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OPTICALLY SELECTIVE ADSORPTION OF α-AMINO ACIDS ON MONTMORILLONITE-Cu-l-LYSINE COMPLEXES IN HIGH-PRESSURE LIQUID CHROMATOGRAPHY

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Abstract—Optically active cationic complexes adsorbed on montmorillonite can be used for the resolution of racemic mixtures. Montmorillonite-Cu-lysine systems were used as a solid phase in high-pressure liquid chromatography for the resolution of the optical isomers of α-amino acids. Selectivity constants >1.5 were measured for phenylalanine and tryptophan. The selectivity constants for the amino acids containing saturated-hydrocarbon side chains were in the range of 1.25–1.44. The montmorillonite-Cu-l-lysine complex displayed a stronger affinity for the l-isomers of α-amino acids than for the d-isomers at pHs near neutrality. Inasmuch as surface-catalyzed peptide formation on clays has been proposed as a step in chemical evolution, this stronger affinity between the clay-Cu-l-amino acid complex and l-amino acids might have been significant in prebiotic evolution. The mechanism of optical resolution probably involved ligand exchange. Optimizing the choice of the optically active ligands and of the chelating cation in the chiral agent may improve the resolution of the optical isomers.

Key Words—Adsorption, Amino acid, Cu-l-lysine, High-pressure liquid chromatography, Montmorillonite, Optically active ligands.

INTRODUCTION

The resolution of racemic α-amino acids by ligand exchange on high-pressure liquid chromatography (HPLC) columns has been carried out using several solid supports, such as organic polymers, reversed phase (n-octadecylsilane or n-octylsilane bound to a silica gel surface; e.g., Lindner *et al.*, 1979), and silica (see, e.g., Davankov, 1981; Kurganov and Davankov, 1981). The complex whose ligand is exchanged must be optically active to bring about resolution between enantiomers. The optically active species used were either covalently bound to the solid phase (e.g., Gubitz *et al.*, 1981) or added to the mobile phase (e.g., Hare and Gil-Av, 1979) in the chromatographic run. Kurganov and Davankov (1981) and Grushka *et al.* (1983), among others, proposed that sorbed complexes are responsible for the resolution in both the silica and the reversed phase systems, even when the chiral species are applied in the mobile phase.

The first partial resolution of optical isomers of metal (III) chelates on small chromatographic columns containing clay was reported by Yamagishi and Soma (1981), Yamagishi (1982), and Yamagishi and Ohnishi (1982). Yamagishi and Ohnishi (1983) were successful in partially resolving the cyclic amino acid proline, as well as other optically active cyclic compounds, but failed in their attempt to resolve acyclic amino acids on similar columns. In the above studies ambient pressure was employed. Although some optical selectivity of α-amino acids on montmorillonite has been claimed (e.g., Bondy and Harrington, 1979), the resolution of racemic mixtures of acyclic α-amino acids (and of cyclic ones with the above-mentioned exception of proline and its derivatives) on montmorillonite loaded with optically active species has not been reported previously.

Some reports indicate that α-amino acids form with transition metal ions (and in particular with Cu^{2+} ions) complexes on clay surfaces that differ from the complexes formed in solution (Bodenheimer and Heller, 1967; Yang and Condrate, 1971, 1972; Heller *et al.*, 1973; Siegel, 1966; Siffert and Kessaissia, 1978). Lysine was reported to adsorb on Cu-montmorillonite at a Cu : lysine ratio of 1:2 (see, e.g., Bodenheimer and Heller, 1967; Yang and Condrate, 1972), and Bodenheimer and Heller (1967) suggested that the complex of lysine with Cu-montmorillonite is more stable than the lysine-Cu complex in solution.

Most α-amino acids form neutral complexes with Cu^{2+}. Lysine can form a biligand complex with Cu^{2+} in which the α-amino group and a carboxylate oxygen are bound to Cu^{2+} and the ε-amino group is protonated (Figure 1). The resulting positively charged Cu-lysine complex should adsorb more strongly on the negatively charged clay surface than copper complexes with neutral or acidic amino acids. Bodenheimer and Heller (1967) reported that the adsorption coefficients of glutamic acid (at pH 3.4), glycine (at pH 9.6) and lysine (at pH 9.7) on Cu-montmorillonite were about 0, 16, and 1600 ml/g, respectively.

In the present study, spray-dried bentonite (SP-bentonite; Mingelgrin and Tsvetkov, 1985) loaded with the Cu-l-lysine complex was utilized as a packing material for the HPLC separation of enantiomers of dif-

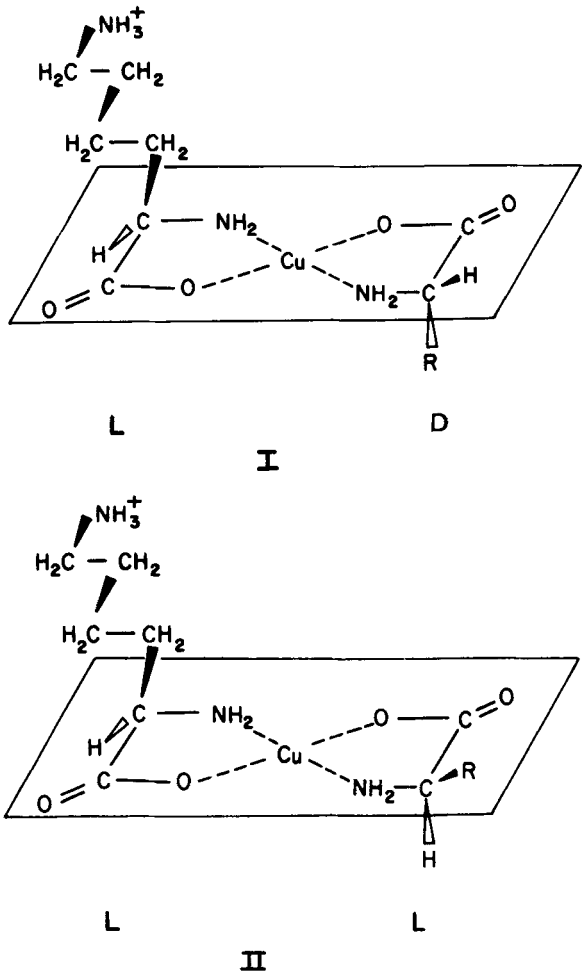

Figure 1. Schematic representation of the mixed Cu-l-lysine-amino acid complexes. I = d-isomer, II = l-isomer.

ferent amino acids. The l-lysine is one of the essential α-amino acids. Its interaction with montmorillonite-transition series ion systems is therefore of interest not only because of its chromatographic potential, but also in areas such as the study of the origin of life.

MATERIALS AND METHODS

Materials

All the examined α-amino acids were manufactured by Sigma Chemical Company (St. Louis, Missouri). Doubly distilled water was used in all experiments. Some relevant properties of the clay used in the present study (bentonite, Fisher B-235, Fisher Scientific Co., Fairlawn, New Jersey) were given by Mingelgrin and Tsvetkov (1985). Because the high-quality bentonite used is composed almost entirely of montmorillonite, the clay is herein referred to as montmorillonite.

Preparation of the stereoselective HPLC columns

An aqueous suspension of the clay was spray-dried to form quasispherical particles having a mean diameter of 7 μm. The resulting spray-dried particles (SP-bentonite) were used as the solid phase in the HPLC runs after they were heated at 520°C for 24 hr. The mode of preparation and the properties of that preheated SP-bentonite were described previously (Mingelgrin and Tsvetkov, 1985). In that study the preheated SP-bentonite was shown to retain the surface properties of the original, well-dispersed, untreated montmorillonite.

Table 1. Dominant ionic forms of lysine in aqueous solution at different pHs.

Form	pH	Ionic structure
I	<2.2	$^{+}NH_3$–$(CH_2)_4$–CH–COOH NH_3^+
II	2.2–8.9	$^{+}NH_3$–$(CH_2)_4$–CH–COO^- NH_3^+
III	8.9–10.5	$^{+}NH_3$–$(CH_2)_4$–CH–COO^- NH_2
IV	>10.5	NH_2–$(CH_2)_4$–CH–COO^- NH_2

One type of column (column 1) was prepared by suspending preheated SP-bentonite in a solution of 17 mM Cu acetate and 34 mM l-lysine (free base) at a 1:25 solid to solution ratio and at pH 7.1. The suspension was allowed to equilibrate while shaking for 24 hr. The solid phase was then collected by centrifugation and resuspended twice in the Cu acetate, l-lysine solution at the above solid to solution ratio. Finally, the extra fine SP-bentonite particles were removed by repeated sedimentation of the SP-bentonite from a dilute solution of copper acetate (1.7 mM) and l-lysine (3.4 mM). The pH of the clay suspension in the dilute solution was 7.4. A paste containing 50% SP-bentonite loaded with Cu-l-lysine was degassed and used as a packing material for the HPLC columns. The packing was performed using first low vacuum and then a Tracor 955 LC pump under a 4000 psi pressure. Two additional types of columns (columns 2 and 3) were prepared by the same procedure as column 1, but the pH of the initial solution was kept at 11 by adjustment with NaOH. During the preparation of the Cu-SP-bentonite-l-lysine stationary phase for column 3, an excess of l-lysine in the initial solution (51 mM instead of 34 mM l-lysine to yield a 3:1 l-lysine:Cu^{2+} ratio) was used.

Preparation of Cu^{2+}-lysine-montmorillonite complexes

Complexes of montmorillonite with Cu^{2+} and l-lysine were prepared at pHs of 2.7, 4.5, and 11.0 from mixed solutions of copper acetate (40 mM) and l-lysine (120 mM). The pH was adjusted with acetic acid and sodium hydroxide. The absence of turbidity in the mixed copper acetate-l-lysine solution at pH 11.0 indicated that no significant precipitation of $Cu(OH)_2$ occurred. The solutions were allowed to equilibrate for 48 hr with oven-dried (105°C) montmorillonite at a clay:solution ratio of 1:25. After centrifugation, the complexes were washed three times with water and freeze-dried.

The pHs selected correspond to different distributions of the ionic forms of lysine (Table 1). Accordingly, the complexes had different colors, from very light blue-green (pH 2.7) to bright blue-violet (pH 11.0). Montmorillonite-Cu^{2+}-d-lysine complexes were also prepared in a similar manner.

Adsorption experiments

Samples of the montmorillonite-Cu-lysine complexes prepared at pH 4.5 and at 11.0 were added to 10 mM solutions of racemic α-amino acids. The resulting 2% suspensions were equilibrated for 24 hr at 5°C and then centrifuged. The con-

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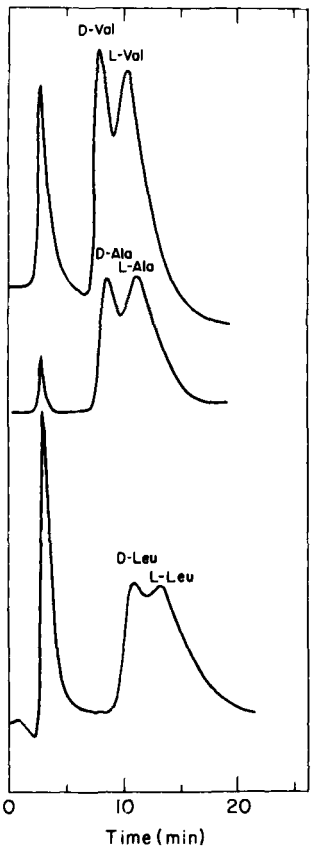

Figure 2. Resolution of d-, l-valine, d-, l-alanine and d-, l-leucine on preheated SP-bentonite-Cu-l-lysine high-pressure liquid chromatography column (5 × 0.46 cm). Eluent = 5 × 10^{-4} M copper acetate and 1 × 10^{-3} M l-lysine solution in water; pH = 5.7; flow rate = 0.2 ml/min; ultraviolet detection: λ = 280 nm.

centration of the two enantiomers in the supernatant was then checked by HPLC (Weinstein, 1982). Equilibration at 5°C minimized the risk of microbial degradation of the l-isomers; indeed, adding Na-azide did not affect the adsorption measurements, demonstrating that microbial degradation did not take place.

Analytical procedures

Chromatograms were obtained on a Tracor HPLC system equipped with a variable-wavelength UV detector model 970A, set at 280 or 240 nm. The concentration of the injected α-amino acids was in the range of 1–3 mM. Elemental analyses of carbon and copper in the Cu^{2+}-lysine-montmorillonite complexes were obtained. The copper was determined by atomic absorption following desorption with ethylenediamine tetraacetic acid (Ure and Barrow, 1970). The *c*-spacing of the complexes was measured by X-ray powder diffraction (XRD) using a Philips X-ray diffractometer type 1030 and a Co target.

RESULTS AND DISCUSSION

Separation of optical isomers of α-amino acids using Cu-montmorillonite-l-lysine HPLC columns

Figures 2 and 3 show chromatograms obtained for several enantiomeric mixtures of α-amino acids on column 1. The capacity ratio (k′) and the selectivity (α) of some α-amino acids are presented in Table 2. The parameters k′ and α are defined as:

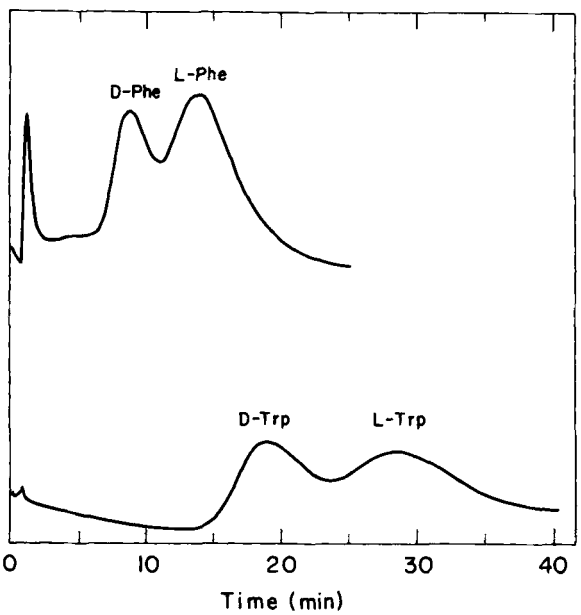

Figure 3. Resolution of d-, l-phenylalanine and d-, l-tryptophan on preheated SP-bentonite-Cu-l-lysine high-pressure liquid chromatography column (5 × 0.46 cm). Eluent = 5 × 10^{-4} M copper acetate and 1 × 10^{-3} M l-lysine solution in water; pH = 5.7; flow rate = 0.5 ml/min; ultraviolet detection: λ = 280 nm.

$$k' = \frac{t_R - t_0}{t_0} \quad (1)$$

and

$$\alpha = \frac{k'_l}{k'_d}, \quad (2)$$

where t_R is the retention time of the amino acid, t_0 is the retention time of noninteracting molecules, and K'_l and k'_d are the capacity ratios of the l- and d-isomers, respectively.

The α values obtained were superior to those achieved using silica columns on which optically active Cu complexes were adsorbed (Kurganov and Davankov, 1981), thereby illustrating the chromatographic potential of the investigated clay-based systems. Optimization of these systems (a better choice of the chelating cation, the chiral ligand or the eluent) should produce considerably better optical resolutions.

The detection procedure employed was based on ultraviolet (UV) absorption. The detected species was a complex of the amino acid with Cu and not the amino acid itself, which, with the exception of the aromatic amino acids, does not absorb at the wavelengths used. The first peak in the chromatograms (Figures 2 and 3) appeared in all experiments at the same retention time (t_0). The peak at t_0 was found to contain only lysine

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and none of the resolved enantiomers. This peak was assigned to the Cu complex with l-lysine, which was released from the montmorillonite surface following the injection of the amino acids. A number of ligand and cation-exchange processes might have been involved in increasing the concentration of the Cu-lysine complex in the mobile phase. For example, ligand exchange between lysine in the Cu-lysine complex and the injected amino acid could have been followed by cation exchange between the adsorbed Cu-lysine complex and both the released lysine and the complex formed by the ligand exchange. The above data and those presented below suggest that a mixed lysine-resolved amino acid-Cu complex was formed by ligand exchange between lysine in $Cu(lysine)_2$ and the resolved enantiomers and that this ligand exchange was responsible for the optical resolution.

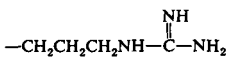
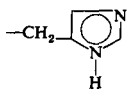
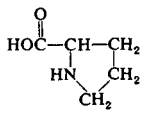
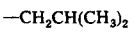
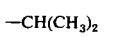
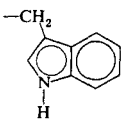
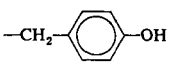
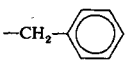
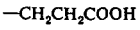
If ligand or cation exchange were involved in the transport of the resolved amino acids through the column, the intensity of the peaks assigned to the various enantiomers should have been a function of the difference between the specific absorbance (ξ) of $Cu(lysine)_2$ and of the Cu complex containing the resolved amino acid at the wave length at which the detection took place. This wave length was chosen so that the ξ values of the mixed Cu-lysine-amino acid complexes will be higher than that of $Cu(lysine)_2$. Detection at wave lengths at which the ξ values of the complexes with the resolved amino acids were lower than that of $Cu(lysine)_2$ should result in a negative peak, and, indeed, such negative peaks were observed. The magnitude of the ξ values of the mixed complexes relative to that of $Cu(lysine)_2$ also affected the ratio between the intensity of the peaks at t_0 and at t_R. Accordingly, the peak at t_0 in the chromatogram of tryptophan (ξ value about an order of magnitude larger than that of $Cu(lysine)_2$) was much smaller than the peaks associated with the enantiomers of tryptophan (Figure 3).

The selectivity constants of the aromatic amino acids were generally higher than those of the other amino acids, and their capacity ratios were significantly higher than those of the amino acids having similar isoelectric points. These results may be explained by the participation of the aromatic ring in the interaction of the Cu-l-lysine-amino acid complex with the montmorillonite surface. The aromatic π-electrons were probably attracted by the acidic sites of the clay (Doner and Mortland, 1969). The interaction of these π-electrons with the surface was most likely dependent on the orientation of the ring relative to the surface. In the l,l-complex, the side chains of the resolved aromatic amino acid and of the lysine projected out of the same side of the plane defined by the Cu atom and the groups bound to it (Figure 1). In the l,d-complex, on the other hand, each of these side chains projected out of a different side of the plane. Both the ϵ-amino group of lysine and the aromatic ring were expected to interact with the clay surface. The orientation of the two side chains relative to the above defined plane (and hence to the clay surface) may, therefore, have had a significant effect on the overall interaction of the Cu-l-lysine-aromatic amino acid complex with the surface. Thus, the difference between the strength of interaction of the side chains of the complexed l- and d-isomers with the surface may have determined to a large extent the selectivity between the aromatic enantiomers.

With the exception of arginine, the l-amino acids adsorbed more strongly than the d-amino acids on Cu-l-lysine-SP-bentonite at pHs close to neutrality. Whereas at lower pHs arginine had high k′ values and $\alpha < 1$ (Table 2), at pH 11.4 (column 3) k'_l of arginine was 4.5 and k'_d was 4.2 ($\alpha \sim 1.07$). Arginine has a relatively long side chain with a guanido group at its end. At pHs below its isoelectric point (11.2), the protonated amino groups on the side chain should adsorb strongly on the clay surface (e.g., Mingelgrin and Tsvetkov, 1985) and thus produce the high k′ values. The relatively great length of the side chain and the strong interaction between the protonated amino groups at its end and the clay surface apparently minimized the role of the asymmetric centers of the Cu complex with arginine in the adsorption. The difference between the retention of l-arginine and d-arginine should therefore have been small and α should have been close to unity. The optical resolution of arginine at low pHs was indeed low ($\alpha = 0.95$; Table 2). The higher retention of the d-isomer may have resulted from the repulsion between the protonated amino groups at the end of the side chains of both lysine and arginine which should be considerably stronger in the Cu-l-lysine-l-amino acid complex than in the l,d-complex (Figure 1). At pHs above the isoelectric point, a smaller portion of the arginine had a protonated side chain, and the k′ values decreased sharply accordingly. The aforementioned mutual repulsion between the side chains of l-lysine and l-arginine in the mixed Cu complex should have also decreased as a result of the lower degree of protonation of the side chains, allowing the selectivity to become somewhat greater than unity.

The resolution of the amino acids with saturated-hydrocarbon side chains increased as the length of the side chain decreased in the following order: leucine < valine < alanine. The hydrophobic side chains probably did not interact strongly with the hydrophilic montmorillonite surface. Accordingly, the retention of the amino acids containing saturated-hydrocarbon side chains was not as high as that of the aromatic amino acids or of the amino acids with a basic side chain (Table 2). The lower k′ values of the d-isomers as compared with those of the l-isomers observed at pH <10 (Table 2 and Figure 4) may be explained for amino acids containing a saturated-hydrocarbon side chain as follows: In the Cu-l-lysine-d-amino acid complex, the saturated-hydrocarbon side chain and the side chain

Table 2. Retention and resolution of some enantiomeric α-amino acids on a preheated SP-bentonite-Cu-l-lysine column.[1]

Compound	Side chain (R) of the amino acid R–CH(NH$_2$)–COOH	Isoelectric point (pH)	Eluent: 0.5 mM copper acetate, 1 mM l-lysine, pH = 5.7		Eluent: 1.7 mM copper acetate, 3.4 mM l-lysine, pH = 5.7		Eluent: 0.5 mM copper acetate, 1 mM l-lysine, pH = 7.3	
			k′	α	k′	α	k′	α
d-arg		11.2	14.0	0.95	—	—	—	—
l-arg			13.3					
d-his		7.5	28.5	1.21	—	—	13.5	1.11
l-his			34.5				15.0	
d-pro		6.3	2.8	1.07	1.8	1.0	3.2	1.19
l-pro	(complete structure)		3.0		1.8		3.8	
d-leu		6.02	2.5	1.32	1.6	1.31	2.8	1.25
l-leu			3.3		2.1		3.5	
d-ala		6.00	1.9	1.47	0.8	1.25	3.4	1.0
l-ala			2.8		1.0		3.4	
Gly	—H	5.97	3.5	—	1.3	—	4.4	—
d-val		5.96	1.8	1.44	1.0	1.4	2.5	1.28
l-val			2.6		1.4		3.2	
d-trp		5.89	14.8	1.55	8.2	1.56	10.5	1.54
l-trp			23.0		12.8		16.2	
d-tyr		5.66	7.3	1.41	—	—	7.5	1.33
l-tyr			10.3				10.0	
d-phe		5.48	6.0	1.55	3.7	1.54	5.7	1.63
l-phe			9.3		5.7		9.3	
d-glu		3.20	2.2	1.14	0.85	1.18	3.7	1.08
l-glu			2.5		1.0		4.0	

[1] Eluent: copper acetate and l-lysine in water; k′ = capacity ratio; α = selectivity constant.

of lysine are on opposite sides of the plane in which the Cu atom and the functional groups bound to it are located (Figure 1), thereby hindering the parallel alignment of the planar part of the complex with the surface. Such a parallel alignment, which is more probable for the l,l-complex, would have probably maximized the attraction between the planar part of the complex and the surface. This attraction may be more important for amino acids containing saturated-hydrocarbon side chains because, as mentioned above, such side chains are expected to contribute to the interaction of the complex with the surface much less than the side chains of the aromatic or basic amino acids.

A more than three-fold increase in the concentration of Cu^{2+} and l-lysine in the eluent did not change the selectivity constants significantly. It decreased, however, the capacity ratios of all amino acids tested (Table 2). This reduction in k′ values may be due to the increase in concentration of $Cu(lysine)_2$, which competes with the Cu-lysine-resolved amino acid complex for adsorption on the clay surface.

Increasing the pH of the eluent from 5.7 to 7.3 did

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not increase the selectivity (Table 2). The capacity ratio of the amino acids containing additional amino groups (histidine and tryptophan) decreased with an increase in pH, probably because of the decrease in the extent of protonation of the additional amino groups. For the amino acids containing a saturated-hydrocarbon side chain (glycine, alanine, valine, and leucine) the capacity ratio increased slightly with increasing pH. Here, the interaction of the side chain (or lack of it) with the surface was probably independent of pH. The α-amino group of lysine in solution is predominantly protonated at both pH 7.3 and 5.7, whereas pH 7.3 is above and pH 5.7 is below the isoelectric point of the amino acids containing saturated-hydrocarbon side chains (Tables 1 and 2). Thus, the tendency of amino acids containing a saturated-hydrocarbon side chain to complex with copper may have increased with pH more than the tendency of lysine to do that, thereby encouraging the ligand exchange of lysine in $Cu(lysine)_2$ by the amino acid at the higher pH. The k′ values of the aromatic tyrosine and phenylalanine were not affected significantly by pH.

A more detailed investigation of the influence of pH on the retention of the enantiomers of valine and leucine was carried out using columns 2 and 3 (see Figure 4). The k′ values of the l-isomers were more sensitive to changes in pH than those of the d-isomers. The order of adsorption of d- and l-valine was actually reversed at high pHs (Figure 4). As was suggested for histidine (Williams, 1972; Brookes and Pettit, 1975), lysine may act at a sufficiently high pH (at which its ϵ-amino group is not protonated) as a tridentate ligand in complexes with Cu^{2+}. Steric hindrance by the side chain of the resolved l-amino acid makes such a ternary interaction between l-lysine and the copper ion less likely in the mixed l,l-complex than in the mixed l,d-complex (Figure 1). Even if lysine did not serve as a tridentate ligand, the deprotonation of its ϵ-amino group at high pH may have affected both the stability of the l-lysine-Cu-amino acid complex and the interaction of this complex with the clay surface differently for the l- and the d-isomers of the resolved amino acids. As the pH rises, hydroxyl ions become increasingly available to serve as ligands in Cu^{2+} complexes together with the amino acids. This availability may strongly influence the behavior of the Cu-l-lysine-resolved amino acids system.

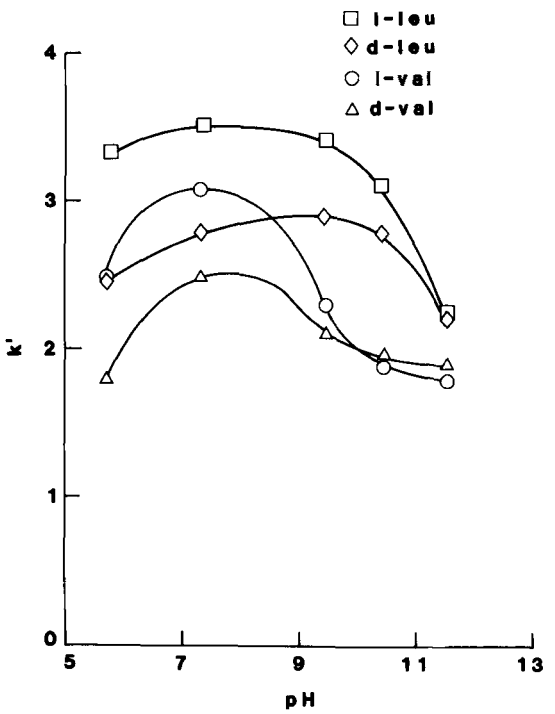

Figure 4. Capacity ratios (k′) of d-, l-valine and d-, l-leucine vs. pH. Solid phase = preheated SP-bentonite-Cu-l-lysine, eluent = 5×10^{-4} M copper acetate and 1×10^{-3} M l-lysine solution in water (pH adjusted with NaOH).

Batch studies

Mingelgrin and Tsvetkov (1985) demonstrated that preheated SP-bentonite has surface properties similar to those of well-dispersed bentonite powder. Thus, the adsorption of some racemic amino acids in aqueous suspensions of bentonite powder-copper-lysine was studied to supplement the experiments with the SP-bentonite-Cu-l-lysine HPLC columns. The effect of pH on some properties of the copper-lysine-bentonite powder complex was also studied. Lysine is a basic amino acid; in an aqueous solution it exists in different ionic forms. Table 1 presents the dominant ionic forms of lysine at different pHs, and Table 3 summarizes some properties of the various Cu-l-lysine-bentonite powder systems prepared. The dominant ionic form of lysine complexed with copper, either adsorbed or in solution, at any given pH is not necessarily the same as the dominant ionic form of free lysine in aqueous solution at that pH. Thus, in interpreting the results summarized in Table 3 one should bear in mind that Table 1 may not describe well the pH dependence of the dominant ionic forms of lysine at the clay surface in the various Cu-lysine-montmorillonite systems. The electric field at the charged clay surface, for example, may have a strong influence on the distribution of the ionic forms of lysine at the surface. In addition, that electric field may also influence the pH at the surface and thus indirectly affect the ionic distribution of lysine there. The pH at the surface of montmorillonite is assumed to be one to two units below that in the bulk liquid phase (e.g., Bailey *et al.,* 1968).

The system prepared at pH 2.7 displayed no evidence of the presence of a copper-l-lysine complex on the clay surface. Its *c*-spacing (air-dried) was 12.6 Å, as was the *c*-spacing of air-dried Cu-bentonite (Table 3). The color of the sample was light green-blue, similar to the color of Cu-bentonite. The ratio of Cu to lysine

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Table 3. Properties of Cu-l-lysine-montmorillonite systems prepared at various pHs.

pH	Amount of: (mmol/g clay) Cu	Lysine	c-spacing[1] (Å)
2.7	0.12	0.44	12.6
4.5	0.26	0.60	13.7
11.0	0.65	1.4	16.1
Cu-bentonite (pH = 4.3)	0.60	0.04[3]	12.6
Cu-bentonite-lysine[2] (pH = 9.7)	0.42	0.74	13.6

[1] Air-dried.
[2] Prepared by Bodenheimer and Heller (1967).
[3] Experimental error or organic C background.

Table 4. Adsorption of racemic amino acids at pH = 12 on the Cu-lysine-montmorillonite systems prepared at pH = 11 and on Cu-montmorillonite.

Compound	Optical purity[1] (%) of supernatant after adsorption on montmorillonite complexes with: Cu-l-lysine	Cu-d-lysine	Cu^{2+}
d,l-leucine	+2.4	−2.6	−1.0
d,l-valine	+2.2	−2.0	−2.0
d,l-glutamic acid	+4.5	−4.3	−0.5

[1] Optical purity = ([l-isomer] − [d-isomer]) × 100/([l-isomer] + [d-isomer]), where square brackets denote molar concentrations in solution.

at the surface (1:3.7) indicates that $Cu(lysine)_2$ was not the dominant adsorbed species. Thus, at this low pH Cu^{2+} and the cationic lysine were probably adsorbed chiefly as separate cations. Accordingly, the sum of the charges of the adsorbed Cu and lysine (both as 2+) was nearly equal to the charge of the copper (as 2+) adsorbed on Cu-bentonite (Table 3). Some of the cation-exchange capacity might have been occupied by protons at that low pH.

The system prepared at pH 4.5 was bright blue. This pH was the equilibrium value obtained without any acetic acid or NaOH addition. The distinct color suggests that at least part of the Cu^{2+} was present on the clay surface as a complex with lysine. The nature of this complex (or complexes) and the extent of complexation were, however, hard to estimate. The fact that both amino groups in the lysine molecule are protonated at pH 4.5 (Table 1) suggests that some free lysine and Cu^{2+} may have existed at the surface of the clay. The concentration of Cu^{2+} at the clay surface (Table 3) indicates that a significant fraction of the exchange capacity was not occupied by $Cu(lysine)_2^{2+}$, supporting the assertion that free lysine ions as well as Cu^{2+} or Cu-lysine complexes other than $Cu(lysine)_2^{2+}$ occupied some exchange sites.

The system prepared at pH 11 was violet-blue, and the ratio of Cu to lysine was about 1:2 (Table 3). At this pH the α-amino groups of lysine are not protonated. The aforementioned decrease in pH at the clay surface relative to the pH in the bulk solution suggests that most of the ε-amino groups of the adsorbed Cu-lysine complex were protonated (Table 1) and that most of the cation-exchange capacity was therefore occupied by $Cu(lysine)_2^{2+}$ (Table 3). The amount of Cu adsorbed per unit weight of clay in the system prepared at pH 11 was somewhat larger than in the Cu-bentonite, suggesting the possible adsorption of a certain quantity of the neutral $Cu(lysine)_2$ complex of type IV (Table 1). Both copper and lysine adsorbed in this system more than twice as much as in the systems prepared at the lower pHs (Table 3). This high density of adsorbate may explain why the *c*-spacing in the high-pH complex was 16.1 Å compared with 13.7 Å for the pH-4.5 complex.

Table 4 presents results of static adsorption experiments at pH 12. Both column and batch experiments demonstrated the preferential adsorption of the d-amino acids on Cu-l-lysine-montmorillonite at sufficiently high pHs (Table 4 and Figure 4) and the preferential adsorption of the l-isomers at lower pHs (Table 2 and Figure 4; the exceptional behavior of arginine was discussed above). Under the conditions defined in Table 4, the l-isomers of the investigated amino acids adsorbed on d-lysine-copper-montmorillonite more strongly than the d-isomers, in agreement with the preferential adsorption of the d-isomers in the corresponding l-lysine-copper-clay system.

Some preferential adsorption of the l-isomers was observed on the Cu-montmorillonite which was used as a reference (Table 4). Copper may exist at the surface of the clay (especially at pHs above neutrality) in a variety of forms such as bi- or polynuclear hydroxides (Stumm and Morgan, 1981). Polynuclear hydroxides of other metals, such as Al, were also observed on clay surfaces (Frenkel and Shainberg, 1980). Inasmuch as complexes of the form $CuA_2B_2C_2$, where A, B and C are three different ligands, have optical isomers (Cotton and Wilkinson, 1966), several adsorbed copper species may be optically active. If, for example, the adsorbed species is $[Cu_2(OH)_2(H_2O)_4]^{2+}$ and the clay acts as a polydentate ligand, optical isomers will exist and optically selective adsorption can take place. This fact may be relevant to prebiotic evolution. Naturally occurring clays may have on their surface chelating cations including copper (see, e.g., Kerr *et al.*, 1950). The results of the present study indicate that the preferred complexation of one enantiomer with a chelating cation on the clay surface may bring about, at pHs close to neutrality, a selective accumulation of the isomers of other amino acids having the same configuration as that enantiomer. Hence, if as suggested by Lahav *et al.* (1978) clay surfaces can enhance peptide formation, the preferred complexation of the l-isomer with adsorbed Cu^{2+} (or other chelating cations) may result in the selective formation of peptides composed of l-amino acids. Practically all of the asymmetric amino acids

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in proteins have the same (l-) configuration at the α-carbon atom. Harada (1968, 1970) demonstrated that aspartic acid and other amino acids may display optically selective crystallization from their saturated solutions in the form of copper complexes on dust, glass, quartz, sand, wool, and cotton powders. On wool and cotton, which have positively charged surfaces, d-isomers crystallized preferentially, whereas on quartz (regardless of its optical form), dust, sand, and glass, all of which have negatively charged surfaces, l-isomers crystallized preferentially. All the above negatively charged surfaces bear some resemblance to the montmorillonite surface.

CONCLUSIONS

SP-bentonite HPLC columns are a sensitive and accurate tool for the investigation of chelating cation-amino acid-clay systems. The relative stability of the copper-lysine-l-amino acid and -d-amino acid complexes at the surface of montmorillonite is strongly dependent on pH. In the pH range prevalent in biological systems, montmorillonite-copper^{2+}-l-lysine-l-amino acid complexes are more stable as a rule than the corresponding l,d-complexes. At higher pHs the l,d-complexes are more stable. Montmorillonite in the form of preheated SP-bentonite particles may be utilized for the resolution of racemic amino acids by HPLC where a Cu-l-(or d-)lysine complex serves as the resolving agent. Enantiomers other than those of lysine may form on clay surface complexes with copper (or other exchangeable cations) that resolve racemic mixtures or optically active organic compounds more efficiently then the presently investigated system. The formation of complexes on a clay surface could have been involved in the selective accumulation of the l-isomers of amino acids in the prebiotic era. HPLC clay columns may be used therefore as models in the study of the origin of life.

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REFERENCES

Bailey, G. W., White, J. L., and Rothberg, T. (1968) Adsorption of organic herbicides by montmorillonite. Role of pH and chemical character of adsorbate: *Soil Sci. Soc. Amer. Proc.* **32,** 222–234.

Bodenheimer, W. and Heller, L. (1967) Sorption of α-amino acids by copper montmorillonite: *Clay Miner.* **7,** 167–176.

Bondy, S. C. and Harrington, M. E. (1979) l-Amino acids and d-glucose bind sterospecifically to a colloidal clay: *Science* **203,** 1243–1244.

Brookes, G. and Pettit, L. D. (1975) Stereoselectivity in the formation of ternary histidinatocopper(II) complexes in the biological pH range: *J. Chem. Soc. Chem. Commun.,* 385–386.

Cotton, F. A. and Wilkinson, G. (1966) *Advanced Inorganic Chemistry:* Interscience, New York, p. 146.

Davankov, V. A. (1981) Resolution of racemates by ligand-exchange chromatography: *Adv. Chromatogr.* **18,** 139–195.

Doner, H. E. and Mortland, M. M. (1969) Benzene complex with copper(II) montmorillonite: *Science* **166,** 1406–1407.

Frenkel, H. and Shainberg, I. (1980) The effect of hydroxy-Al and hydroxy-Fe polymers on montmorillonite particle size: *Soil Sci. Soc. Amer. J.* **44,** 626–629.

Grushka, E., Leshem, R., and Gilon, C. (1983) Retention of amino acid enantiomers in reversed-phase liquid chromatography: *J. Chromatogr.* **255,** 41–50.

Gubitz, G., Jellenz, W., and Santi, W. (1981) Separation of the optical isomers of amino acids by ligand exchange chromatography using chemical bonded chiral phases: *J. Chromatogr.* **203,** 377–394.

Harada, K. (1968) Optical resolution of dl-aspartic acid copper complex by the use of biopolimers: *Nature* **218,** 199.

Harada, K. (1970) Origin and development of optical activity of organic compounds on the primordial Earth: *Naturwiss.* **57,** 114–119.

Hare, P. E. and Gil-Av, E. (1979) Separation of d- and l-amino acids liquid chromatography: Use of chiral eluents: *Science* **204,** 1226–1228.

Heller-Kallai, L., Yariv, S., and Riemer, M. (1973) Effect of acidity on the sorption of histidine by montmorillonite: *Proc. Int. Clay Conf., Madrid, 1972,* J. M. Serratosa, ed., Div. Ciencias C.S.I.C., Madrid, 651–662.

Kerr, F., Hamilton, P. K., Pill, R. J., Wheeler, G. V., Lewis, D. R., Burkhardt, W., Reno, D., Taylor, G. L., Mielenz, R. C., King, M. E., and Shieltz, N. C. (1950) Analytical data on reference clay materials. *Amer. Petrol. Inst. Prelim. Rept.* **7,** Columbia University, New York, 76–83.

Kurganov, A. A. and Davankov, V. A. (1981) Ligand-exchange chromatography of racemates. XVI. Microbore column chromatography of amino acid racemates using N,N-tetramethyl-(R)-propanediamine 1,2-copper(II) complexes as chiral additives to the eluent: *J. Chromatogr.* **218,** 559–567.

Lahav, N., White, D., and Chang, S. (1978) Peptide formation in the prebiotic era: thermal condensation of glycine in fluctuating clay environments: *Science* **201,** 67–69.

Lindner, W., Le Page, J. N., Davies, G., Sietz, D. E., and Karger, B. L. (1979) Reversed-phase separation of optical isomers of dansyl amino acids and peptides using chiral metal chelate additives: *J. Chromatogr.* **185,** 323–344.

Mingelgrin, U. and Tsvetkov, F. (1985) Adsorption of dimethyanilines on montmorillonite in high pressure liquid chromatography: *Clays & Clay Minerals* **33,** 285–294.

Siegel, A. (1966) Equilibrium binding studies of zinc-glycine complexes to ion-exchange resins and clays: *Geochim. Cosmochim. Acta* **30,** 757–768.

Siffert, B. and Kessaissia, V. S. (1978) Contribution au mechanism d'adsorption des α-amino acides par la montmorillonite: *Clay Miner.* **13,** 255–270.

Stumm, W. and Morgan, J. J. (1981) *Aquatic Chemistry. An Introduction Emphasizing Chemical Equilibria in Natural Waters:* 2nd ed., Wiley, New York, p. 239.

Ure, A. M. and Barrow, W. L. (1970) Copper, zinc, manganese in EDTA soil extracts: *Anal. Chim. Acta* **52,** 247–257.

Weinstein, S. (1982) Resolutions of d- and l-amino acids by HPLC with copper complexes of N,N-dialkyl α-amino acids as novel chiral additives: A structure-selectivity study: *Angew. Chem. Suppl.,* 425–433.

Williams, D. R. (1972) Thermodynamic considerations in

coordination. X. Potentiometric and calorimetric investigation of copper(II) histidine complexes in solution: *J. Chem. Soc., Dalton Trans.* **1,** 790–797.

Yamagishi, A. (1982) Clay column chromatography: Partial resolution of metal(III) tris(acetylacetonate) on a Δ-nickel(II) tris(1,10 phenanthroline) montmorillonite column: *Inorg. Chem.* **21,** 3393–3396.

Yamagishi, A. and Ohnishi, R. (1982) Clay column chromatography for optical resolution: Partial resolution of tris-(acetylacetonato) metal(III) on a Λ-tris(1,10-phenanthroline)ruthenium(II) montmorillonite column: *J. Chromatogr.* **245,** 213–218.

Yamagishi, A. and Ohnishi, R. (1983) Chromatographic resolution of cyclic organic compounds on a Λ-tris(1,10-phenanthroline)ruthenium(II) montmorillonite column: *Angew. Chem., Int. Ed.-Eng.* **22,** 162–163.

Yamagishi, A. and Soma, M. (1981) Optical resolution of metal chelates by use of adsorption on a colloidal clay: *J. Amer. Chem. Soc.* **103,** 4640–4642.

Yang, S. D. and Condrate, R. A. (1971) The IR spectra of lysine, adsorbed on several cation substituted montmorillonites: *Clays & Clay Minerals* **20,** 75–82.

Yang, S. D. and Condrate, R. A. (1972) Infrared spectra of α-alanine adsorbed on Cu-montmorillonite: *Applied Spectroscopy* **26,** 102–104.

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