

# pH PROFILE OF THE ADSORPTION OF NUCLEOTIDES ONTO MONTMORILLONITE

## *II. Adsorption and Desorption of 5'-AMP in Iron-Calcium Montmorillonite Systems*

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**Abstract.** The interaction of 5'-AMP with montmorillonite saturated with various ratios of two metals found ubiquitously on the surface of Earth, that is, iron and calcium, is investigated. Adsorption and desorption of the nucleotide were studied in the pH range of 2–12 at three levels of addition: 0.080, 0.268 and 0.803 mmole 5'-AMP per gram of clay. Two desorption stages were employed – H<sub>2</sub>O wash and NaOH extraction (pH = 12.0). 5'-AMP was preferentially adsorbed on the Fe-containing clays relative to the Ca clay. The nucleotide was fully recovered by the two desorption stages, mostly by the NaOH extraction. The evidence at hand indicates that 5'-AMP reaction with clay is affected by electrostatic interactions involving both attraction and repulsion forces. Some specific adsorption, possibly the result of covalent bonding and complex formation with the adsorbed ion, cannot be ruled out for iron but does not appear to operate for calcium. Changes in pH cause varying degrees of attraction and repulsion of 5'-AMP and may have been operating on the primitive Earth, leading to sequences of adsorption and release of this biomolecule.

## 1. Introduction

The interaction of nucleotides with montmorillonite was shown to be pronouncedly affected by the adsorbed ion (Lawless *et al.*, 1984, this issue). Lawless and Edelson (1980) found specific preference for adsorption of 5'-AMP by Zn-montmorillonite relative to several other di- and monovalent adsorbed ions (iron was not included, however, in their study). Rishpon *et al.* (1982) found that ADP and ATP are not adsorbed by Na-montmorillonite, but that they are readily adsorbed by Zn- and Mg-montmorillonite. They, as others, observed that the pH has a large effect on the amount

adsorbed and found a maximum in the adsorption at pH 7.0 for both ATP and ADP reacting with Zn-montmorillonite. The pH range studied, however, was relatively narrow.

Graf and Lagaly (1980) found that clays (e.g., montmorillonite, kaolinite, and illite) have strong preferences for ATP over AMP. They explained this by suggesting that adsorption was done by replacement-exchange of edge-hydroxyl groups on the clay with the anionic phosphate group. In the case of AMP, the adenosine moiety prevented close contact of the phosphate group with the aluminium at the edge of the clay. In the case of ATP, this steric hindrance was lower, since the phosphate group was more remote from the adenosine moiety and larger amounts could be adsorbed. They also found clear evidence for hydrolysis of ATP in the presence of montmorillonite and other clays, possibly because of the reduction of the activation energy for hydrolysis when ATP was adsorbed. Graf and Lagaly's study has raised questions about the ability to displace and recover the adsorbed nucleotide.

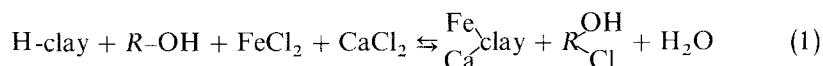
In view of the various suggestions regarding the involvement of clays in certain stages of the origin and evolution of life (Lawless *et al.*, 1985, this issue) and particularly their role in the concentration, orientation, and possible polymerization of nucleotides, and the various questions raised above, we have conducted a detailed study of the interaction of 5'-AMP with iron, iron-calcium, and calcium montmorillonite clays. Whereas most previous studies dealt with monoionic clays not frequently found under natural conditions, the present study addressed a series of mixed-ion clays. Iron and calcium were selected as the adsorbed ions because they are abundant elements of Earth's crust: they may have been present as soluble ions in the primitive environment, and, thus, they could have been frequently encountered as adsorbed ions on clays at very early stages of Earth's evolution. Nucleotide adsorption on, and desorption from, the clays were measured over a wide range of pH values, and the mechanisms of adsorption and modes of chemical interaction were studied throughout this pH range.

## 2. Experimental

### 2.1. MATERIALS

A reference montmorillonite (SWy-1) from the Clay Minerals Society repository was used. The exchangeable ions were converted to various combinations of Fe and Ca, using the quantitative ion-exchange method (Banin, 1973, Gerstl and Banin, 1980). This method entails titrating a mixture of freshly prepared H<sup>+</sup>-montmorillonite and OH<sup>-</sup>-saturated anion-exchange resin with the chloride salt of the desired cation or combination of cations. The added cations replace H<sup>+</sup> from the clay surface, and the Cl<sup>-</sup> exchanges with OH<sup>-</sup> from the resin; OH<sup>-</sup> and H<sup>+</sup> then neutralize each other. Some OH<sup>-</sup> attaches also to the edge-aluminium, saturating the anion-adsorbing sites. The titration is usually terminated at a pH of about 5.5. If there is any possibility of the cation precipitating, even at this pH, the end-point is taken at a lower pH. At the end-point, the electrical conductivity (EC) is at a minimum between 10 and 100  $\mu\text{mho/cm}$ ,

indicating that the soluble ion concentration in solution is rather low (<0.1 meq/L). The reaction can be schematically presented as follows:



where *R* represents the anion-exchange resin. This method makes it possible to prepare a mixed-ion clay at a predetermined composition, one in which the mixing of the ions on the surface of the clay is highly random: (Most other methods of preparation of mixed-ion smectites result in 'demixing' of the adsorbed ions and formation of localized clusters of ions of the same kind). It also prevents or diminishes the hydrolysis of the adsorbed ions which, in the widely used leaching method of preparation, occurs during the removal of the excess salt from the clay.

For the present experiments ferrous-chloride solution was prepared from fresh ferrous-chloride salt of AR grade, immediately before the titration. The solution was kept at low temperature and in the darkness, and the reaction system was continuously purged with N<sub>2</sub> gas to prevent iron oxidation. The converted clay suspension was separated from the OH-Cl-resin by filtration, centrifuged, the sedimented paste freeze-dried, and the dry clay lightly crushed. The adsorbed metals were extracted from an aliquot of the clay by sequential three-stage leaching with H<sub>2</sub>O, 0.5 M CaCl<sub>2</sub>, and 0.5 M HCl. It was found that during the drying stage, iron oxidation took place, converting 50–70% of the adsorbed iron to the ferric form. The values for total extractable metals from the five clays are given in Table I.

TABLE I  
Extractable metal content of the five Fe/Ca clays

Designation	Extractable metals, mmole g <sup>-1</sup>	
	Ca	Fe
100% Ca	0.3460	0.0382
25% Fe + 75% Ca	0.2414	0.0910
50% Fe + 50% Ca	0.1860	0.1939
25% Fe + 25% Ca	0.0818	0.2887
100% Fe	0.0382	0.3760

The converted clay was kept in powdered form, and, at least several days before the experiment, an aqueous suspension was prepared at a concentration of 2 mg of clay per milliliter. The 5'-AMP (free-acid form) was purchased from Boehringer Mannheim GmbH (W. Germany); solutions of the required concentration were prepared freshly for each experiment. Reagents used were of analytical reagent quality throughout.

## 2.2. PROCEDURES

The following protocol was used in this study:

*Adsorption stage:* Three milliliters of the clay suspension were transferred to a 10-ml glass centrifuge test tube. The pH was adjusted to the required value with a few drops of either HCl or NaOH solution, using various concentrations at various pH ranges to ensure minimal change in the suspension volume. Two milliliters of the appropriate 5'-AMP solution were added, (diluting the clay suspension to a concentration of 1.2 mg of clay per milliliter). Three initial concentrations of 5'-AMP were used: 0.080, 0.268 and 0.803 mmole 5'-AMP per gram of clay, giving ratios of mmole 5'-AMP to meq. adsorbed metal of approximately 1 : 10, 1 : 3, and 1 : 1, respectively. The pH was then rechecked and adjusted, if necessary. The test-tubes were shaken for 24 hr in a controlled-temperature room ( $t = 20^{\circ}\text{C} \pm 1^{\circ}\text{C}$ ) for equilibration. A preliminary experiment showed only slight differences in adsorption when measured after 7, 24, or 48 hr of equilibration. After 96 hr, somewhat increased apparent adsorption was measured; this may have been, however, a result of chemical or bacterial decomposition of the 5'-AMP that remained in solution.

At the end of the equilibration period, the pH was again measured, recorded, and used as the value characterizing the systems (pH changes during equilibration were observed, especially in the intermediate range of 4–7). The suspension was then centrifuged for 15 minutes at 11,800 RCF (relative centrifugal force) in a Sorvall superspeed centrifuge with a SM-24, 28° angle head and for 5 min at 1,700 RCF in an IEC clinical centrifuge with a horizontal rotor to enable complete separation of the supernatant solution from the sediment.

*Desorption stages: Water wash* – Five milliliters distilled water were added to the clay sediment in the test tube, and the clay was redispersed by vigorous mechanical shaking, followed by gentle shaking for 2 hr. The pH was then remeasured, the clay sedimented, and the solution separated for analysis using the same procedure as in the adsorption stage. *NaOH extraction* – The procedure of the first desorption stage (water wash) was followed except that after addition of the water to the clay sediment, the pH was adjusted to 12 with NaOH and the clay was shaken for 24 hr.

The 5'-AMP concentration was determined in the solutions from the three stages described above, after appropriate dilution, by UV absorption at 258 nm: analyses were performed within 1-2 days of the separation of the solution from the clay. The experimental series included three 5'-AMP standards, adjusted to pH's 2, 7, and 12 and six clay blanks adjusted to pH's 2, 4, 6, 8, 10, and 12. The UV readings of the 5'-AMP-treated clay samples were corrected by subtracting from them the readings for the clay blanks; the latter usually ranged from 0% to 5% of the sample readings. The 5'-AMP concentration in solution was then obtained by comparison to the standards. The amount of 5'-AMP adsorbed to the clay was calculated from the difference between the initial and final concentrations of 5'-AMP in the solution.

The absolute magnitude and variability of the 'carry-over' effect caused by solution entrapped in the clay sediment and carried from one stage to the next was evaluated. The average carry-over for 28 test tubes in one experiment was found to be  $A = 0.249$  g of solution with a standard deviation,  $\sigma = 0.088$  g. Since the total solution mass was 5 g, this amounts to  $A = 5\%$  and  $\sigma = 1.8\%$  of the total mass. Considering the other sources of variability and the level of uncertainty in the procedure, this source was not considered large enough to justify the tedious weighing procedure to obtain an exact correction for each test tube. However, a correction, using the average carry-over value shown above, multiplied by the measured solution concentration, was introduced in the concentrations measured in the water-wash when the overall recovery of 5'-AMP was calculated. The carry-over correction was significant only in cases in which only slight adsorption took place in the adsorption stage.

### 3. Results and Discussion

*Adsorption vs. pH:* Figures 1 through 5 show the change in amount of 5'-AMP adsorbed as a function of pH onto the five Fe-Ca clays. In general, the adsorption diminished as the pH rose, and in none of the clays studied was a pronounced specific adsorption at the neutral pH range found. The shape of the adsorption versus pH curves was generally similar for all the clays, but significant differences were found between them with respect to the amount adsorbed at a given pH; these differences will be discussed in detail below.

The general shape of the adsorption curve can be shown to be closely related to the net molecular charge of the 5'-AMP molecule, schematically presented in Figure 6. High adsorption is observed at the pH range from 2 to 4.5, and it is interpreted to be at least partly related to cation exchange where the positive edge of the 5'-AMP molecule, the protonated N1 site, is attached to the basal surface of the clay and displaces some of the exchangeable cations. However, since 5'-AMP is a zwitterion in this pH range (Figure 6), anion-adsorption is taking place as well. In relation to this, it is noticeable that at the iso-electric point (IP) of 5'-AMP, estimated to be at pH 2.8–3.0, there is always measurable adsorption onto the clay (Figures 1–5). This shows that even though the overall net charge is zero at the IP, the 5'-AMP molecule still has charged sites that have enough spatial separation so that they may individually participate in adsorption processes. This has been found also to be the case for the adsorption of other large organic molecules such as polypeptides and proteins (Harter, 1977).

At the pH range of 4.5 to 5.5, the adsorption decreases somewhat: at this range 5'-AMP is monoanionic (Figure 6) and apparently interacts via the phosphate moiety with positive charges at the edges of the clay particles or adsorbs through ligand exchange with coordinated  $\text{OH}^-$  or  $\text{H}_2\text{O}$  on the solid surface as does inorganic phosphate (Hingston *et al.*, 1972). Previous studies of 5'-AMP adsorption on Ca-montmorillonite by Graf and Lagaly (1980) were conducted at pH 5–6; little adsorption was observed there, consistent with the observations here. Following this region, a clear minimum in adsorption occurs in the pH range of 6 to 11, where the

**5'-AMP + 100% Ca CLAY**

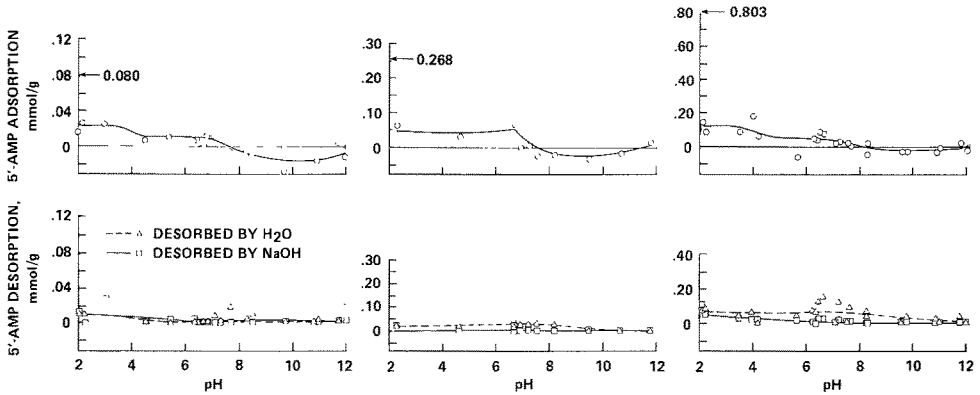


Fig. 1. pH profile of adsorption of 5'-AMP on a 100% Ca-montmorillonite and its subsequent desorption by H<sub>2</sub>O wash and NaOH (pH = 12.0) extraction. Arrows in the adsorption plot indicate initial level of addition of 5'-AMP.

molecule is a di-anion: in most cases (Figures 1–5) there is actual repulsion of 5'-AMP from the clay shown as *negative* values of adsorption. This observation will be discussed in more detail in the next paragraph. Note that within this range, Zn<sup>++</sup> and Cu<sup>++</sup> clays show a maximum in the adsorption (Lawless *et al.*, 1984, this issue). Presumably, Zn and Cu have specific ability to bond with 5'-AMP at this pH range, on the surface of the clay, whereas iron and calcium do not.

At pH's above 11, a small increase in adsorption is observed (Figures 1–5). At first observation this reversal in adsorption trend is puzzling. It may be speculated that the

**5'-AMP + (25% Fe + 75% Ca) CLAY**

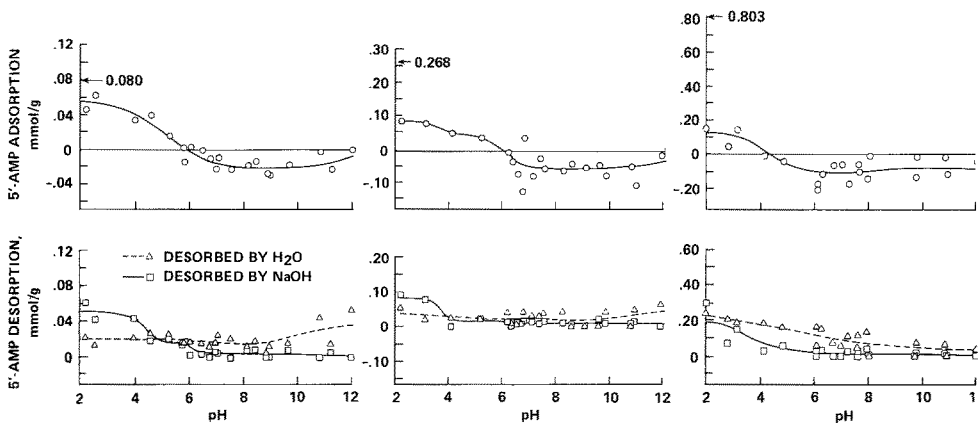


Fig. 2. pH profile of adsorption and desorption of 5'-AMP on (25% Fe + 75% Ca)-montmorillonite (see legend for Figure 1).

5'-AMP + (50% Fe + 50% Ca) CLAY

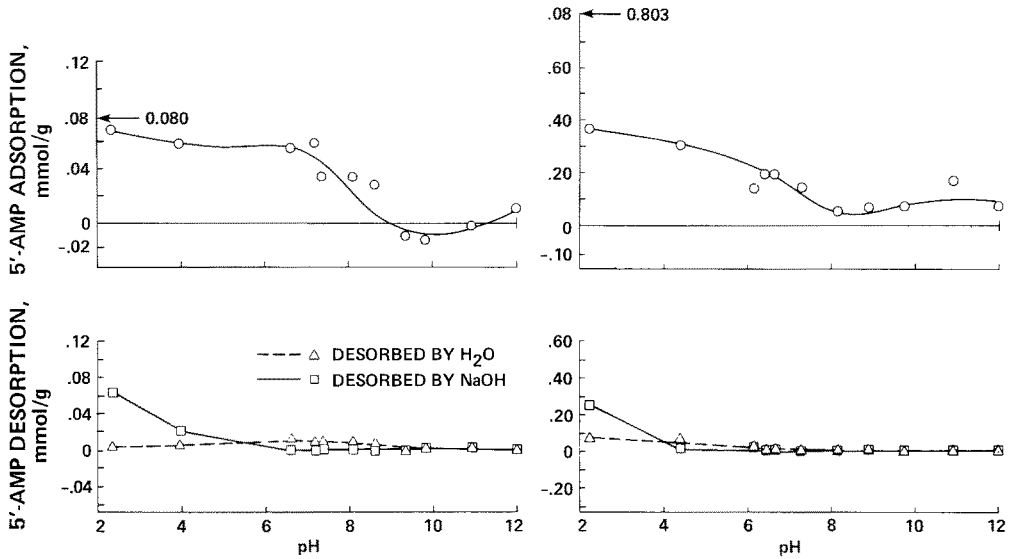


Fig. 3. pH profile of adsorption and desorption of 5'-AMP on (50% Fe + 50% Ca)-montmorillonite (see legend for Figure 1).

electrostatic forces that caused repulsion of the doubly charged phosphate moiety of the 5'-AMP in the pH range 6–11 do not operate as strongly on the monoanionic charge-site developing on the ribose moiety (Figure 6). Another possible cause may be the change in surface chemistry of the clay owing to selective dissolution of aluminum at

5'-AMP + (75% Fe + 25% Ca) CLAY

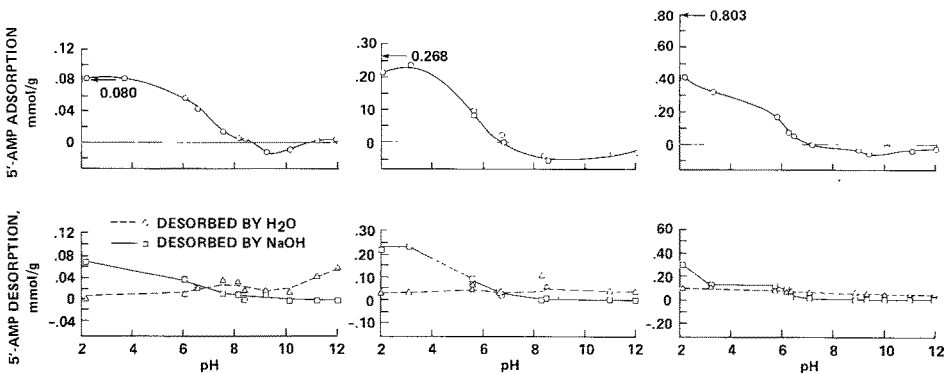


Fig. 4. Profile of adsorption and desorption of 5'-AMP on (75% Fe + 25% Ca)-montmorillonite (see legend for Figure 1).

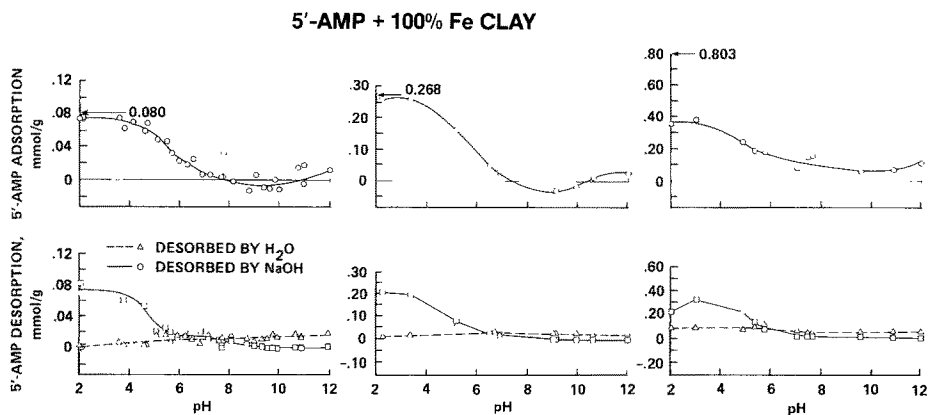


Fig. 5. pH profile of adsorption and desorption of 5'-AMP on 100% Fe-montmorillonite clay (see legend for Figure 1).

the edges at high pH (Jackson, 1964), which again enables ligand exchange and adsorption of the phosphate (Hingston *et al.*, 1972).

*Negative Adsorption:* In some of the systems studied (Figures 1–5), a 'negative adsorption' of 5'-AMP was measured. It resulted from the experimental observation that  $C_e$ , the equilibrium concentration of 5'-AMP in the supernatant following interaction with the clay, was higher than  $C_i$ , the calculated initially added concentration. ( $C_i$  is defined as the 5'-AMP concentration which, according to calculation, should have been observed immediately after mixing of the 5'-AMP solution with the clay suspension and before any interaction between clay and 5'-AMP took place). When  $C_e$  is smaller than  $C_i$  the interaction between the organic molecule and the clay is termed adsorption: when  $C_e$  is larger than  $C_i$ , it is termed negative adsorption, showing negative values for the amounts adsorbed.

One possible explanation for the observation of negative adsorption may be as follows: some clay component was leached-out, which increased UV absorption and caused  $C_e$  to be, apparently, larger than  $C_i$ . However, the UV readings for the reacted clays were corrected by subtracting from them the readings for clay blanks (See the Procedures section). Unless there is increased leaching from the clay in the presence of 5'-AMP, this correction should have eliminated this effect.

Assuming, for the sake of this discussion, that the negative adsorption observations were not due to leaching from the clay, they may result from repulsion of the anionic 5'-AMP molecule, by electrical double layer forces, from the vicinity of the negatively charged clay particles (de Haan, 1964), as was observed for inorganic anions, such as  $Cl^-$  and  $NO_3^-$  (e.g., Schofield and Talibuddin, 1948; Banin, 1972). For the systems studied here, negative adsorption was observed in the pH range 6 to 11, in which 5'-AMP is in the dianionic form. This observation is particularly interesting because, for phosphate alone, a specific positive adsorption is usually found on clays at this pH



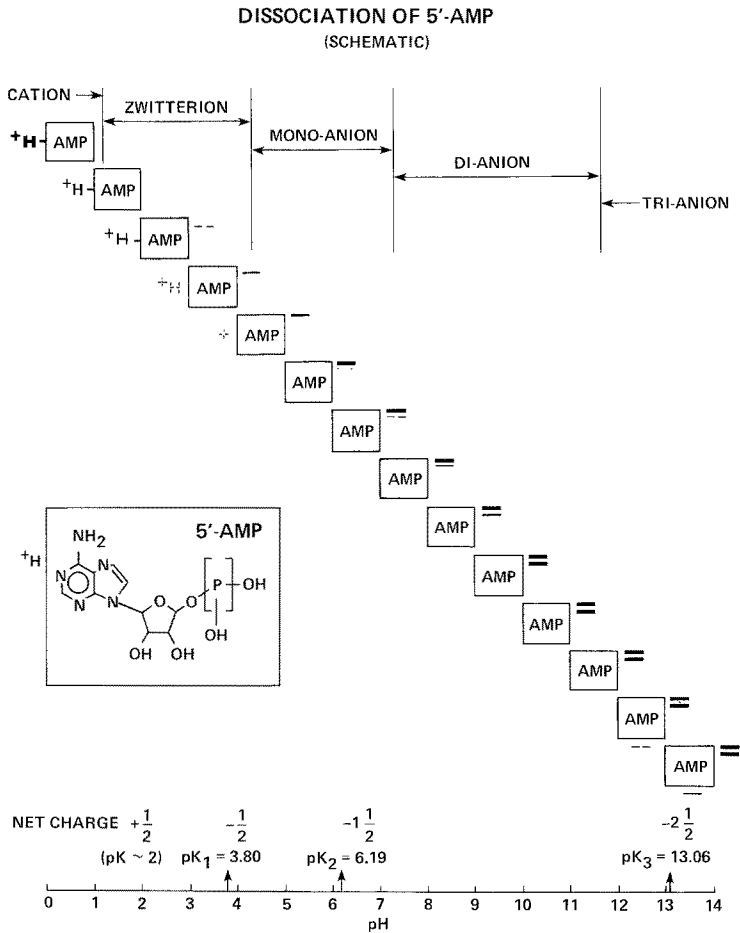


Fig. 6. Schematic presentation of 5'-AMP dissociation and net charge throughout the pH range 0 to 14.

range. Although further experimentation is needed to confirm this hypothesis, it may be suggested that because of the large molecular size of 5'-AMP, specific adsorption at the hydroxy-metal edge sites is less favored than for phosphate and electrostatic repulsion, which also operates in the case of phosphate, becomes predominant.

To estimate the amount of negative adsorption predicted by double-layer theory, various calculations have been performed. These calculations (Banin, 1984) show that the maximal repulsion to be expected, under the most extreme assumptions (full double-layer depth of 1,000 Å, full exposure of surface area of the clay) may cause negative adsorption at a level of up to 30% of the initial concentration. The values observed in this study were mostly in the range of 5–15% of the initial concentration, probably since conditions for full double-layer repulsion did not exist.

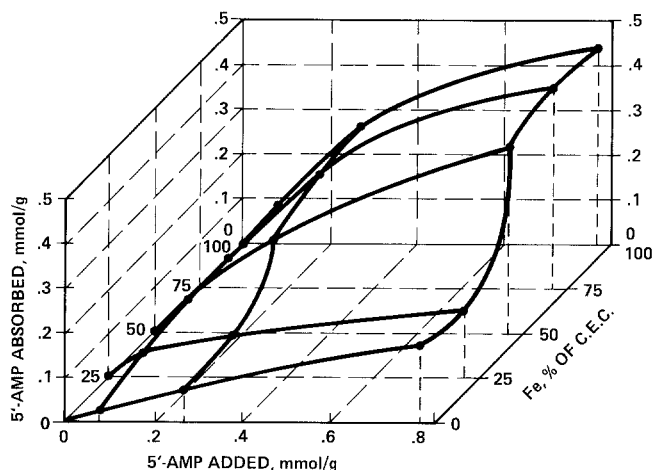


Fig. 7. Adsorption of 5'-AMP on Fe-Ca-montmorillonites, at the pH range of 2.0 to 2.5, as a function of the adsorbed iron content (% of cation exchange capacity (C.E.C.)), and level of 5'-AMP addition.

*Desorption:* The total recovery of 5'-AMP in the two desorption steps (water wash plus NaOH extraction) was generally around 100% of the amount adsorbed. When deviations from complete recovery were observed, they were of random nature (ranging between 85% and 115% of the adsorbed) and considered to be due to experimental variation. No indication for breakdown of the 5'-AMP molecule during adsorption was found. At the low end of the pH range (2–5) most of the adsorbed 5'-AMP was desorbed by the NaOH extraction. At higher pH values, a more significant proportion was desorbed by the initial water wash; however, it shall be noted that the amount adsorbed was lower at the higher pH range. Presumably, the mechanism of desorption by the NaOH extraction involves the following steps: (1) a change of the 5'-AMP molecule to the dianionic and trianionic forms, in which it is only slightly adsorbed, and (2) increased competition by  $\text{OH}^-$  groups in the solution to anion-adsorption of 5'-AMP on the clay.

*Effect of exchangeable iron on adsorption:* As noted above, adsorption of 5'-AMP increased, at a given pH, as the adsorbed iron content of the clay increased. A three-dimensional plot of adsorption as a function of 5'-AMP addition and nominal adsorbed Fe content on the clay is shown in Figure 7 for the low pH range (pH = 2.0–2.5). It is seen that for each clay, the amount adsorbed as a function of level of addition, first increases and then levels off. This is typical for an adsorption process where the clay has a limited adsorption capacity for 5'-AMP. The limiting capacity is clearly cation-dependent and increases with iron content. However, the increase is not proportional to iron content: the 50% Fe-clay adsorbed practically as much 5'-AMP as the 100% Fe-clay at the highest level of 5'-AMP addition. Thus, another limitation to adsorption may be present; one possibility is that adsorption is limited by steric hindrance and interferences at the adsorbing sites on the surface of the clay.

TABLE II

Metal release and 5'-AMP adsorption on 100% Ca and 100% Fe montmorillonites at the low pH range (pH = 2.0–2.5)

5'-AMP added	5'-AMP adsorbed	Ca released	Fe released	Molar Ratio <sup>a</sup>	pH
mmole g <sup>-1</sup>					
100% Ca montmorillonite					
				Ca/5'-AMP	
0.000	0.000	0.363	0.001	—	2.57
0.080	0.027	0.331	0.001	12.3	2.23
0.268	0.062	0.306	0.001	4.9	2.31
0.803	0.117	0.311	0.001	2.7	2.25
Initial in clay:		0.346	0.038	—	—
100% Fe montmorillonite					
				Fe/5'-AMP	
0.000	0.000	0.000	0.180	—	2.36
0.268	0.080	0.032	0.130	1.63	2.15
0.268	0.258	0.047	0.122	0.47	2.25
0.803	0.400	0.145	0.105	0.26	2.08
Initial in clay:		0.038	0.376	—	—

<sup>a</sup> Molar ratio = [(mmole metal released/g clay)/(mmole 5'-AMP adsorbed/g clay)].

In this pH range (2.0–2.5) 5'-AMP carries both positive and negative charge and can be adsorbed by nonspecific electrostatic interaction with the surface of the clay. The portion of adsorption which involves cation-exchange may be related to cation release from the clay. Data on metal release from two of the clays are given in Table II. It is seen from this data that the adsorption process, even at low pH, is not solely and simply a stoichiometric ion exchange process. The addition of the acid and adjustment of pH to 2.0–2.5 was sufficient to displace and solubilize all of the adsorbed calcium from the 100% Ca-clay. Still, not all of the added 5'-AMP was adsorbed nor did 5'-AMP adsorption reach the cation exchange capacity even at the highest level of addition. Other cations must then compensate the excess negative charge of the clay. These include hydrogen, and ions released from the clay lattice at low pH, primarily aluminum and magnesium. In the 100% Fe clay, a much smaller proportion of the iron was displaced and solubilized by the addition of the acid, but more 5'-AMP was adsorbed than in the 100% Ca clay. Apparently the presence of adsorbed iron increased the adsorption capacity of the clay for 5'-AMP and facilitated adsorption not involving displacement of the iron. This is supported by the observation that as more 5'-AMP was added (and adsorbed), less iron was released to the solution (Table II). This may suggest a preference for adsorption of an Fe-5'-AMP molecular pair as yet undefined. More work is required to clarify the metal release dynamics during 5'-AMP adsorption.

#### 4. Conclusions

Five forms of the AMP molecule (monocation, zwitterion, and mono-, di-, and trianion) which are present in varying proportions as pH changes, appear to interact with montmorillonite clay through primarily electrostatic attraction-repulsion mechanisms leading to positive and negative adsorption. It does not appear that specific 5'-AMP-metal complexation is taking place at a significant level throughout the whole pH range, and the adsorption seems to be due mostly to cation exchange, anion exchange, and anion specific adsorption on the clay surface. Regarding their effects on 5'-AMP adsorption on montmorillonite, iron and calcium are similar, in this respect, to most other metal ions studied in the present series, but are different from Zn and Cu. However, the amount of 5'-AMP adsorbed by the clay at a given pH increases as the iron content increases, implying that iron facilitates adsorption. There are indications that the amount adsorbed in the mixed Fe/Ca-clay and the pure Fe clay is limited by both the amount of iron present and the specific surface area per site of the clay.

The variation of nucleotide adsorption onto clays in the pH range of 4–9 may have had important ramifications in relation to abiotic evolution. For example, fluctuations in moisture content of clays, which affect the pH of the system (the drier the clay, the more acidic it is), may cause cyclical adsorption-desorption processes, leading to enhanced interactions between molecular species. Alternating inputs of acidic fumarolic gases into clay beds, which were exposed also to inputs of organic molecules, may have caused increased adsorption of the organics at the low pH range and their release at another time or location as a result of change in pH; the clay thus acted as a concentrating carrier for the organic molecules, with built-in release mechanisms.

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