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SMECTITE INTERACTIONS WITH RIBOFLAVIN

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Abstract—Smectite with polyvalent cations on the exchange complex readily absorbed riboflavin from aqueous solution to a limit of about 0.5 mmole/g. The shape of the adsorption isotherms was of the Langmuir type. An exception was Na^+-smectite which provided an S-shaped isotherm and smaller amounts of adsorption. Ca^{2+}-vermiculite had no interlamellar adsorption of riboflavin, suggesting that this mineral does not swell sufficiently to permit such adsorption. Adsorption isotherms, X-ray powder diffraction data, and UV-visible spectroscopic data suggest that the mechanisms of interaction between smectite and riboflavin may involve a combination of ion-dipole, charge transfer, hydrogen bonding, and physical effects.

Key Words—Adsorption, Charge-transfer complex, Riboflavin, Smectite, UV-visible spectra, Vermiculite.

INTRODUCTION

The mechanisms of clay-organic interactions range from physical types of low energy to chemical kinds involving many kilocalories (Mortland, 1970). Suggestions have been made that ubiquitous clays may have played a role in the prebiotic evolution of macromolecules (Bernal, 1951). The great adsorption properties of certain clay as well as their ability to catalyze certain chemical reactions would undoubtedly have been involved in any such scenario.

The interactions of various pyrimidines, purines, and nucleosides with clays have been studied mostly by Brindley and co-workers (e.g., Lailach *et al.*, 1968; Thompson and Brindley, 1969). More recently, nucleotide adsorption was investigated by Lawless and Edelson (1980) who found important effects of the nature of the exchange cation of clays (particularly Zn^{2+}) on nucleotide adsorption, and by Graf and Lagaly (1980) who found that certain clay minerals affected ATP decomposition. A variety of interactions with clays is possible, including protonation on acid clay surfaces, complexation with exchange cations (particularly transition metal cations), and less energetic interactions such as hydrogen bonding and physical forces (Mortland, 1970).

Flavins are organic compounds that act as coenzymes which are extremely important in metabolic functions, particularly in carbohydrate metabolism, and as part of charge-transfer systems. Riboflavin (vitamin B_2) is a molecule composed of an isoalloxazine moiety with an attached D-ribityl sugar. Kim *et al.* (1981) adsorbed riboflavin on silica gel and, when employing it as a chromatographic column, they were able to separate enantiomers of 6- to 14-helicene. In the adsorbed state, the flavin has the ability to discriminate between various molecules through charge-transfer interactions resulting in chiral recognition via the sugar fragment.

The enzyme systems in which flavins act as co-factors are comprised of a protein, flavin, and metal ions. The whole complex, plus a substrate, constitutes a single resonance system with respect to electron transfer. The metal cations may bind the flavin to the protein, as well as participate in electronic changes within the system. Whereas the metal ion is not required for the reaction of reduced enzyme with two-electron acceptors, it is directly involved in one-electron reaction as in ferricyanide and cytochrome (Wagner and Folkers, 1964). In a more primitive milieu, it is possible to picture a simpler system where a phyllosilicate matrix might have substituted for the proteins and exchanged cations (transition metal cations) on the mineral surface for the metal portion of the "enzyme." A flavin moiety might then have been adsorbed at this surface in an arrangement perhaps not greatly different from that in an enzyme.

The objectives of this work were first to characterize the nature of riboflavin interactions with clays, and then to see to what degree the riboflavin-clay complexes may function in charge-transfer reactions and in chiral-discrimination interactions.

EXPERIMENTAL METHODS

The smectite used in these experiments was from Upton, Wyoming, and was obtained from Ward's Natural Science Establishment as API No. 25 reference sample. The <2-μm fraction of the material 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 were obtained by weighing 50 mg of clay into 100-ml volumetric flasks and then adding amounts of a 0.0005 M riboflavin solution to give

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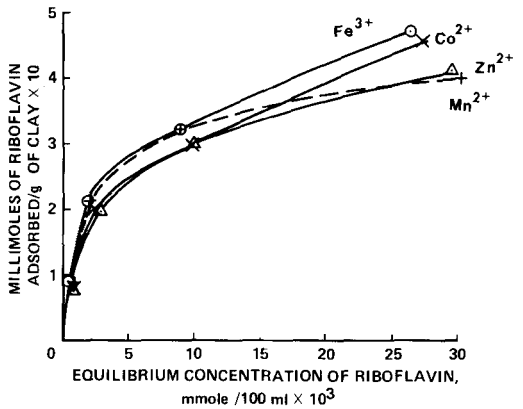


Figure 1. Adsorption isotherms of riboflavin on Fe^{3+}-, Co^{2+}-, Zn^{2+}-, and Mn^{2+}-smectite.

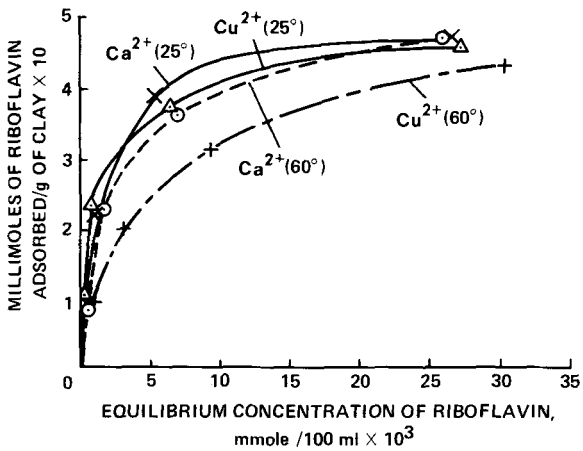


Figure 3. Adsorption isotherms of riboflavin on Cu^{2+}- and Ca^{2+}-smectite at two temperatures.

0.1, 0.25, 0.5, and 1.0 mmole of riboflavin per g of clay. Water was then added to give a total volume of 100 ml. The pH of the system was not adjusted because any salt, acid, or base added would affect ionic strength and cause ion exchange with the counter ion on the clay.

Samples were equilibrated in the dark for 24–72 hr and frequently shaken. After the clay settled, equilibrium concentrations of riboflavin were determined with a Cary 14 spectrophotometer by measuring the optical density of the riboflavin peak at 2670 Å. The pH of the equilibrium solutions was between 6.2 and 7.0.

X-ray powder diffraction (XRD) data were obtained by placing water suspensions of the clays onto glass slides and evaporating the water off on a warm surface. A Philips diffractometer, equipped with Co radiation, was used to obtain 001 reflections.

UV-visible spectra of riboflavin-clay complexes were obtained by evaporating water suspensions of the clay of equal volume onto quartz plates, placing the plates into a riboflavin solution for 1–12 hr, washing the plates with distilled water to remove all unadsorbed riboflavin, and then placing the plates in larger quartz cells containing water. A cell containing a clay film with no complex on a quartz plate was used in the reference beam. Spectra were obtained with a Cary 14 spectrophotometer. Resulting spectra were differential in nature because optical adsorptive properties of the clay were cancelled due to the equal quantities of clay in the sample and reference beams. Spectra of dried samples were obtained by heating the plates at 75° for 12 hr and then covering the clay films with mineral oil to prevent rehydration and to reduce light scattering effects.

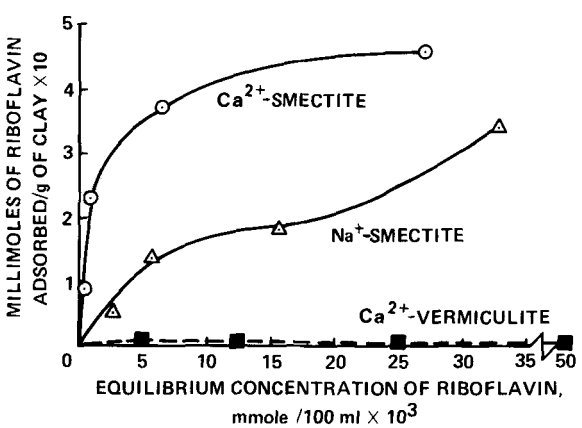


Figure 2. Adsorption isotherms of riboflavin on Ca^{2+}- and Na^{+}-smectite and on Ca^{2+}-vermiculite.

DISCUSSION OF RESULTS

Figures 1–3 present adsorption isotherms of riboflavin on a variety of homoionic swelling clays. The isotherms for Fe^{3+}-, Co^{2+}-, Zn^{2+}-, and Mn^{2+}-smectite in Figure 1 show rather similar characteristics and are indeed comparable with those of the Cu^{2+}- and Ca^{2+}-smectites appearing in Figures 2 and 3. Counter ions were not displaced into solutions, thus adsorption via ion exchange did not occur. The isotherms approach a maximum of 0.4 to 0.5 mmole per g of clay regardless of the nature of the exchangeable cation for these polyvalent cations. Those values along with the XRD data prove that interlamellar adsorption occurs. The isotherm for Na^{+}-smectite in Figure 2 has a different shape and has lower amounts of riboflavin adsorption, which suggests that the degree of swelling of the clay mineral in water is critical in its effect on the adsorption isotherm. For all the polyvalent smectites, swelling in water proceeds to about 20 Å and no farther regardless of the amount of water present. The Na^{+}-smectite swells continuously so that the clay platelets are separated by great distances. Apparently the close proximity of the

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Table 1. Langmuir equation constants and d(001) spacing for adsorption of riboflavin on several homoionic smectites.

Clay	a_s (mmole/g)	log K	d(001) at maximum adsorption (air dried) (Å)
Cu^{2+} -smectite	0.50	2.82	15.1
Mn^{2+}-smectite	0.42	2.72	15.5
Zn^{2+} -smectite	0.46	2.49	15.3
Co^{2+} -smectite[1]	0.35	2.76	N.D.
Fe^{3+} -smectite[1]	0.38	2.77	15.7
Ca^{2+} -smectite	0.48	2.77	15.5
Na^{+} -smectite[2]	N.D.	N.D.	15.5–14[3]

[1] Data points on isotherms at 1 mmole/g addition not utilized because of deviation from Langmuir plot.
[2] Langmuir equation parameters not calculated because of lack of obedience.
[3] Highly interstratified.

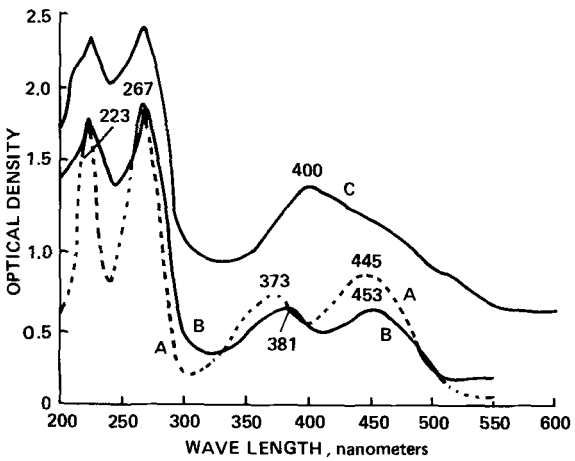


Figure 4. (A) UV-visible spectra of riboflavin in H_2O (0.0025 mmole/100 ml); (B) complexed with Cu^{2+}-smectite (film on quartz plate in H_2O); (c) sample B heated to 75°C and covered with mineral oil.

clay platelets (10 Å) in the polyvalent smectites confers a stabilizing influence upon the riboflavin within the interlamellar regions, perhaps through hydrogen bonding and physical interactions between the riboflavin and the two opposing silica layers, in addition to riboflavin-cation ineractions. For the Na^{+}-smectite, interaction would be with only one surface and a weakly complexing cation, resulting in a weaker binding on the whole. Figure 2 also shows the results for Ca^{2+}-vermiculite where very little riboflavin is adsorbed. The lack of adsorption of riboflavin on the vermiculite is explained by the fact that vermiculite will swell to only about 15 Å in water; this distance between the two opposing mineral layers (~5 Å) is too small to permit interlamellar adsorption of the riboflavin. The above results suggest that the swelling properties of the clays are a major factor in the adsorption of riboflavin.

Figure 3 shows the effect of temperature on the adsorption of riboflavin on Cu^{2+}- and Ca^{2+}-smectite. The temperature effect seems to be the greater for the Cu^{2+}-clay. The enthalpy (ΔH) of adsorption (calculated from the Clausius-Clapeyron equation) between the levels of adsorption 0.2–0.4 mmole per g is 7–6 and 3–1 kcal/mole for the Cu^{2+}- and Ca^{2+}-smectites, respectively. These data suggest an effect of cation in the adsorption of riboflavin on swelling clays.

When the isotherm data were plotted in accordance with the Langmuir equation, good conformance was generally obtained. The equation can be expressed

$$c/a = 1/Ka_s + c/a_s$$

where c is the equilibrium concentration of riboflavin, a is the number of moles of riboflavin adsorbed per g of clay, a_s is the maximum amount of adsorption possible, and K is the Langmuir adsorption coefficient. Table 1 presents the constants K and a_s calculated from these plots. In general, some uniformity of these values is evident. They are in fact similar to constants obtained for the adsorption of some nucleotides on Zn^{2+}-smectite reported by Lawless and Edelson (1980). Table 1 also presents the d(001) values for most of the air-dried, clay-riboflavin complexes. The spacing averages about 15.5 Å, and because the clay itself has a 9.5-Å basal spacing, 6 Å must represent one dimension of the riboflavin molecule adsorbed on the interlamellar surfaces of the clay minerals. When Cu^{2+}-smectite is complexed with lumichrome, the isoalloxizine moiety of riboflavin, a d(001) spacing of 13.8 Å is obtained, suggesting that the ribityl carbohydrate of riboflavin is involved in whatever molecular orientation determines the d(001) spacing. A likely orientation would place the ribityl sugar interacting with one silica sheet via H-bonding while the isoalloxizine interacts with the other via π electrons and H-bonding. It may be that the ribityl group itself defines the interlamellar spacing because 15.5 Å is close to values obtained for monolayers of several sugars in smectite (Theng, 1974). In all likelihood, the 15.5-Å spacing and the maximum adsorption level of 0.5 mmole per g represent the complex upon complete packing of the interlayer space with one layer of riboflavin.

Figures 4–6 are UV-visible spectra of some riboflavin complexes with clays. Figure 4 shows the spectrum (A) of riboflavin dissolved in water. Spectrum B is that of riboflavin complexed with Cu^{2+}-smectite on a quartz plate and immersed in water, and spectrum C is the same sample after heating in an oven at 75°C for several hours and then coated with mineral oil to prevent rehydration and to reduce light scattering. The spectrum of the copper-clay-riboflavin complex in water shows some small upward shifts of all the peaks. Stronger hydrogen bonding with the clay than with water and different environmental conditions, could cause these red shifts (Song, 1981). After heating the complex, a

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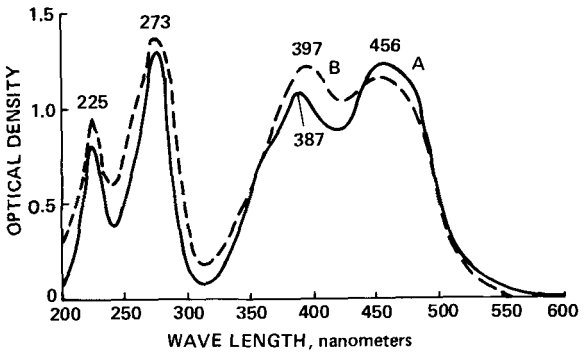


Figure 5. (A) UV-visible spectra of riboflavin complexed with Ca^{2+}-smectite on a quartz plate in H_2O; (B) sample A heated to 75°C and covered with mineral oil.

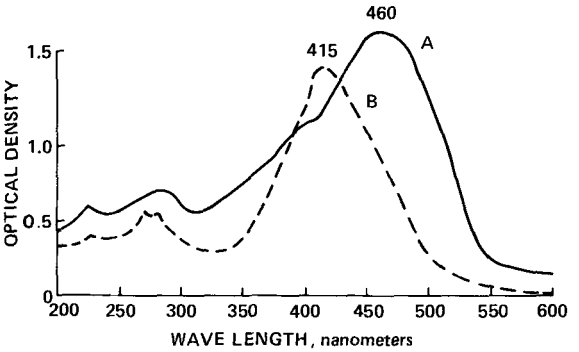


Figure 6. (A) UV-visible spectra of riboflavin complexed with Fe^{3+}-smectite on a quartz plate in H_2O; (B) sample A heated to 75°C and covered with mineral oil.

dramatic shift was noted in the visible region to a broad peak centered at 400 nm, a value very similar to that of "charge-transfer" chelates of flavoquinone-Cu^{2+} in water described by Hemmerich *et al.* (1965). This change in spectrum could result from removal of some directly coordinated water molecules from the Cu^{2+} and riboflavin taking their places in a ligand position with the Cu^{2+}. Figure 5 shows the same kind of experiment with Ca^{2+}-smectite-riboflavin complexes. Complexation of the riboflavin results in small red shifts of the peaks in the water system, whereas drying followed by heat treatment has little effect. Again, these small shifts could be caused by different environmental conditions in the clay compared with H_2O. Because charge-transfer complexes between riboflavin and the exchangeable Ca^{2+} are not expected, these results are not surprising. Figure 6 shows a similar experiment for Fe^{3+}-smectite complexation with riboflavin. Here, the hydrated system (spectrum A) is quite different from uncomplexed riboflavin (A in Figure 4) with a broad peak centered near 460 nm, which shifts to 415 nm after heating and dehydration. Another effect of riboflavin complexation by Fe^{3+}-smectite is the almost complete disappearance of the two peaks in the ultraviolet region. Again, these spectra are reminiscent of charge-transfer complexes described by Hemmerich *et al.* (1965). Whereas the UV-visible spectra are difficult to interpret in a rigorous fashion, they certainly establish that the nature of the exchange cations influences the status of riboflavin, and that it complexes to some degree with polyvalent cations on the smectite surface.

CONCLUSIONS

Riboflavin adsorbs readily within the interlamellar spaces of smectite from water solution. When polyvalent cations are on the cation-exchange complex, Langmuir-type adsorption isotherms are obtained. With Na^{+}-smectite, an S-shaped isotherm was obtained with lower levels of riboflavin adsorption. In all likelihood, the limited swelling of smectite when saturated with polyvalent cations (~20 Å) conferred additional stability on adsorbed riboflavin, possibly because of interaction with both opposing silica sheets. Ca^{2+}-vermiculite did not adsorb riboflavin within its interlamellar space because its limited swelling (~15.0 Å) excluded the riboflavin. A maximum of about 0.5 mmole of riboflavin per g of clay can be intercalated into smectite. This amount apparently represents a complete monolayer packed into the interlamellar regions of smectite.

Ultraviolet-visible spectra of clay-riboflavin complexes suggest charge-transfer complexes when the exchange cations are in the transition group (i.e., Cu^{2+} and Fe^{3+}), when compared with published spectra in homogeneous solution. Because Ca^{2+}-smectite adsorbs riboflavin as readily as any of the transition cation-clay systems, other kinds of riboflavin-clay interactions must also be operative. Such mechanisms could include coordination, ion-dipole, hydrogen bonding of the sugar moiety with surface oxygens, physical forces, and π-electron interaction of the isoalloxizine moiety with surface oxygens of the silica sheet as well as with adjoining or interleaved riboflavin molecules.

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Резюме—Смектит с поливалентными катионами на обменных комплексах легко абсорбировал рибофлавин из водных растворов до величины около 0,5 моля/грамм. Форма адсорбционных изотерм была типа Лангмюра. Исключением являлся Na^+-смектит, для которого получились изотермы типа S и меньшие значения адсорбции. Ca^{2+}-вермикулит не проявлял межслойной адсорбции рибофлавина, указывая на то, что этот минерал не разбухается в достаточной степени, чтобы допустить такую адсорбцию. Адсорбционные изотермы, данные по порошковой ренигеновской дифракции и по ультрафиолетовой и видимой спектроскопии указывают на то, что механизм взаимодействия между смектитом и рибофлавином может включать комбинацию взаимодействия ион-диполь, передачу заряда, водородную связь, и физические эффекты. [E.G.]

Resümee—Smektit mit polyvalenten Kationen am Austauschkomplex adsorbierte Riboflavin bis zu 0,5 × 10^{-3} Mol/g sehr leicht aus wässrigen Lösungen. Die Form der Adsorptions isotherme war vom Langmuir-Typ. Eine Ausnahme bildete Na^+-Smektit, der eine S-förmige Isotherme lieferte und geringere Mengen adsorbierte. Ca^{2+}-Vermiculit zeigte keine Zwischenschichtadsorption von Riboflavin. Dies deutet darauf hin, daß dieses Mineral nicht genügend quillt, um eine derartige Adsorption zu erlauben. Adsorptions isothermen, Röntgenpulverdiffraktometerdaten und optische Spektren (UV, sichtbar) deuten darauf hin, daß der Mechanismus der Wechselwirkung zwischen Smektit und Riboflavin eine Kombination von Ionen-Dipol-, Charge transfer-, Wasserstoffbrückenbindungs-, und physikalischen Effekten umfaßt. [U.W.]

Résumé—De la smectite avec des cations polyvalents sur le complexe d'échange a facilement absorbé le riboflavin d'une solution aqueuse jusqu'à une limite de 0,5 mmoles/g. La forme des isothermes d'adsorption était du type Langmuir. Une exception était la smectite-Na^+ qui a fourni un isotherme en forme de S et de plus petites quantités d'adsorption. La vermiculite-Ca^{2+} n'avait aucune adsorption interlamellaire de riboflavin, suggérant que ce minéral ne gonfle pas suffisemment pour permettre une telle adsorption. Les isothermes d'adsorption, les données de diffraction des rayons-X, et les données spectroscopiques visibles à l'UV suggèrent que les mécanismes d'interaction entre la smectite et le riboflavin pourraient impliquer une combinaison d'effets ion-dipole, de transfert de charge, de liens hydrogène, et physiques. [D.J.]

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