

# MONTMORILLONITE: A MULTIFUNCTIONAL MINERAL CATALYST FOR THE PREBIOLOGICAL FORMATION OF PHOSPHATE ESTERS

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**Abstract.** Reaction of diiminosuccinonitrile (DISN) with 3'-AMP in the presence of alkali- and alkaline earth-montmorillonites results in the formation of 2',3'-cAMP in aqueous solution. Little or no 2',3'-cAMP is produced when metal ion concentrations equivalent to that of the metal ion associated with the homoionic clays are used instead of montmorillonite. Yields comparable to those obtained with DISN are obtained when diaminomaleonitrile (DAMN) is used in place of DISN as the condensing agent. DAMN, a compound which is more stable than DISN in aqueous solution, is oxidized to DISN on the surface of the clay by  $Fe^{+3}$  in the clay lattice. DISN, the true condensing agent, is thus generated in the presence of the bound 3'-AMP on the montmorillonite surface. The montmorillonite catalyzes the DISN-mediated formation of 2',3'-cAMP and this product, which binds much less strongly than does the 3'-AMP, is desorbed from the clay surface. This research established that the montmorillonite performs four different functions in its role as catalyst: (1) Binding one of the substrate molecules (3'-AMP) (2) Activating the second substrate (DAMN) (3) Catalyzing the formation of 2',3'-cAMP (4) Releasing the reaction product so another substrate molecules can bind to the montmorillonite.

## 1. Introduction

The laboratory demonstration of the oligomerization of unblocked monomers in aqueous solution using prebiological conditions is both a fundamental and recalcitrant problem in the field of chemical evolution. In none of the reported polymerizations does the proposed prebiological reaction proceed in the presence of water using reagents that may have been readily available on the primitive earth. For example, anhydrous reaction conditions are required to prepare oligopeptides from amino acids (Fox and Dose, 1972). In one approach to the synthesis of oligoribonucleotides, anhydrous heating in the presence of both a nucleotide triphosphate and the condensing agent cyanamide is required (Rao *et al.*, 1980). The condensation of activated ribonucleotides to oligonucleotides proceeds regiospecifically under mild conditions in aqueous solution, but the reaction requires the presence of a preformed RNA template and the 2-methylimidazolidine derivative of the phosphate, reactants that may not have been present on the primitive earth (Inoue and Orgel, 1982). The condensation of some ribonucleotide diphosphates occurs in the absence of a template to give oligomers that are linked by the pyrophosphate bond. This reaction proceeds in aqueous solution and requires the use of the imidazolidine derivative of the phosphate grouping as the activating agent (Schwartz and Orgel, 1985; Schwartz, 1986).

In previous studies from this laboratory we reported that diiminosuccinonitrile (DISN), a compound which is formed readily by the oxidation of a tetramer of

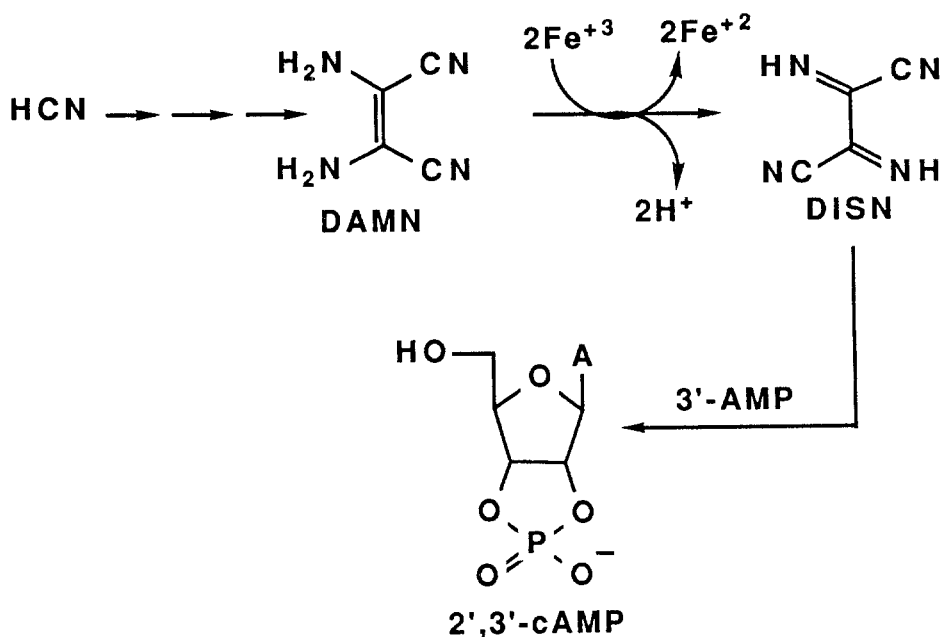


Fig. 1. A chemical system for the formation of the phosphodiester bond in aqueous solution. HCN condenses to DAMN which in turn is oxidized to DISN. DISN is the condensing agent which effects the intramolecular formation of the phosphodiester bond in 3'-AMP to generate 2',3'-cAMP.

hydrogen cyanide (diaminomaleonitrile, DAMN) effects the cyclization of 3'-ribonucleotides to the corresponding 2',3'-cyclic nucleotides in aqueous solution (Ferris *et al.*, 1982; Ferris *et al.*, 1984) (Figure 1). This model system for the formation of the phosphodiester linkage proceeds in aqueous solution at 60°C and is catalyzed by divalent metal ions. Thus it fulfills the prerequisites of a generally applicable prebiological reaction in that it takes place in aqueous solution, it utilizes a plausible prebiotic condensing agent and the reaction proceeds at moderate temperatures (0–80°C). A major drawback of this approach is that the condensing agent (DISN) is hydrolyzed by water and consequently an excess is required to achieve modest yields of the 2',3'-cyclic nucleotide.

It was felt that montmorillonite clays may serve to bind and concentrate the nucleotides so that if it were possible to generate the DISN on the surface of the montmorillonite, it would react efficiently with the proximate bound nucleotide to generate a higher yield of the corresponding cyclic nucleotide (Ferris and Hagan, 1986). This possibility was explored using Zn<sup>2+</sup>-montmorillonite since nucleotides bind to it strongly in aqueous solution (Lawless *et al.*, 1985; Ferris and Hagan, 1986). Higher yields of 2',3'-cAMP were observed when the reaction of 3'-AMP and DISN was performed in the presence of Zn<sup>2+</sup>-montmorillonite, but the yields were only slightly higher than when a concentration of aqueous Zn<sup>2+</sup> corresponding to that of the Zn<sup>2+</sup> bound to the clay was used (24.3% versus 17.8%) (Ferris and Hagan, 1986).

If DAMN was substituted for DISN, low yields of 2',3'-cAMP (< 5%) were obtained when homoionic montmorillonites with transition metal ions as the exchangeable cation were investigated (Hagan, 1985). Since it has been reported that 5'-AMP binds to a series of homoionic montmorillonite clays (Lawless *et al.*, 1985; Banin *et al.*, 1985) it was anticipated that 3'-AMP would also bind to these clays. The effect of a series of homoionic clays on the cyclization of 3'-AMP was investigated in the present study and it was observed that the formation of 2',3'-cyclic AMP proceeds efficiently in the presence of some alkali- and alkaline earth-montmorillonites under conditions where no reaction is observed in the absence of montmorillonite. A preliminary report of some of these findings has been published (Ferris *et al.*, 1986).

## 2. Experimental\*

### A. MATERIALS AND METHODS

The materials and methods used in this research were described in Ferris and Hagan (1986). All high performance liquid chromatography (HPLC) was performed with a Waters Microbondapak C-18 column.

### B. REACTIONS

#### 1. General Procedure for the Formation of 2',3'-cAMP

A 2 ml solution of 0.05M 3'-AMP in 0.2M PIPES (pH 6.35) was added to a 8 ml screw cap test tube containing 200 mg of montmorillonite and the mixture was shaken on a Vortex mixer for 30 sec and allowed to stand at room temperature for 16–20 h. This mixture was centrifuged and 40  $\mu$ l of the supernatant was diluted to 10 ml and the UV absorbance at 260 nm was measured. The extent of 3'-AMP binding to the montmorillonite was determined by the decrease in absorbance when compared with the absorbance of a comparable dilution of the stock solution. To the 3'-AMP and montmorillonite mixture was added 2 ml of either 12.2 mM DAMN (final concentration 0.0061 M) or 9.44 mM DISN (final concentration 0.0047 M) in 0.2M PIPES. The final concentration of 3'-AMP was 0.025 M. The mixture was shaken for 30 sec on a Vortex mixer and heated at 60°C for 3 h. The mixture was then cooled to room temperature, the pH measured, 4 ml of 0.2 M EDTA was added and then the tube was shaken on the Vortex mixer. After 20 min the mixture was centrifuged, a portion of the supernatant was filtered and 1 ml of the filtrate was diluted to 10 ml with distilled water. The diluted sample (40  $\mu$ l) was used for HPLC analysis (Ferris *et al.*, 1984).

\* Abbreviations: DAMN, diaminomaleonitrile; DISN, diiminosuccinonitrile; 3'-AMP, 3'-adenylic acid; 5'-AMP, 5'-adenylic acid; 2',3'-cAMP, 2',3'-cyclic adenylic acid; ApAp, adenylyl-3', 5'-adenosine-3'-phosphate; (Ap)<sub>4</sub>, adenylyl-3', 5'-adenyl-3', 5'-adenyl-3', 5'-adenosine-3'-phosphate; PIPES; 1-4-piperazinebis(ethanesulfonic acid).

TABLE I  
Decomposition of DAMN on native Montmorillonite<sup>a</sup>

Native Montmorillonite (mg)	DAMN		Loss	
	0.2M PIPES		No buffer	
	Final pH	(%)	Final pH	(%)
0	6.5	3.4	5.7	4.7
50	6.5	28.9	5.5	24.6
100	6.4	42.7	5.2	30.7
150	6.4	51.5	5.1	36.8
200	6.3	59.9	5.0	43.1

<sup>a</sup> The analysis of DAMN was performed by HPLC. The reaction conditions were the same as those described in the general experimental procedure except no 3'-AMP was used.

### 3. Results and Discussion

Reaction variables including the concentrations of 3'-AMP, DAMN and DISN, the quantity of clay and the presence or absence of 0.2 M PIPES buffer were varied in a systematic fashion to determine the factors which affect the yield of 2',3'-cAMP. Studies on the decomposition of DAMN by the Fe<sup>+3</sup> located in the aluminosilicate lattice of the native montmorillonite, i.e., the mineral sample that is only processed by grinding and washing with distilled water before use, showed that the oxidation proceeded more efficiently in the presence of 0.2 M PIPES buffer than with no buffer (Table I). It has not been determined whether this phenomenon is due to the pH variation in the absence of buffer or whether PIPES increases the accessibility of interlamellar surfaces (Ferris and Hagan, 1986).

The yield of 2',3'-cAMP was found to be directly dependent on the amount of Na<sup>+</sup>-montmorillonite used (Table II). It was decided to use 200 mg of montmorillonite because it gave a yield of 2',3'-cAMP that could be accurately measured by HPLC. Previous studies established that in the presence of 0.2 M PIPES 90  $\mu$ mole of DAMN are oxidized per gram of native montmorillonite (Ferris and Hagan, 1986), so the 200 mg. of clay should oxidize 18  $\mu$ mole of DAMN. The extent of DAMN oxidation is less than this value when 200 mg of montmorillonite was used, but it was greater than the expected values of 9 and 4.5  $\mu$ mole when 100 and 50 mg. of clay were used respectively (Table II). A comparable amount of DISN was used (18.8  $\mu$ mole) to facilitate the comparison between DAMN and DISN as condensing agents.

The yield of 2',3'-cAMP was not changed appreciably by the presence of 0.2 M PIPES buffer when DISN was used directly as the condensing agent (Table II). A 3.3-fold higher yield of the cyclic AMP was observed in the presence of PIPES when DAMN was used as the condensing agent. It can be seen from the data in Table I that the decomposition of DAMN is slightly greater in the presence of 0.2M PIPES but the difference is not a factor of 3.3 in magnitude.

The extent of the catalysis by various homoionic montmorillonites was then inves-

TABLE II  
Variation in 2',3'-cAMP yield with the amount of Na<sup>+</sup>-Montmorillonite<sup>a</sup>

Na <sup>+</sup> Montmorillonite (mg)	3'-AMP Adsorbed (μmol)	Final pH	DAMN Reacted (μmol)	DISN Reacted (μmol)	Yield 2',3'-cAMP (%) <sup>b</sup>
50	4.4	6.4	9.7	—	2.3
50	3.7	6.4	—	18.8	5.1
100	8.0	6.4	12.5	—	4.5
100	7.8	6.4	—	18.8	9.0
200	13.9	6.3	15.9	—	9.8
200	14.8	6.3	—	18.8	16.3
200	8.2	6.0	15.3	—	3.0 <sup>c</sup>
200	9.4	6.2	—	18.8	11.5 <sup>c</sup>

<sup>a</sup> The reaction conditions are described in the general experimental procedure using 100 μmol of 3'-AMP except the amount of Na<sup>+</sup>-montmorillonite was varied.

<sup>b</sup> Yield based on DAMN or DISN reacted.

<sup>c</sup> Reactions performed in the absence of PIPES.

tigated. The best yields of 2',3'-cAMP were obtained using Na<sup>+</sup>- and Ca<sup>2+</sup>-montmorillonite (Table III). The transition metal montmorillonites bind 3'-AMP more strongly than the alkali and alkaline earth derivatives of the clay but give lower yields of 2',3'-cAMP. The yield of 2',3'-cAMP obtained with the native clay is comparable with that obtained using Na<sup>+</sup>- or Ca<sup>2+</sup>-montmorillonite, a finding consistent with the report that this smectite has Na<sup>+</sup> and Ca<sup>2+</sup> as its principal exchangeable cations (American Petroleum Institute, 1951; Ferris *et al.*, 1979). It was established that the formation of 2',3'-cAMP was not catalyzed by the exchangeable cation associated with the montmorillonite. In reactions performed under the same conditions except the soluble metal ion was used in place of the metal ion-montmorillonite complex (Table V), no 2',3'-cAMP was detected except for the small yields observed using Ca<sup>2+</sup> and Cu<sup>2+</sup>. The virtual absence of metal ion catalysis clearly established the catalytic role of the clay surface in the formation of 2',3'-cAMP. This catalytic effect is much greater than was observed previously with Zn<sup>2+</sup>-montmorillonite (Ferris and Hagan, 1986).

The most extensive mineral catalysis was observed with homoionic montmorillonites in which Na<sup>+</sup>, K<sup>+</sup> and Ca<sup>2+</sup> are the exchangeable cations. These clays are the least effective in binding the 3'-AMP. Up to this point in our research the strategy had been to equate potential catalysis with strong binding so minerals were screened on the basis of their affinity for organic reactants. The transition metal montmorillonites, which are the most effective in binding nucleotides, may indeed be good catalysts for the formation of the phosphate ester bond but they may also form complexes with DISN (Ferris *et al.*, 1984) and catalyze the hydrolysis of DISN or the DISN-AMP adduct. This is one possible explanation of the low yields of 2',3'-cAMP using transition metal montmorillonites.

The nature of the binding of 3'-AMP to montmorillonite is not clear. It was con-

TABLE III  
Reaction of 3'-AMP and DISN in the presence of Montmorillonite<sup>a</sup>

Homoionic Clay	3'-AMP ( $\mu\text{mol}$ )	Final pH	3'-AMP Adsorbed ( $\mu\text{mol}$ )	2',3'-cAMP <sup>b</sup> (%)
Native	10	6.25	6.1	1.1
	50	6.30	18.1	6.8
	50	6.30	18.2	6.6
	100	6.25	22.9	10.5
	100	6.25	22.4	11.7
	200	6.36	29.0	14.0
Na <sup>+</sup>	10	6.25	6.9	1.6
	50	6.28	12.7	8.9
	100	6.30	14.8	16.3
	200	6.30	16.6	21.7
K <sup>+</sup>	10	6.05	7.7	1.3
	100	6.10	20.3	16.3
Ca <sup>+2</sup>	50	6.40	17.4	9.4
	100	6.36	24.3	15.7
	200	6.40	26.2	21.3
Fe <sup>+3</sup>	50	6.20	35.3	3.3
	100	6.12	43.6	7.9
Cu <sup>+2</sup>	100	6.14	52.7	3.0
Zn <sup>+2</sup>	50	6.30	46.8	0.7
	100	6.36	68.5	6.2
	200	6.36	75.4	13.2

<sup>a</sup> The general procedure outlined in the Experimental Section was used except the quantity of 3'-AMP was varied.

<sup>b</sup> Yield based on DISN added, except when 10  $\mu\text{mol}$  3'-AMP was used and then it is based on 3'-AMP.

cluded that the binding of 5'-AMP to montmorillonite in the pH 4.5–5.5 range is by the interaction of the negatively charged phosphate group to the positive charges at the edges of the clay particles or by ligand exchange with coordinated water (Lawless *et al.*, 1985; Banin *et al.*, 1985). The nature of the binding to homoionic montmorillonites in the pH 6–7 range used in the present study was not investigated.

The actual mechanism of the montmorillonite catalysis of 2',3'-cAMP formation has not been established but two postulates have been formulated on the basis of the available data. The first is based on the observation that the yields of 2',3'-cAMP in the solution phase reactions are very low, suggesting that the rate of the bimolecular reaction between DISN and 3'-AMP is slow relative to the hydrolysis of DISN. Since the clay-mediated formation of 2',3'-cAMP proceeds under conditions where no cyclic nucleotide can be detected in the absence of montmorillonite, the clay en-

TABLE IV  
Reaction of 3'-AMP and DAMN in the presence of montmorillonite<sup>a</sup>

Montmorillonite	3'-AMP ( $\mu\text{mol}$ )	Final pH	3'-AMP Adsorbed ( $\mu\text{mol}$ )	DAMN Reacted <sup>b</sup> ( $\mu\text{mol}$ )	2',3'-cAMP <sup>c</sup> (%)
Native	10	6.10	7.5	17.4	0.6
	50	6.24	20.5	15.4	5.2
	50	6.24	20.5	15.4	5.3
	100	6.23	26.5	15.1	10.2
	100	6.22	26.9	15.1	9.5
Na <sup>-</sup>	10	6.24	6.9	15.7	0.8
	50	6.26	12.6	16.0	4.8
	100	6.26	13.9	15.9	9.8
	200	6.26	16.0	14.0	20.3
K <sup>+</sup>	10	6.05	7.6	15.5	0.5
	100	6.08	20.3	15.2	8.2
Ca <sup>-2</sup>	10	5.90	6.9	d	0.8
	50	6.40	17.0	20.6	4.7
	100	6.35	23.7	18.6	8.3
	200	6.35	28.0	14.2	8.6
Fe <sup>+3</sup>	50	6.20	35.4	13.7	4.5
	100	6.12	43.5	15.4	10.4
Cu <sup>-2</sup>	100	6.15	52.4	26.0	4.4
Zn <sup>+2</sup>	10	6.02	9.3	d	0
	50	6.28	48.3	17.4	0
	100	6.30	66.2	17.7	2.5
	200	6.32	73.0	17.8	5.6

<sup>a</sup> Reaction performed as described in the general procedure in the Experimental except the 3'-AMP concentration was varied.

<sup>b</sup> Unreacted DAMN determined by HPLC analysis.

<sup>c</sup> Yield based on DAMN reacted, except when 10  $\mu\text{mol}$  3'-AMP is used and then it is based on 3'-AMP.

<sup>d</sup> Not determined.

hances the reaction between DISN and 3'-AMP. The enhanced reaction rate may be due to the concentration of the reactants on the mineral surface. While the adsorption of 3'-AMP on montmorillonite has been measured, the corresponding binding of DISN has yet to be established.

Catalysis of the cyclization of the 3'-AMP-DISN adduct is a second explanation for the role of montmorillonite. The precedent for this explanation comes from our observation that divalent metal ions enhance the yield of 2',3'-cAMP in the reaction of DISN or BrCN with 3'-AMP (Ferris *et al.*, 1984). It was proposed that the divalent cations bind to the 3'-AMP-DISN adduct thus shielding the negative charge on the phosphate group and enhancing its electrophilic character. The rate of the attack of the 2'-hydroxyl group is greater because of the increased electrophilicity of the phos-

TABLE V  
Metal ion catalysis of the DISN-mediated formation of  
2',3'-cAMP<sup>a</sup>

Metal Ion <sup>b</sup>	Yield 2',3'-cAMP (%)
—	0
Na <sup>+</sup>	0
Ca <sup>+2</sup>	3.4
K <sup>+</sup>	0
Fe <sup>+3</sup>	0 <sup>b</sup>
Zn <sup>+2</sup>	0 <sup>c</sup>
Cu <sup>+2</sup>	3.9 <sup>c</sup>

<sup>a</sup> Yield based on DISN as the limiting reagent. An amount of metal ion equivalent to the amount of exchangeable cation associated with 200 mg of homoionic montmorillonite was used, 0.0175 M for M<sup>+2</sup> and 0.035 M for M<sup>+1</sup>. The reaction conditions were the same as those outlined in the general procedure for the formation of 2',3'-cAMP.

<sup>b</sup> A precipitate formed which did not dissolve upon addition of EDTA. The recovery of 3'-AMP was 36% indicating loss by adsorption on the precipitate.

<sup>c</sup> A precipitate formed which dissolved on adding EDTA after termination of the reaction.

phate group. A similar enhanced rate of cyclization of 2',3'-cAMP is possible at the edges of the montmorillonite where exposed Al<sup>+3</sup> sites may serve as Lewis acid catalysts (Paecht-Horowitz, 1976). Thus the cyclization step will proceed more rapidly at the mineral edge than it does in solution. Although the two mechanistic proposals are not fully established and do not preclude the possibility of other reaction intermediates, these models do suggest two ways that clay minerals may have catalyzed the formation of the phosphate ester bond on the primitive earth.

The yields of 2',3'-cAMP obtained when DAMN was used as the condensing agent generally parallel those obtained with DISN with the exception of Fe<sup>+3</sup>-montmorillonite (Table IV). The montmorillonites with alkali metal exchangeable cations gave high yields of 2',3'-cAMP. The comparable trend in the yields confirms our previous conclusion the DAMN is oxidized to DISN by the clay. DAMN may be subject to other reaction processes as well since the product yield is slightly less than when it is the condensing agent.

The more extensive catalysis observed with the Fe<sup>+3</sup>-montmorillonite using DAMN as the condensing agent as compared with DISN (Tables III and IV) is difficult to understand. It is not due to the generation of larger amounts of DISN by the exchangeable Fe<sup>+3</sup> because as can be seen in Table IV the amount of recovered DAMN is about the same with every montmorillonite except Cu<sup>+2</sup>. From these data we conclude that the exchangeable Fe<sup>+3</sup> is not reacting with the DAMN and the only oxidant is the Fe<sup>+3</sup> bound in the lattice of the montmorillonite. This finding indicates that the oxidation potential of the exchangeable Fe<sup>+3</sup> is less than



TABLE VI  
AMP binding to Montmorillonite clays<sup>a</sup>

Homoionic Clay	AMP Adsorption (%)	Soluble 2',3'-cAMP (%)	Increase upon EDTA Wash (%)		
			2',3'-cAMP	2'-AMP	3'-AMP
none	—	100	—	—	—
Na <sup>+</sup>	12	81	-2.6	2.7	3.9
Native	17	78	1.3	1.9	3.2
Ca <sup>2+</sup>	22	71	3.2	2.4	3.9
Zn <sup>2+</sup>	36	60	-1.4	11.1	16.7

<sup>a</sup> A mixture of 2',3'-cAMP (0.025 M) and montmorillonite (100 mg) in 1 ml of 0.2 M PIPES (pH 6.4) was allowed to stand at room temperature for 24 h and the mixture was then centrifuged. The extent of adsorption (column 2) represents the difference between the initial concentration of 2',3'-cAMP and the sum of the supernatant concentrations of 2',3'-cAMP, 2'-AMP and 3'-AMP determined by HPLC analysis of a 0.3 ml aliquot that was diluted with 0.3 ml of 0.2 M PIPES and 0.6 ml of 0.2 M EDTA. The percent 2',3'-cAMP in the supernatant is given in column 3. The clay and the supernatant were again mixed, 1 ml of 0.2 M PIPES buffer and 2 ml of 0.2 M EDTA were added, and the mixture was centrifuged. The amount of each AMP isomer was determined by HPLC and the increase after EDTA treatment is given in columns 4–6. About 90% of the initial 2',3'-cAMP was accounted for after EDTA treatment.

that of the lattice-bound Fe<sup>+3</sup>. This is not a surprising result because there are theoretical (Aronowitz *et al.*, 1982) and experimental studies which are in agreement with the observation that lattice-bound Fe<sup>+3</sup> is a stronger oxidizing agent than Fe<sup>+3</sup> in solution or bound to an ion exchange resin. What is usual is the observation that Fe<sup>+3</sup> which is cation-exchanged to montmorillonite is a weaker oxidant than soluble Fe<sup>+3</sup> or Fe<sup>+3</sup> bound to an ion-exchange resin. In our previous studies we demonstrated that both of the latter forms of Fe<sup>+3</sup> oxidized DAMN to DISN (Ferris *et al.*, 1982) but our present studies suggest that the exchangeable Fe<sup>+3</sup> associated with montmorillonite does not. The reason for this diminished reactivity is not known but it may have been an important factor on the primitive earth where some organic sediments may not have been oxidized by the Fe<sup>+3</sup> bound to minerals.

Binding studies established that 2',3'-cAMP is not adsorbed on to montmorillonite under the reaction conditions used in this research. The investigation of 2',3'-cAMP binding is complicated by its montmorillonite-catalyzed hydrolysis (Table VI). It is clear that 3'-AMP binds more strongly to Zn<sup>+2</sup>-montmorillonite than does 2',3'-cAMP when the extent of adsorption in Tables III and VI respectively is compared. While the extent of adsorption of AMP isomers on Na<sup>+</sup>, Ca<sup>+2</sup>, and native montmorillonite given in Table VI is comparable to that for 3'-AMP given in Table III, the amount of 2',3'-cAMP bound to the clay is less than the total quantity of nucleotide adsorbed as shown by the elution of greater amounts of 2- and 3'-AMP on treatment with EDTA (Table VI).

The absence of 2'-AMP as a reaction product (yield less than 0.2% with Na<sup>+</sup>, Ca<sup>+2</sup>, and native-montmorillonite) in the experiments reported in Table III is further evidence for the facile desorption of 2',3'-cAMP from the clay. The binding studies in Table VI demonstrate that montmorillonite catalyzes the hydrolysis of

2',3'-cAMP and that in the absence of montmorillonite no hydrolysis takes place. Hydrolysis of 2',3'-cAMP is known to give a 1:1.5 ratio of 2'- and 3'-AMP respectively (Usher and Yee, 1979) so the failure to observe 2'-AMP establishes that 2',3'-cAMP does not bind to montmorillonite. The stronger binding of 3'-AMP thus serves to protect the product from hydrolysis by blocking adsorption sites that would otherwise be available to catalyze the breakdown of the 2',3'-cAMP.

#### 4. Minerals, HCN, and the Prebiotic Formation of Phosphate esters

The possible role of clays in processes leading to the origins of life has been a subject of conjecture since 1947 when Bernal first suggested in a lecture that clays may have served to concentrate and orient small molecules for subsequent reactions and then protect the intercalated reaction products from degradation (Bernal, 1949). This concept has been extended by Cairns-Smith (1982) in his postulate that life processes were originally effected by clays and that contemporary organic compounds took over the 'clay life' at a later evolutionary stage. There are no experimental reports of clay catalysis in prebiological systems that approach the chemical complexity proposed by Bernal and Cairns-Smith. The polymerization of alanine adenylate on sodium montmorillonite has been reported (Paecht-Horowitz, 1976) but there has been one report where it was not possible to reproduce these findings (Brack, 1976). It has not been possible to repeat the synthesis of the amino acid adenylates using the zeolite Decalco F as catalyst (Warden *et al.*, 1974).

In the montmorillonite-catalyzed reaction reported herein the smectite exhibits many of the characteristics of an enzyme (Figure 2). It binds one of the substrate molecules (3'-AMP) (Step A), activates the masked form of the condensing agent (oxidizes DAMN to DISN) (Step B), it catalyzes the reaction (Step C) between DISN and 3'-AMP, and the product formed (2',3'-cAMP) is desorbed from the catalytic surface. These findings provide direct experimental support for the postulate that minerals may have served as prototypical enzymes on the primitive earth. It might be argued that this system does not model the catalysis observed with enzymes because the  $\text{Fe}^{+2}$  generated in the montmorillonite lattice is not converted back to  $\text{Fe}^{+3}$  in the course of these transformations. However, this conversion is likely to have occurred on the primitive earth by the photochemical expulsion of an electron from the  $\text{Fe}^{+2}$  (Braterman *et al.*, 1983) or by the oxidation of  $\text{Fe}^{+2}$  by photochemically generated radicals (Banin and Rishpon, 1979), thereby completing the reaction cycle.

It is significant that catalysis is observed with the unprocessed (native) montmorillonite. While it is not claimed that this particular montmorillonite was present on the primitive earth, it is reassuring to observe that the laboratory procedures used for the generation of homoionic species were not essential for catalysis. That catalysis was observed with  $\text{Na}^{+}$ - and  $\text{Ca}^{+2}$ -montmorillonite is also significant. Since nucleotides bind more strongly to montmorillonites in which transition metals are the exchangeable cations (Lawless *et al.*, 1985; Ferris and Hagan, 1986), it was assumed that such

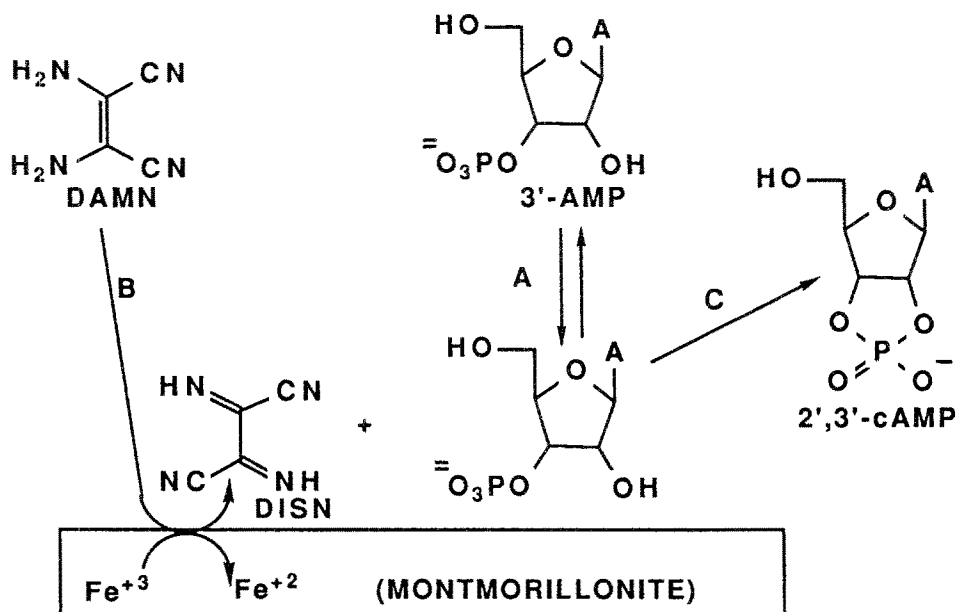


Fig. 2. Montmorillonite catalysis of the solution phase process shown in Figure 1. A. The reversible binding of 3'-AMP to montmorillonite. B. DAMN reacts with lattice-bound Fe<sup>3+</sup> in montmorillonite to generate DISN proximate to 3'-AMP. C. Montmorillonite catalysis of the reaction of DISN and 3'-AMP to generate 2',3'-cAMP which is released from the montmorillonite surface.

clays would be better catalysts. While it is not difficult to imagine primitive earth environments where transition metal ions constituted an appreciable concentration of the exchangeable cations, the prevalence of these environments was undoubtedly much less than those where the alkali and alkaline earth cations predominated.

These studies do not establish that montmorillonites were the most efficient or the only mineral catalysts on the primitive earth. Insoluble phosphates (Miller and Parish, 1964; Acevedo and Orgel, 1986), iron and manganese carbonates (Joe *et al.*, 1986) and iron oxide-hydroxides (Holm, 1985) are a few of the other classes of minerals which are currently being investigated. But the present work does provide experimental verification for the Bernal postulate (1949) that minerals may have had a central role in primitive earth processes.

The use of DISN as a condensing agent follows directly from our studies on the prebiotic chemistry of HCN. In these earlier studies it was established that representatives of the three major classes of nitrogen-containing biological molecules, purines, pyrimidines and amino acids, may be produced directly from HCN in a 'one-pot' reaction (Ferris and Hagan, 1984 and references therein). The central intermediate in all this chemistry is DAMN, a compound which forms directly from HCN in mildly alkaline solutions (Sanchez *et al.*, 1967). The oxidation of DAMN to DISN by Fe<sup>3+</sup> and other oxidants is a well-established chemical reaction, so if it is assumed

that HCN was the source of nitrogen-containing biomolecules on the primitive earth, then it follows that DISN was also formed in the presence of mild oxidants.

The principal thrust of the present research was to establish whether montmorillonite will catalyze the formation of the phosphate ester bond. However, the formation of the 2',3'-cAMP does constitute a pathway to RNA oligomers since it has been possible to effect the polymerization of this monomer (Verlander *et al.*, 1973; Verlander and Orgel, 1974). As the long-range goal of the research is to explore the clay-catalyzed oligomerization of nucleotides we investigated the polymerization of ApAp in the presence of  $Zn^{+2}$ -montmorillonite (C.-H. Huang, unpublished results from this laboratory). The objective of the study was to see if it was possible to form higher oligomers using reaction conditions where there was an ApAp associated with each  $Zn^{+2}$  cation of the montmorillonite. It can be calculated from the charge: surface area ratio of the montmorillonite that each  $Zn^{+2}$  would be, on the average, separated by 14–22 Å. In the interlayer between two clay platelets, the  $Zn^{+2}$  cations associated with both platelets would be separated by 10–15.5 Å. Inspection of a molecular model of ApAp indicated that the distance between its 3'-phosphate and 5'-OH groupings is about 11 Å so some ApAp molecules bound to the montmorillonite may be in close proximity. However, no  $(Ap)_4$  was detected upon addition of DISN or 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide to a solution of ApAp and a suspension of the nucleotide bound to montmorillonite. The only new product that was detected had an HPLC retention time close to that of ApAp and it was assumed to be the corresponding cyclic phosphate (ApA-2',3'-cAMP). The facile intramolecular cyclization of the 2'-hydroxyl group proximate to the phosphate probably proceeds much more rapidly than the bimolecular reaction with another nucleotide even though the nucleotide is bound on an adjacent site on the clay surface. Currently studies are in progress using 5'-AMP, since it is expected that the formation of 3',5'-cAMP will not be as facile as that of the formation of 2',3'-cAMP from 3'-AMP and thus it may be possible to observe the formation of oligomers of pA.

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