D-GLUCOSAMINE SORPTION ON Cu(II)-MONTMORILLONITE AS THE PROTONATED AND NEUTRAL SPECIES

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Abstract—The adsorption of D-glucosamine (2-amino-2-deoxy-D-glucose) in the protonated or neutral form on homoionic Cu^{2+}-bentonite was investigated by infrared (IR) and electron spin resonance (ESR) spectroscopy and chemical analyses of solution. The results show that the interaction of the glucosamine hydrochloride with the clay complexed a small fraction of interlayer metal ions with neutral amino sugar molecules. In contrast, the adsorption of free glucosamine was much more favorable and resulted in interlayer copper(II) complexes, mainly those in which the ligand was coordinated through amino and deprotonated hydroxyl groups. Part of the amino sugar molecules, however, were transformed into glucosammonium, replacing copper as the saturating ion.

Key Words—Adsorption, Copper, D-glucosamine, Electron spin resonance, Infrared spectroscopy, Montmorillonite, Protonation.

INTRODUCTION

The amino sugars glucosamine and galactosamine are important constituents of the soil nitrogen pool (Parsons, 1981; Stevenson, 1982). An accumulation of these compounds has been observed in the smallest particle-size fraction of soil (Benzing-Purdie, 1984), and strong acid hydrolysis has been required for their release, indicating a close physical association of these compounds with clays (Belluomini *et al.*, 1986). A study aimed at understanding the adsorption of glucosamine hydrochloride on Ca- and Na-montmorillonite has indicated that the charged sugar was adsorbed by a cation-exchange mechanism (Parfitt, 1972). On the other hand, amino sugars are known to be very effective ligands for metal chelation (Micera *et al.*, 1985; Lerivrey *et al.*, 1986). To obtain greater insight into the interactions which take place between amino sugars and clays in soil, this study of the glucosamine-Cu^{2+}-montmorillonite system was undertaken. The adsorption of this amino sugar, in either the charged or neutral form, from aqueous solutions was examined by chemical analyses and spectroscopy.

EXPERIMENTAL

Materials

The <2-μm size fraction of bentonite from Ponza, Italy, was obtained by sedimentation. X-ray powder diffraction (XRD) analysis showed the clay to be predominantly montmorillonite, with traces of illite and quartz. The structural formula, obtained by dissolution of a sample with HF in a Perkin-Elmer digestion bomb and chemical analysis using a Beckman Spectraspan IV spectrometer, is as follows:

$$R^+_{0.737}(Si_{7.856}Al_{0.144})(Al_{3.133}Fe^{3+}_{0.254}Mg_{0.593})(OH)_4O_{20}.$$
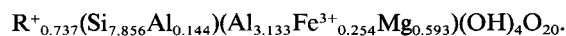
The surface area, determined by the ethylene glycol monoethyl ether (EGME) method (Carter *et al.*, 1965), is 643 m^2/g. The Cu^{2+}-exchanged samples were prepared by the usual procedure: the clay was immersed into 1 N solutions of the metal chloride for 24 hr, centrifuged, and then washed with deionized water until a test for chloride in the supernatant solution was negative. The clay was then dried at room temperature. The cation-exchange capacity (CEC) was determined by ammonium and calcium exchange (Chapman, 1965). Both methods gave a value of 74.6 meq/100 g clay.

Chemicals

D-glucosamine hydrochloride was obtained from the Sigma Company. The neutral form of the amino sugar was prepared according to the procedure reported by Appelman-Lippens *et al.* (1985).

Adsorption experiments

The adsorption of both charged or uncharged glucosamine on the homoionic clay was carried out in triplicate by equilibrating 50 mg of Cu-montmorillonite in 20 ml of aqueous solution of amino sugar over the concentration ranges listed in Tables 1 and 2. Samples were shaken at room temperature for 24 hr, as no additional adsorption took place after longer equilibration times. After equilibrating (pH values are given in Tables 1 and 2), the suspensions were centrifuged and the supernatants analyzed by the method of Schloss (1951) to determine the total glucosamine concentration. The amount of adsorbed amino sugar was calculated from the difference in concentration before and

Table 1. Adsorption of glucosamine hydrochloride on copper montmorillonite.

pH[1]	Added sugar (mmole/100 g)	Adsorbed sugar (mmole/100 g)	Released Cu (mmole/100 g)
4.72	30	6	2.58
4.62	50	8	3.78
4.61	70	10	4.54
4.54	100	10	5.42
4.43	130	14	6.92
4.43	176	16	8.32
4.40	198	18	9.12
4.38	220	20	9.92

[1] Equilibrium reading.

Table 2. Adsorption of free glucosamine on copper montmorillonite.

pH[1]	Added sugar (mmole/100 g)	Adsorbed sugar (mmole/100 g)	Released Cu (mmole/100 g)
8.02	30	30	5.99
8.05	50	30	6.11
8.07	70	40	7.73
8.11	100	64	9.21
8.19	130	74	12.48
8.39	176	90	18.71

[1] Equilibrium reading.

after equilibration. The total amount of Cu^{2+} ions (in either the free or complexed form) released from the clay during adsorption was determined by the colorimetric neocuproin method (Nebesar, 1964). Samples suitable for IR (self-supporting films) or electron spin resonance (ESR) (powders) analysis were obtained by contacting 50 mg of clay with an aqueous solution containing glucosamine or glucosamine hydrochloride with stirring for 24 hr. After separating the solution, the clay fraction was washed three times with distilled water, centrifuged, resuspended into distilled water, and, finally, air-dried. The samples for XRD analysis were prepared similarly on glass slides.

Physical measurements

Infrared (IR) spectra were recorded with a Perkin-Elmer 683 spectrophotometer. Electron spin resonance (ESR) spectra (X-band frequency) were recorded at 298 K using a Varian E 9 spectrometer. The pH of solutions was measured by a Radiometer PHM 84 pH-meter. XRD data were obtained on a Philips PW 1730 instrument equipped with an Olivetti M-20 computer, using CoKα radiation.

RESULTS

The analytical data for the adsorption from solutions of glucosamine hydrochloride are summarized in Table 1. The amount of copper(II) ions released into solution was found to be proportional to the amino sugar sorption. In fact, whatever the given equilibrium concentration, the molar ratio between adsorbed glucosamine and desorbed copper was always near 2, indicating that two amino sugar molecules replaced a copper ion in the interlayer. Because of the rather low adsorption levels, IR bands of adsorbed glucosamine were not detected. On the other hand, the ESR spectra (Figure 1, spectrum b) showed, besides the presence of hydrated copper(II), complexes with the adsorbed amino sugar, although the latter were never predominant species. In particular, the ESR parameters of the complexes ($g_{\parallel}$ = 2.30, $A_{\parallel}$ = 170 × 10^{-4} cm^{-1}) were very similar to those measured in aqueous solution for the complex in which two neutral ligand molecules coor-

dinate to the metal ion through the amino groups (CuL_2, see Micera *et al.*, 1985). Consistent with the low extent observed for the adsorption, the Cu-montmorillonite exhibited, after interaction with a solution containing an amount of glucosamine hydrochloride equal to the CEC, only a minor increase of the d(001) value to 13.6 Å, compared with 12.6 Å for the untreated Cu-clay.

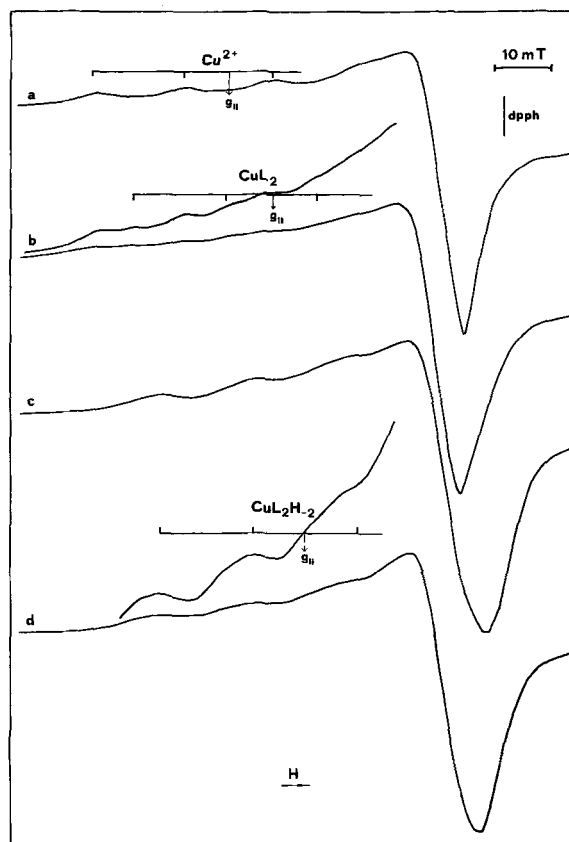Different behavior was observed when the neutral

Figure 1. X-band electron spin resonance spectra recorded at room temperature on Cu-bentonite after heating at 120°C (a), Cu-bentonite treated with an amount of glucosamine hydrochloride equal to the cation-exchange capacity (CEC) (b), and Cu-bentonite, treated with an amount of glucosamine equal to CEC, before (c) and after washing (d). Spectrum b was recorded on a Cu/Ca-bentonite exchanged with Cu^{2+} and Ca^{2+} in a 1:10 molar ratio.
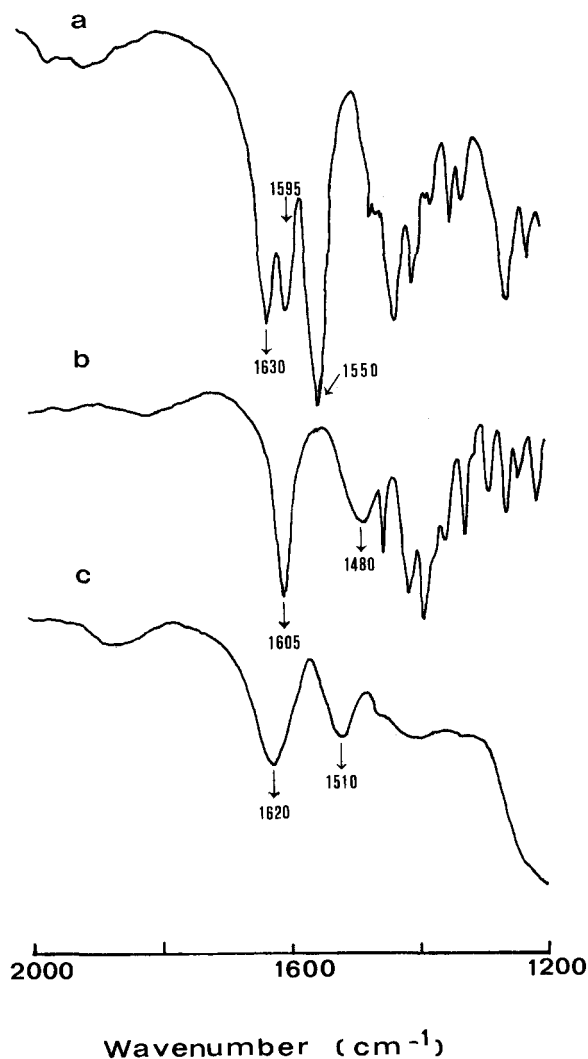
Figure 2. Infrared spectra of: (a) glucosamine hydrochloride and (b) glucosamine (KBr pellets); (c) Cu-bentonite after adsorption from a solution containing an amount of glucosamine equal to the cation-exchange capacity (self-supporting film).

glucosamine was equilibrated with the Cu-clay (Table 2). Here, more glucosamine was adsorbed and much more copper(II) was released, although the molar ratio between adsorbed sugar and released copper was always > 2. IR spectra (Figure 2) showed two strong bands at 1510 and 1620 cm^{-1}, which were absent from the spectra of either glucosamine or glucosammonium hydrochloride, but which are typical for glucosammonium ion bound to montmorillonite (Parfitt, 1972). Accordingly, XRD showed that the clay, after being exposed to a solution containing an amount of glucosamine equal to the CEC, had a d(001) value of 15.0 Å, an increase of 2.4 Å compared with that of untreated sample.

The ESR spectra of the Cu-clay contacted with glu-

cosamine (Figure 1, spectrum c) substantiated that, before washing, most of the copper ions were in a complexed form ($g_\| = 2.24$ and $A_\| = 190 \times 10^{-4}$ cm^{-1}). These complex species were probably identical to those reported in aqueous solution involving the coordination of two anionic ligands, forming a chelate with copper(II) through the amino and the deprotonated C-1 hydroxyl groups (CuL$_2$H$_{-2}$, see Micera *et al.*, 1985). By washing the sample with distilled water, part of the copper was removed, and signals of the CuL$_2$ complexes appeared due to the release of CuL$_2$H$_{-2}$ complex into solution and/or to the decrease of pH.

DISCUSSION AND CONCLUSIONS

Glucosammonium ion is a weak acid, with pK$_a$ = 7.70. Thus, only the protonated form exists in solution at pHs as low as 4.4–4.8. The well-defined stoichiometry of the exchange process in the low pH range clearly suggests that the metal displacement was largely a consequence of the adsorption of the protonated amino sugar. Analogously, Parfitt (1972) observed that glucosammonium replaced sodium and, to a lesser degree, calcium. The situation is more complicated in the present study, because part of the adsorbed amino sugar took part in metal complexation in the form of neutral glucosamine. The occurrence of a complexation process implies that the proton released by each coordinating molecule became an exchange ion, unless it was replaced by a cation from solution. Because of the monovalent charge of the glucosammonium ion, however, small amounts of the amino sugar were adsorbed via the cation-exchange mechanism, and the extent of metal complexation was limited by the low pH. Under similar conditions Parfitt (1972) observed that about 85% of the sodium was replaced by glucosammonium.

The slight tendency of Cu(II) to be exchanged by glucosammonium ion was demonstrated by the fact that remarkably larger amounts of the amino sugar were adsorbed from basic solutions. Probably, neutral molecules were adsorbed but underwent protonation to yield glucosammonium ions.

The protons involved in the transformation of neutral sugar molecules into cations were donated by water (most likely water bound to the interlayer copper ions) or by other sugar molecules which took part in complex formation. In the absence of complexation the extent of adsorbed and protonated sugar should have been limited to an amount equivalent to the CEC, whereas the copper ions should have been hydrolyzed to the hydroxide, thus behaving as two-proton donors. In the second case, a larger amount of amino sugar adsorption was to be expected. In fact, at pH >7 the ligand formed complexes with copper by coordinating through the amino group and the adjacent deprotonated C-1 hydroxyl. The release of acidity from this metal-ion-assisted dissociation may have been responsible for the protonation of other glucosamine molecules and the

formation of glucosammonium as the saturating ion. In the limiting case, four molecules of glucosamine (two complexing and two protonated) could have been adsorbed for each interlayer copper ion. In this regard, stoichiometric adsorption of neutral base molecules to give charged copper(II) complexes in the clay interlayer have been observed, e.g., with ethylamine (Farmer and Mortland, 1965). In the present study, however, the situation was more complicated because the copper-glucosamine CuL_2H_{-2} complex being neutral probably diffused into solution.

The experimental data show that copper was released in the basic media and that the molar ratios between adsorbed glucosamine and desorbed metal ion >2. On the other hand, the ESR spectra indicate that the CuL_2H_{-2} species formed on the clay. Thus, the proposed complexation process was, without doubt, working in our system. This does not rule out that part of copper (i.e., that not involved in complex formation) formed ESR-silent uncomplexed polymeric species, e.g., $Cu(OH)_2$, in the high pH systems.

In conclusion, despite the complexity of the processes occurring in the clays and the incomplete knowledge of the complexation phenomena occurring therein, the present results indicate that amino sugars may be involved in complex formation with interlayer metal ions. Similar behavior is expected for other divalent transition metal ions and analogs of glucosamine (Radomska *et al.,* 1988). Thus, amino sugar compounds may take part in processes which govern the mobility of microelements in soil.

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