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SMECTITE INTERACTIONS WITH FLAVOMONONUCLEOTIDE[1]

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Abstract—Adsorption isotherms and UV-visible and Mössbauer spectroscopic data point to specific interactions between flavomononucleotide (FMN) and Fe^{3+}-smectite. The maximum amount of FMN adsorption was 0.3 mmole/g of Fe^{3+}-smectite giving a 1:1 molar proportion of Fe^{3+} and FMN. The results suggest a Fe^{3+}-FMN complex residing at the smectite surface. Other homoionic smectites (Cu^{2+}, Zn^{2+}, and Ca^{2+}) exhibited lower levels of adsorption and less apparent specific interaction.

Key Words—Adsorption, Flavomononucleotide, Mössbauer spectroscopy, Organo-clays, Smectite, UV-visible spectroscopy.

INTRODUCTION

Flavins are co-factors for a large number of enzymes involved in oxidation-reduction reactions. Whereas flavins may be catalytically active on their own, such activity and specificity may be much greater when they are present as the co-factor in an enzyme system.

Lawless and Edelson (1980) found clay interactions with nucleotides to be profoundly influenced by the nature of the exchangeable cation. In investigating the adsorption of 2′-, 3′-, and 5′-adenosine monophosphate (AMP) by various homoionic smectites, they found that the 5′-AMP isomer was preferentially adsorbed by Zn^{2+}-smectite over the other two. Smectites saturated with transition metal cations, Zn^{2+}-clay in particular, adsorbed 5′-AMP to differing degrees, but smectites saturated with alkali metals did not adsorb any of the nucleotide.

The work reported here is a characterization of the interactions between flavomononucleotide (FMN) and smectite. Earlier work by Mortland and Lawless (1983) was concerned with riboflavin-clay interactions. Riboflavin is the same as FMN except that it has no phosphate group on the sugar moiety. Riboflavin was found to be adsorbed on polyvalent cation-saturated smectite to a maximum level of about 0.5 mmole/g of clay. The shape of these isotherms was of the Langmuir type. Information from UV-visible spectroscopy suggested that charge-transfer complexes were formed when transition metal cations (i.e., Fe^{3+} and Cu^{2+}) occupied the exchange sites on the clay. In addition, hydrogen bonding and physical forces were also important components of riboflavin-clay interactions because Ca^{2+}-smectite adsorbed as much riboflavin as clays saturated with transition metal cations. As expected, however, these forces did not produce much change in the electronic spectrum of the adsorbed riboflavin. Ca^{2+}-vermiculite adsorbed no riboflavin between the clay layers, undoubtedly because its more limited swelling in water (14.5 Å vs. ~20 Å for Ca^{2+}-smectite) did not permit penetration of the large riboflavin molecule into the internal surfaces.

EXPERIMENTAL METHODS

The smectite used in these experiments was from Upton, Wyoming, and was obtained from Wards Natural Science Establishment as API No. 25 reference sample. The <2-μm fraction was treated with the chloride salts of the desired cations in great excess of the cation-exchange capacity, and then dialyzed against distilled water until there was a negative test for chloride in the dialysate. The clay was then freeze-dried and stored for use.

Adsorption isotherms at 20°C were obtained by weighing 50 mg of clay into 100-ml volumetric flasks and then adding amounts of a 0.001 M FMN solution to give 1, 0.5, 0.2, and 0.1 mmole FMN/g of clay. Water was then added to give a total volume of 100 ml. The pH of the various systems was not adjusted because any salt, acid, or base added would affect ionic strength

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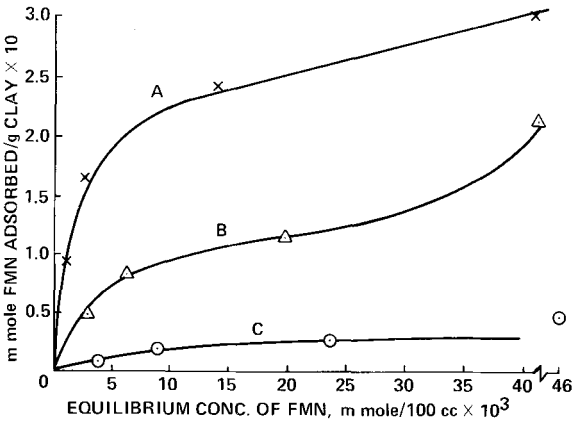


Figure 1. Adsorption isotherms of flavomononucleotide (FMN) on (A) Fe^{3+}-, (B) Cu^{2+}-, and (C) Ca^{2+}-smectite.

and cause ion exchange and/or precipitation with counter ions on the exchange complex of the clay.

UV-visible spectra were obtained by evaporating water suspensions of the clay onto quartz plates, placing the plates into an FMN solution for a few hours, washing them with distilled water to remove all unadsorbed FMN, and then placing them in larger quartz cells containing water. A cell containing a clay film with no complex was used in the reference beam. Spectra were obtained with a Cary 14 spectrophotometer. Resulting spectra were differential in nature because optical absorptive properties of the clay were cancelled. Spectra of dried samples were obtained by heating the plates at 75°C for 12 hr and then covered the films with mineral oil to prevent rehydration and to reduce light scattering effects.

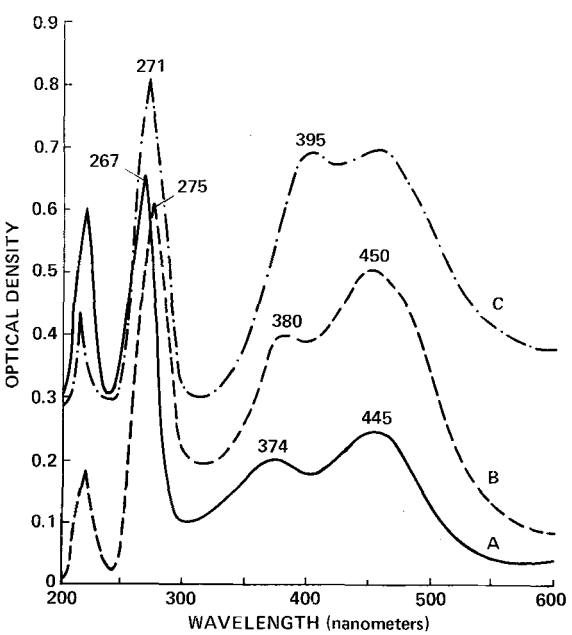


Figure 2. UV-visible spectra of flavomononucleotide (FMN) on (A) H_2O (0.0025 mmole/100 ml), (B) complexed with Cu^{2+}-smectite (film on quartz plate in H_2O), (C) sample B heated to 75°C and covered with mineral oil.

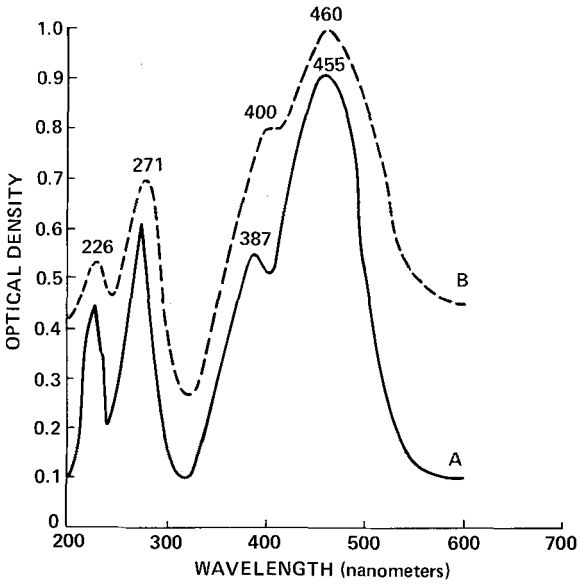


Figure 3. UV-visible spectra of flavomononucleotide (FMN) (A) complexed with Fe^{3+}-smectite on a quartz plate in H_2O, (B) sample A heated to 75°C and covered with mineral oil.

Mössbauer spectra of Fe^{3+}-smectite-flavin complexes were obtained by preparing $^{57}Fe^{3+}$-smectite by ion exchange of this iron isotope with Na^+ on the clay. Some of the $^{57}Fe^{3+}$-smectite was permitted to adsorb FMN and riboflavin from solution. After washing with distilled water, the $^{57}Fe^{3+}$-smectite, with and without the flavin complexes, was air dried, ground, and incorporated into boron nitride for analysis.

DISCUSSION OF RESULTS

Figure 1 shows the adsorption isotherms of FMN on (A) Fe^{3+}-, (B) Cu^{2+}-, and (C) Ca^{2+}-smectites. The isotherm for Zn^{2+}-smectite was very similar to that of Cu^{2+}-smectite. Just as isotherms of riboflavin adsorption (Mortland and Lawless, 1983) on smectites saturated with polyvalent cations were characterized by their similarities, those of FMN were distinguished by their singularities. The iron-saturated smectite gave a Langmuir-type isotherm with a maximum adsorption of about 0.3 mmole FMN/g of clay. X-ray powder diffraction gave a 001 spacing of 15.3 Å at this level of adsorption. The Cu^{2+}- and Zn^{2+}-smectite adsorption isotherms are distinctly different showing much lower amounts of adsorption. Ca^{2+}-smectite showed very little affinity for the FMN, adsorbing only small quantities. Thus, the isotherms suggest specific complexation with the Fe^{3+}-smectite with less energetic

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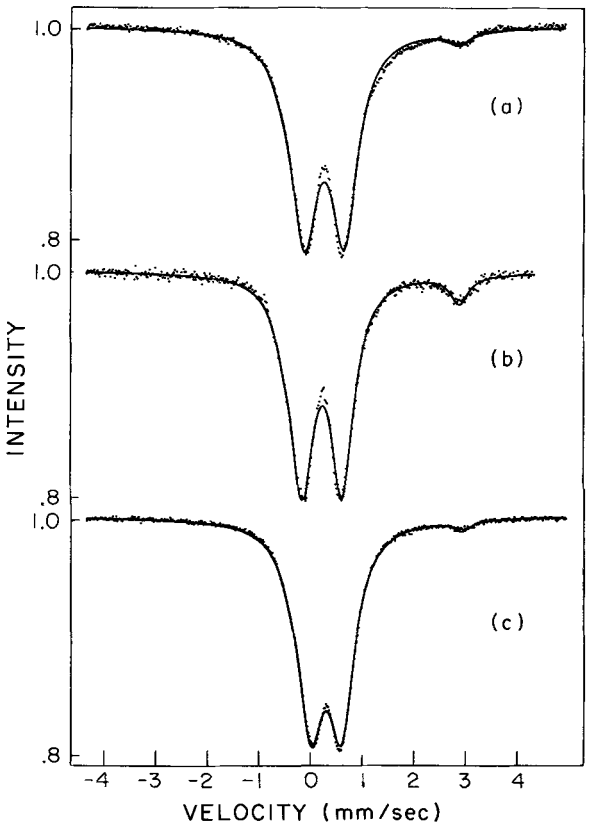


Figure 4. Mössbauer spectrum of (a) air-dried $^{57}Fe^{3+}$-smectite, (b) Mössbauer spectrum of riboflavin–$^{57}Fe^{3+}$-smectite complex, and (c) Mössbauer spectrum of flavomononucleotide (FMN)–$^{57}Fe^{3+}$-smectite complex.

interactions with Cu^{2+}- and Zn^{2+}-clay, and even less with Ca^{2+}-clay. Analyses of the equilibrium solutions showed only traces of iron indicating that iron was not displaced from the clay and suggesting that ion exchange was not a phenomenon associated with FMN adsorption.

UV-visible spectra of FMN complexed with Cu^{2+}- and Fe^{3+}-smectite are shown in Figures 2 and 3. Attribution of the two visible and two ultraviolet bands was made to the $\pi \rightarrow \pi^*$ type transitions by Pill-Soon Song (1981). On comparing the spectrum of free FMN (spectrum A, Figure 2) with those of the Cu^{2+}- and Fe^{3+}-clay-FMN complexes, changes in relative intensity of some bands can be seen as well as small red shifts of the two visible bands (~374 and 445 nm). The latter can be attributed to hydrogen bonding (Pill-Soon Song, 1981) between FMN and the mineral surface. These spectra do not show the dramatic changes noted for riboflavin complexes with Cu^{2+}- and particularly Fe^{3+}-smectite (Mortland and Lawless, 1983) which these authors suggested were due to the formation of charge-transfer complexes.

Mössbauer spectra of Fe^{3+}-smectite alone (Figure 4a) complexed with riboflavin (Figure 4b) and complexed with FMN (Figure 4c) were obtained utilizing $^{57}Fe^{3+}$ as the isotope on the exchange sites. The spectra indicate that ferric iron is the major oxidation state in all three materials, with only small amounts of ferrous iron (Table 1). Except for the small difference in the amount of Fe^{2+}, the spectral parameters of the riboflavin–iron-smectite complex and the iron-smectite are similar. On the other hand, the spectral parameters of the FMN–iron-smectite are quite different from the iron-smectite, suggesting formation of an iron-FMN interaction on the clay.

DISCUSSION

Adsorption isotherms, UV-visible, and Mössbauer spectra suggest a very specific interaction between FMN and Fe^{3+}-smectite. It is likely that this interaction is between the Fe^{3+} on the clay and the phosphate group on the sugar moiety of the FMN. The earlier work on riboflavin (Mortland and Lawless, 1983), suggested an interaction between the isoalloxizine moiety and transition metal cations on the clay complex probably via the No. 5 nitrogen. This close relationship with the aromatic system of the isoalloxizine group resulted in greater fundamental electronic perturbation of the resonating system and gave the resulting UV-visible spectra. For FMN, if interaction with Fe^{3+} is via the phosphate group, the isoalloxizine moiety should be more distantly related to the Fe^{3+} with less effect on the UV-visible spectrum. For riboflavin–Fe^{3+}-smectite complexes, UV-visible spectra suggest that charge transfer complexation took place (Mortland and Lawless, 1983), whereas the Mössbauer spectrum (Figure 4) indicates little difference from the air-dried Fe^{3+}-smectite except for a modest increase in Fe^{2+}. Thus, charge-transfer

Table 1. Mössbauer spectral parameters of $^{57}Fe^{3+}$-smectite complexed with riboflavin and flavomononucleotide (FMN).

Sample	Temperature (°K)	Fe(III)			Fe(II)		
		ΔEq[1]	I.S.[2]	%	ΔEq[1]	I.S.[2]	%
Fe^{3+}-smectite	77	0.78	0.29	94	3.5	1.17	6
Fe^{3+}-smectite + riboflavin	77	0.77	0.282	90	3.4	1.22	10
Fe^{3+}-smectite + FMN	77	0.60	0.32	97	3.4	1.28	3

[1] Quadrupole splitting in mm/sec.
[2] Isomer shift relative to iron metal at room temperature in mm/sec.

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complexation may not produce great changes in Mössbauer spectra in the riboflavin systems, whereas Fe^{3+}-smectite interaction with the phosphate group of FMN gives the observed changes in the Mössbauer parameters.

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Резюме—Изотермы адсорбции и данные по ультрафиолетово-видимой и Мессбауеровской спектроскопиях указывают на специфические взаимодействия между флавомононуклеотидом (ФМН) и Fe^{3+}-смектитом. Максимальные количества адсорбированного ФМН были 0,3 ммоль на грамм Fe^{3+}-смектита, что давало молярные пропорции Fe^{3+} и ФМН равные 1:1. Результаты указывают на то, что Fe^{3+}-ФМН комплекс находится на поверхности смектита. Другие гомоионные смектиты (Cu^{2+}, Zn^{2+}, и Ca^{2+}) показывали более низкие уровни адсорбции и менее видимое специфическое взаимодействие. [E.G.]

Resümee—Adsorptionsisotherme sowie sichtbare UV- und Mössbauerspektren deuten auf spezifische Wechselwirkungen zwischen Flavomononukleotiden (FMN) und Fe^{3+}-Smektit hin. Die maximale FMN-Adsorption betrug 0,3 mMol/g Fe^{3+}-Smektit, was ein molares Verhältnis von 1:1 für Fe^{3+} und FMN ergibt. Die Ergebnisse deuten auf einen Fe^{3+}-FMNkomplex auf der Smektitoberfläche hin. Andere homoionische Smektite (Cu^{2+}, Zn^{2+}, und Ca^{2+}) zeigten niedrigere Adsorptionsmengen und eine weniger offensichtliche spezifische Wechselwirkung. [U.W.]

Résumé—Les isothermes d'adsorption et les données spectroscopiques d'UV-visible et de Mössbauer indiquent des interactions spécifiques entre la flavomononucléotide (FMN) et la smectite-Fe^{3+}. L'adsorption maximum d'adsorption FMN était 0,3 mmole/g de smectite-Fe^{3+}, donnant une proportion molaire 1:1 de Fe^{3+} et de FMN. Les résultats suggèrent un complexe Fe^{3+}-FMN résidant à la surface de la smectite. D'autres smectites homoioniques (Cu^{2+}, Zn^{2+}, et Ca^{2+}) ont exhibé des niveaux d'adsorption plus bas et une interaction specifique moins apparente. [D.J.]

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