

# SPECIFIC CO-ABSORPTION OF PURINES AND PYRIMIDINES BY MONTMORILLONITE (CLAY-ORGANIC STUDIES XV)

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**Abstract**—The co-absorptions of various purines and pyrimidines from aqueous solutions by Na- and Ca-montmorillonite are studied in the range of pH 1–6. The pyrimidines, thymine and uracil, which are not absorbed from solutions of these compounds alone, are appreciably absorbed from solutions containing also adenine or 2,6-diaminopurine, are weakly absorbed from solutions containing hypoxanthine, and are not absorbed from solutions containing purine, cytosine, and caffeine. The specific co-absorption is tentatively attributed to hydrogen-bond formation between the molecules in solution rather than to an overlapping of the molecular configurations (vertical stacking). The montmorillonite-organic associations have a basal spacing of about 12.5 Å which permits only a single molecular layer between successive silicate layers.

## INTRODUCTION

THE ABSORPTION of purines, pyrimidines and nucleosides by montmorillonite from dilute aqueous solutions containing only one organic compound at a time has been studied in relation to the exchangeable cations on the clay and the ambient pH of the medium, (Lailach, Thompson and Brindley, 1968*a,b*). The absorption takes place mainly by a cation exchange reaction between the inorganic cations on the clay and protonated organic molecules in the solution when the ambient pH is near the  $pK_a$  value for the protonation of the organic material, and consequently takes place mainly under acidic conditions. If more than one organic compound is present in solution, the absorption behavior cannot be deduced from the absorptions of the compounds separately because of their possible interactions with each other, either in solution or after absorption in/on the clay. Extensive studies by Ts'o *et al.* have shown that important interactions may take place in aqueous solutions between purines, pyrimidines and their nucleosides (Ts'o *et al.* 1963, 1964; Chan *et al.* 1964; Schweizer *et al.* 1965) and they have deduced that the interaction is a vertical stacking of the molecules with a partial ring overlap, in preference to horizontal hydrogen bonding. If a similar vertical stacking should occur in the absorbed state, it would be easily measurable by X-ray diffractometry of the samples under equilibrium conditions.

Preliminary experiments have shown that

thymine, and, to a lesser extent, uracil are absorbed by montmorillonite together with adenine, but not alone. Since an interaction between two protonated molecules is improbable because of electrostatic repulsion, co-absorption experiments have been undertaken under conditions where only one molecular species is likely to be protonated. Attention has been given, therefore, to the possible co-absorption of the very weak bases thymine and uracil together with other purines or pyrimidines under conditions where the latter are normally absorbed.

## EXPERIMENTAL

Homoionic Ca- and Na-montmorillonite with a cation exchange capacity of 100 meq/100 g have been prepared as described previously (Lailach *et al.* 1968*a*). The organic compounds were of the highest purity available on the market supplied by Nutritional Biochemical Corporation, Cleveland; Calbiochem, Los Angeles; Cyclo Chemical Corporation, Los Angeles;  $C^{14}$ -labeled thymine and uracil were purchased from New England Nuclear Corporation, Boston.

In the absorption experiments, aliquots of an unlabeled purine or pyrimidine solution, of  $C^{14}$ -labeled thymine or uracil solution, of HCl and water were mixed together. To each mixture an aliquot of a montmorillonite suspension was added. After 15 hr at room temperature the suspensions were centrifuged with a Sorvall SS-1 high speed centrifuge. A part of the sedimented

clay and associated liquid was filled into glass capillaries (supplied by Unimex-Caine Corporation, Chicago) for X-ray powder diagrams made with a modified Norelco powder camera, dia. 11.46 cm. From the supernatant solution two aliquots were diluted with acidic and alkaline buffer solution, respectively, for the UV-spectrophotometric determination of the concentrations of the two bases in the supernatant, using four different wavelengths. From these values and the values of absorbance measured with standards of the two compounds at the same wavelengths the concentrations of the two organic compounds were calculated. Two aliquots of the supernatant solution also were used to determine the concentration of the labeled compound by measuring the radioactivity with a Nuclear Chicago Mark I scintillation counter. The experimental error of the concentration measurements is estimated as  $\pm 2$  per cent. The equilibrium pH was measured in the supernatant solution with a Beckman Expandomatic pH meter.

#### RESULTS AND DISCUSSION

Adenine is absorbed nearly quantitatively by Na- and Ca-montmorillonite in the pH range 1-5. Depending on the amount of adenine absorbed by the montmorillonite, the initial interlayer cations of the montmorillonite, and the pH of the aqueous medium, a varying percentage of the montmorillonite forms a phase with a basal spacing of about 12.5 Å. The montmorillonite of this phase

has within its 3.1 Å thick interlayer space principally purinium or pyrimidinium cations, protons, and water in a mono-layer. In addition it may also contain Na<sup>+</sup>-ions but, because of the problems of co-ordination, is less likely to have Ca<sup>2+</sup>-ions (Lailach *et al.* 1968a).

Thymine and uracil are not absorbed by Na- and Ca-montmorillonite at pH's above pH1, but together with adenine they are absorbed to an extent depending on various conditions which are summarized in Table 1.

In the first place, a high proton concentration is necessary. As discussed previously, (Lailach *et al.* 1968a), the proton concentration in the interlayer space may be significantly higher than that in the ambient solution because the clay itself becomes partially an H-clay. However, this is clearly not a sufficient explanation because a high proton concentration alone does not give rise to thymine absorption. Evidently the adenine plays an important role, either through some more-or-less direct complexing with the thymine molecules, or through the formation of the 12.5 Å montmorillonite phase. Ca-montmorillonite in water gives a basal spacing of 18-20 Å and Na-montmorillonite in water a > 40 Å basal spacing, and in neither case is thymine absorbed.

Table 2 summarizes further experimental data showing absorption data for thymine and for uracil when other bases than adenine are used. Also shown are the estimated proportions of the 12.5 Å phase (or 12.8 Å phase in the case of caffeine).

Table 1. Influence of pH and interlayer cations on the c-absorption of adenine and thymine by montmorillonite from aqueous solutions 1.3 mM of each, adenine and thymine

Equilibrium pH ( $\pm 0.1$ )	Montmorillonite in the 12.5 Å phase* Estd. %	Available adenine absorbed (%)	meq absorbed adenine/100 g clay	Available thymine absorbed (%)	meq absorbed thymine/100 g clay
<u>Ca-montmorillonite</u>					
1.2	60	100	23.2	30.4	7.1
2.0	45	100	23.2	23.0	5.3
3.8	10	100	23.2	8.6	2.0
5.6	< 10	90.0	20.9	0	0
<u>Na-montmorillonite</u>					
2.0	60†	100	23.5	13.2	3.1
3.7	10‡	100	23.5	4.6	1.1
4.3	70	100	23.5	2.3	0.5
5.3	75	97.5	22.9	1.0	< 0.2
6.1	75	91.0	21.4	0	0

\*Values estimated from the relative intensities of the basal reflections of the fully expanded and 12.5 Å phases in the X-ray powder diagrams of the suspensions. Error is about  $\pm 10$  per cent.

†X-ray powder diagram indicates poor crystallinity of the 12.5 Å montmorillonite phase.

‡X-ray powder diagram indicates extremely poor crystallinity of the 12.5 Å montmorillonite phase.

Table 2. Co-absorption of thymine and uracil, respectively, with various other bases by Ca-montmorillonite from aqueous solutions 1.3 mM of each of the two bases

Protonated base	Equilibrium pH ( $\pm 0.1$ )	Total montmorillonite present as 12.5 Å phase* (%)	meq absorbed/100 g montmorillonite ( $\pm 0.3$ )				Equilibrium concentration ( $\times 10^{-5}$ M)			
			thymine	uracil	protonated base	protonated base	thymine	uracil	protonated base	protonated base
—	2.0	—	0	0	—	130	130	—	—	
Adenine	2.0	45	5.3	1.7	23.2	100	120	<1	<1	
2,6-Diaminopurine	2.0	60	5.6	2.7	23.2	99	114	<1	<1	
Hypoxanthine	2.0	<10	0.5	0.4	23.2	127	128	<1	<1	
Purine	{2.0	35	<0.1	0	23.2	130	130	<1	<1	
	{2.0	85	<0.2	0	46.3	129	130	1	1	
Cytosine	{2.0	<10	<0.1	0	22.4	130	130	10	10	
	{2.0	25	0.8	0	44.4	125	130	26	26	
Caffeine	{2.0	<10 (12.8 Å)	0	—	23.2	130	—	<1	<1	
	{2.0	100 (12.8 Å)	0	—	45.8	130	—	3	3	
—	1.2	—	0	0	—	130	130	—	—	
Adenine	1.2	60	7.1	3.1	23.2	90	113	<1	<1	
2,6-Diaminopurine	1.2	80	6.9	2.7	23.2	91	114	<1	<1	
Guanine	1.2	45	1.8	1.0	23.2	120	124	<1	<1	
Hypoxanthine	{1.2	<10	<0.1	—	23.2	130	—	<1	<1	
	{1.2	45	0.5	—	23.2	127	—	<1	<1	
Purine	{1.2	100	0.6	—	46.0	126	—	1	1	
	{1.2	<10	<0.2	—	21.7	129	—	8	8	
Cytosine	{1.2	20†	0.4	—	41.9	128	—	26	26	

\*Values estimated from Debye-Scherrer powder diagrams of the equilibrated suspensions; error is about  $\pm 10$  per cent.

†Broad reflection from 12.5 to 19 Å indicating poorly developed organophilic phase.

One sees that a notable proportion of the 12.5 Å phase is formed with adenine and with 2,6-diaminopurine, and also with purine and with caffeine when the concentration of the organic base is double that normally used. However, only with adenine and with 2,6-diaminopurine is there a significant absorption of thymine or of uracil. Therefore a molecular interaction seems to be more clearly the cause of the thymine and uracil absorptions than some favorable condition(s) arising from the 12.5 Å phase as such.

A possible absorption mechanism is hydrogen-bond formation between the protonated and non-protonated bases as shown in the following diagrams (Fig. 1), which are compatible with the interaction energies calculated by Pullman *et al.* (1966) for the interaction of non-protonated bases.

Further experimental data showing the extent of thymine absorption are given in Table 3. These data correspond to an equilibrium pH of 2.0 and under these conditions the available adenine is taken up almost completely as adeninium ions. With increasing amounts of adenine taken up, (column 2), the fraction of 12.5 Å phase (column 1) increases until the montmorillonite is wholly in this phase and the thymine absorbed likewise increases though not proportionally. Also when the equilibrium concentration of the thymine is increased, (column 5) from 102, to 222, to  $562 \times 10^{-5}$  M with 100% of 12.5 Å phase and with roughly equal amounts of adenine absorbed, the amount of absorbed thymine increases, but not proportionately; the thymine absorbed is always less than the adenine absorbed in meq/100 g clay.

In relation to the nature of the association between thymine and adenine when co-absorption occurs, emphasis must be placed on the increase of thymine absorbed as the proportion of 12.5 Å

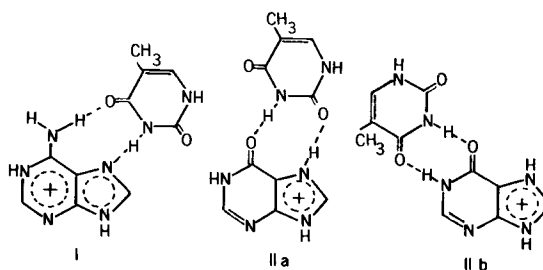


Fig. 1. Possible co-absorption complexes: I, adenine-thymine; II, a, b, hypoxanthine-thymine.

phase increases. This basal spacing is incompatible with vertical stacking of the absorbed species and permits only a horizontal (or inplane) association of the molecules.

The interlayer space occupied by adenine and thymine together is always of the order of 40 per cent of the available space in the 12.5 Å complex. For adenine alone, the highest observed absorption, 75 meq/100 g clay, corresponds to the occupation of about 55 per cent of the available space. The somewhat smaller occupation of space when thymine is absorbed may be due partly to steric problems in placing the adeninium cations near cation absorption sites. A similar explanation may be suggested for the fact that only a fraction of the adeninium ions can have associated thymine molecules. A further consideration is that the H-bond interaction energy between thymine and adenine, plus the van der Waals interaction energy between thymine and the silicate layers may be insufficient to liberate more strongly bonded water molecules. This may be the explanation why the smaller molecules of uracil are co-absorbed to a lesser extent than the larger thymine molecules.

Table 3. Influence of the amount of absorbed adenine, the proportion of 12.5 Å montmorillonite phase, and the concentration of thymine on the co-absorption of thymine and adenine by Ca-montmorillonite (equilibrium pH =  $2.0 \pm 0.1$ )

Per cent of total montmorillonite present as 12.5 Å phase ( $\pm 10\%$ ) (1)	meq absorbed base/100 g montmorillonite ( $\pm 0.3$ )		Equilibrium concentration ( $\times 10^{-5}$ M)	
	Adenine (2)	Thymine (3)	Adenine (4)	Thymine (5)
45	23.2	3.9	< 1	43
25	11.6	3.7	< 1	109
45	23.2	5.3	< 1	100
85	34.8	6.3	< 1	95
100	46.2	4.9	1	102
45	23.2	6.5	< 1	223
100	46.2	6.8	1	222
100	38.7	11.1	< 1	562

## CONCLUSIONS

This study shows that co-absorption of the separately non-absorbable molecules thymine and uracil can take place in association with the strongly absorbed molecules adenine and 2,6-amino-purine by the expanding clay mineral montmorillonite. The phenomenon is complex as regards the energy relations involved, but a primary reason appears to be H-bond interaction between the protonated and non-protonated molecules in the interlayer space of montmorillonite. The formation of the 12.5 Å phase of the montmorillonite appears to be an important factor in the overall process. A basal spacing of this magnitude is not compatible with the vertical stacking interaction deduced by Ts'o *et al.* for molecular interactions in solutions.

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*Postscript:* Since submission of this paper for publication, the authors' attention has been directed to a publication by M. M. Mortland, (*J. Agr. Food Chem.* **16**, 706-7 (1968)), "Pyridinium-montmorillonite complexes with ethyl N,N-Di-n-propylthiolcarbamate (EPTC)" which probably result from a hydrogen bond between the protonated nitrogen of pyridinium and the carbonyl of EPTC. The mechanism is the same as that independently suggested in the present paper.

**Résumé**—On étudie les co-absorptions de différents purines et pyrimidines à partir de solutions aqueuses par le Na- et Ca-montmorillonite, dans la gamme de pH 1-6. Les pyrimidines, thymine et uracil qui ne sont pas absorbés à partir des solutions de ces composés à eux seuls, sont absorbés de manière importante à partir de solutions contenant également l'adenine ou le 2,6-diaminopurine, sont absorbés de manière peu importante à partir des solutions contenant l'hypoxanthine et ne sont pas absorbés à partir de solutions contenant le purine, le cytosine et le caféine. La coabsorption spécifique est attribuée en premier lieu à la formation d'une liaison d'hydrogène entre les molécules en solution plutôt qu'à un recouvrement des configurations moléculaires (tassement vertical). Les associations montmorillonite-organiques ont un espacement basal d'environ 12,5 Å qui permet une seule couche moléculaire entre des couches successives de silicate.

**Kurzreferat**—Die Koabsorptionen verschiedener Purine und Pyrimidine aus wässrigen Lösungen durch Na- und Ca-Montmorillonit wurden in einem pH Bereich von 1-6 studiert. Die Pyrimidine Thymin und Uracil, die aus Lösungen dieser Verbindungen allein nicht absorbiert werden, erfahren beträchtliche Absorption aus Lösungen, die ebenfalls Adenin und 2,6 Diaminopurin enthalten, während sie aus Hypoxanthin enthaltenden Lösungen schwach, und aus Purin, Cytosin und Caffein enthaltenden Lösungen nicht absorbiert werden. Die spezifische Koabsorption wird provisorisch eher der Bildung einer Wasserstoffbrücke zwischen den Molekülen in der Lösung als einer Überlagerung molekularer Konfigurationen (Vertikalstapelung) zugeschrieben. Die Montmorillonit-Organisch Assoziationen haben einen Basisabstand von ca. 12,5Å, was nur eine einzelne Molekülschicht zwischen aufeinanderfolgenden Silikatschichten gestattet.

**Резюме**—Ко-абсорбции различных пуринов и пиримидинов из водных растворов посредством Na- и Са-монтмориллонита подвергались исследованиям в диапазоне pH 1–6. Пиримидины, тимин и урацил, которые не абсорбируются из растворов только этих соединений, в значительной степени абсорбируемы из растворов, также содержащих аденин или 2, 6-диаминопурин, слабо абсорбируемы из растворов, содержащих гипоксантин и не абсорбируемы из растворов, содержащих пурин, цитозин и кофеин. Удельная ко-абсорбция пробно приписывается к связанным водородом формациям между молекулами в растворе, а не к перекрытию молекулярных конфигураций (вертикальное группирование). Монтмориллонит-органические ассоциации обладают основным расстоянием прибл. в 12,5 Å, что допускает лишь единичный молекулярный слой между последовательными силикатными слоями.