

## A NATURAL CLAY ORGANIC COMPLEX FROM ANDALUSIAN BLACK EARTH

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**Abstract**—Protein complexes of smectites in soils are difficult to detect if the usual smectite tests show no peculiarities. Andalusian black earths are typical examples. Investigation of the alkylammonium derivatives, however, allows detection of adhered macromolecules which might be protein-like although this cannot be proved exactly.

Investigation of artificial clay-protein complexes reveals different types of clay protein interactions. Calcium smectites adsorb proteins mainly on the external surfaces, the macromolecules being anchored in the interlayer spaces. Sodium smectites give partial crystalline products in which the silicate layers are distributed in the protein matrix.

Exchange of alkylammonium ions can be used as a tool for detection of the protein. If this is adsorbed on external surfaces (calcium smectites) the increased layer separation during the cation exchange enables the macromolecules to slip between the layers and the basal spacing of the alkylammonium derivatives are changed in characteristic ways. The partial crystalline sodium clay-protein complexes are reorganized by alkylammonium ions to regular structures. The proteins are not displaced completely from the silicate surfaces so that the basal spacing of the alkylammonium derivatives are enhanced in comparison with pure montmorillonite.

### INTRODUCTION

Micro-organisms and plants act as continuous sources of a wide variety of monomeric, oligomeric and polymeric organic compounds. Most of these compounds are adsorbed by soil minerals. The interaction covers all bonding types from physical sorption to cation exchange and chemical reactions. Physical adsorption of monomeric compounds is mostly reversible and most of the sorbate-sorbent-complexes are destroyed during the different preparation processes of the soil. Polymeric molecules often are irreversibly adsorbed—not in the thermodynamic sense but due to kinetic hindrance of the desorption. Clay minerals strongly adsorb polymeric molecules, especially cationic polymers which can be bound by cation exchange. The sorption isotherms are steep; even from very dilute solutions large amounts of polymers are adsorbed. Among the different macromolecular systems in soils proteins and protein degeneration products are very important due to their basic groups ( $-\text{NH}_3^+$ ,  $-\text{NR}_3^+$ , guanidium, imidazol, pyrrolidinium, etc.) which can be bound by cation exchange.

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Protein adsorption on clays has been extensively discussed in the literature (Ensminger and Gieseck 1941; Mortland and Gieseck, 1952; Talibudeen, 1954; Lynch and Cotnoir, 1956; McLaren *et al.*, 1958; Pink, 1962; Armstrong and Chesters, 1964). Differences of adsorption data reported in the literature are caused by different methods of preparation and by the fact that reaction of clays with proteins proceeds sometimes very fast, sometimes very slowly.

In connection with our experiments on layer charge determination of swelling clay minerals we often found hints of adsorbed macromolecules on the clay minerals by unusual spacings of the alkylammonium derivatives. An Andalusian black earth revealed this phenomenon quite clearly; the properties were very similar to those of synthetic montmorillonite-protein complexes.

### EXPERIMENTAL

#### *Andalusian black earth*

The Vertisol is a very gray soil, little permeable, of very coarse texture and slightly alkaline reaction, low content of organic matter and characteristic mull humus.  $\text{CaCO}_3$  content is of the order of 15% throughout the profile. It is a clayey soil with high

Table 1. Profile of Andalusian black earth (Carretera Sevilla-Granada, Sevilla-Lantejuela)

Nr.	Horizon	Depth (cm)	Description
13	Ap	0-25	Very dark gray; clayey; moderately or strongly developed, thick granular-crumb structure; friable to firm; slightly porous; little permeable; calcareous; slightly organic;
14	Ap/A <sub>1</sub>	25-55	Very dark gray (10YR3/1), clay; moderately developed, thick subangular blocky structure; firm, compact; little permeable; calcareous; slight organic;
15	(B) <sub>1</sub>	55-70	Very dark gray; clay; thick and medium prismatic-blocky structure, moderately to strongly developed; firm, compact; very little permeable; calcareous.
16	(B) <sub>2</sub>	70-125	Same characteristics as in preceding horizon, with strongly developed thick prismatic structure.
17	(B) <sub>2</sub> /C <sub>1</sub>	125-160	Dark gray and brown, clay; strongly developed thick subangular blocky structure; firm; compact, very little permeable; calcareous;
18	B <sub>2</sub> /C <sub>2</sub>	160-180	Brown, gray brown and dark gray clay; moderately developed, medium subangular blocky structure; firm to friable; compact; very little permeable; calcareous.

Table 2(a). Andalusian black earth: particle size distribution, organic material, N-content and CaCO<sub>3</sub> (Weight Per cents)

Horizon	Depth (cm)	Coarse sand	Fine sand	Silt	Clay	O.M.*	N†	C/N‡	CaCO <sub>3</sub>
Ap	0-25	9.30	24.20	10.70	54.30	1.55	0.07	12.80	15.40
Ap/A <sub>1</sub>	25-55	10.90	21.30	11.80	54.20	1.12	0.08	8.10	15.70
B <sub>1</sub>	55-70	17.40	20.60	6.30	54.10	1.02	0.06	9.80	14.60
B <sub>2</sub>	70-125	6.80	31.10	6.20	53.90	0.91	0.06	8.80	15.80
B/C <sub>1</sub>	125-160	10.70	23.90	8.40	55.90	0.50	0.03	9.60	13.00
B/C <sub>2</sub>	>160	9.70	22.40	6.30	60.20	0.26	0.02	7.50	12.20

\* By KMnO<sub>4</sub>.

† Micro Kjeldahl.

‡ Without carbonate C.

Table 2(b). Andalusian black earth: exchangeable cations

Horizon	Depth (cm)	pH	Exchangeable cations (meq./100 g)				CEC (meq./100 g)
			Ca	Mg	Na	K	
Ap	0-25	7.5	55.2	4.9	—	0.9	61.0
Ap/A <sub>1</sub>	25-55	7.6	54.6	5.7	0.6	0.5	61.4
B <sub>1</sub>	55-70	7.7	51.5	6.2	1.3	0.5	59.4
B <sub>2</sub>	70-125	7.9	50.5	7.2	2.7	0.4	60.8
B/C <sub>1</sub>	125-160	7.7	52.4	8.3	7.0	0.4	68.1
B/C <sub>2</sub>	>160	7.5	51.3	7.4	5.6	0.4	64.7

smectite and illite content and a base exchange capacity of 60 meq./100 g (Gonzalez Garcia and Perez Rodriguez, 1970). Detailed information is given in Tables 1 and 2.

#### Fractionating of the black earths

Carbonates were removed by treatment with a buffer solution of sodium acetate-acetic acid at pH = 5. The suspension in water was fractionated by the usual sedimentation procedure and the 2  $\mu$ m clay fraction separated. The suspension was flocculated with ammonium acetate, washed and air dried.

#### Preparation of alkylammonium derivatives

About 50–100 mg of the clay fraction were treated with 2–3 ml of *n*-alkylammonium chloride solutions at 65°C for 3 days; then washed 3 times with ethanol-water and again suspended in 2–3 ml of alkylammonium chloride solution for 3 days at 65°C. The samples were washed 6 times with 2–3 ml ethanol and 20 times with 2–3 ml ethanol-water (1:1) and air-dried. Before the X-ray investigations in Debye-Scherrer cameras the samples were carefully dried at 0.1 Torr and 40°C and then at 10<sup>-3</sup> Torr and 40°C for 20 hr.

#### Alkanol complexes of the alkylammonium derivatives

Small amounts of alkylammonium derivatives were mixed with not more than the same volume of alkanol (larger excess has to be avoided!) and tempered for 24 hr at 65°C. The samples were cooled slowly to room temperature and X-rayed in Debye-Scherrer powder cameras (dia 114.6 mm).

#### Preparation of montmorillonite-protein complexes

Montmorillonite-protein complexes were prepared from montmorillonite from Niederbayern (Südchemie, Germany) and Wyoming (A.P.I. No. 25, Wards, USA). Homoionic calcium and sodium forms were

obtained by treating 50 g of the natural product extensively (i.e. 3 times for calcium and 5 times for sodium) with 500 ml 0.1 N calcium or sodium chloride solutions. The excess salt was removed by dialysis.

The proteins used were bovine serum albumen (Cohn Fract. V, 15.2% N; Serva, Heidelberg, Germany), lysozyme (3  $\times$  cryst., 17.1% N, Serva) and salmine sulfate (pract., 20.1% N, Fluka, Switzerland). Adsorption of the proteins was measured by direct determination of protein loss in solution (photometrically by biuret reaction) and by C and N analyses of the dried montmorillonite-protein complexes.

For preparation of the protein complexes 20 g calcium montmorillonite was dispersed in 500 ml water. For the sodium montmorillonite 5 g sodium montmorillonite was dispersed in 5 l water (and more) in order to get a very dilute dispersion with complete separation of the silicate layers. Five grammes albumen or lysozyme in 500 ml water were slowly added. pH was not adjusted but was always 5.0–5.5 (experiments at different pH-values indicated the adsorption on montmorillonite to be less pH-dependent than on hydrophilic silica (MacRitchie, 1972)). After 120 hr at 4°C in darkness (to avoid growth of algae) the montmorillonite-protein complexes were separated by filtration, washed with water and dried in the same way as the natural soils.

## RESULTS

#### Unusual properties of the alkylammonium derivatives of the soil clay fraction

The clay fraction of the soil is a mixture of illite and smectite and some kaolinite. It reveals no unusual behavior if the usual soil tests are applied, in contrast to a natural organic clay complex described by Moum *et al.* (1973). The smectite expands with ethylene glycol and does not collapse with potassium chloride. Drying in high vacua decreases the basal

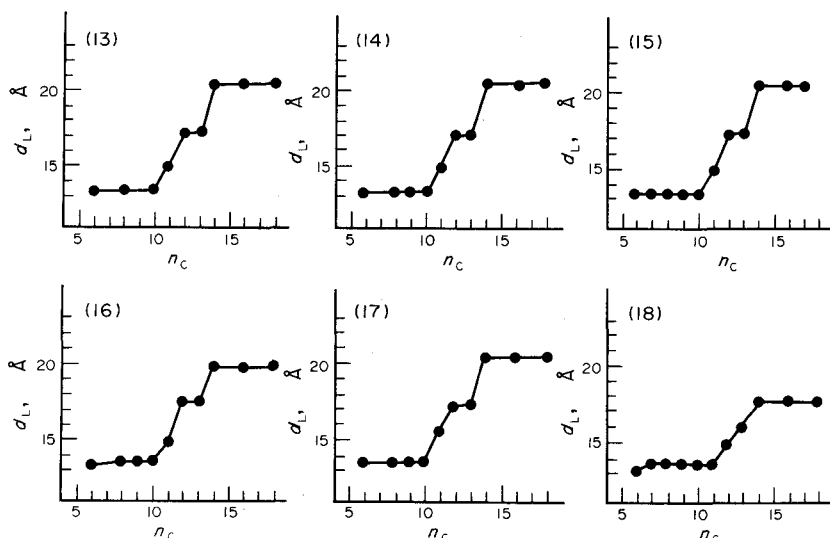


Figure 1. Basal spacings  $d_L$  of *n*-alkylammonium derivatives of soils samples No. 13–18 (Table 1, 2) from Andalusian black earth ( $n_c$  is the number of carbon atoms in the alkyl chain).

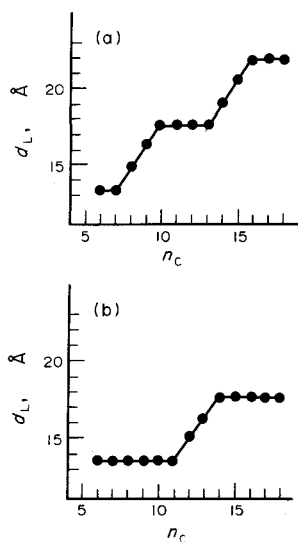


Figure 2. Typical basal spacings of alkylammonium smectites. (a) high layer charge (about 0.4 eq./ $(\text{Si}, \text{Al})_4\text{O}_{10}$ ). (b) low layer charge (about 0.3 eq./ $(\text{Si}, \text{Al})_4\text{O}_{10}$ ).

spacing to about 10.2 Å. C- and N-analyses indicate about 2–3% organic material.

The basal spacings of the *n*-alkylammonium derivatives (after careful washing and drying) are shown in Figure 1. Their variation with chain length is very unusual. Among our investigation of about 200 smectites we did not find similar curves (Lagaly and Weiss, 1976). The peculiarity is revealed by comparison with the spacings of a low and a high charge smectite (Figure 2). The three plateaus at basal spacings of 13.5 Å, 17.7 Å and 22 Å correspond to monolayers, bilayers and pseudotrimolecular layers of alkylammonium ions ( $n_C$  carbon atoms) between the silicate layers (Lagaly and Weiss, 1970, 1971). Only one of the soil samples, No. 18 on the basis of the profil, shows the expected variation of the basal spacing. The broad transition range from monolayer to bilayer structures (from  $n_C = 11$  to  $n_C = 14$ ) indicates a low charge smectite with heterogeneous charge distribu-

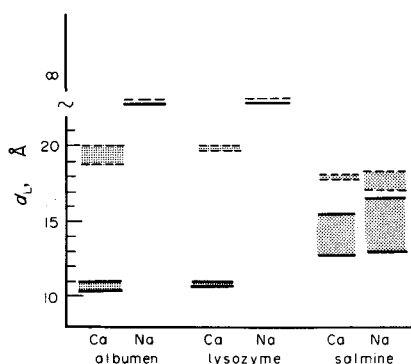


Figure 4. Basal spacings of calcium and sodium montmorillonite protein complexes under equilibrium solution (-----) and dried in high vacuum ( $p \leq 10^{-2}$  Torr) at 40°C (—).

tion (Lagaly and Weiss, 1970; Lagaly and Weiss, 1976). The interlayer cation density varies randomly between 0.28 and 0.33 eq./ $(\text{Si}, \text{Al})_4\text{O}_{10}$ .

In all other samples the basal spacings start to increase beyond 13.6 Å at  $n_C = 10$  indicating an upper limit of cation density of 0.36 eq./ $(\text{Si}, \text{Al})_4\text{O}_{10}$ . The plateau corresponding to the bilayer structure extends only from  $n_C = 12$ –13, and has spacings (17.2–17.5 Å) lower than those for typical bilayer structures (17.7 Å). From  $n_C = 13$  to  $n_C = 14$  the spacing increases to the 20–21 Å-plateau. This would indicate a smectite or vermiculite with a layer charge of at least 0.55 eq./ $(\text{Si}, \text{Al})_4\text{O}_{10}$  in contrast to the data from the monolayer/bilayer transition.

The narrow limits of the second plateaus and their comparatively low basal spacings, the extended plateaus at 20–21 Å and the sharp transition between the plateaus indicate the unusual properties of these soil smectites. This behavior is related to adsorbed organic material. Oxidation by  $\text{H}_2\text{O}_2$  leads to basal spacings identical to those of sample No. 18 with no peculiarities. The results reported resemble observations on synthetic montmorillonite-protein complexes.

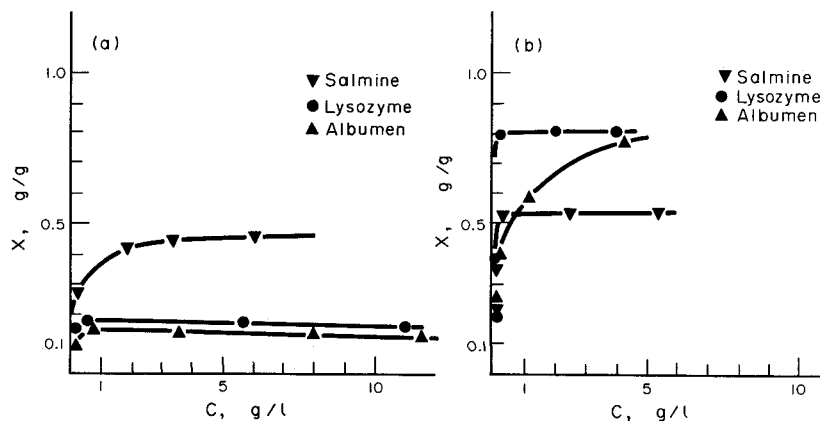


Figure 3. Adsorption isotherms of albumen, lysozyme and salmine on calcium (a) and sodium montmorillonite (b) (pH of solution 5.0–5.5).  $C$ : equilibrium concentration of proteins (g/l).  $X$ : amount protein adsorbed (g/g dried montmorillonite, 1 g albumen = 10.9 meq.N, 1 g lysozyme = 12.2 meq.N, 1 g salmine = 14.4 meq. N).

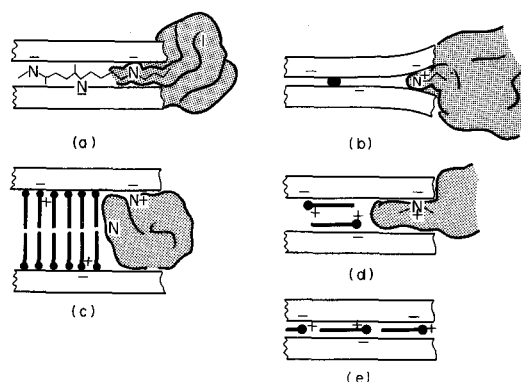


Figure 5. Protein adsorption by calcium montmorillonite. (a) Salmine, which unfolds and penetrates between the layers, (b) albumen and lysozyme, which are adsorbed on external surfaces and anchored in the interlayer spaces, (c) interaction of alkylammonium ions with calcium montmorillonite protein complexes in alkylammonium chloride solutions, (d) alkylammonium derivatives of montmorillonite protein complexes, (e) alkylammonium derivatives of pure montmorillonite for comparison.

*Model system: adsorption of albumen, lysozyme and salmine on montmorillonite*

Calcium and sodium montmorillonites take up large amounts of albumen, lysozyme and salmine. The adsorption isotherms are steep (Figure 3). At high protein concentration the adsorption decreases and unusual adsorption isotherms with a maximum are observed, presumably caused by strong solvent-solution interactions at high concentrations of macromolecules. There are large differences between the calcium and the sodium clays. Calcium montmorillonite adsorbs markedly larger amounts of salmine (660 meq. N/100 g) than lysozyme and albumen. The basal spacings (13.9–15.4 Å) show that the salmine molecules have slipped between the silicate layers. Lysozyme and albumen are adsorbed in maximum amounts of about 200 and 150 meq. N/100 g. The basal spacing of the dried complexes lie between 10 Å and 11 Å (Figure 4).

Sodium clay adsorbs about 700 meq. N/100 g of salmine (sharp basal spacings of 17.1–18.3 Å) and in contrast to calcium clay about 1000 meq. N/100 g of lysozyme and albumen. Even if the lysozyme and albumen samples were carefully dried, (001)-reflexions could not be observed in Debye-Scherrer powder cameras and by Kratky experiments.

*Binding of proteins by calcium montmorillonite*

Calcium smectites expand in water to 20 Å; the protein molecules have to penetrate between the layers which are separated by 10 Å at most. Only some proteins such as salmine with minor complexity and capability of unfolding can penetrate between the layers relatively fast as previously stated by Talibudeen (1954) (Figure 5(a)). The reaction may be promoted by the high basicity of salmine (isoelectric points: salmine, pH = 12; lysozyme, pH = 10.5; albumen, pH = 5.2). The large amounts of lysozyme and albumen are adsorbed on the external surfaces,

probably anchored in the interlayer spaces (Figure 5(b)). The anchoring opens the interlayer spaces in the outer regions, increasing  $d_{001}$  to somewhat above 10 Å. It cannot be excluded that proteins penetrate deeper into some interlayer spaces so that packets of 10 Å layers become separated by interlayer spaces with larger separations. The quality of the (001)-reflexions is too poor for a conclusive decision. The results with alkylammonium ions (see below) support the first model.

In slow processes even proteins with high complexity and without complete unfolding may penetrate into the interlayer spaces upon expanding the layers to more than 20 Å. Definite experimental results are difficult to obtain; long reaction times often lead to bacteriological decomposition of proteins and to intercalation of degeneration products.

*Binding of proteins by sodium montmorillonite*

Most sodium smectites expand completely under water, the basal spacing increases to hundreds of Å. Due to complete separation of the silicate sheets proteins can be adsorbed on the whole area of the surfaces. The adsorption is not very specific; proteins with different molecular weight, composition and complexity are adsorbed.

Salmine macromolecules bring together the layers in relatively high order with sharp (001)-reflexions of spacings of 17.1–18.3 Å. The spacings and the amount adsorbed lie in the same range as those of calcium montmorillonite.

Lysozyme and albumen are adsorbed by sodium clay in higher amounts (about 1000 meq. N/100 g) than by calcium clay (about 200 meq. N/100 g). The X-ray diagrams show that the layers do not collapse. Even if the complexes were carefully dried, (001)-reflexions could not be observed in Debye-Scherrer powder cameras (dia 114.6 mm) or by the Kratky technique. At first glance one might imagine that the basal spacings are very large. But it is improbable that the dried samples have high spacings because there is not enough organic material to maintain the layers in large separation. One has to assume that flocculation of the colloid solution in which the silicate layers are surrounded by proteins yields less ordered structures with protein coils of different sizes between not parallel layers (Figure 6). (This process may be considered as interfacial coagulation of proteins according to MacRitchie, 1972.) By special experimental conditions the collapse of the layers during flocculation can be controlled in such a way that ordered structures are formed (McLaren *et al.*, 1958; Armstrong and Chesters, 1964). For comparison with natural samples the disordered protein complexes are more appropriate.

*Alkylammonium derivatives of montmorillonite-protein complexes*

There are characteristic differences between the alkylammonium derivatives of the montmorillonite-



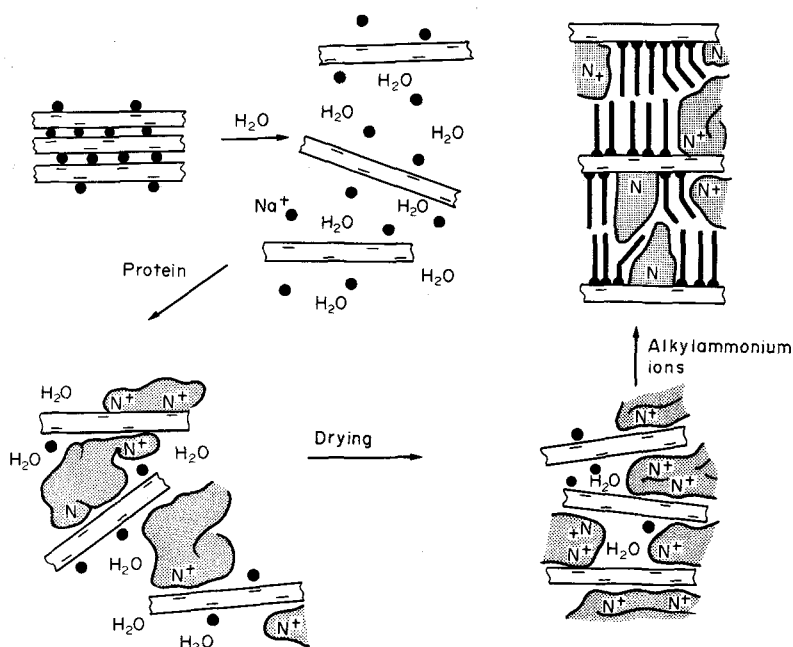


Figure 6. Protein adsorption by sodium montmorillonite leading to less ordered complexes and interaction with alkylammonium ions.

protein complexes and those of the pure montmorillonite (Figure 7). The spacings increase above 13.6 Å at  $n_c > 8$  (protein-complexes) and  $n_c > 11$  (pure montmorillonite). The second plateau lies at increased spacings (18.1 Å). The third plateau at 22 Å is not observed with pure montmorillonite. Without experience on these complexes one would be surprised by the sharp transitions between the plateaus ( $\Delta n_c = 1$ ). Among more than 200 smectites (Lagaly and Weiss, 1976) we found only 3 with similar sharp transitions. One could calculate a cation density of 0.42 eq./ $(\text{Si, Al})_4\text{O}_{10}$  from the transition between the 13.6 Å-plateau and the 18.1 Å-plateau at  $n_c = 8$  and of 0.58 eq./ $(\text{Si, Al})_4\text{O}_{10}$  from the transition between the 18.1 Å-plateau and 22 Å-plateau at  $n_c = 13$ . (The pure montmorillonite has a cation density of 0.25–0.33 eq./ $(\text{Si, Al})_4\text{O}_{10}$  calculated from the transition range from  $n_c = 11$  to  $n_c = 16$ ).

The peculiarity of the protein complexes becomes further evident in swelling experiments. Alkylammonium montmorillonites react with *n*-alkanols to give alkanol complexes with basal spacings linearly increasing from about 41 Å for  $n_c = 11$  to about 58 Å for  $n_c = 18$  (chain length of alkylammonium ion and alkanol the same). The protein complexes behave differently (Figure 8). Up to  $n_c = 13$  the basal spacings remain unchanged. For  $n_c > 13$  the series of (001)-reflexions become nonintegral; only  $d_{001}$  is near the spacings of the complexes of pure montmorillonite. Often an additional (001)-series is observed with spacings of about 22 Å, i.e. spacings of the unreacted alkylammonium clays. With prolonged reaction times (from several days to weeks) the intensity of 22 Å-series decreases. Differences are observed under alkylammonium chloride solutions (pH = 5.5), too (Figure

8). Sodium montmorillonite protein complexes give similar results.

#### Interaction of alkylammonium ions with montmorillonite-protein complexes

**Calcium montmorillonite.** The increased layer separation of calcium montmorillonite during the reaction with alkylammonium ions enables larger parts of the protein molecules (adhered at the external surfaces (Figure 5(b)) to penetrate between the layers. In the alkylammonium chloride solution the layer separation is about 30–40 Å as a consequence of formation of alkyl chain bilayers and, therefore, in the

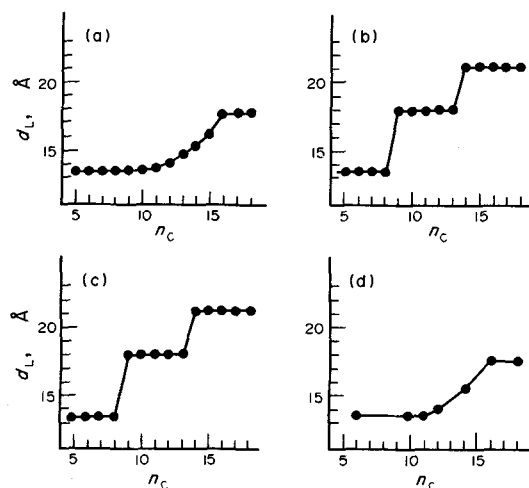


Figure 7. Basal spacings of alkylammonium derivatives from calcium montmorillonite (a), calcium montmorillonite albumen complexes (b), calcium montmorillonite lysozyme complexes (c) and calcium montmorillonite salmine complexes (d).

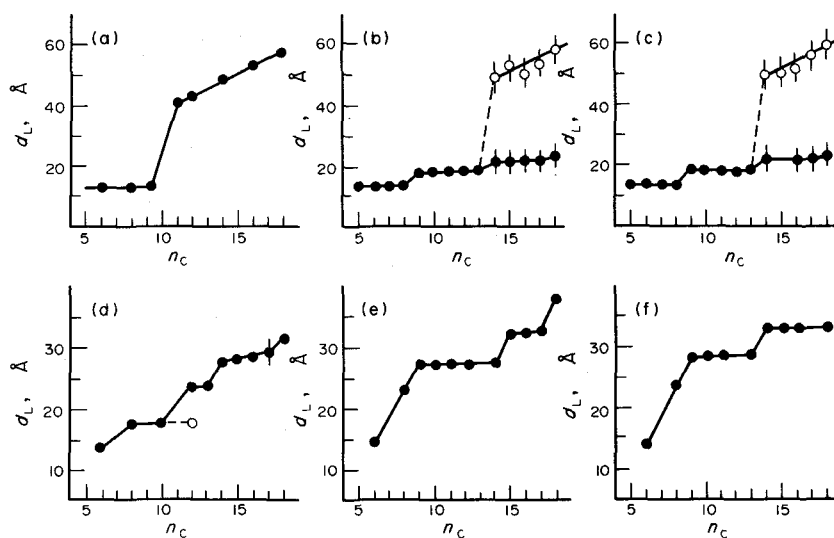


Figure 8. Swelling behaviour of alkylammonium derivatives of calcium montmorillonite protein complexes. (a) Basal spacings of the alkanol complexes of pure montmorillonite (integral (001)-reflexions). (b) Basal spacings of the alkanol complexes of albumen montmorillonite (two (001)-series for  $n_c > 13$ ). (c) Basal spacings of the alkanol complexes of lysozyme montmorillonite (two (001)-series for  $n_c > 13$ ). (d) Basal spacing under 0.1 N alkylammonium chloride solution, pure montmorillonite. (e) Basal spacings under 0.1 N alkylammonium chloride solution, albumen montmorillonite. (f) Basal spacings under 0.1 N alkylammonium chloride solution, lysozyme montmorillonite.

order of the smallest diameter of albumen (about 30–40 Å) and lysozyme (about 30 Å, Fasold, 1972; cf. Armstrong and Chesters, 1964). Since in alkylammonium chloride solution the spacing of the protein complexes and of pure montmorillonite are nearly the same, the spacing of the protein complexes is determined by ordered domains of alkyl chain bilayers (Figure 5(c)).

The enhanced adsorption of proteins at the interlayer spaces becomes evident if the alkylammonium complexes are washed and dried: the basal spacings are increased in comparison to pure alkylammonium montmorillonite. They appear to be higher charged montmorillonites but are really produced by occupation of interlayer sites by protein molecules (Figure 5, (d), (e)). From Figure 7 one estimates about 25% of the internal area is occupied by protein. Since the spacings lie between 17 and 25 Å, protein binding appears to be restricted to the peripheral regions of the interlayer spaces. Probably basic groups of proteins replace alkylammonium ions. Since the area/basic group is certainly larger than the area of an alkylammonium ion, the spacings are enhanced in comparison to pure alkylammonium montmorillonite.

Salmine complexes give spacings identical with those of pure montmorillonite (Figure 7(d)). It seems that salmine molecules do penetrate between the layers of calcium clay (Figure 5(a)) but on the other hand are easily displaced by the alkylammonium ions.

*Sodium montmorillonite.* Unlike the random distribution of the silicate layers of sodium montmorillonite in the protein matrix (Figure 6), the alkylammonium derivatives have relatively sharp (001)-reflexions with basal spacings similar to those of the alkyl-

ammonium derivatives of calcium montmorillonite-protein complexes. The X-ray diagrams show enhanced background and an intensive small angle scattering. The alkylammonium ions are capable of reorganizing the system to ordered structures: formation of the alkylammonium bilayers forces parallel orientation of the silicate layers with mostly regular separations (Figure 6) and more or less strong (001)-reflexions. Organizing to regular structures does not displace completely the protein molecules from the silicate surface. The basal spacings make evident that larger parts of the protein molecules remain bound in interlayer regions.

The enhanced small angle scattering indicates that the reorganization by alkylammonium ions does not comprise all of the silicate layers; some of the layers remain in the partial crystalline state. Since the intensity of the (001)-reflexions is nearly as great as in case of calcium montmorillonite, the amorphous part may not exceed 10–20%.

Organization of macromolecular systems by alkylammonium ions not only has been observed with proteins but also with amorphous high molecular phosphates, polyglucosides (Weiss and Michel, 1958; 1960; 1962),  $\text{SiO}_2$ -gels and in a very special case, with a mica-like silicate from lungs of silicosis patients (Weiss, 1963).

## DISCUSSION

For soil investigations the results from calcium montmorillonite are of most interest because pure sodium clays are abundant only in very special soils. The investigation reported concerns calcium montmorillonites with large amounts of proteins adsorbed

which collapse to about 10 Å by drying *in vacuo*. The protein is strongly adsorbed at the external surfaces and probably anchored between the silicate layers at the periphery of the interlayer spaces (Figure 5(b)). It can be detected by reaction with alkylammonium ions.

We described the behavior of sodium montmorillonite as a model for complexes with large organic molecules and diffuse (001)-reflexions for the following reasons: it cannot be excluded that in soils even calcium smectites are transformed into protein complexes with mostly diffuse (001)-reflexions, because a wide variety of proteins occur in soils. Due to the long time of alteration processes in soils, even very slowly penetrating macromolecules can expand the lattice to less regular structures. In addition several organic compounds in soils are able to displace the calcium cations by cation exchange reactions producing less ordered organic derivatives. Examples are amino derivatives of carbohydrates such as chitosamine (glucosamine, decomposition product of chitine) and chondrosamine (galactosamine). Chitosammonium smectites take up large amounts of water, the (001)-reflexions become diffuse and remain diffuse in the not completely dried state. Alkylammonium ions displace the chitosammonium ions from the interlayer space.

Among other macromolecules in soils, polyuronides and humic acids and their different precursors such as fulvic acids may be mentioned. Due to their negative charge they may be not as strongly adsorbed on clays as protein-like macromolecules. Several mechanisms were proposed in order to explain the adsorption of humic and fulvic acids (Schnitzer and Khan, 1972): anion exchange reactions by substitution of OH-groups on external surfaces of clays by OH-groups of the macromolecules (Greenland, 1971); hydrogen bonds between hydration water of exchangeable metal cations and macromolecules (Mortland, 1970; Theng and Sharpenseel, 1975; Theng, 1976); bridging by polyvalent cations (Edwards and Bremmer, 1967); competition of macromolecules and water for ligand positions around exchangeable cations (Schnitzer and Kodama, 1972). Bridging between clay and negatively charged macromolecules by diamino- or diammonium compounds represents a further possibility. (Air dried sodium montmorillonite shows in presence of sodium huminate and dodecyldiammonium ions no (001)-reflexions, intensive drying gives products with low spacings (13–14 Å.) Swelling and exchange of longer chain alkylammonium ions are prevented.) Large amounts of humic material can also be bound on clay surfaces by SiO<sub>2</sub>-gels which make stable amorphous coatings on clay particles (Jackson, 1969; McKyes *et al.*, 1974) and strongly adsorb fulvic acids.

During our investigation of large collections of smectites (Lagaly and Weiss, 1976) we found several samples with low amounts of organic material which gave usual basal spacings with small alkylammonium

ions. With the longest alkylammonium ions the (001)-reflexions disappeared and enhanced diffuse scattering was observed. Evidently treatment with sufficiently long alkylammonium ions opens the interlayer spaces to the extent that externally adsorbed macromolecules penetrate between the layers under formation of less ordered structures. The reaction with alkylammonium ions has a contrary effect as described above. The alkylammonium ions do not reorganize an "amorphous" system to an ordered structure but enable the macromolecules to penetrate between the layers making the layer separation very irregular. A strong interaction of the macromolecules with the silicate layers overcomes the "ordering power" of the alkylammonium ions.

In conclusion, the similarities of the results from the soil clays and from the prepared clay-protein complexes are at least consistent with the assumption that the natural clays are complexes with large organic molecules which might well be protein-like although this cannot be specially proved.

#### REFERENCES

- Armstrong, D. E. and Chesters, G. (1964) Properties of protein-bentonite complexes as influenced by equilibrium condition: *J. Soil Sci.* **98**, 39.
- Edwards, C. A. and Bremmer, J. M. (1967) Microaggregates in soils: *J. Soil Sci.* **18**, 64.
- Ensminger, L. E. and Gieseking, J. E. (1941) The adsorption of proteins by montmorillonite clays and its effect on base-exchange capacity: *J. Soil Sci.* **51**, 125–132.
- Fasold, H. (1972) *Struktur von Proteinen*: Verlag Chemie, Weinheim.
- Gonzalez Garcia, F. y Perez Rodriguez, J. L. (1970) Constitución y propiedades fisicoquímicas de las arcillas de suelos del valle de Guadalquivir: *Anales Edaf. y Agrobiol.* **XXIX**, 791–820.
- Greenland, D. J. (1971) Interactions between humic and fulvic acids: *J. Soil Sci.* **111**, 34.
- Jackson, M. L. (1969) *Soil Chemical Analysis*: Prentice-Hall, Englewood Cliffs.
- Lagaly, G. and Weiss, Armin (1970) *Inhomogeneous charge distribution in mica-type layer silicates*: Reunion Hispano-Belga de Minerales de la Arcilla, Madrid, 1970, 179–187.
- Lagaly, G. and Weiss, Armin (1971) Anordnung und Orientierung kationischer Tenside auf Silicatoberflächen, Teil IV: Anordnung von n-Alkylammoniumionen bei niedrig geladenen Schichtsilicaten: *Kolloid Z. Z. Polymere* **243** (1971), 48–55.
- Lagaly, G. and Weiss, Armin (1976) The layer charge of smectitic layer silicates: *Proc. Internat. Clay Conf. Mexico, 1975*, Applied Publ. Ltd., Wilmette, Illinois, USA, 1976, 157–172.
- Lynch, D. L. and Cotnoir, L. J. (1956) The influence of clay minerals on the breakdown of certain organic substrates: *Soil Sci. Soc. Am. Proc.* **20**, 367–370.
- MacRitchie, F. (1972) The adsorption of proteins at the solid/liquid interface. *J. Colloid Interface Sci.* **38**, 484–488.
- McLaren, A. D., Peterson, G. H. and Barshad, I. (1958) The adsorption reactions of enzymes and proteins on clay minerals: *Soil Sci. Soc. Am. Proc.* **22**, 239–244.
- McKyes, E., Sethi, A. and Yong, R. N. (1974) Amorphous coatings on particles of sensitive clay soils: *Clays and Clay Minerals* **22**, 427–33.
- Mortland, M. M. (1970) Clay organic complexes and interactions: *Adv. Agron.* **22**, 75.



- Mortland, M. M. and J. E. Gieseking (1952) The influence of clay minerals on the enzymatic hydrolysis of organic phosphorus compounds. *Soil Sci. Soc. Am. Proc.* **16**, 10–13.
- Pink, L. A. (1962) Adsorption of proteins, enzymes and antibiotics by montmorillonites: *Proc. 9th Nat. Clay Conf.*, 520–529.
- Schnitzer, M. and Kodama, H. (1972) Reactions between fulvic acids and  $\text{Cu}^{2+}$ -montmorillonite: *Clays and Clay Minerals* **20**, 359–367.
- Schnitzer, M. and Khan, S. U. (1972) *Humic Substances in the Environment*. M. Dekker, Inc., New York.
- Talibudeen, O. (1954) Complex formation between montmorillonoid clays and amino acids and proteins: *Trans. Faraday Soc.* **51**, 582–590.
- Theng, B. K. G. (1976) Interactions between montmorillonite and fulvic acid: *Geoderma* **15**, 243–251.
- Theng, B. K. G. and Scharpenseel, H. W. (1975) The adsorption of  $^{14}\text{C}$ -labelled humic acid by montmorillonite. *Proc. Internat. Clay Conf.*, Mexico City, 1975; Applied Publ. Ltd., Wilmette, Illinois, USA, 1976, p. 643–653.
- Weiss, Armin und Michel, E. (1958) Über Kationenaustausch und innerkristallines Quellungsvermögen bei kettenförmigen Polyphosphaten: *Z. Anorg. Allgem. Chemie* **296**, 313–332.
- Weiss, Armin und Michel, E. (1962) Über die zweidimensionale, geordnete Quellung von Salzen der Pektinsäure: *Z. Naturforsch.*, **17b**, 133–154.
- Weiss, Armin und Michel, E. (1960) Zur Kenntnis der innerkristallinen Quellung von Poly-D-mannuronsäure (Alginsäure): *Z. Naturforsch.*, **15 b**, 807–89.
- Weiss, Armin (1963) Isolierung und Konstitutionsermittlung des quellungsfähigen Phosphatsilicates aus Lungen Silikosekranker: *Beitr. Silikose Forsch.* **5**, 93–111.