pH PROFILE OF THE ADSORPTION OF NUCLEOTIDES ONTO MONTMORILLONITE

I. Selected Homoionic Clays

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Abstract. The effect of adsorbed ions and pH on the adsorption of several purine and pyrimidine nucleotides on montmorillonite was studied. The cations used to prepare homoionic montmorillonite were Na^+, Mn^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+}. The nucleotides studied were 5'-, 3'-, and 2'-AMP, and 5'-CMP in the pH range 2 through 12. The results show that preferential adsorption amongst nucleotides and similar molecules is dependent upon pH and the nature of the substituted metal cation in the clay. At neutral pH, it was observed that 5'-AMP was more strongly adsorbed than 2'-AMP, 3'-AMP, and 5'-CMP. Cu^{2+} and Zn^{2+} clays showed enhanced adsorption of 5'-AMP compared to the other cation clays studied in the pH range 4–8. Below pH 4, the adsorption is attributed to cation and anion exchange adsorption mechanisms; above pH 4, anion exchange may also occur, but the adsorption (when it occurs) likely depends on a complexation mechanism occurring between metal cation in the clay exchange site and the biomolecule. It is thus proposed that homoionic clays may have played a significant role in the concentration mechanism of biomonomers in the prebiotic environment, a prerequisite step necessary for the formation of biopolymers in the remaining steps leading to the origin of life.

1. Introduction

It is widely accepted that the origin of life process began with the formation of important biomonomers from simpler molecules contained in the prebiotic environment (Miller and Orgel, 1974). This thesis is supported by a large body of experimental results (Miller and Orgel, 1974; Lemmon, 1970; Day, 1984). The subsequent steps of chemical evolution are equally significant and may include *selection* of a small group of biomonomers from those available, *concentration* of the biomonomers, and the *condensation* of these biomonomers to bipolymers of increasing complexity and information content.

Although the organic chemistry of the putative primitive Earth environment has been the subject of extensive experimental investigation, the influence of inorganic chemistry on prebiotic organic chemistry has not been well studied. However, an

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extensive literature on the coordination of metal ions and nucleotides exists and has been reviewed (Barton and Lippard, 1978; Marzilli *et al.*, 1978; Martin and Mariam, 1979). Because inorganic chemistry, manifested through complexation reactions of metal cations, is an essential feature of the structure, stability, and functioning of biomolecules involved in today's life processes, it is reasonable to hypothesize that they were essential to the very first biomolecules, the precursors of those complex ones now observed.

Clays are inorganic minerals with excess surface charges, acting as natural ion exchangers. They contain accessible metal cations held between alumino-silicate layers of the clay crystal. The ubiquitous clay minerals are found in meteorites (Bunch and Chang, 1980), are believed to be present in primitive environments, such as the planet Mars (Toulmin *et al.*, 1977; Banin and Rishpon, 1979), and are detected early in the geological history of the Earth (Rutten, 1971). Bernal (1951) was the first to suggest that clays may have played a significant role in chemical evolution, both as a surface upon which to concentrate biomonomers and as a catalyst in subsequent condensation reactions. More recently this has been the focus of other investigations in the field (Lahav and Chang, 1976; Theng, 1974; Paecht-Horowitz *et al.*, 1970; Paecht-Horowitz, 1973; Ostroschenko and Vasilyeva, 1977; Friebele *et al.*, 1980; Lawless and Levy, 1979; White and Erickson, 1980), and the thesis was expanded in a book by Cairns-Smith (1982).

Most clays with monovalent cations do not readily adsorb nucleotides at the nearly neutral pH (7.5 ± 1) thought to have prevailed in the primitive ocean or in other, smaller water bodies. However, Lailach and Brindley (1969) and Lailach *et al.* (1968) have shown that homoionic montmorillonite clays containing divalent cations adsorb significantly larger amounts of various nucleosides and their corresponding bases. Similarly, it has been shown that montmorillonite containing divalent exchangeable cations may have high adsorption capacity for 5'-nucleotides (Lawless and Edelson, 1980). In that study it was found that montmorillonite with an alkali metal at the exchange site (Na^+, Li^+, or K^+) showed no adsorption of 5'-nucleotides, but montmorillonite with a divalent cation, especially Zn^{2+} and Cu^{2+}, at the exchange site did show adsorption. These adsorption studies were conducted at pH 7.5 only.

In this and the following paper we report the results of a more thorough investigation of the adsorptive interaction of nucleotides with montmorillonite clay over a wide pH range (2–12). The first paper deals with the behavior of various nucleotides interacting with one homoionic clay (Zn-montmorillonite) and the interaction of several homoionic clays with one nucleotide (5'-AMP); the second paper focuses on clays having controlled mixed ionic composition (Fe + Ca) reacting with 5'-AMP. The results increase the understanding of the mechanisms of clay-nucleotide interaction and lend credence to the thesis that clays could have played a significant role in the selective concentration of biomonomers in the primitive environments on Earth.

2. Experimental

2.1 MATERIALS

Montmorillonite of the Wyoming bentonite type was obtained from Fisher Scientific (Pittsburgh, PA) and was primarily in sodium form. The nucleotides 5'-AMP, 3'-AMP, 2'-AMP, and 5'-CMP were obtained as the di-sodium salt from P. L. Biochemicals (Milwaukee, Wis.). The metals used were in the chloride form and were of analytical reagent quality (Fisher Scientific). All aqueous solutions were prepared using doubly distilled, ion-exchanged water. Any other reagents used were of the highest commercial purity available.

2.2 PROCEDURES

Exchange of the zinc into the cation-exchange sites of montmorillonite clay was done using two methods of preparation. The first is the quantitative ion-exchange process for clays of Banin (Banin, 1973; Gerstl and Banin, 1980). In future discussions it is referred to as the 'titration' procedure. This method of clay preparation is described in detail in Part II of this series (Banin *et al.*, 1984). In addition to zinc-clay, the following metal substituted clays were prepared by the titration method: Na^+, Mn^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, and Cu^{2+}. The second method of clay preparation is referred to as the 'leaching' procedure. In this method, a suitably sized sample of montmorillonite (5–10 g) was repeatedly washed with the desired 0.5 M metal halide solution. The clay was then washed with distilled water until a negative test for chloride was obtained in the washings (Lawless and Edelson, 1980). The clays produced by both procedures were freeze dried and stored in powder form. After stripping the metal cation from the clay with 1.0 M ammonium nitrate, the exchangeable cation content of each clay was determined by atomic adsorption spectrometry or by X-ray fluorescence, or both.

Adsorption -pH profiles, millimoles of nucleotide adsorbed per gram of clay versus pH, were run for all nucleotides of this study on zinc-clay and for 5'-AMP with the other metal-clays prepared. To each preweighed clay sample was added a known volume of nucleotide solution. For the experiments dealing with adsorption of nucleotides onto zinc clay, the suspension contained 7.5–10 mg of clay per milliliter and the solution initially contained the same number of moles of nucleotide as the number of moles of adsorbed zinc in the whole system (a 1 : 1 ratio). For some of the experiments, the nucleotide to zinc ratio was changed to 1 : 10 by increasing the clay content to 50 mg of clay per milliliter and simultaneously decreasing the nucleotide molarity. In those experiments where 5'-AMP was adsorbed onto different homoionic clays, the ratio of initial moles of 5'-AMP added to moles of cation on the clay was always 1 : 1. The suspensions were shaken for 30 sec using a vortex mixer and then sonicated for another 30 sec. The pH of this mixed suspension was adjusted to the desired value using microliter volumes of concentrated acid (1 N HCl) and base (1 N NaOH), or dilutions of these where necessary. The suspensions were allowed to equilibrate at $20° \pm 1 °C$

with intermittent shaking. The pH was measured again after 18 hr, and this final value was used.

The equilibrated sample mixtures were centrifuged on an analytical desk-top centrifuge (3300 RPM). The supernatant was decanted and filtered using 0.5 µm fluoropore filters (Millipore Filter Corp.). The pH of the decanted samples was then readjusted to 7.5, and the nucleotide concentration was determined by ultraviolet absorbance measurements at the wavelength of maximum absorption (λ max) for the nucleotide under study. The amount of nucleotide adsorbed was calculated from the difference between the initial and final concentrations of the supernatant solution. The precision of the data is estimated to be ca. $\pm$ 10%. Curve-fitting to the data points was done by a combination of computer least-square and manual curve-fitting techniques.

3. Results and Discussion

1. Adsorption of Various Nucleotides on Zinc-Montmorillonite.

The method of clay preparation affected the zinc loading of the clay: 0.5 mmole by the leaching technique and 0.33–0.37 mmole by the quantitative ion-exchange technique. In the leaching procedure, the loading of zinc is larger than the cation-exchange capacity (CEC), possibly because of the presence of hydrolyzed zinc species. On the other hand, in the quantitative ion-exchange procedure, zinc loading lower than the CEC may have been obtained, because the clay edge sites may have remained saturated with Al^{3+} at the pH point at which the titration was stopped (Banin *et al.*, 1984, this issue). Thus the 'true' capacity of the clay is probably somewhere between these two values.

The clays prepared by the two methods gave similar results for 5'-AMP, as shown by the curves of Figure 1, and also for 5'-CMP (not shown). This suggests that valid comparisons can be done using clay prepared by either method. Figures 1–3 show the adsorption of the purine nucleotides 5'-, 3'-, and 2'-AMP, respectively. For all three isomers there is a relatively strong adsorption at low pH values (2–3). At these low pH values, the nucleotide molecule is both negatively and positively charged (see Figure 6 in Banin *et al.*, 1985, this issue), and adsorption may occur by anion and cation exchange. As the pH increases, the charge on the molecule becomes more negative and adsorption diminishes (a detailed analysis of this trend will be given in Part II of this series (Banin *et al.*, 1985, this issue)). For the 3'- and 2'-isomers, this trend continues throughout the pH range studied, so that the maximum adsorption occurs at the lowest pH values. For 5'-AMP, however, there is a notable exception: in the pH range of 6–8, a peak in the adsorption is observed. In this range, the transition from monoanion to the dianion form of 5'-AMP occurs due to dissociation of the second proton of the phosphate group ($pK_{2a} = 6.67$). It has been observed that the transition metal cations form stronger complexes with dianionic nucleotides than with monoanionic nucleotides (Taylor and Diebler, 1976). With regard to the 2'- and 3'-isomers of AMP
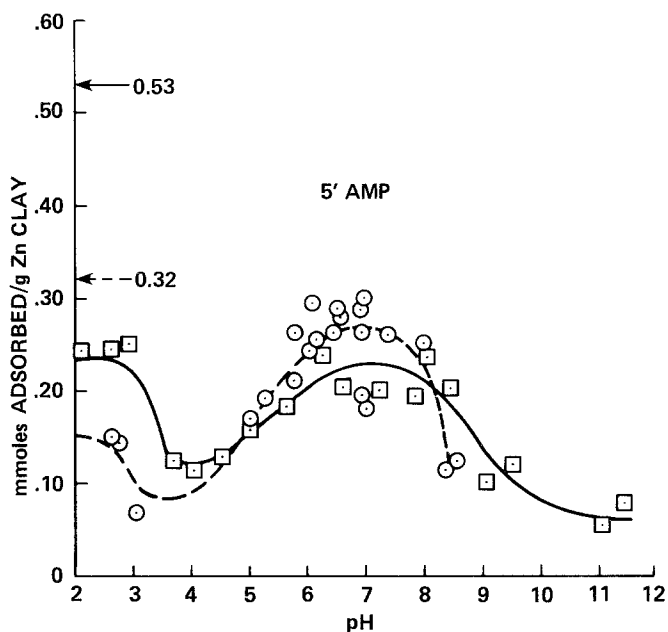
Fig. 1. Adsorption of 5'-AMP, at various pH values on Zn-montmorillonite prepared by two methods: titration (⊙------⊙) and leaching (⊡————⊡). (See text for details of the methods). The initial level of 5'-AMP addition, mmole/g clay is indicated by the arrows.
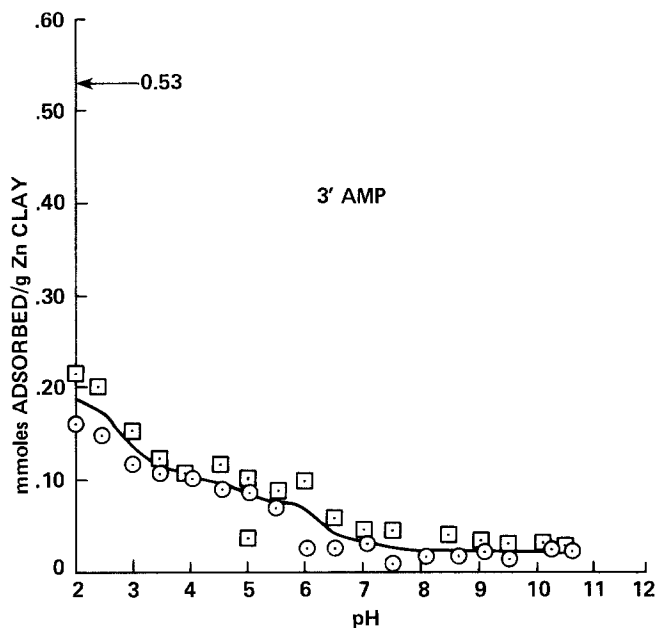

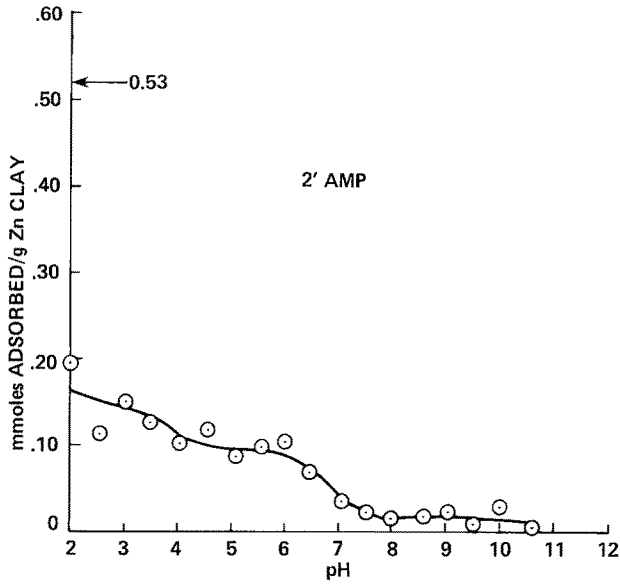Fig. 2. pH-Adsorption profile of 3'-AMP on Zn-montmorillonite.

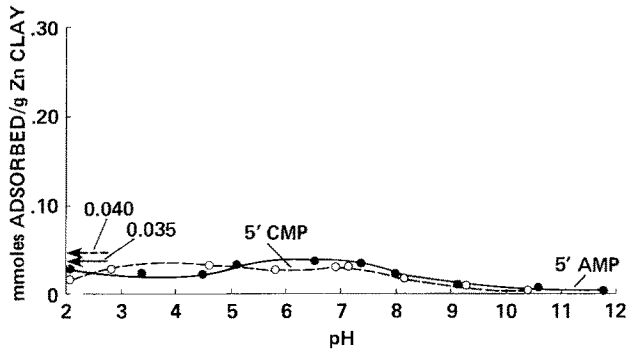Fig. 3.   pH-Adsorption profile of 2′-AMP on Zn-montmorillonite.

Fig. 4.   pH-Adsorption profile of 5′-AMP and 5′-CMP on Zn-montmorillonite with a 1 : 10 mole ratio of nucleotide added to adsorbed zinc.
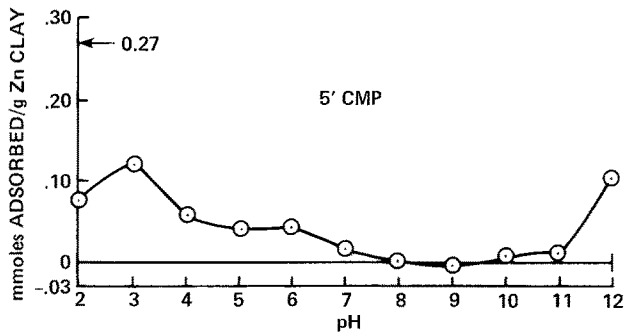

Fig. 5.   pH-Adsorption profile of 5′-CMP on Zn^{2+}-montmorillonite with a 1 : 1 mole ratio of nucleotide added to adsorbed zinc.

only limited experimental results exist (Martin and Mariam, 1979). However, semi-empirical molecular orbital calculations (Liebmann *et al.*, 1982) have shown that the 5'-AMP:Zn complex is energetically more favorable than either the 2'- or 3'-AMP:Zn complexes. This theoretical study is in qualitative agreement with experimental observations (Martin and Mariam, 1979). Therefore, we suggest that 5'-AMP is adsorbed preferentially onto Zn^{2+}-clay (relative to the 2'- and 3'-isomers) due to complex formation.

A comparison of the adsorption behavior of a purine nucleotide (5'-AMP) and a pyrimidine nucleotide (5'-CMP) was also done (Figures 1, 4, and 5). At low levels of nucleotide addition (1 : 10 molar ratio of nucleotide to adsorbed zinc, Figure 4), relatively strong adsorption for both nucleotides is observed throughout the whole pH range, and nearly 100% of the added nucleotides was adsorbed. Although not shown, the same was observed for 2'- and 3'-AMP. Comparing Figures 1 and 5 where added nucleotide concentration was higher (molar ratio of nucleotide to Zn^{2+} is 1 : 1), a similar magnitude of adsorption of the two nucleotides was found at low pH values (2–3). In this pH range, electrostatic ion exchange is suggested as the cause for adsorption for both nucleotides. In the pH range of 6–8, however, the pyrimidine nucleotide (5'-CMP, Figure 5) does not exhibit significant adsorption, whereas the purine nucleotide (5'-AMP, Figure 1) does. As reported, complexation of the pyrimidine nucleotide can occur only through the phosphate moiety (a similar constraint holds for 2'- and 3'-AMP), and thus the significant adsorption of 5'-AMP at neutral pH values apparently reflects its ability to form a bridging complex with the metal cation which involves the available N-7 purine ring position in addition to the phosphate moiety (Martin and Mariam, 1979).

II. Adsorption of 5'-AMP onto Various Homoionic Montmorillonite Clays.

The variation of 5'-AMP adsorption with pH on various homoionic clays is shown in Figures 6 and 7. It is seen that the metal clays can be divided into two distinct groups based on differences in adsorption in the pH range 4 to 8. The first group consists of Na^+, Mn^{2+}, Fe^{3+}, Co^{2+}, and Ni^{2+} clays (Figure 6), and the second group consists of Zn^{2+} and Cu^{2+} clays (Figure 7). Note in Figure 6 that the initial addition of 5'-AMP to the Na^+-clay is larger than the additions to the other clays. This is so because a mole ratio of 1 : 1 (added 5'-AMP to adsorbed metal cation) has been used: when one considers the ratio of meq of 5'-AMP added to meq of metal cation on the clay, the ratio for Na^+ clay comes closer to the others. However, it still remains slightly higher, because in the ion-exchange preparation procedure for homoionic clays, a greater amount of sodium can be added without hydrolysis and precipitation relative to the transition metal ions used. Both of these reasons are applicable also in explaining the variation of initial millimoles of 5'-AMP added for Mn^{2+}, Co^{2+}, Fe^{3+}, and Ni^{2+} clays.

For the group of metal clays of Figure 6, the adsorption of 5'-AMP shows a continuous decrease over the pH range studied from low to high pH values. This trend can be correlated with the charge on the 5'-AMP molecule which, as stated earlier, is
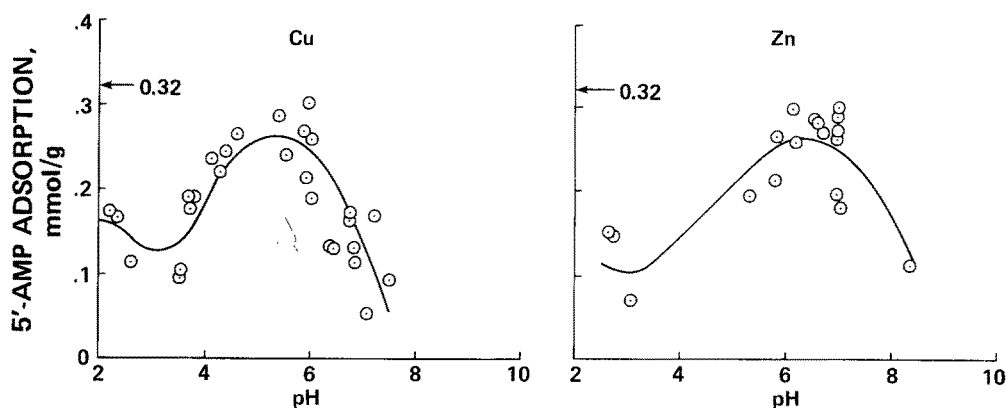
Fig. 6.   Profile of adsorption (mmole 5'-AMP adsorbed per gram of clay) versus pH for homionic-substituted montmorillonite clays of Na^+, Fe^{3+}, Mn^{2+}, Co^{2+}, and Ni^{2+}.

dependent on the state of protonation of the N-1 nitrogen on the purine ring and the oxygens of the phosphate moiety of the molecule (Banin *et al.*, 1984). In the pH range of 1 to 4, the zwitterionic 5'-AMP molecule could adsorb onto the clay by electrostatic ion-exchange mechanisms, both cationic substitution of 5'-AMP for the metal cation in the clay cation-exchange site and anion substitution at the edges of the clay. These may operate simultaneously. At pH values greater than about 4–5, the 5'-AMP molecule has a net negative charge and any adsorption observed may be due to anion exchange (Frieble *et al.*, 1980; Graf and Lagaly, 1980) or complexation (or both) between the cationic metal on the clay and 5'-AMP. It would appear from the adsorption-pH profiles of the group of metal clays with Na^+, Mn^{2+}, Co^{2+}, and Ni^{2+} that the ion-exchange mechanisms of adsorption in this case are more important than the complexation mechanism. Fe^{3+}-clay, even though it resembles the other members of this group, is different. On closer inspection, it is observed that Fe^{3+}-clay systematically adsorbs more 5'-AMP throughout the pH range studied. This phenomenon is addressed in detail in Banin *et al.* (1985).

Copper and zinc belong to a second group with regard to adsorption behavior for 5'-AMP (Figure 7) showing increased adsorption of 5'-AMP at pH values above 4. Several concepts may be invoked in an attempt to explain this behavior. These would involve preferred coordination geometry, steric considerations, and the strength of the analogous metal-5'-AMP complexes in solution. Some of these characteristics are summarized in Table I. The coordination geometry frequently found for complexes of Mn^{2+}, Fe^{3+}, Co^{2+} and Ni^{2+} is octahedral. For Cu^{2+} complexes, two preferred coordination geometries are found, square planar and distorted octahedral; Zn^{2+} prefers a tetrahedral geometry in its complexes (Cotton and Wilkinson, 1980). This does not provide a clear explanation for the experimentally observed trends in adsorption, because the two metals, Cu^{2+} and Zn^{2+}, which show larger adsorption of 5'-AMP have different preferred coordination geometries. Steric considerations may not seem to be a major factor in explaining the strength of binding of 5'-AMP to

TABLE I

Metal cation characteristics

Cation	$\text{Log } K \frac{M^{n+}}{M \cdot 5'\text{-AMP}}$ [a]	Frequently Found Coordination Geometry [b]	Effective Ionic [c] Radii, pm
Na^+	0.5	—	116
Mn^{2+}	2.35	Octahedral	81
Fe^{3+}	—	Octahedral	69
Co^{2+}	2.56	Octahedral	79
Ni^{2+}	2.84	Octahedral	83
Cu^{2+}	3.18	Square planar	71
		Distorted octahedral	87
Zn^{2+}	2.72	Tetrahedral	74

[a] Martell, A. E. and Smith, R. M.: 1974, *Critical Stability Constants*, Plenum Press, N.Y., Vol. 2.

[b] Cotton, F. A. and Wilkinson, G.: 1980, *Advanced Inorganic Chemistry*, Interscience Publishers, New York, pp. 590, 739, 758, 768, and 811.

[c] Huheey, J. E.: 1978, *Inorganic Chemistry*, Harper & Row, N.Y., pp. 71–74.

different divalent transition metals in aqueous solution since there are only small variations in ionic diameters of the cation. However, when forming a metal-ligand complex in the interlamellar spaces of the clay, even small differences in size and configuration of the complex resulting from small variations in metal ion diameters may be amplified and translated into significant differences in adsorption. The Irving-Williams series which is frequently cited to explain stability trends for divalent metal complexes of the first transition series in solution (Huheey, 1982), predicts an enhanced adsorption of 5'-AMP onto Cu^{2+}-clay relative to the homoionic clays of Mn^{2+}, Co^{2+} and Ni^{2+} as observed, but fails in the case of Zn^{2+}-clay.

Other studies (Eichhorn, 1973) have indicated differences in binding behavior of various metals with 5'-AMP, 5'-ADP, 5'-ATP, and their analogues. As concluded by Eichhorn (1973), the binding of a metal ion to a specific site on a biomolecule such as 5'-AMP, which contains a variety of potential electron donor sites, occurs to produce the most stable complex between the metal and the biomolecule. It appears that Zn^{2+} and Cu^{2+}, when adsorbed on smectite clay surfaces, are able to form complexes with 5'-AMP that are more stable than those for Mn^{2+}, Fe^{3+}, Ni^{2+}, and Co^{2+} clays. This stability probably reflects a variety of geometric, steric, and energetic factors between the metal on the surface of the clay and 5'-AMP that affect the stability of the complex formed. Clearly more crystallographic (i.e., solid-state) and solution structural studies are needed to resolve fully the differences in adsorption behavior observed.

The results of these experiments suggest strongly that clays substituted with divalent metal ions may have played an important role in the processes that led to the origin of life. Clays could have provided a surface for adsorption of nucleotides by two mechanisms: at low pH values, ion exchange (both cationic and anionic) of the charged biomolecule at charge sites of the clay is suggested, while at intermediate range pH
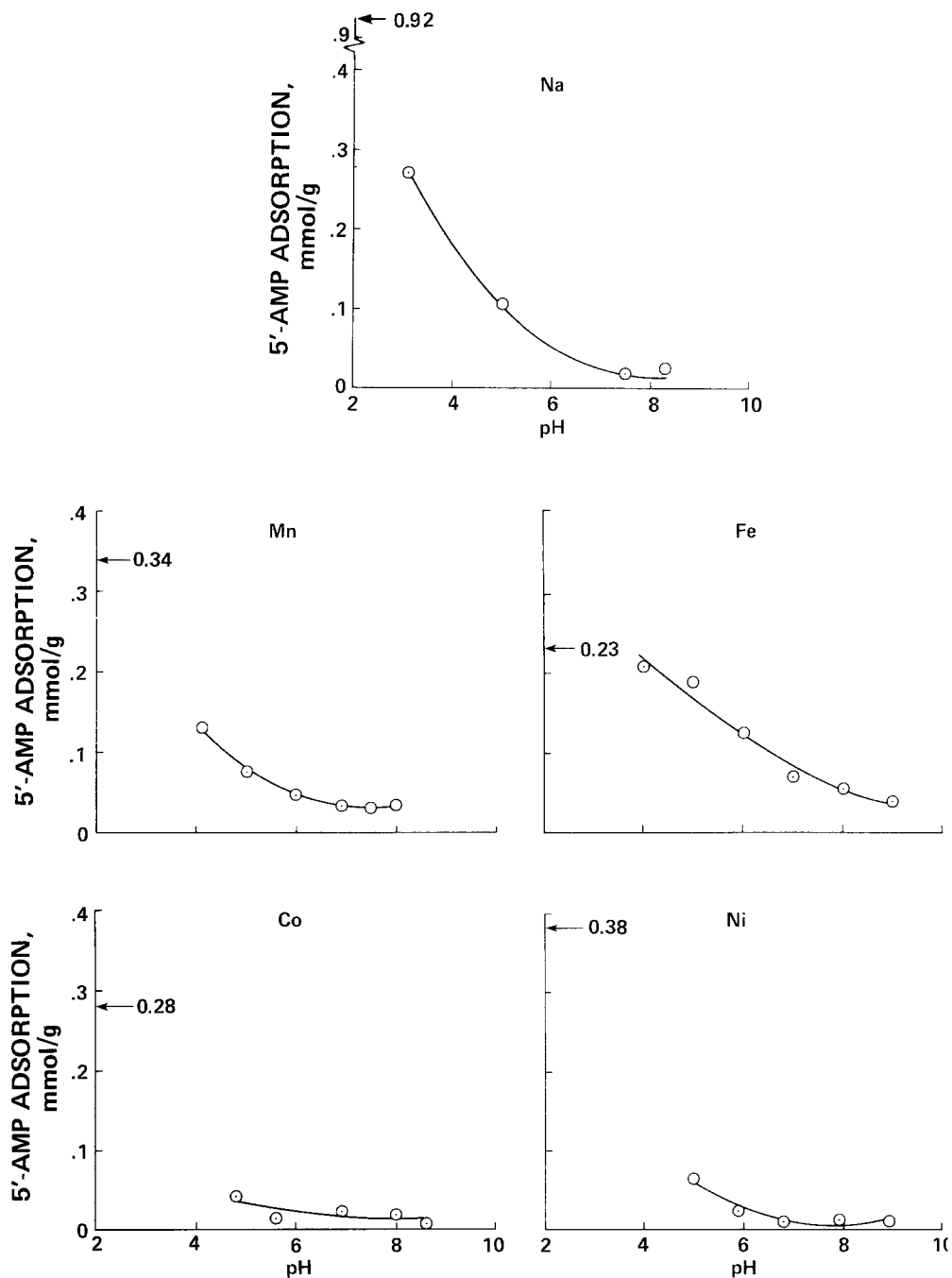
Fig. 7. Profile of adsorption (mmole 5'-AMP adsorbed per gram of clay) versus pH for homoionic-substituted montmorillonite clays of Cu^{2+} and $Zn2^{+}$.

values, a complexation mechanism between the nucleotide and the metal in the clay cation exchange site is proposed. In the intermediate pH range, a significant selectivity of the zinc clay for 5'-AMP over the 2'- and 3'-AMP isomers was observed. This neutral or intermediate pH range represents the physiological and putative primordial pH range (Paecht-Horowitz, 1973). In the biochemistry of life, the 5'-nucleotides occur more abundantly (e.g., free nucleotides in cells) (Lehninger, 1975), even though chemical evolution experiments have shown that in the phosphorylation of nucleosides roughly equal amounts of 2'-, 3'- and 5'-nucleotide isomers are formed (Ponnamperuma and Mack, 1965). Obviously a selection mechanism is required, and it is suggested that selective adsorption by clays may have provided such a mechanism.

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