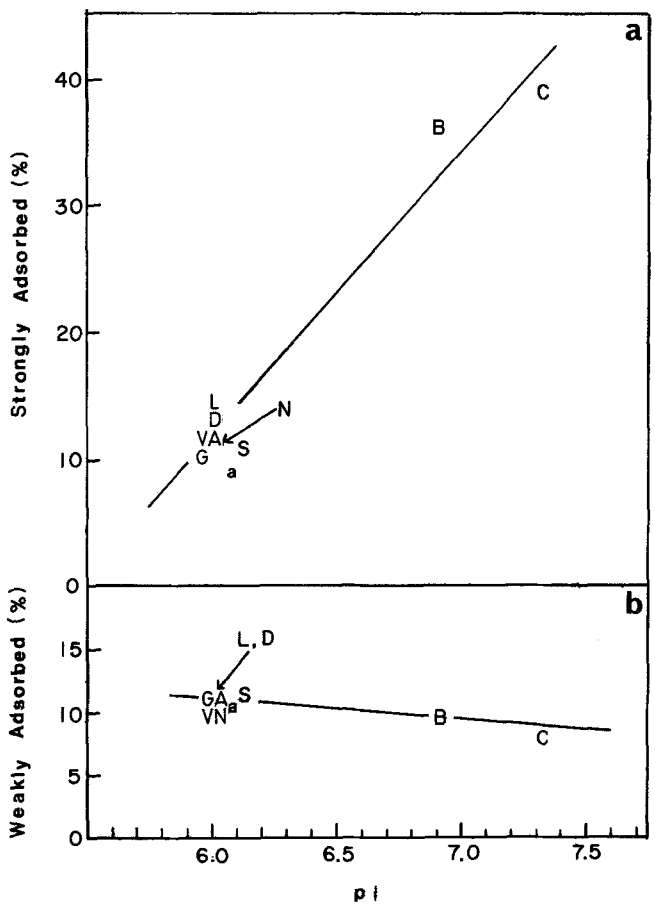

Fig. 3. The relationship between quantities of amino acids which are strongly adsorbed (a) and weakly adsorbed (b) at pH 3 on Na-montmorillonite, and their isoelectric points – From Friebele *et al.* (1980). Symbols: G, glycine; S, sarcosine; A, α-alanine; B, β-alanine; a, α-aminobutyric acid; C, γ-aminobutyric acid; V, valine; N, norvaline; L, L-isoleucine; D, D-alloisoleucine.

TABLE III

Adsorption of protein and non-protein amino acids on Na-montmorillonite (100% CEC, or 200 meq total amino acids (Friebele *et al.*, 1980) and 200 mg Na-montmorillonite)

pH	Amino Acid	Adsorbed			Non-Adsorbed	Total
		Strongly	Weakly	Total		
C_2						
3.0	Glycine	11.0%	11.0%	22.0%	77.9%	99.9%
	Sarcosine*	11.0	11.3	22.3	72.7	95.0
7.0	Glycine	1.1	6.4	7.5	87.7	95.2
	Sarcosine	1.3	4.1	5.4	83.8	89.2
10.0	Glycine	1.7	4.9	6.6	92.2	98.8
	Sarcosine	0.7	4.5	5.2	94.5	99.7
C_3						
3.0	DL-α-Alanine	11.6	10.8	22.4	78.6	101.0
	β-Alanine	36.3	9.6	45.9	57.6	103.5
7.0	DL-α-Alanine	1.0	5.0	6.0	88.3	94.3
	β-Alanine	2.3	7.2	9.5	86.6	96.1
10.0	DL-α-Alanine	0.8	5.1	5.9	96.4	102.3
	β-Alanine	0.8	4.9	5.7	91.0	96.7
C_4						
3.0	DL-α-Aminobutyric Acid	9.0	10.5	19.5	84.7	104.2
	DL-γ-Aminobutyric Acid	39.0	8.0	47.0	52.5	99.5
7.0	DL-α-ABA	1.8	7.9	9.7	92.1	101.8
	DL-γ-ABA	2.8	9.4	12.2	89.1	101.3
10.0	DL-α-ABA	0.5	5.1	5.6	98.7	104.3
	DL-γ-ABA	0.6	5.6	6.2	94.6	100.8
C_5						
3.0	DL-Valine	11.8	10.4	22.2	74.4	96.6
	DL-Norvaline	11.4	10.3	21.7	77.7	99.4
7.0	DL-Valine	1.1	6.1	7.2	87.1	94.3
	DL-Norvaline	1.0	5.8	6.8	87.3	94.1
10.0	DL-Valine	0.7	4.5	5.2	93.1	98.3
	DL-Norvaline	0.7	4.5	5.2	93.5	98.7
C_6						
3.0	L-Isoleucine	13.7	11.2	24.9	75.5	100.4
	D-Alloisoleucine	12.8	11.3	24.1	75.0	99.1
7.0	L-Isoleucine	1.2	7.0	8.2	88.7	96.9
	D-Alloisoleucine	1.4	7.1	8.5	89.5	98.0
10.0	L-Isoleucine	0.7	4.7	5.4	94.9	100.3
	D-Alloisoleucine	0.8	4.6	5.4	95.1	100.5

* Although sarcosine contains three carbon atoms, it is placed in the C-2 group because it is structurally related to glycine.

fractions was estimated by an amino acid analyzer (Durrum D–500). The total recovery ranged from between 91 to 99% of the initial amount. The amount of amino acids adsorbed estimated by direct and indirect methods differ significantly.

The adsorption of amino acids on the clay surface takes place mainly through cationic exchange, ion-dipole and coordination interactions, hydrogen bonding, and physical forces. The extent to which these various mechanisms occurs depends upon the isoelectric point, dipole moment, and molecular size and shape of the molecules in solution at a given pH.

The observation by many workers (Talibudeen, 1954; Sieskind and Wey, 1959; Sieskind, 1960; Cloos *et al.*, 1966; Jaffe, 1975; Shimoyama and Ponnamperuma, 1980) that adsorption of amino acids increases with decreasing pH and increasing amino acid concentration indicates that the major mechanism of adsorption at low pH is cationic exchange, because the amino acids are in cationic form. Fiebele *et al.* (1980) observed an additional mechanism of adsorption at low pH on Na-montmorillonite. The quantity of an amino acid which is strongly adsorbed (tightly held against washing with water) on the clay at pH 3 increases with increasing isoelectric point of the amino acid (see Figure 3a and Table III). Thus, amino acids in the strongly adsorbed fraction are adsorbed by cationic exchange. The quantity of amino acids weakly adsorbed (washed from the clay with pH-adjusted water) is independent of the isoelectric point of the amino acid (Figure 3b). Hydrogen bonding between the amino group and the oxygen of interlayer water molecules is a possible mechanism of adsorption for the weakly adsorbed fraction.

Cloos *et al.* (1966) reported two mechanisms of amino acid adsorption occurring on hydrogen montmorillonite under neutral conditions: proton transfer and zwitterion association (the presence of zwitterions was later confirmed with infrared studies by Fripiat *et al.*, 1966). The quantity of molecules which were bound by the latter mechanism and removed from the clay surface by washing with water, followed increasing K_1 values of the amino acids. Under the conditions of the primitive oceans, which may have been slightly alkaline due to dissolved ammonium ions (Bada and Miller, 1968), significant concentration of amino acids by adsorption on clays can take place even though most of the molecules are in zwitterion form and cationic exchange occurs only to a limited degree. Jaffe (1975) noted that at pH 7.6 and amino acid concentration of 0.002 N, 17% of the total amino acids are adsorbed on montmorillonite. Greenland *et al.* (1962) noted that no cations are liberated when amino acids and peptides are adsorbed on sodium and calcium clays at neutral pH. In Table III the major portion of adsorbed amino acids at pH 7 and pH 10 are weakly adsorbed, probably by hydrogen bonding. Under the neutral conditions of the primitive oceans, the major mechanisms responsible for adsorption would have been zwitterion association, hydrogen bonding, ion-dipole interactions, and van der Waals forces.

An important aspect of amino acid adsorption on clays in chemical evolution is the orientation of adsorbed molecules in the interlayer space of the clay minerals. Amino acids generally form single layer complexes as they are incorporated into the interlayer space. (Talibudeen, 1955; Greenland *et al.*, 1962 and 1965a; Cloos *et al.*, 1966;

Hsu, 1977). Contraction of the interlayer space to a distance slightly smaller than the thickness of the adsorbed molecules has been observed by X-ray diffraction studies and explained by the above authors by a 'keying' of the amino acid molecules into hexagonal holes of the clay surface.

Hsu (1977) performed X-ray diffraction studies of various amino acids adsorbed on montmorillonite and found that increasing the number of carbon atoms between functional groups of the amino acid does not increase the interlamellar thickness of the clay. On the other hand, increasing the size of the side chain of α-amino acids does increase the interlamellar spacing. He concluded that the adsorbed molecules are oriented so that the main chain of the amino acid (from one functional group to the other) lies parallel to the basal surface of the clay. Thus, the alkyl group substituted at the α-carbon projects into the interlamellar space at a large angle. His results were supported by the additional finding that the interlamellar spacing of the clay with the peptides, di-alanine and tri-alanine intercalated is the same as that when α-amino acid monomers are adsorbed.

Fripiat *et al.* (1966) detected zwitterions of glycine and β-alanine adsorbed on montmorillonite with infrared studies of clay films, and they suggested a specific orientation of these molecules on the clay surface. Upon heating the film (140–244°C), they found the formation of a secondary amide linkage. This experiment simulated somewhat the condition of a drying ocean beach and may indicate the importance of zwitterion association and orientation on the clay surface in chemical evolution.

2.2.2. *Preferential Adsorption of Amino Acids*

One of the most interesting questions concerning the role of clay minerals in chemical evolution is whether clays can preferentially adsorb certain types of organic molecules. Specifically, do clays adsorb (and thux concentrate) biological compounds more easily than nonbiological ones? Friebele *et al.* (1980) recently investigated the question of whether clays selectively adsorb protein amino acids over non-protein amino acids. They mixed Na-montmorillonite with five different pairs of protein and non-protein amino acids and analyzed the quantities of amino acids in three fractions: nonadsorbed (indirect), weakly adsorbed (wash) and strongly adsorbed (remaining on clay, extracted with 1 N HCl). By estimating quantities of amino acids in all fractions and obtaining complete recovery (100% ± 5%), they were assured that error due to loss or degradation was insignificant. Their results, shown in Table III, show that there is little difference in the adsorption of most protein and non-protein amino acids by Na-montmorillonite at differing hydrogen ion concentrations. The exception occurs at low pH, and to a slight degree at neutral pH, for the pairs containing an amino acid having more than one carbon atom separating the amino and carboxyl groups. The β- and γ-amino acids are preferentially adsorbed over their α-amino acid counterparts. Sieskind and Wey (1959) obtained similar results with mixtures of single amino acids and montmorillonite, and noted a linear relationship

between adsorption of amino acids at low pH and the number of carbon atoms separating functional groups.

The amino basis for differential adsorption of protein and non-protein amino acids appears to be differences in their isoelectric points, which results in different degrees of cationic exchange occurring at low pH (β-alanine and γ-aminobutyric acid have larger pI's than their α-amino acid counterparts). In this case, the difference in dipole moments of the molecules does not appear to influence their adsorption by Na-montmorillonite since there are no constant differences in the quantities of α- and non-α-amino acids in the adsorbed fractions over all hydrogen ion concentrations. Jaffe (1975) also observed, in mixtures of a large number of amino acids and montmorillonite, that adsorption is dependent upon the isoelectric point.

From these results, we can speculate that preferential adsorption by clays would result in removal of non-α-amino acids from the primitive ocean by adsorption, leaving α-amino acids in solution to react. The larger the distance between functional groups, the larger number of molecules removed (Sieskind and Wey, 1959) from the primordial soup. This differentiation would occur to a large extent only if the primitive ocean were acidic; it would occur to a smaller degree under neutral conditions,

TABLE IV

The binding of leucine, aspartate, and glucose to bentonite

^{3}H-labeled compound	10^4 M Additions of unlabeled compound	Binding	
L-Leucine	–	26.8	±3.3
L-Leucine	L-Leucine	2.7*	±0.4
L-Leucine	D-Leucine	25.3	±2.1
D-Leucine	–	4.1	±0.6
D-Leucine	L-Leucine	3.2	±0.6
D-Leucine	D-Leucine	3.6	±0.7
L-Aspartate	–	2.2	±0.3
L-Aspartate	L-Aspartate	0.2*	±0.1
L-Aspartate	D-Aspartate	1.7	±0.3
D-Aspartate	–	0.3	±0.1
D-Aspartate	L-Aspartate	0.4	±0.1
D-Aspartate	D-Aspartate	0.3	±0.1
D-Glucose	–	2.6	±0.3
D-Glucose	D-Glucose	0.5*	±0.2
D-Glucose	L-Glucose	2.6	±0.2
L-Glucose	–	0.23	±0.05
L-Glucose	D-Glucose	0.11	±0.03
L-Glucose	L-Glucose	0.20	±0.02

* In some experiments, nonradioactive D- and L-compounds were present at 10^{-4} M concentration. Binding is expressed as picomoles of labeled compounds per 10 mg of bentonite. Standard errors of the mean of 6 to 18 individual determinations are presented. * = binding of stereoisomers is significantly different $P < 0.05$. Students 2-tailed t-test. From Bondy and Harrington (1979).

with any significant net change in the distribution of these amino acids requiring a large amount of time.

The fact that proteins contain only L-optical isomers of amino acids and no D-isomers poses a problem to the process of chemical evolution, since amino acids outside of biological systems tend to exist in the racemic state. Clay minerals might provide a mechanism for the concentration of homogeneous optical isomers if they exhibited selective adsorption of L-amino acids over D-amino acids. This ability to differentiate between configurations of optical isomers would depend upon the presence of an asymmetric center on the clay mineral surface, which has not been confirmed. The possibility of chirality existing throughout kaolinite layers resulting from interlayer shifts and vacant octahedral sites in successive layers has been discussed by Bailey (1963).

Jackson (1971b) reported that the quantity of L-phenylalanine adsorbed on kaolinite was significantly greater than the quantity of D-phenylalanine adsorbed at pH 5.8. However, his indirect method of measurement, which was to take optical density measurements of the equilibrium solutions, could be subject to considerable error, as demonstrated before. This seems evident in the fact that the difference between the estimated quantities of D- and L-phenylalanine adsorbed was only slightly larger than the standard error of the measurements. Thus, the validity of this reported selective adsorption is questionable. (Other work concerning preferential adsorption of enantiomers will be discussed in the polymerization section).

More recently, Bondy and Harrington (1979) studied the adsorption of leucine, aspartic acid, and glucose enantiomers on bentonite using tritiated molecules. Their results, obtained by measuring radioactivity on the clay, are shown in Table IV. A summary of their results reveals that the addition of L-leucine greatly decreased the adsorption of L-(^{3}H) leucine, while the addition of D-leucine did not. However, in a similar experiment, neither the addition of L-leucine nor D-leucine inhibited adsorption of D-(^{3}H) leucine. (Similar results were obtained with L-aspartic acid and with D-glucose). This inconsistency in the results requires explanation, for if the L-isomer is preferentially adsorbed by a factor of ten, as these results imply, the adsorption of the L-isomer should inhibit adsorption of both of the tritiated D- and L-isomers. These findings have been questioned by Wellner (1979) on the theoretical grounds that bentonite has no chirality, and that differences in the amino acid isomers themselves could not be responsible for differential binding. Repeating these adsorption experiments with labelled amino acid isomers, Youatt and Brown observed that the apparent preferential absorption of isomers can result from the binding of radioactive decomposition products and isotope dilution effects. Thus, this indirect method of observing only the adsorption of labelled isomers in the presence of unlabelled isomers gave results falsely implying preferential adsorption.

Direct measurements of racemic mixtures of amino acid enantiomers adsorbed on Na- montmorillonite have indicated that there is no large preferential adsorption of either enantiomer by Na-montmorillonite (Friebele *et al.*, 1981a and b). The measurements were a part of the experiments presented in Table III; therefore, there is little

doubt that the distribution of the amino acid enantiomers between the clay and the solution is due to abiotic processes, since complete recovery of the amino acids was obtained. Enantiomer ratios which were obtained by gas chromatography and a newer method employing chiral eluants in high performance liquid chromatography (Hare and Gil-Av, 1979; Gil-Av *et al.*, 1980) are given for the adsorbed and non-adsorbed fractions in Tables V and VI.

The existence of a statistically significant difference between the standard and sample D:L ratios is denoted by an asterisk. In the strongly and weakly adsorbed fractions showing a significant difference, the D:L ratios are less than one, indicating a 0.5–2.0% greater quantity of the L-enantiomer adsorbed than D-enantiomer. This small difference is larger than the standard deviations for the chromatographic method, and thus greater than variations inherent in the analysis.

In order to be sure that these differences are not due to contamination, we calculated mass balances of the enantiomers adsorbed and nonadsorbed, obtaining a total quantity of D- and L-amino acids for each experiment. Since the experiments were begun with racemic mixtures, the total quantity of each enantiomer should be equal, within experimental error. An example of the mass balance calculated for valine is

TABLE V

Gas chromatographic analysis of amino acid enantiomers adsorbed and nonadsorbed on Na-montmorillonite

Sample	Valine			Norvaline			α-Aminobutyric Acid		
	D:L	σ	n	D:L	σ	n	D:L	σ	n
Standard	1.008	0.022	10 (A)				1.010	0.024	5 (A)
	1.011	0.014	10 (B)	1.006	0.031	10 (B)			
pH 3									
Adsorbed S	0.977*	0.013	5 (A)	0.986*	0.020	4 (B)	1.005	0.002	3 (A)
Adsorbed W	0.973*	0.026	3 (A)	1.017	0.011	3 (B)	0.985	0.012	4 (A)
Nonadsorbed	1.020*	0.005	3 (A)	1.006	0.027	6 (B)	1.014	0.015	4 (A)
pH 7									
Adsorbed S	0.996	0.013	6 (A)	0.979*	0.016	5 (B)	0.996	0.005	4 (A)
Adsorbed W	1.031*	0.021	3 (A)	0.995	0.013	4 (B)	0.993	0.032	3 (A)
Nonadsorbed	1.005	0.011	3 (A)	0.997	0.023	5 (B)	1.021	0.038	3 (A)
pH 10									
Adsorbed S	1.015	0.021	4 (B)	0.986	0.035	4 (B)	0.925*	0.046	7 (A)
Adsorbed W	1.008	0.021	3 (B)	1.011	0.025	3 (B)	0.988*	0.004	3 (A)
Nonadsorbed	1.010	0.011	3 (B)	1.00	0.012	3 (B)	1.012	0.021	4 (A)

S– Strongly adsorbed; W – Weakly adsorbed
*– Statistically significant difference between standard and sample D:L Ratios
n– Number of GC analyses
(A)– Sterile conditions not used
(B)– Sterile conditions used

TABLE VI

D-L ratio of α-alanine adsorbed and nonadsorbed on Na-montmorillonite

Sample		a. Analysis by gas chromatography			b. Analysis by liquid chromatography		
		D:L	σ	n	D:L	6	n
Standard	(A)	1.028	0.029	10	0.997	0.016	15
	(B)	1.005	0.014	4			
pH 3							
Adsorbed S	(A)	1.022	0.009	5			
Adsorbed W	(A)	1.018	0.011	5			
Nonadsorbed	(A)	1.024	0.006	4			
pH 7							
Adsorbed S	(B)	0.976*	0.028	5	1.001	0.017	10
Adsorbed W	(B)	1.013	0.028	4	0.999	0.007	7
Nonadsorbed	(B)	1.013	0.020	6	1.003	0.018	4
pH 10							
Adsorbed S	(B)	0.953*	0.012	4	0.983*	0.008	10
Adsorbed W	(B)	0.979*	0.021	4	0.982	0.042	5
Nonadsorbed	(B)	1.011	0.006	3	1.008	0.015	8

S– Strongly adsorbed; W – Weakly adsorbed
*– Statistically significant difference between standard and sample D:L Ratios
n– Number of analyses
(A)– Sterile conditions not used
(B)– Sterile conditions used

given in Table VII. In all experiments, except for experiment B at pH 3, the difference in the total measured quantities of D- and L-valine is less than the variation in μmoles expected from the analytical method. Thus, we are assured that no significant loss or degradation of either enantiomer took place during the course of the experiments.

Although replicates of the experiments were performed, the reproducibility of the results was not thoroughly tested because some of the data sets were rejected as invalid when total measured D- and L-isomer quantities did not balance. Two replicate experiments of the adsorption of (D, L) valine at pH 3 gave D:L ratios as follows: strongly adsorbed, 0.977 and 0.979, weakly adsorbed, 0.973 and 0.992, and nonadsorbed, 1.020 and 1.001, respectively.

Even with the knowledge that full recovery of amino acids was obtained and total recovered quantities of D- and L-enantiomers were equal within experimental error, it is difficult to be fully confident that these very small differences in the adsorbed fractions, which suggest preferential adsorption, are real. To determine whether the small differences in D:L ratios could be due to some artifact in the gas chromatographic analyses, enantiomer analysis of some of the samples was performed by liquid chromatography using a reversed phase column and an elution buffer containing a

TABLE VII

Quantities of valine enantiomers measured by gas chromatography in three fractions in adsorption experiments

	Fraction	μM D	μM L	Δ μM	σ μM
	pH 3.0				
	SA	5.81	5.99	−0.18**	0.040
	WA	5.11	5.29	−0.18**	0.070
Exp. A	Total adsorbed	10.92	11.28	−0.36	
	Non adsorbed	37.42	36.98	+0.44**	0.090
	Total	48.34	48.26	+0.08	0.200
	SA	5.66	5.84	−0.18*	0.024
	WA	5.40	5.50	−0.10*	0.034
Exp. B	Total adsorbed	11.06	11.34	−0.28*	0.058
	Non adsorbed	35.23	35.66	−0.43*	0.25
	Total	46.29	47.0	−0.71	0.31
	pH 7.0				
	SA	0.547	0.553	−0.006	0.004
	WA	3.09	3.01	+0.08**	0.031
Exp. A	Total adsorbed	3.64	3.56	+0.086	
	Non adsorbed	43.46	43.64	−0.18	0.240
	Total	47.10	47.20	−0.10	0.275
	pH 10.0				
	SA	0.601	0.599	+0.002	0.006
	WA	2.30	2.31	−0.010	0.024
Exp. B	Total adsorbed	2.90	2.90	−0.008	
	Non adsorbed	43.05	43.05	0	0.24
	Total	45.95	45.95	−0.008	0.27

* D-L > Standard Deviation.
** A statistically significant difference between sample and standard D:L.

copper-L-proline complex (Friebele *et al.*, 1981b). Comparative results from GC and LC analyses of samples from the adsorption experiments are shown in Table VI. In the experiment at pH 7, the D:L ratios of all fractions are very close to 1.0, according to the LC analyses, and the slight predominance of L-α-alanine in the adsorbed fraction suggested by the GC analyses is not confirmed. In the pH 10 experiment, however, both GC and LC analyses suggest a larger quantity of the L-enantiomer in the adsorbed fraction. Thus, the LC data do not consistently confirm or deny the validity of the small preferential adsorption of L-amino acids by clays found with GC.

While clay has no known asymmetric centers, some adsorption studies have been made with a mineral which does have chiral surfaces–quartz. Since quartz does not

have the large surface area or sorptive properties of clay, amino acids are adsorbed in very minute quantities under aqueous conditions. Adsorption experiments with ^{3}H-labeled L-alanine and ^{14}C-labeled D-alanine hydrochlorides in anhydrous dimethylformamide on l- and d-quartz indicate a 1.0–1.8 % greater adsorption of L-alanine by l-quartz, and of D-alanine by d-quartz (Bonner *et al.*, 1974; Bonner *et al.*, 1975). Although consistent results were obtained from repeated experiments and numerous measurements, an indirect method of counting radioactivity of the supernatant or equilibrium solution was used. In further work, Kavasmaneck and Bonner (1977) studied the adsorption of alanine derivatives on l- and d-quartz in various organic solvents. After adsorption of (R, S)-alanine isopropyl ester on l-quartz in chloroform, they analyzed nonadsorbed and 'desorbed' fractions for enantiometric composition by gas chromatography. Differences in the amounts of L- and D-alanine derivatives in the supernatant ranged from 0.5 to 1.5 %. The desorbed molecules were removed in four stages with two different solvents; the largest difference in enantiomers desorbed at any one stage was 8–9 %, but if the quantities in all the desorption stages are totaled, the difference in the total amounts of the two enantiomers desorbed approaches zero.

While quartz does not have the sorptive properties of clay minerals, and adsorption of amino acids on quartz and on clays takes place under different conditions, the studies of enantiomer adsorption on quartz are of interest to this review. The chiral quartz mineral represents an 'optimum' surface for selection of enantiomers, but, according to these studies, preferential adsorption of amino acid isomers on quartz is quite small, if it exists at all. Thus, even if a clay mineral were shown to have a small number of asymmetric centers distributed throughout the layers, the work of Bonner *et al.*, (1974 and 1975), and Kavasmaneck and Bonner (1977) casts some doubt as to whether preferential adsorption of amino acid isomers would occur even on a 'chiral clay'.

2.2.3. *Peptides and Proteins*

The adsorption of peptides has been studied by Greenland *et al.* (1962, 1965a and b). They observed that adsorption increases on montmorillonite and illite at neutral pH with increasing molecular weight of the peptide (from diglycine to tetraglycine), and that there is a linear relationship between calculated free energies of adsorption and molecular weight of the peptides. Also, as peptides increase in molecular weight the entropy factor becomes favorable to adsorption, and for a peptide as large as tetraglycine, it becomes positive due to desorption of water molecules. The desorption of water molecules from the adsorbed phase is indicated by a decrease in the interlayer water volume detected by X-ray diffraction.

Hsu (1977) found that an L-alanine tripeptide was adsorbed in greater amounts than an L-alanine dipeptide at low pH on different cationic montmorillonites. He attributed the greater adsorption to increased van der Waals forces of the larger molecule and to a more favorable entropy effect per mole for the larger peptide induced by desorption of more water molecules.

Using X-ray diffraction, Hsu (1977) determined that single amino acids and the alanine dipeptide form a single layer complex on the clay, but that the alanine tripeptide forms single and double layers. A characteristic of peptide adsorption isotherm on Fe- and Mg-montmorillonites is a plateau of adsorption occurring at a value less than cation exchange capacity of the clay – possible evidence for a complex between the peptides and a multivalent cation.

Studies of adsorption of proteins on clay minerals by cation exchange (McLaren *et al.*, 1958; Armstrong and Chesters, 1964; Albert and Harter, 1973) have shown that the adsorption is dependent on solution pH, with highest adsorption occurring around the isoelectric point of the proteins. Thus, protein adsorption on clay differs in this respect from amino acid adsorption, which is greatest at a pH lower than the isoelectric point. An explanation of the high adsorption of proteins at a pH near the isoelectric point is that the positive charge density of the protein molecules is less at the isoelectric pH than at lower pH.

The release of a corresponding quantity of sodium ions upon the adsorption of proteins on Na-montmorillonite, na-illite, and Na-biotic vermiculite (Albert and Harter, 1973) indicates a cationic exchange mechanism for adsorption. Hsu also described adsorption of a (D, L)-alanine polypeptides by cationic exchange, noting a decreasing adsorption with decreasing pH, and also a slight decline at alkaline pH. The plateau in adsorption occurred at only 20% cationic exchange capacity of the clay. He postulated that ion exchange occurs with the terminal NH_3^+ group anchoring the polypeptide. The remainder of the polypeptide chain is attracted to the clay surface by van der Waals forces and covers many exchange sites. Hsu detected double and triple layers of polyalanine adsorbed on Na-montmorillonite and only single layers on the Mg^{++} and Fe^{+++} forms, presumably because of the stronger attractive forces between layers in the latter two. Armstrong and Chesters (1964) observed bentonite layer expansions up to 64 Å, indicating two layers of adsorbed lysozyme.

Important points of adsorption of peptides and proteins on clays in regard to chemical evolution are: (a) larger peptides are adsorbed more easily than smaller ones; (2) adsorption is greatest at a pH near the pI of the peptide or protein molecule; and, (3) clays have a large capacity for adsorbing proteins and peptides, and they expand to incorporate double layers of the macromolecules in the interlayer space. Thus, clays could have concentrated peptides and proteins in the primordial ocean. The extent of concentration depends upon whether the isoelectric points of the peptides or proteins in question were near the neutral or slightly alkaline pH of the primordial ocean. Larger peptides would be adsorbed preferentially over smaller peptides – a fact which could affect the sequence of events in chemical evolution.

2.2.4. *Nucleic Acid Bases, Nucleosides, and Nucleotides*

Nucleic acid bases are adsorbed on clays in much the same way as amino acids. The adsorption reaction is pH dependent, with cationic exchange being the major mechanism of adsorption at low pH. Proton transfer also plays an important part in the acidic and neutral pH ranges (Lailach *et al.*, 1968a). Adsorption of the bases can

occur through interactions with inorganic or transition metal ions on the clay surface at higher pH (Lailach *et al.*, 1968a and b), with differences in the aromatic character of the bases providing selective associations through resonance structures with these cations. In addition, the size and shape of the molecules partially determined the quantity of adsorption on clays. Of course, van der Waals forces will be greater for larger molecules and will increase their interaction with the clay surface, but adsorption of larger molecules may also be inhibited because of steric limitations. Thompson and Brindley (1969) studied the adsorption of nucleic acids and nucleosides on illites and found the same adsorption mechanisms occurring as an montmorillonite. The degree of adsorption depends upon the pK_a of the compound, the solution pH, and the inorganic cation on the illite. In contrast to the adsorption isotherms for montmorillonite, however, there is a definite maximum adsorption for the nucleic acid bases and nucleosides on illite. The pH at which maximum adsorption occurs on each cationic form of illite varies linearly with pK_a of the bases (Figure 5), there being no dependence upon the structure of the molecules being adsorbed. It was observed, however, (Figure 5) that the maximum adsorption depends upon the organic molecular constitution as well as on pK_a, with purine nucleosides being adsorbed more than pyrimidine nucleosides. It was suggested that the larger size of purine molecules leads to greater adsorption because of van der Waals forces (Thompson and Brindley, 1969).

The biological nucleic acid bases are not adsorbed equally on clays. Thymine, uracil, and their nucleotides and nucleotides are not adsorbed on montmorillonite (Shaw, 1965; Lailach *et al.*, 1968a and b), while other bases, nucleosides and nucleo-

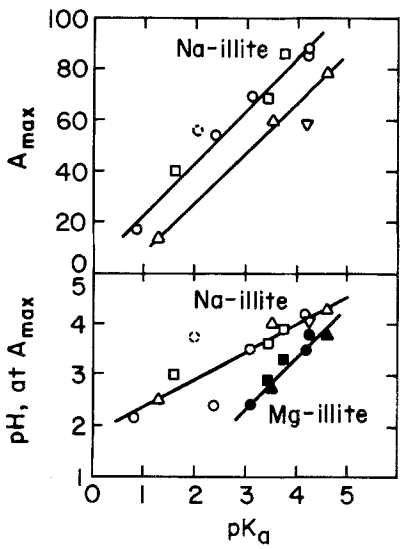

Fig. 4. Relationship between pH giving maximum adsorption, A_{max} the maximum adsorption, and pK_a values of compounds used. ○, purines; △, pyrimidines; □, purine nucleosides; ▽, pyrimidine nucleoside – From Thompson and Brindley, 1969.

tides are adsorbed. Shaw found that only adenine triphosphate (ATP) among four nucleotides (ATP, GTP, CTP, and UTP) was adsorbed on the clay surface at low pH. (Otroshchenko and Vasilyeva (1977) observed that uracil nucleotides are adsorbed on volcanic basalt in smaller quantities than other nucleotides.) These observations can be explained by the difference in chemical structure of these bases: uracil and thymine have no amino groups, while the other bases do. As a result, the pK_a's of thymine and uracil are extremely low (0 and 0.5 respectively), and they cannot be adsorbed in the pH range between 1.7 and 11 (Lailach *et al.*, 1968a). The differences in basicity of the nucleic acid bases affects not only the cationic exchange reaction, but also the formation of ion-organic complexes on the clay surface at a pH above 5, since the more basic molecules form stronger complexes with ions on the clay (Lailach *et al.*, 1968b).

The specific adsorption of adenine nucleotides observed by Shaw was supported by the work of Odom *et al.* (1979). They reported that adenine nucleotides are adsorbed in greater quantities than thymine nucleotides on different homoionic clays containing monovalent, divalent, and metal ions, an observation that may have a potential significance for the origin of the genetic code. It is interesting to note the coadsorption of bases by montmorillonite. Lailach and Brindley (1969) found that in the presence of adenine, thymine and uracil are adsorbed on montmorillonite by hydrogen bond formation with adenine. Measurements of the basal spacing of the clay layers containing these bases indicated the presence of a single layer of cation-base assemblages; thus, the bases could not be stacked upon each other. They are associated through horizontal hydrogen bonding instead. The co-adsorption on the clay surface, together with the preferential ATP adsorption observed by Shaw (1965), leads to an interesting consideration: that only the ATP-UTP or ATP-TTP pairs which are hydrogen bonded can be adsorbed on clay minerals and might form poly (A) and poly (U) (also poly (AU), or poly (A) and poly (T) (also poly AA)). There has been speculation that adenine and uracil were more abundant than guanine and cytosine on the primitive Earth (Crick, 1968) and that the initial genetic code was based on a poly (AU) nucleotide rather than poly (AUGC) (Ishigami 1974).

Nucleosides and nucleotides are adsorbed much less than nucleic acid bases (Lailach *et al.*, 1968a and b; Thompson and Brindley, 1969). This is because the nucleic acid bases, which are planar molecules, are more easily intercalated into the interlayer space than the non-planar, anionic nucleosides and nucleotides (Lailach *et al.*, 1968b). Nucleosides are adsorbed more on Li- and Na-montmorillonites, which have more widely dispersed layers than clay systems containing divalent cations (Lailach *et al.*, 1968b). Some of the nucleotides (especially ATP) can be adsorbed on the clay surface in spite of their anionic character and large size. It is known that an anionic polymer, fluvic acid, is adsorbed on montmorillonite, at both interlayer and external sruface positions (Schnitzer and Kodama, 1966 and 1967).

Graf and Lagaly (1980) studied the adsorption and desorption of ATP, ADP, and AMP on various clays and silicates by measuring flourescence of the equilibrium solutions to which luciferase had been added, after dilution with water and/or buffer. They found that the recovery rate of AMP (the amount not adsorbed) was con-

siderably higher than that of ATP. They concluded that clays exhibit a strong adsorptive preference for ATP over AMP. In comparison, Odom *et al.* (1979) noted that the adsorption of ADP is significantly higher than that of AMP on Na-, Ca-, and Mg-bentonites and kaolinites.

Anion exchange of phosphate ions for hydroxyl groups linked to aluminum ions at the clay edges was proposed as the major mechanism of adsorption of nucleotides (Graf and Lagaly, 1980). The difference in the quantities of AMP and ATP adsorbed were explained by the difference in the distances between the terminal phosphate and bulky adenosine group in the two molecules. In AMP, steric hindrance could affect the anion exchange.

If AMP and ATP are both present with the clay, there seems to be a cooperative interaction during adsorption: the adsorption of AMP is remarkedly increased by the presence of ATP. Graf and Lagaly (1980) suggested that adsorbed ATP molecules enlarge the edge-edge and edge-face distances in the clays, creating new sites for AMP adsorption. These studies were performed with clays coagulated in high NaCl concentrations in order to avoid problems with clay particles in spectrophotometric measurements. The effect of this condition on the adsorption of these molecules is unknown, but it must certainly decrease the number of available adsorption sites available to the molecules in solution. Although the coagulated condition of the clays does not have much relevance to the condition of clays in the primitive ocean, where the clays were probably dispersed and suspended, it might simulate a drying pond, lake, or shoreline.

2.2.5. *Fatty Acids*

Hedges (1977) discovered that stearic acid is adsorbed on kaolinite and montmorillonite to a much greater degree than L-valine or D-glucose. This was attributed to the lower solubility of fatty acids, which is increased in saline solutions such as seawater. Apparently, the stearic acid simply precipitates on the clay particles.

2.2.6. *Concentration of Organics by Clay Minerals*

To what extent can organic compounds in a dilute solution such as the primordial ocean be concentrated by clay minerals? The concentration factor will depend upon such factors as particle size and surface area of the clay, cation exchange capacity, and initial concentration of solute in the aqueous phase. Although the concentration of organic molecules in the primitive oceans was probably variable and is difficult to estimate, we may calculate a concentration factor using the results of amino acid adsorption experiments. At pH 7, a total of 6.6 mM of α- and β-alanine was strongly adsorbed by 200 mg of Na-montmorillonite (See Table III). We can take a moderate value of 700 m^2/g for the clay surface area and assume a monolayer of the amino acid molecules lying in the interlayer position, whose spacing is approximately 5 Å. Thus, the interlayer volume of the 200 mg clay used is 3.5×10^{-2} cm^3 (considering the edge area insignificant). With 6.6 mM of α- and β-alanine adsorbed, the interlayer concentration is 188 mM/cm^3. The initial concentration of alanine molecules is 200 mmoles

in 40 ml of solution, or 5 mM/cm^3. Therefore, the concentration factor by cation exchange on the clay mineral is roughly 38 times.

If we also consider the amount adsorbed by physical forces, the concentration is even greater. According to Table III, the quantity of α- and β-alanine in the weakly adsorbed fraction is 24.4 mM. This is equivalent to an interlayer concentration of 700 mM/cm^3, and a concentration factor of 140 times. Thus the combined concentration factor due to the mechanisms of cation exchange and physical forces at neutral pH is approximately 180 times.

The capacity of clays to concentrate nucleic acid bases is even higher than concentration of amino acids. This is due to the high pK values of certain bases. Lailach *et al.* (1968a) found that 10–80 % of adenine, adenine derivatives, and cytosine present in solution was adsorbed by Na-, Ca-, Mg-, and Li-montmorillonites at pH 7–8. Using the same surface area and interlayer dimensions, we can calculate that the bases were concentrated from 1 μM/cm^3 to 150 to 1200 μM/cm^3, depending upon pH. This is equal to a concentration factor of 150 at pH 8 and of 1200 at pH 7. This data shows that montmorillonite can concentrate more adenine and cytosine on a molar basis than neutral amino acids. In addition, if uracil and thymine were adsorbed by co-adsorption, as the evidence of Lailach and Brindley (1969) suggests, this phenomenon could be an important factor in the question of whether polynucleotides or proteins evolved first in the protein synthesis system. Of course, we are assuming that the bases and amino acids were present in the primitive oceans at similar concentrations. If this were not the case, then clays might have compensated for lower concentrations of nucleic acid bases in the oceans by concentrating them at a higher rate.

2.3. Polymerization

The formation of biologically important macromolecules such as peptides and polynucleotides from monomeric units requires dehydration-condensation reactions, which would be unfavorable in the seas of the primitive Earth where life is postulated to have begun. These macromolecules are thermodynamically less stable than the corresponding monomers under aqueous conditions (Lehninger, 1970), and the presence of water shifts the reaction equilibrium toward the reactant monomers.

The condensation of amino acids can be aided by several energy sources or chemical agents. Although high energy input by ultraviolet light, electric discharge, and heat on the primitive Earth would promote polymerization, it would also accelerate the decomposition of the molecules produced. However, adsorption of these molecules on the clays would afford them protection from degradation. Condensing agents such as hydrogen cyanide derivatives and polyphosphates can also promote the condensation reaction. Condensing agents react with amino acids to produce activated intermediates, in which the carboxyl carbon has a net positive charge. This intermediate subsequently undergoes nucleophilic attack by the amino nitrogen of a free amino acid, resulting in peptide bond formation. Classified with the condensing

agents are the amino acyl adenylates, activated amino acids similar to activated intermediates formed in the presence of polyphosphates.

It is possible that clays might play several roles in promoting the reactions which produce macromolecules. The first role is that of catalyst; i.e., the surface of the clay somehow initiates or promotes condensation reactions. Fripiat and Cruz-Crumplido (1974), reviewing clays as catalysts, suggested that amino acid polymerization on clays can proceed via carboxyl activation. This can occur (on kaolinite, at least) by the reaction of an ionized carboxyl group with an Al^{+3} or $(AlOH)^{+}$ ion, followed by the attack of an amino group. Fripiat and Cruz-Crumplido also described the importance of the H_3O^{+} ion (generated by the cationic electrostatic field on the clay surface) in the formation of amino acid zwitterions which can polymerize when heat is added. A second function is to immobilize and organize adsorbed molecules and polymerization products on the clay surface, and in so doing, to protect the reaction products from decomposition. Finally, it is thought that clays might cause preferential polymerization, due to selective interactions with adsorbed monomers and steric restrictions in the interlayer space. The following discussion of polymerization of biological monomers in the presence of clays will be organized according to the energy source or chemical agents used to promote polymerization.

2.3.1. *Polymerization of Amino Acids*

We will begin with the simplest aid to the condensation reaction: heat. As discussed in the previous section, the zwitterion form of amino acids adsorbed on the clay surface may undergo peptide formation when the clay-amino acid complex is heated to temperature of 140 to 244°C. (Fripiat *et al.*, 1966). Degens and Matheja (1970) reported the formation of polymers containing aspartic acid and glutamic acid from mixtures of amino acids and kaolinite heated at 80°C for seven days. In other experiments conducted at 140°C for short (63 hr) and long (90 days) time periods, polymeric products with molecular weights ranging from 1000 to 2000, and containing principally asp and glu were formed in a mixture of 10 amino acids and kaolinite. Kaolinite was found to be a much more effective 'catalyst' than montmorillonite in this experiment. Other researchers (Flores and Bonner, 1974; McCullough and Lemmon, 1974) have been unable to obtain polymerization of amino acids with kaolinite using heat as a source of energy. Degens and Matheja theorized that the apparent peptide bond formation is brought about by carboxyl activation, i.e., the positively charged Al octahedral surface attracts the carboxyl group of the amino acid, which becomes activated and undergoes subsequent attack by an amino group to produce the peptide bond.

Simulating a simple fluctuating primitive geologic system, Lahav *et al.* (1978) subjected mixtures of glycine and Na-kaolinite or Na-bentonite to wetting-drying and temperature fluctuations (25°–94°C) for a number of cycles and observed the formation of oligo-peptides up to five glycine residues in length. Only trace amounts of diglycine formed in heated mixtures without clays. Wetting and drying cycles, when added to temperature fluctuations, enhanced the quantity and the chain length of

peptides formed. They suggested that the monomers and peptides were redistributed on the clay during the wetting cycle, thus allowing greater contact between monomers and peptides for further polymerization. Lawless and Levi (1979) studied the effect of different cationic forms of bentonite in this reaction during wetting-drying/heating. The effectiveness of the cations as 'catalysts' is in the order $Cu^{++} > Ni^{++} > Zn^{++} > Na^{+}$. However, the fact that these metal forms of clays are very rare in nature diminishes the similarity between conditions in these experiments and those in a primitive tidal lagoon or small lake.

White and Erickson (1980) investigated the effect of another type of catalyst, the dipeptide histidyl-histidine, in the oligomerization of glycine during fluctuating moisture and temperature cycles on kaolinite. Up to 52 nM of additional glycine were polymerized per nM of histidyl-histidine used – definite proof of catalytic action. The proposed reaction mechanism is the formation of an amino acid imidazolide by the rection of the imidazole ring and activated amino acyl groups provided by the wetting/drying cycles of amino acids on the clay surface. The activated intermediate then reacts with the amino group of a free amino acid to produce the peptide bond, releasing the catalyst.

White and Erickson noted that increasing the quantity of catalyst does not enhance the oligomeization reaction; they suggested that the yield of glycine oligomers might be limited by the number of glycine monomers activated by the clay surface.

Flegmann and Scholefield (1978) analyzed the capacity of clays in polymerization reactions using theoretical and experimental approaches. They heated amino acids with and without kaolinite at 90°C for 30 to 60 days and observed no polymerization taking place. They then evaluated the thermodynamic feasibility of condensation reactions on clay surfaces by calculating the free energy of condensation in solution and comparing that to the free energy of the ion exchange reaction that would replace an amino acid monomer on the clay with a dipeptide. Their conclusion was that the thermodynamic barrier to 'surface condensation' at 90°C is not lower than that for solution condensation. However, they did not evaluate the thermodynamics of condensation actually taking place on the clay, probably because a specific mechanism whereby the clay would lower the energy requirement for condensation is not known.

Aminonitriles, which have been known as one of the products of abiotic syntheses in simulated primordial atmospheres, can produce amino acids upon hydrolysis (Ponnamperuma and Woeller, 1967) and can also form peptides without taking a pathway to amino acids. Akabori *et al.* (1956) proposed that polymerization of aminoacetonitrile can take place on a solid surface, followed by hydrolysis to polyglycine and ammonia. Then side chains could be introduced into the polyglycine. Hanafusa and Akabori (1959) demonstrated the formation of di- and tri-glycines by heating aminoacetonitrile at 120°–140°C for several hours in the presence of kaolinite. In similar work. Losse and Anders (1961) synthesized an alanine polymer from α-aminopropionitrile by heating it in the presence of acidic daly (the equivalent of H-montmorillonite). Hanafusa and Akabori stated that the solid surface of the clay seems to play a role in this reaction, since no amino acids were formed in the absence of clays.

Akabori *et al.* (1956) introduced side chains into polyglycine dispersed on kaolinite using aldehydes, a basic catalyst, and heat, obtaining serine and theonine residues after acid hydrolysis. They postulated that the methylene group of polyglycine adsorbed on the clay is more susceptible to the condensation reaction with aldehyde, yielding β-hydroxy amino acid residues, than polyglycine in solution.

Degens and Matheja (1970) reported the formation of peptides (but no free amino acids) after heating mixtures of urea, kaolinite and other organic molecules such as succinic acid, propionic acid, glycol, and glycerol. The amino acid composition of the peptide products showed a dependence upon the organic molecules added.

In different experiments, Ventilla and Egami (1977) heated formaldehyde and hydroxylamine to 100°C in solutions of transition metal ions with and without kaolin, and obtained amino cids and their oligomers. There was little difference in the quantity of products formed when kaolinite was present and when it was absent; thus kaolinite apparently has no catalytic function in this reaction.

Thus far, in simple systems where amino acids are heated in the presence of clay, the empirical results lead to confusion about the role of clay in popypeptide formation. Certainly, the type of reactants, the conditions used, and treatment of the clay prior to experiments (Hawthorne and Solomon, 1972) will affect the quantity and quality of polymerization products. The fact that peptides can be formed by heating amino acids at 65–85° for a period of days (Rohlfing, 1976) weakens the postulate that the presence of clays is required for peptide formation to occur in heated aqueous systems.

2.3.2. *Preferential Polymerization*

The question of preferential polymerization of free amino acid enantiomers in the presence of kaolinite and thermal energy has been raised. Degens et al. (1970) reported that 25 % of L-aspartic acid was polymerized, while only 3 % of the D-isomer and 14 % of the racemic mixture were polymerized in aqueous solutions heated to 90°C for 32 days in the presence of kaolinite. The quantity of polymerized amino acids (determined by biuret and total amino acids – free amino acids) fluctuated in each group over time. For instance, the quantity of material polymerized from L-, D-, and (D, L) aspartic acid after 21 days was only about 1 % for all three. To support their results, Degens *et al.* (1970) and Jackson (1971a and b) presented results indicating the preferential adsorption of L-phenylalanine (already described) and also the preferential polymerization of L-aspartic acid and L-serine, over the corresponding D-isomers on kaolinite. This preferential polymerization of aspartic acid in the presence of kaolinite was re-examined by Flores and Bonner (1974), who determined enantiomeric composition of fractions extracted from clay, and by McCullough and Lemmon (1974) who used L-asp-2-C^{14} to search for polymer formation and also measured optical rotation of the supernatant. These investigators could observe neither the preferential adsorption of L-aspartic acid nor any peptide formation under the same experimental conditions used by the former investigators. The failure of other researchers to repeat the preferential polymerization under identical conditions

emphasizes the importance of obtaining results by a direct confirmation of the compounds of interest isolated from the reaction mixture. Although claims of a large preferential adsorption and polymerization of amino acid enantiomers on clays have been made, these experimental results are difficult to explain in terms of clay structure and clay-amino acid interactions.

Although heat is the most 'natural' condensing agent, chemical agents such as cyanate derivatives (formed in prebiotic synthesis experiments) or polyphosphate also initiate the condensation reaction. One experiment incorporated both types of condensing agents, with the naturally occurring mineral, apatite ($CaPO_4OH$) as the source of phosphate. Flores and Leckie (1973) obtained dipeptides by heating cyanate, apatite, and glycine in the solid state at 95°C for a period of days. Probably condensation was brought about by formation of an amino acyl phosphate intermediate. Similar activated molecules are the amino acyl adenylates, which contain a phosphoanhydride bond between the carboxyl group of an amino acid and the phosphate group of the adenosine monophosphate. These compounds, found in contemporary living systems, are precursors of polypeptides in the protein synthesis system.

Taking a clue from the biosynthetic pathway of proteins, Lewinsohn *et al.* (1967) and Paecht-Horowitz and Katchalsky (1967) proposed a role for aminoacyl adenylates in the prebiotic formation of polypeptides under neutral to alkaline conditions at room temperature. Banda and Ponnamperuma (1971) obtained peptides from the condensation of amino acid adenylates in alkaline solution. These studies were extended with the use of montmorillonite (Paecht-Horowitz *et al.*, 1970; Paecht-Horowitz, 1971, 1973, 1974). In the absence of the clay mineral, polycondensation took place, producing peptides up to 12 monomeric units long. The presence of montmorillonite resulted in not only a higher yield of the polymers but also longer carbon skeletons. In the copolymerization of alanyl adenylate and seryl adenylate, peptides up to approximately 100 units long were reported. The polymerization on montmorillonite was further distinguished by the presence of a discrete series of peptides, which was not found in the absence of the clay mineral.

Paecht-Horowitz *et al.* proposed different mechanisms for polycondensation in the presence and absence of montmorillonite, based on the size distribution of the peptides synthesized. They postulated that in the presence of the clay mineral, the aminoacyl adenylates react to produce polymers without being hydrolyzed, whereas in the absence of the clay mineral the hydrolysis of the adenylates is a necessary step to initiate the reaction. Furthermore, they suggested that the aminoacyl adenylate is adsorbed on the face of the clay close to the edge, with the adenylic acid group protruding from the clay interlayer space. As polymerization proceeds, the lengthening peptide chain 'creeps' over the face of the clay and remains protected from hydrolysis. Studies in which the face of the clay was completely covered with histidine, and the edge covered with a hexametaphosphate, showed that the amino acid adenylate must be attached to the clay at two points in order for maximum polymerization to occur: by the NH_3^+ group at the face of the clay, and by the phosphate group at the edges of the clay (Paecht-Horowitz, 1977).

After studying the copolymerization of a variety of amino acid adenylates on montmorillonite, Paecht-Horowitz (1973) observed a selective interaction; i.e., that peptide bonds between certain amino acids occurred at a higher frequency than others. It was suggested that montmorillonite could have been a prebiotic template for polypeptide synthesis which 'selected' peptide sequences. However, Steinman and Cole (1967) also observed different frequencies of amino acid sequences (corresponding closely with those observed in proteins) in dipeptides formed in solution in the absence of clay with the condensing agent dicyanamide. Thus, whether or not montmorillonite plays a role in determining peptide sequence during polymerization is unclear.

Paecht-Horowitz and Katchalsky (1973) have reported further that the formation and polymerization of alanyl adenylate is possible from a mixture of free alanine and adenosine triphosphate (ATP) in the presence of montmorillonite and zeolite (Decalso F) at neutral pH at 37°. They concluded that Decalso F aided the synthesis of alanyl adenylate, and that montmorillonite catalyzed subsequent peptide formation. However, Warden *et al.* (1974) have presented negative results for adenylate synthesis and peptide formation under the same conditions as those in experiments performed by Paecht-Horowitz and Katchalsky (1973). The only difference in the experiment by Warden *et al.*, was the use of Decalso instead of Decalso F. Apparently, since Warden *et al.* (1974) observed polymerization of preformed amino acyl adenylates on montmorillonite, aminoacyl adenylates are necessary for the formation of peptides in this system, rather than a mixture of amino acids and ATP.

2.3.3. *Polymerization of Nucleotides*

Paecht-Horowitz (1974) reported no polynucleotide formation in the same experiment in which peptides were synthesized from aminoacyadenylates. Apparently, polynucleotide synthesis also requires activated molecules or condensing agents. Burton *et al.* (1974) studied the condensation of nucleoside phosphoramidates, activated derivatives which should easily form phosphodiester bonds, on montmorillonite. Upon heating the dried clay-phosphoramidate complex, they obtained small quantities of dinucleotides; however, no polymerization occurred in the presence of water, and the presence of the clay seemed to have no qualitative or quantitative effect on the reaction. Ibanez *et al.* (1971) investigated the formation of oligonucleotides in the presence and absence of montmorillonite by reacting a mixture of thymidine monophosphate (TMP) and cyanamide at neutral pH at about 90°C. In this experiment, cyanamide catalyzed the formation of oligomers up to four units in length in the absence of montmorillonite. However, oligomers up to five units were detected in the presence of the clay mineral, with a lower yield of di- and trinucleotides. These results suggest that the role of montmorillonite is to aid in the synthesis of longer chain oligomers at the expense of shorter chains. It is likely that the oligomerization reaction requires the presence of cyanamide even when montmorillonite is used.

Most available studies show that clays along do not act as 'catalysts' or condensing agents in the polymerization of free amino acids or nucleotides under aqueous con-

ditions. Clay minerals can provide protons, acting as a Bronsted acid, and they can accept electrons as a Lewis acid in the catalysis of organic chemical reactions (Solomon, 1968; Hawthorne and Solomon, 1972). Clays do not affect the unfavourable net fill energy of dehydration reactions although they may effect activation barriers. The energy barrier of the reaction must be lowered, either by using activated monomers or condensing agents, or adding energy. If polymerization is initiated by the presence of a condensing agent or energy source, the effect of clay minerals is apparently to promote the reaction by protecting the reaction products and providing a space in which there is greater concentration and interaction between reactants. Thus, polymers having longer chain lengths are formed when clays are present than when the reaction takes place in the absence of clays.

In a very thoughtful paper, Lahav and White (1980) have discussed the possible role of clays in the formation of semi-ordered oligomers, and the process by which template directed replication of the oligomers might take place in the clay interlayer space. They do not attribute the role of catalyst to the clay in the polymerization of monomers on the clay surface, but merely see it as a system of fluctuating temperature and moisture in which molecules are protected and redistributed, and in which longer molecules are selectively retained. 'Order', or consistent differences in the oligomer sequence, would result from selective interaction between the monomers themselves. Lahav and White do suggest that clays might contain some structural heterogeniety which might act directly as templates, repeatedly producing certain sequences of amino acids in peptides. Although this suggestion was also made by Cairns-Smith (1971), there are no experimental results to confirm this. Another idea which Lahav and White put forward is the enhancement of oligonucleotide condensation on clays by the presence of oligopeptides, which have been shown to form on clay surfaces. Additionally, template directed synthesis of peptides could occur with oligonucleotides adsorbed on the clay surface. Experimental evidence indicating that homopolyribonucleotides enhance the condensation of amino acids in a fluctuating clay environment will soon be published (White and Erickson, 1980, as quoted by Lahav and White, 1980). However, it is never suggested that clay minerals are direct catalysts in condensation reactions, only that they provide a better environment, including some order or structure, in which polymerization reactions are likely to occur.

3. Conclusion

This review has described and evaluated the information that is presently available on the interaction between clay minerals and the most important organic molecules in biological systems. In summary, the role of clays in the origin of life, according to the experimental results to data, can be described as follows. First, clay minerals facilitate, aid, or speed synthesis reactions in which biological monomers are formed from gaseous constituents of the primordial atmosphere. The presence of clays does not change the direction of the reaction path but apparently promotes the synthesis

reaction. Second, clay materials do adsorb and concentrate by several orders of magnitude, these monomers from aqueous solution. The adsorption of amino acids and nucleic acid bases occurs to a maximum degree by cation exchange at low pH, but adsorption is appreciable at neutral to slightly alkaline pH, where other adsorption mechanisms such as hydrogen bonding and zwitterion association predominate. The latter condition is more similar to conditions which are thought to exist in the primitive oceans. Selective adsorption of biological monomers occurs only if there are large differences in the isoelectric points of the monomers. Thus, basic molecules are adsorbed more than neutral or acidic ones; no distinction is made in the adsorption of biological and non-biological molecules. Although several researchers have claimed to observe preferential adsorption of amino acid and sugar isomers, attempts to repeat their work have been unsuccessful. In thorough, careful work on this problem, there has been no conclusive evidence of asymmetric adsorption of isomers by clays. Finally, clays probably promote polymerization of biological monomers by concentrating them, providing containment and a surface for immobilization of the reacting molecules, and protection for reaction products in the interlayer space. There is no evidence of catalysis or direct participation in condensation reactions between biological monomers by clays.

The role of clays in chemical evolution envisioned by Bernal as selective adsorber, concentrator, catalyst for polymerization, and organizer of molecules for their replication has been only partially borne out by experimental research. The data yielded from laboratory experiments shows that clays are ionically charged surfaces where exchange of inorganic cations for organic cations in solution takes place. Organic molecules are also adsorbed by hydrogen bonding, ion-dipole interactions, or van der Waals forces, becoming more concentrated on the clay surfaces than in the surrounding aqueous medium. The reactions of chemical evolution producing first monomers and then polymers might have been promoted by clays simply because of the greater proximity of the concentrated molecules adsorbed on clays and the redistribution of reactants and reaction products by the fluctuating wet/dry cycles of clay environments. The possible role of clays in selecting oligomer sequences, promoting condensation of mononucleotides, and acting as a template to direct replication of those sequences is clearly a possibility which needs to be reserched more fully in the laboratory. The interaction between peptides and oligonucleotides and their monomers in the confines of the clay interlayer spaces is an especially interesting area of chemical evolution which should be studied.

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