Adsorption of Amino-Acids and Peptides by Montmorillonite and Illite

Part 1.—Cation Exchange and Proton Transfer

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Adsorption isotherms are presented for a range of naturally occurring neutral and acid amino-acids on hydrogen montmorillonite and of basic amino-acid and peptide cations on sodium and calcium montmorillonite and illite. Differences in the isotherms show that the electrostatic bonding which results from proton transfer and cation exchange respectively is supplemented by physical adsorption forces. The magnitude of the physical adsorption forces is determined by the molecular weight and by the shape of the adsorbed molecules. X-ray diffraction analyses of the wet complexes showed that with the exception of lysine, the intercalation of the basic amino-acid cations by montmorillonite led to a decrease in the basal spacing. There was a corresponding reduction in the ease of desorption of the amino-acids in aqueous KCl solutions.

When glycine and its di-, tri- and tetra-peptides are adsorbed by hydrogen montmorillonite the dominant adsorption mechanism is a proton transfer reaction.[1–3] However, other forces, which increase with the molecular weight of the adsorbed species, act in addition to proton transfer. These additional forces include polar and van der der Waals interactions. In the present paper, an attempt is made to distinguish the contributions of each to the adsorption process. The adsorption of a range of naturally occurring neutral and acid amino-acid dipolar ions by hydrogen montmorillonite, and of basic amino-acid cations by sodium and calcium montmorillonite, has been examined, as well as the adsorption of these compounds by another clay mineral, illite.

EXPERIMENTAL

MATERIALS

The montmorillonite used was described previously.[3] The illite sample came from County Grundy, Illinois, and was obtained from the Illinois Clay Products Co., Joliet, Illinois. After preliminary saturation of the clay with sodium ions, the less than 2 μ equivalent spherical diameter (e.s.d.) fraction was separated by mechanical dispersion in distilled water, followed by repeated sedimentation and decantation. X-ray powder photographs of the clay obtained from these suspensions showed that illite and approximately 3 % of quartz were present. The asymmetry of the 10 Å basal reflection suggested that the clay might be interlayered with an expanding lattice mineral.[4] The extent of interstratification was shown to be small by comparison of the external surface area of 106 m^2/g, obtained by nitrogen adsorption, with the total surface area 150 m^2/g, determined

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from the adsorption of cetyl pyridinium bromide.[5] The internal surface area of approximately 44 m^2/g indicated that one in fifteen of the interlamellar regions could be penetrated by water and other adsorbates.

Reagent-grade DL-α-alanine, β-alanine, β-phenyl-DL-α-alanine, p-aminobenzoic acid, DL-aspartic acid, L-glutamic acid and L-arginine hydrochloride were obtained from British Drug Houses. DL-leucine and L-carnosine (β-alanyl-L-histidine) were obtained from L. Light and Co. and DL-serine from Aldrich Chemical Co. Inc. Nutritional Biochemicals Co. supplied DL-arginine hydrochloride and California Biochemical Research Corp. supplied DL-histidine hydrochloride and L-lysine hydrochloride. All compounds contained less than 0·015 equiv. ($Na^+ + Ca^{2+} + Mg^{2+}$) per mole.

METHODS

The procedures used for preparing homoionic clay suspensions and for obtaining the adsorption isotherms have been described previously.[3] To determine the amount of adsorbed amino-acid or peptide cation retained against washing with an excess of N KCl solution the amino-acid-clay complexes obtained from the equilibrium experiments were shaken with a 100-fold excess of N KCl for 20 h, centrifuged and the amino-acid or peptide retained by the centrifugate measured. The error due to the presence of entrained solution containing replaced amino-acid was less than 1 mequiv./100 g. The analytical procedures and X-ray techniques were as described earlier [3] except that in the Kjeldahl procedure for nitrogen, a digestion time of 45 min was used..

RESULTS

The results of the equilibrium experiments are expressed as adsorption isotherms in fig. 1-5, which also show the amounts of sodium or calcium released on adsorption

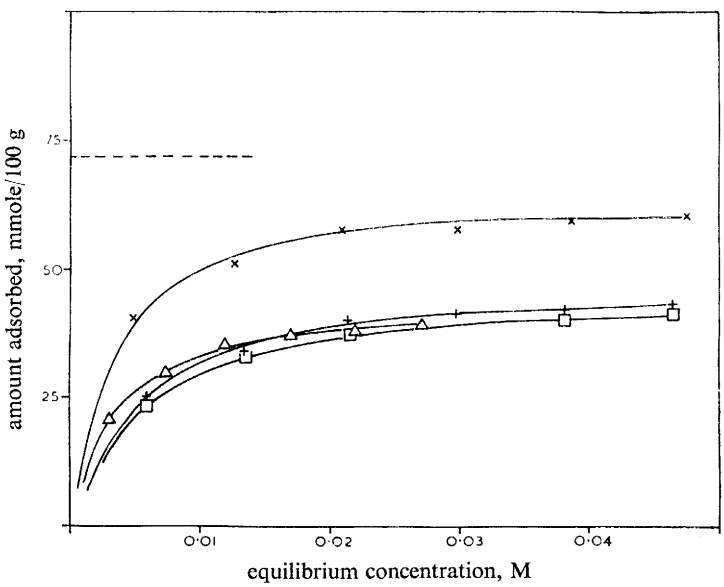

FIG. 1.—Adsorption isotherms for α-alanine, +, β-alanine, ×, leucine, △, and serine, □, on H_3O^+ montmorillonite at 25°C. The dashed line represents the amount of titrateable H_3O^+.

of the amino-acid or peptide cations. The analytical error involved in estimating the amount of amino-acid or peptide adsorbed from the change in solution concentration was between 2 and 6 %. An additional source of error arises because no

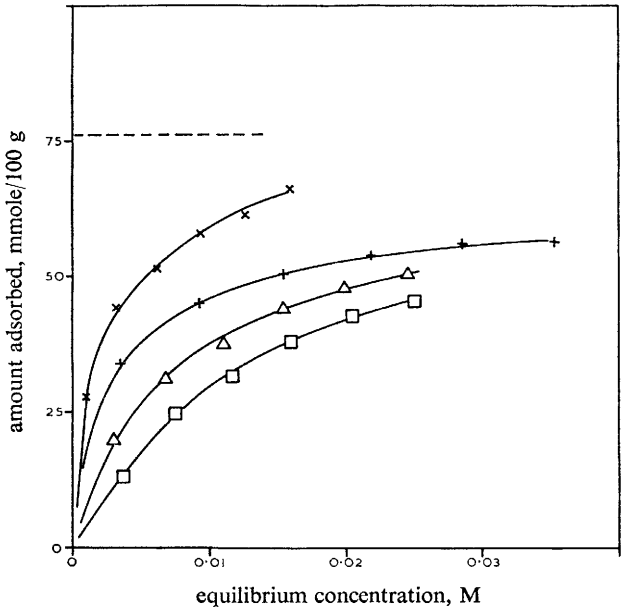

FIG. 2.—Adsorption isotherms for aspartic acid, □, glutamic acid, △, phenylalanine, +, and p-aminobenzoic acid ×, on H_3O^+ montmorillonite at 25°C. The dashed line represents the amount of titrateable H_3O^+.

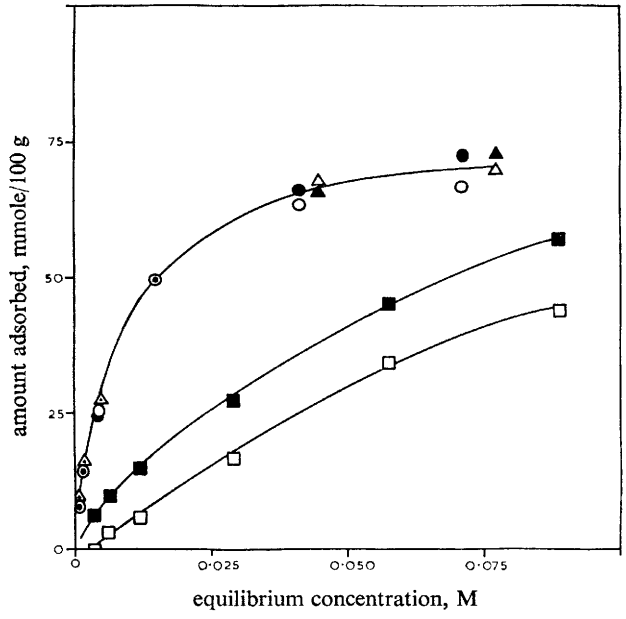

FIG. 3.—Adsorption isotherms for DL-arginine, ○, L-arginine, △, and glycine, □, cations on Ca^{2+} montmorillonite at 20°C. The solid markers represent the amount of Ca^{2+} liberated on adsorption of these cations.

allowance was made in calculating the results for the amount of water desorbed during the adsorption process. This error is less than 2 % for the range of concentrations studied. The results of the X-ray analyses of the wet and dried complexes obtained from the equilibrium experiments are presented in table 2.

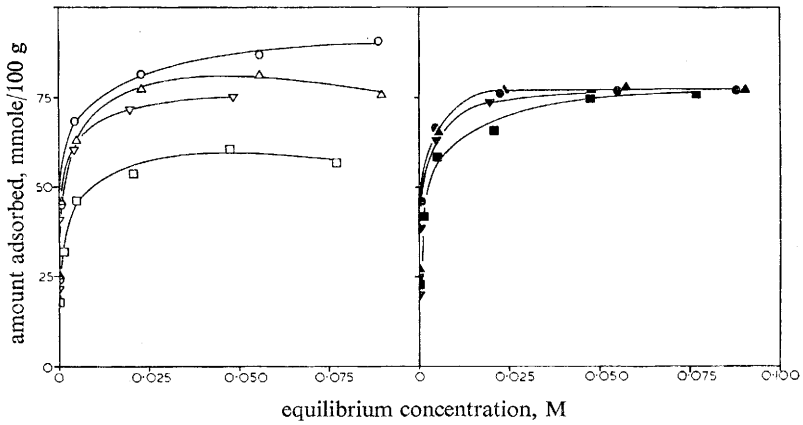

FIG. 4.—Adsorption isotherms for L-arginine, △, histidine, ○, and lysine, □, at 20°C and for carnosine, ▽, at 25°C on Na^+ montmorillonite. The solid markers represent the amount of Na^+ liberated on adsorption of these cations.

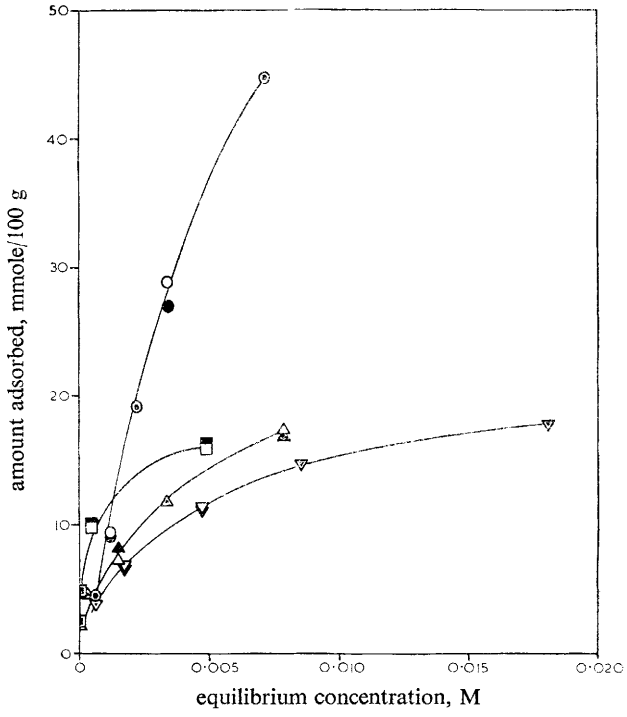

FIG. 5.—Adsorption isotherms for carnosine on Na^+ illite, □, and histidine on Ca^{2+} montmorillonite, ○, Na^+ illite, △, and Ca^{2+} illite, ▽, at 25°C. The solid markers represent the amount of Na^+ or Ca^{2+} liberated on adsorption of these cations.

TABLE 1.—CHARACTERISTICS OF CLAY SUSPENSIONS

substrate		amount of clay in suspension g/100 ml	pH	[Cl] N	cation exchange capacity mequiv./100 g	titratable hydrogen or exchangeable cation mequiv/100g
montmorillonite						
sodium		2·6	6·16	$3{\cdot}5\times10^{-5}$	88	87·3 (incl. 5 of Ca^{2+})
calcium		6·4	6·60	5×10^{-5}	90	89
hydrogen *	*a*	2·0	2·86			72·0
	b	2·1	2·95			76·4
illite						
sodium		16		$<10^{-5}$	29·3	29·2
calcium		8		$<10^{-5}$	29·2	29·0

* used for the adsorption of (*a*) α-alanine, β-alanine, leucine and serine; (*b*) aspartic, glutamic and p-aminobenzoic acids and phenylalanine.

TABLE 2.—X-RAY DIFFRACTION RESULTS FROM ANALYSES OF WET AND DRIED COMPLEXES OF MONTMORILLONITE WITH THE AMINO-ACIDS AND PEPTIDES STUDIED

adsorbate	amount adsorbed mmole/100 g	condition of complex	*d*(001) Å	*d*(002) Å	*d*(003) Å	*d*(004) Å	*d*(005) Å	*d*(001)-9·5 Å
α-alanine	44	wet	14·1					
	44 *	dry	13·7	6·55		3·20		4·2
β-alanine	60	wet	13·2 (w)					
	60 *	dry	13·00	6·50	4·35	3·22		3·5
leucine	39	wet	15·3					
	39 *	dry	14·8		4·90	3·64	2·93	5·3
serine	42	wet	15·0 (v.w.)					
	42 *	dry	13·20			3·24		3·7
aspartic acid	46	wet	13·5					
	46 *	dry	12·90	6·35		3·19		3·4
glutamic acid	31	wet	13·5 (v.w.)					
	31 *	dry	13·20	6·60		3·23		3·7
p-aminobenzoic acid	66	wet	14·9					
	66 *	dry	13·00	6·41		3·18		3·5
phenylalanine	56	wet	15·70	7·77	5·15		3·07	
	54 *	dry	13·85	6·80	4·35	3·39	2·72	4·4
arginine	76	wet	16·6					
	76 *	dry	13·60		4·43	3·40		4·1
carnosine	75	wet	13·7					
	75 *	dry	13·52		4·38	3·32		4·0
histidine	91	wet	13·7					
	91 *	dry	13·52		4·38	3·32		4·0
lysine	61	wet	>100					
	61 *	dry	13·60			3·34		4·1

m = medium w = weak v.w. = very weak

* These values are slightly low, as some further adsorption of amino-acid would have occurred during the drying of the complex plus entrained solution.

DISCUSSION

ADSORPTION BY HYDROGEN MONTMORILLONITE

In contrast to the results obtained when non-basic amino-acids are adsorbed by calcium clays,[3, 6] adsorption by hydrogen clays gives rise to L-type (Langmuir) isotherms, and not linear ones. This is due to the fact that adsorption occurs primarily at the exchange sites. The process may be represented by the equation

$$RH^{\pm} + \text{clay } H_3O \rightleftharpoons \text{clay } RH_2 + H_2O, \quad (1)$$

for which the equilibrium constant is

$$K_t = \frac{a'_{RH_2}}{a'_{H_3O}a_{RH^{\pm}}} = \frac{N_{RH_2^+}}{N_{H_3O^+}[RH^{\pm}]} \frac{\gamma'_{RH_2^+}}{\gamma'_{H_3O^+}\gamma_{RH^{\pm}}} = K_n K_\gamma,$$

where the a', N and γ' terms denote the surface activities, concentrations (in mequiv/100 g) and activity coefficients respectively of the species indicated by the subscripts, and the other terms have their usual significance. The proton transfer reaction (1) is directly comparable with the reverse of the first dissociation of the amino acid or peptide cation,

$$RH_2^+ + H_2O \rightleftharpoons RH^{\pm} + H_3O^+ \quad (2)$$

for which the equilibrium constant is K_1 and obtainable from the literature.[7, 8] K_t will exceed $1/K_1$ insofar as physical adsorption forces contribute to the adsorption process, and differences in the product K_tK_1 will reflect the relative magnitudes of these forces for different amino acids. Differences in the values of K_t indicate differences due to basicity in addition to those due to other forces. The relative contributions of basicity and physical adsorption forces can therefore be derived from the values of $K_nK_1K_\gamma$ for the different compounds studied. In table 3 the values of K_1 taken from the literature [7, 8] and of K_n and K_1K_n are presented. K_n was determined by plotting $1/[RH^+]$ against $N_{H_3O^+}/N_{RH_2^+}$, when a straight line was obtained (in a few instances there was a departure from linearity at high surface coverages), the slope of which is K_n. No procedure is yet available for evaluating the surface activity coefficients. However, when K_nK_1 is plotted against the molecular weights of the amino acids or peptides (fig. 6), all but one of the points lie very close to a single straight line. A

TABLE 3.—THE MASS ACTION QUOTIENTS FOR PROTON TRANSFER ADSORPTION BY HYDROGEN MONTMORILLONITE TOGETHER WITH THE FIRST DISSOCIATION CONSTANTS OF THE CATIONS AND THE PRODUCT K_nK_1

adsorbate	K_n M^{-1}	$K_1 \times 10^3$ M	K_nK_1
glycine	34 *	4·60	0·16
glycyl glycine	730 *	0·85	0·62
diglycyl glycine	2100 *	0·55	1·15
triglycyl glycine	1800 *	0·89	1·60
α-alanine	123	4·48	0·55
β-alanine	312	0·25	0·08
leucine	179	4·70	0·84
serine	111	6·17	0·68
aspartic acid	60	7·95	0·48
glutamic acid	114	7·95	0·90
phenylalanine	272	2·63	0·72
p-aminobenzoic acid	415	4·38	1·82

* These values differ from those given previously [3] by the factor 55·5, the molal concentration of liquid water.

similar linear increase with molecular weight is found for adsorption on calcium montmorillonite [3, 6] when only physical adsorption forces are involved. This suggests that the values of the activity coefficient ratios K_γ are similar for the various amino acids and peptides studied, as might be expected. The deviations from the general relation may be due to different values for K_γ, but they are also explicable in terms of the expected influences of the molecular parameters on the adsorption process.

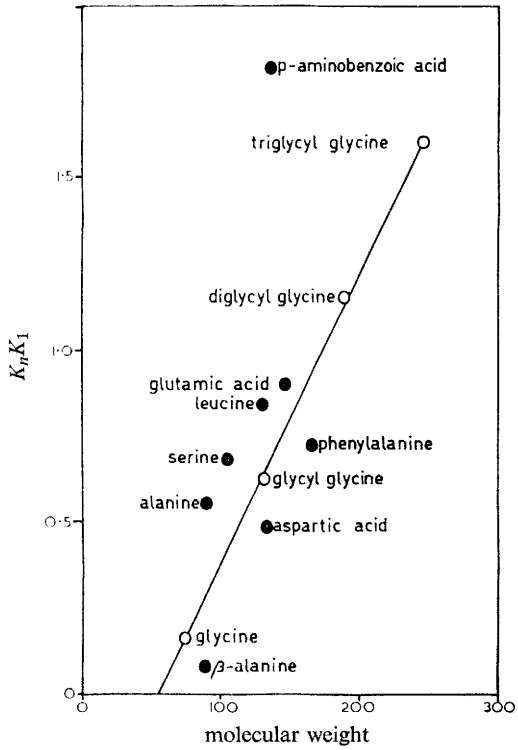

FIG. 6.—The variation in K_nK_1 with molecular weight for the adsorption of amino-acids and peptides by H_3O^+ montmorillonite. The values of K_nK_1 for glycine and its peptides were calculated from earlier data.[3]

The exceptionally large value of K_nK_1 for p-aminobenzoic acid may be attributed to the interaction of the benzene ring with the clay surface, intimate contact being possible because of the planar nature of the p-aminobenzoic acid molecule. p-Aminobenzoic acid is the only compound studied which does not exist predominantly as the dipolar ion in aqueous solution. However, this property is not likely to have as great an effect on the value of K_nK_1 as would the planar character of the molecule. The comparison of K_nK_1 for phenylalanine (0·72) with that for p-aminobenzoic acid (1.82) shows that the shapes of the molecules play an important part in determining their physical adsorption, and that van der Waals forces are effective over very short distances only. The adsorption of planar aromatic molecules may also be assisted by the interaction of the π electrons of the nucleus with the surface oxygens.[9] Assuming that the benzene ring of intercalated phenylalanine is placed centrally in the interlamellar space and parallel to the surfaces, the distance between the tops of the surface oxygen atoms and the faces of the benzene rings as determined from the X-ray

data for the dried phenylalanine complex (table 2) is approximately 0·6 Å. This distance is apparently too great for efficient van der Waals bonding of the phenyl group of phenylalanine to the oxygen surfaces compared with the benzene ring of p-aminobenzoic acid.

When the values of K_n are compared with those of K_nK_1 it is seen that the differences in K_n are much greater than in K_nK_1, e.g., K_n for triglycyl glycine is about 60 times as large as for glycine, whereas K_nK_1 is only 10 times as large. This indicates that basicity of the amino-acid is more important to the adsorption energy than the physical adsorption forces, as Talibudeen [1] and Sieskind [2] have stated, but the contribution of physical adsorption forces is by no means negligible and will undoubtedly be extremely important for very large molecules which can collapse to the clay surface.

ADSORPTION OF THE BASIC AMINO-ACID AND PEPTIDE CATIONS

Fig. 3-5 show that adsorption was invariably accompanied by the liberation of approximately equivalent amounts of the exchangeable cation. The cation exchange equilibria and equilibrium constants are as follows:

adsorption by sodium clays,

$$RH_2^+ + \text{clay Na} \rightleftharpoons \text{clay } RH_2 + Na^+, \tag{3}$$

$$K_a = K_rK_\gamma,$$

where

$$K_r = N_{RH_2^+}[Na^+]/N_{Na^+}[RH_2^+];$$

adsorption by calcium clays,

$$2RH_2^+ + \text{clay Ca} \rightleftharpoons \text{clay } 2RH_2 + Ca^{2+} \tag{4}$$

and

$$K_s = N^2_{RH_2^+}[Ca^{2+}]/N_{Ca^{2+}}[RH_2^+]^2.$$

The adsorption of weakly basic amino-acids from the hydrochloride solutions by calcium montmorillonite differs in detail from (4) above. Considerable hydrolysis occurs in solution:

$$RH_2^+ + H_2O \rightleftharpoons RH^\pm + H_3O^+ \tag{5}$$

so that the overall exchange reaction is

$$RH_2^+ + H_3O^+ + \text{clay Ca} \rightleftharpoons \text{clay } H_3O,\ RH_2 + Ca^{2+} \tag{6}$$

and

$$K_s' = \frac{N_{RH_2^+}N_{H_3O^+}}{N_{Ca^{2+}}} \frac{[Ca^{2+}]}{[RH_2^+][H_3O^+]}.$$

The adsorption of glycine, for instance, was accompanied by a marked decrease in pH and the liberation of Ca^{2+} equivalent to the amount of H_3O^+ and amino-acid cation adsorbed (fig. 3).

The determination of K_s' for the adsorption of glycine by calcium montmorillonite required the values of $N_{H_3O^+}$ and $[H_3O^+]$. These were obtained by subtraction of $N_{RH_2^+}$ from the amount (in mequiv./100 g) of Ca^{2+} liberated, and from the pH of the supernatant solutions respectively.

The symbols in eqn. (3) to (6) are defined earlier. However, the units of concentration and surface concentration were g ion/l. and mg ion/100 g respectively. K_γ was assumed to be constant in the interpretation of the results. The values K_r, K_s

and K_s' were determined by plotting the ratio of the concentrations in the solution phase (e.g., $[Na^+]/[RH_2^+]$) against the corresponding ratio in the adsorbed phase (e.g., $[Na^+]/[N_{RH_2^+}]$). The amount of exchangeable cation remaining on the surface was obtained by difference. The region of maximum curvature of the isotherm for the adsorption of carnosine by sodium illite (Fig. 5) was not determined with sufficient accuracy to warrant this type of analysis. For the remainder of the isotherms the error probably does not exceed 60 % for any single point.

For the purposes of comparison of the present data with those of Cowan and White,[10] non-standard free energy values have been calculated using the van't Hoff isotherm,

$$-\Delta G^m = RT \ln K_m. \quad (7)$$

Cowan and White [10] studied the adsorption of an homologous series of n-alkylammonium cations by sodium montmorillonite, a system closely analogous to the present, so that comparison of the non-standard free energies is probably valid. They found that the n-octylammonium ion (molecular weight 130) had a free energy of cation exchange in this system of 1290 cal/g ion and was the first of the series to be adsorbed in excess of the exchange capacity. The excess amine was adsorbed as the uncharged molecule, adsorption being accompanied by a decrease in pH due to the hydrolysis of the cation. All the amino-acid cations adsorbed by sodium montmorillonite in the present work have molecular weights greater than that of the n-octylammonium ion, but only carnosine (molecular weight 226) has a larger free energy of cation exchange (table 4). The amino-acid cations are more readily hydrolyzed

TABLE 4.—THE MASS ACTION QUOTIENTS AND FREE ENERGIES FOR ADSORPTION BY CATION EXCHANGE OF AMINO-ACID OR PEPTIDE CATIONS

molecular weight	arginine 174		histidine 155		lysine 146		carnosine 226		glycine 76	
	K	$-\Delta G^m$ cal/g ion	K	$-\Delta G^m$ cal/g ion	K	$-\Delta G^m$ cal/g ion	K	$-\Delta G^m$ cal/g ion	K	$-\Delta G^m$ cal/g ion
Na montmorillonite	8·1	1240	8·2	1250	3·4	720	12·0	1470		
Ca montmorillonite	5350	5080	10^4	5460					83	2600
Na illite			2·3	490						
Ca illite			2860	4700						

than the n-octylammonium ion. However, none of the amino-acids or carnosine is adsorbed in excess of the exchange capacity. This is probably because (i) the products of hydrolysis are water-soluble, whereas the uncharged amines adsorbed above the exchange capacity are sparingly soluble in water and (ii) the chains of the n-alkylamines pack closely together, probably in a highly ordered manner.[11] This allows efficient van der Waals interaction between the adsorbed molecules. For the adsorption of n-alkylammonium ions above pentylammonium there is a constant increment in $-\Delta G^m$ of 400 cal/CH_2-group (m.w. = 14). By comparison, the increment in $-\Delta G^m$ for histidine and its β-alanyl peptide carnosine is only 240 cal/β-alanyl group (m.w. = 71). It is evident from these data that the shape of the adsorbed cations is an important factor in determining the extent of van der Waals interaction. This was illustrated earlier by the adsorption of p-aminobenzoic acid and phenylalanine by hydrogen montmorillonite.

COMPARISON OF ADSORPTION ON MONTMORILLONITE AND ILLITE

Inspection of table 4 reveals that the free energy of exchange of the histidine cation with sodium montmorillonite is greater than with sodium illite. This is also true of

the corresponding calcium clays and probably results from the fact that each intercalated histidine cation interacts with two internal surfaces. The ion-to-surface contributions to the adsorption energy will consequently be greater than for adsorption on external surfaces. The internal surface area of montmorillonite is greater than 90 % of the total area while this proportion is approximately 30 % for the illite used. In contrast to this, adsorption energies due to physical forces alone are greater on illite than montmorillonite.[6]

DESORPTION OF AMINO-ACID OR PEPTIDE CATIONS

Carnosine, histidine and arginine were retained against a 20 h extraction with a 100-fold excess of 1N KCl to an extent ranging from 28 to 58 % of the amount initially present (table 5). On the other hand, lysine was almost completely extracted from the highly expanded complex which it formed with montmorillonite. These observations accord with the limited intracrystalline swelling of the complexes other than that of lysine. The data of Mortland [12] show that the adsorption of K^+ by vermiculite is restricted in the presence of organic cations including those of basic amino-acids, histidine being the most effective. The desorption of amino-acid cations from illite is relatively unrestricted.

TABLE 5.—THE AMOUNTS OF ADSORBED AMINO-ACIDS AND PEPTIDES RETAINED AGAINST EXTRACTION WITH KCl SOLUTION

substrate	adsorbed cation	amount present initially	amount retained against 1 N KCl
		mmole/100 g	
Na montmorillonite	arginine	76	21
	histidine	66	22
	lysine	75	1
	carnosine	75	33
Ca montmorillonite	histidine	45	26
Na illite	histidine	17	2
	carnosine	16	1
Ca illite	histidine	18	1

X-RAY RESULTS

The results of the X-ray diffraction studies of the dried complexes (table 2) showed that all the compounds examined formed single layer complexes with montmorillonite. Δ values, obtained by subtracting 9·5 Å (the assumed thickness of the aluminosilicate layer) from the basal spacing for the dried complexes,[13] are also given in table 2. Only for leucine and p-aminobenzoic acid were the Δ values equal to the minimum thickness of the adsorbed cations derived from molecular models. For all other compounds the Δ values were less than the minimum molecular thickness. The maximum extent to which the molecules could be keyed into the hexagonal depressions of the oxygen surfaces of montmorillonite was determined from molecular models as described previously.[3] The models could be fitted into the surface to give contractions greater by about 0·2 Å than those actually observed (0 to 1·2 Å). This is in agreement with the earlier work [3] and substantiates the conclusion that it is not necessary to invoke other mechanisms [1] such as C—H . . . O—Si hydrogen bonding to interpret the apparent contraction of the thickness of these molecules on intercalation by montmorillonite.[3, 14]

The data given show that non-ionic forces play some part in the adsorption of amino-acids and peptides by cation exchange and proton transfer reactions. The

molecular weight and the shape of the adsorbed molecules largely determine the magnitude of the physical adsorption contribution. The carboxyl groups of aspartic and glutamic acids did not restrict the adsorption of these compounds by proton transfer (fig. 6), although adsorption of organic acids at higher pH values, where dissociation of the carboxyl groups is significant, would be expected to be restricted by repulsion of the $-COO^-$ groups by the clay surface.

With the exception of lysine, the intercalation of amino-acid cations by montmorillonite causes the collapse of the interlamellar space, so that the surface migration of the cations, and therefore the rates of adsorption and desorption, are restricted.

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