Clays and Clay Minerals, Vol. 43, No. 6, 647–655, 1995.

ADSORPTION OF TYROSINASE ONTO MONTMORILLONITE AS INFLUENCED BY HYDROXYALUMINUM COATINGS

A. NAIDJA,[1] A. VIOLANTE,[2] AND P. M. HUANG[1]

[1] Department of Soil Science, University of Saskatchewan
51 Campus Drive, Saskatoon, Saskatchewan S7N 5A8 Canada

[2] Dipartimento di Scienze Chimico-Agrarie, Università di Napoli
"Federico II," Via Università 100, 80055 Portici, Napoli, Italy

Abstract—In soil environments, the surfaces of clay minerals are often coated with hydrolytic products of Al. However, limited information is available on the effect of hydroxyaluminum coatings on the interlayering of enzymes for montmorillonite. The objective of this study was to compare the adsorption of tyrosinase onto montmorillonite as influenced by levels of hydroxyaluminum coatings. Tyrosinase is one of the strongest catalysts in the transformation of phenolic compounds. Adsorption of tyrosinase onto Ca-montmorillonite (Ca-Mte) and different hydroxyaluminum-montmorillonite complexes ($Al(OH)_x$-Mte), containing 1.0, 2.5 and 5.0 mmol coated Al/g clay, was studied both in the absence and in the presence of a phosphate buffer at pH 6.5 and 25°C. Except for Ca-Mte in the absence of phosphate where the adsorption isotherm was of C type (linear), the adsorption isotherms were of L type (Langmuir). More tyrosinase molecules were adsorbed onto Ca-Mte than onto the $Al(OH)_x$-Mte complexes, both in the absence and in the presence of phosphate. This indicated the easy accessibility of the enzyme to the uncoated Ca-Mte surfaces. The presence of phosphate did not significantly affect the amount of tyrosinase adsorbed onto Ca-Mte, but substantially reduced the adsorption of tyrosinase onto $Al(OH)_x$-Mte complexes. The higher the level of hydroxyaluminum coatings, the lower the amount of tyrosinase was adsorbed. Because of their affinity to the aluminous surfaces, phosphate ions evidently competed strongly with tyrosinase for $Al(OH)_x$-Mte complexes adsorption sites. The intercalation of tyrosinase by Ca-Mte was indicated by the increased d-spacing of the complex as the amount of the enzyme adsorbed increased. The infrared spectra of tyrosinase-Ca-Mte complex showed that the amide II band of tyrosinase at 1540 cm^{-1} was practically unaffected by adsorption. The amide I band at 1654 cm^{-1} was shifted toward a higher frequency, indicating a slight perturbation in the protein conformation. This perturbation became more noticeable in the presence of $Al(OH)_x$-Mte complexes. The data indicated that hydroxyaluminum coatings play an important role in retarding the adsorption of tyrosinase by montmorillonite, and phosphate effectively competes with tyrosinase for the adsorption sites on $Al(OH)_x$-Mte complexes.

Key Words—Adsorption, Clay minerals, Enzymes, Hydroxyaluminum-montmorillonite complex, Interlayering, Tyrosinase.

INTRODUCTION

In soil environments, extracellular enzymes contribute to a portion of the biological activity and some enzymes are held onto clays and humic substances (Burns 1990). Numerous studies have been carried out on the adsorption of proteins including enzymes onto phyllosilicates (Talibudeen 1955; McLaren *et al* 1958; Morgan and Corke 1976; Garwood *et al* 1983). Within acidic soil environments, clays are usually coated by iron or aluminum hydroxides (Barnhisel and Bertsch 1989). Limited information is available on the interaction of enzymes with iron and/or aluminum coated clay minerals (Fusi *et al* 1989; Gianfreda *et al* 1991, 1992). Tyrosinase (EC 1. 14. 18. 1), a copper-containing polyphenol oxidizing enzyme, is present in litter and soil (Kiss *et al* 1975; Burns 1978) and it has an important role in the polymerization of phenolic compounds and the transformations of humic substances (Sjoblad and Bollag 1981). This enzyme is widely distributed in plants (Janovitz-Klapp *et al* 1990) and responsible for the undesirable melanosis, a blackening of the agricultural products (Chen *et al* 1991). However, the adsorption mechanism of tyrosinase onto both the "clean" and the coated montmorillonite still remains to be uncovered. Such information is essential to elucidate the activity of clay-immobilized enzymes, which is still obscure (Skujins 1978; Boyd and Mortland 1990). Therefore, the objective of the present study was: 1) to investigate the influence of the different levels of hydroxyaluminum coatings onto montmorillonite on the adsorption of tyrosinase both in the absence and the presence of a phosphate buffer, 2) to examine whether or not tyrosinase is intercalated into Ca-montmorillonite and $Al(OH)_x$-montmorillonite complexes, and 3) the effect of tyrosinase adsorption by both the "clean" and the coated clay on the protein conformation.

MATERIALS AND METHODS

Tyrosinase

Tyrosinase (EC 1. 14. 18. 1) from mushroom was obtained from Sigma Chemical, USA. The enzyme was stored desiccated in the refrigerator at −5°C until use.

Table 1. Influence of the level of hydroxyaluminum coatings on certain properties of Ca-montmorillonite.

Adsorbent[1]	CEC (cmol kg^{-1})	d_{001} (25°C) (Å)	d_{001} (110°C) (Å)	d_{001} (500°C) (Å)	S_{EGME} (m^2 g^{-1})	S_{BET} (m^2 g^{-1})	[2]D (Å)	[3]10^3 V (cm^3 g^{-1})
Ca-Mte	103	14.9	13.0	9.6	707	23.1	146	84
$Al(OH)_x$-Mte_1	76	15.2	13.3	10.9	467	24.2	343	207
$Al(OH)_x$-$Mte_{2.5}$	40	16.0	14.3	13.0	241	28.9	468	338
$Al(OH)_x$-Mte_5	18	17.4	16.1	15.3	200	37.1	489	407

[1] Ca-Mte = Ca-montmorillonite, $Al(OH)_x$-Mte = hydroxyaluminum-montmorillonite complexes; the Al coatings were obtained at the level of 1, 2.5 and 5 mmol Al/g clay.
[2] D: average pore diameter.
[3] V: Total pore volume.

Preparation of Ca-montmorillonite (Ca-Mte)

The montmorillonite sample (SWy-1 Crook County, Wyoming, USA) was obtained from the Source Clay Repository of the Clay Mineral Society.

The purification treatments and the homoionic Ca saturation of montmorillonite were previously described by Naidja and Huang (1994).

Preparation of hydroxyaluminum-montmorillonite ($Al(OH)_x$-Mte) complexes

The $Al(OH)_x$-Mte complexes containing 1.0, 2.5 and 5.0 mmol coated Al/g clay [$Al(OH)_x$-Mte_1, $Al(OH)_x$-$Mte_{2.5}$, and $Al(OH)_x$-Mte_5] were prepared by addition of an appropriate aliquot of 1.0 M $AlCl_3$ to 5.0 g of montmorillonite suspension followed by adjustment of the pH to 7.0 with dropwise addition of 0.5 M NaOH. The suspensions were stirred for 2 h, dialyzed against deionized distilled water until Cl^- free and then freeze-dried.

The cation exchange capacity (CEC) of Ca-Mte and $Al(OH)_x$-Mte complexes were determined according to the method of Alexiades and Jackson (1965).

Determination of surface area

a) The external surface area (S_{BET}) of Ca-Mte and $Al(OH)_x$-Mte complexes was determined using the BET equation (Brunauer *et al* 1938) and multipoints N_2 gas adsorption isotherms. A microprocessor controlled sorption meter type Autosorb 1 (Quantachrom Corp., New York, USA) was used. The samples were degassed for 12 to 16 h at 25°C under vacuum. Surface areas were determined at liquid N_2 temperature (−196°C) using N_2 as adsorbate and He as the carrier gas. The total volume of pores was calculated from the total volume of the adsorbate and the average pore diameter was calculated from the ratio of the total volume and the BET surface area (Lowell and Shields 1991).

b) The total surface area (S_{EGME}) of Ca-Mte and $Al(OH)_x$-Mte complexes was determined by ethylene glycol monoethyl ether (EGME) method (Eltantawy and Arnold 1973).

Adsorption isotherms

Four grams of Ca-Mte or $Al(OH)_x$-Mte complexes were suspended into 1 L boiled-deionized-distilled (BDD) water containing 10 drops of toluene and dispersed by sonication for 3 min. Two sets of clay samples were prepared at pH 6.5, in the absence and in the presence of phosphate buffer. The buffer was prepared by mixing 50 mL of 0.1 M KH_2PO_4 with 13.9 mL of 0.1 M NaOH and diluting the final volume to 100 mL with deionized distilled water (Weast 1974). In the absence of buffer, the pH was adjusted by addition of 0.1 M HCl or 0.1 M NaOH. Immediately before use, tyrosinase (2 mg/mL) was prepared in two different solutions: 1) in water adjusted pH to 6.5 with 0.1 M NaOH and 2) in phosphate buffer at the same pH. In different polysulfone flasks containing 1 mL of clay sample suspensions (2 mg clay), different amounts of enzyme (from 0.05 to 3.0 mg) were added. The suspensions were shaken at 25°C until equilibrium was established. The equilibrium was reached in 1 h. After centrifugation at 19,000 *g*, the amount of tyrosinase remaining in solution was determined by spectrophotometry at 280 nm (Fusi *et al* 1989) using Beckman DU 650 microprocessor controlled spectrophotometer.

X-ray diffraction analysis

X-ray diffractograms (XRD) was acquired from a Rigaku D/MAX-RBX diffractometer (Rigaku Company Tokyo, Japan), with CuKα radiation filtered by graphite monochromator at the settings of 50 kV and 150 mA. Oriented samples (clays or enzyme-clay complexes) were smeared onto glass slides and maintained at a relative humidity (RH) of 50% and room temperature. Those heated for 2 h at different temperatures (110–500°C) were kept dry in a desiccator containing anhydrous silica gel until examination. The basal spacings, d_{001}, at 25, 110 and 500°C are presented in Table 1.

Infrared analysis

Fourier Transform infrared (FTIR) spectra of Ca-Mte or $Al(OH)_x$-Mte complexes before and after ad-

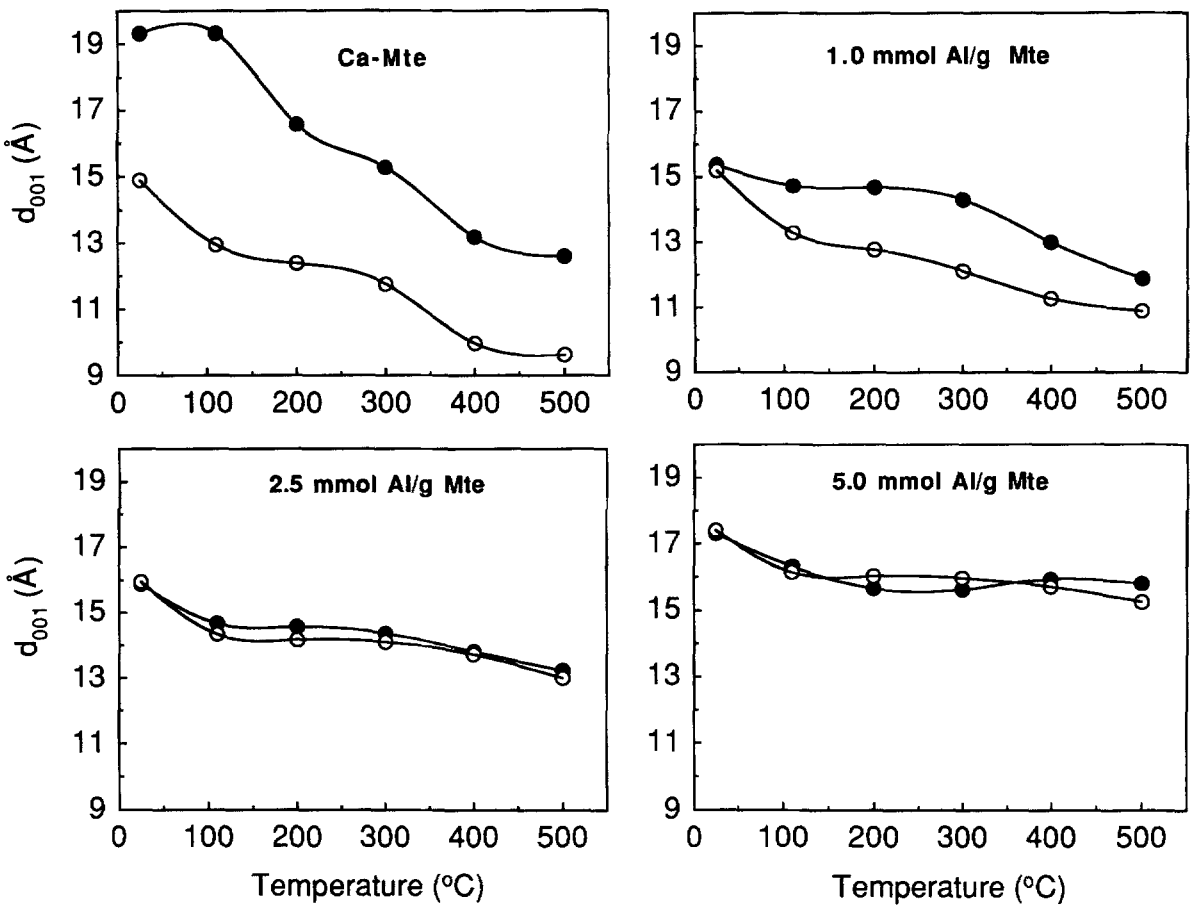


Figure 1. Effect of temperature on the d_{001} spacing of the tyrosinase-Ca-montmorillonite and tyrosinase-Al(OH)$_x$-montmorillonite complexes at the levels of Al coatings of 1.0, 2.5 and 5.0 mmol Al/g clay. ○ before adsorption and ● after adsorption of tyrosinase. Mte stands for montmorillonite.

sorption of tyrosinase were recorded from a KBr disk, which contain 1% of sample by weight, using Biorad 3240 SPS, microprocessor controlled spectrophotometer (Minnesota, USA). The spectra were referenced against the spectrum of pure KBr and expressed in units of percent absorbance. The spectrum of the Ca-Mte or Al(OH)$_x$-Mte was subtracted from that of the respective enzyme-clay complex to obtain the differential FTIR spectrum of adsorbed tyrosinase.

Differential thermal analysis (DTA)

Differential thermal analysis (DTA) data of selected samples was obtained from a Netasch thermal analyzer programmed from 25 to 900°C at a rate of 10°C/min, using alumina as the reference material.

RESULTS AND DISCUSSION

Properties of the clay minerals

Table 1 shows the effect of the level of hydroxyaluminum coatings on certain properties of Ca-montmorillonite. The d-spacing of Al(OH)$_x$-Mte complexes gradually increased with increasing levels of the coating from 1.0 to 5.0 mmol Al/g clay. This clearly indicated the intercalation of hydroxyaluminum species into the interlayers of montmorillonite. Furthermore, upon a preheating treatment up to 500°C (Table 1 and Figure 1) the d-spacing of Mte collapsed to 9.6 Å. The d-spacing of Al(OH)$_x$-Mte complexes remained higher than 9.6 Å according to the level of the coatings such as 10.9 Å for the complex formed at 1.0 mmol Al/g clay and 15.3 Å for the complex formed at 5.0 mmol Al/g clay. This indicated the presence and the stability of the intercalated aluminous material into the montmorillonite. The increase of the level of Al(OH)$_x$ coatings greatly reduced the cation exchange capacity (CEC) of the clay. Thus, the positively charged species of Al(OH)$_x$ in the interlayer spaces neutralize a large portion of the negative charge of montmorillonite. The intercalation of Al(OH)$_x$ species into the montmorillonite constitute a blocking material (Barnhisel and Bertsch 1989) preventing the retention of ethylene gly-

https://doi.org/10.1346/CCMN.1995.0430601 Published online by Cambridge University Press

col monoethyl ether (EGME) by the clay surfaces. This resulted in a substantial decrease of the specific surface area, which was measured by EGME, of the clay with increasing the amount of $Al(OH)_x$ intercalated (Table 1). Conversely, the BET surface area and the porosity (Total volume and average diameter of pores) increased with increasing the level of $Al(OH)_x$ coatings (Table 1). This indicates the easy accessibility of N_2 molecules to the interstitial cavities when a higher amount of $Al(OH)_x$ polymers was adsorbed onto the internal and the external surfaces of montmorillonite. The absence of micropores was indicated by the t-method (Lowell and Shields 1991). The total surface area of pores corresponds to the mesopores surface area.

Adsorption isotherms

Figure 2 shows the adsorption isotherms of tyrosinase onto Mte and onto $Al(OH)_x$-Mte complexes at 25°C and pH 6.5 both in the absence and in the presence of a phosphate buffer. Except for Ca-Mte in the absence of phosphate where the adsorption isotherm was of C type (linear), the adsorption isotherms were of L (Langmuir) type. According to the best fitting, using least square regression program (Table 2), the adsorption data over the range of concentrations studied conformed to two equations: 1) linear type, only in the case of Ca-Mte in the absence of buffer where the amount adsorbed was proportional to the equilibrium concentration of the adsorbate, and 2) Langmuir type:

$$X = XmKC/(1 + KC) \quad [1]$$

for the other adsorbents (Ca-Mte in the presence of a buffer and $Al(OH)_x$-Mte complexes both in the absence and in the presence of a buffer). In the above equation, X is the amount of adsorbate taken up per unit mass of adsorbent, Xm is the maximum amount of adsorbate that may be bound, C is the equilibrium concentration of adsorbate (mg/mL) and K is a constant related to the adsorption energy. Therefore, the adsorption parameters (X_m and K) were estimated (Table 2). Adsorption isotherm of tyrosinase onto Ca-Mte in the absence of phosphate was linear and corresponded to the C type as classified by Giles *et al* (1960). Thus, constant repartition of tyrosinase onto montmorillonite surfaces with increasing enzyme concentration indicated the easy accessibility of protein molecules to the external and internal clay surfaces. The presence of phosphate slightly suppressed the adsorption of tyrosinase onto montmorillonite and the isotherm fitted better as a L (Langmuir) type (Figure 2).

In the absence of phosphate, the adsorption isotherms of tyrosinase onto $Al(OH)_x$-Mte complexes corresponded to L (Langmuir) type after Giles *et al* (1960) (Figure 2) with an adsorptive capacity (X_m) ranging from 354 mg/g to 429 mg/g depending on the level of coatings (Table 2). The reason that the total surface area of $Al(OH)_x$-Mte complexes decreased and the BET surface area increased (Table 1) with increasing the level of coatings (1.0, 2.5, 5.0 mmol Al/g clay) was discussed above. No clear trend was observed for the effect of the level of coatings on the adsorptive capacity (X_m). The lack of correlation between the surface area and the sorptive capacity of Mte and $Al(OH)_x$-Mte complexes was also indicated by Gianfreda *et al* (1992) for the adsorption of urease onto Mte and $AI(OH)_x$-Mte complexes. The adsorption of enzymes by clays is far from a simple static deposition of the adsorbate molecules onto the adsorbent surfaces. Some intrinsic factors inherent to the clay surfaces include specific adsorption sites of Mte and $Al(OH)_x$-Mte complexes surface and the steric hindrance that varies with the level of $Al(OH)_x$ coatings might contribute to these interactions.

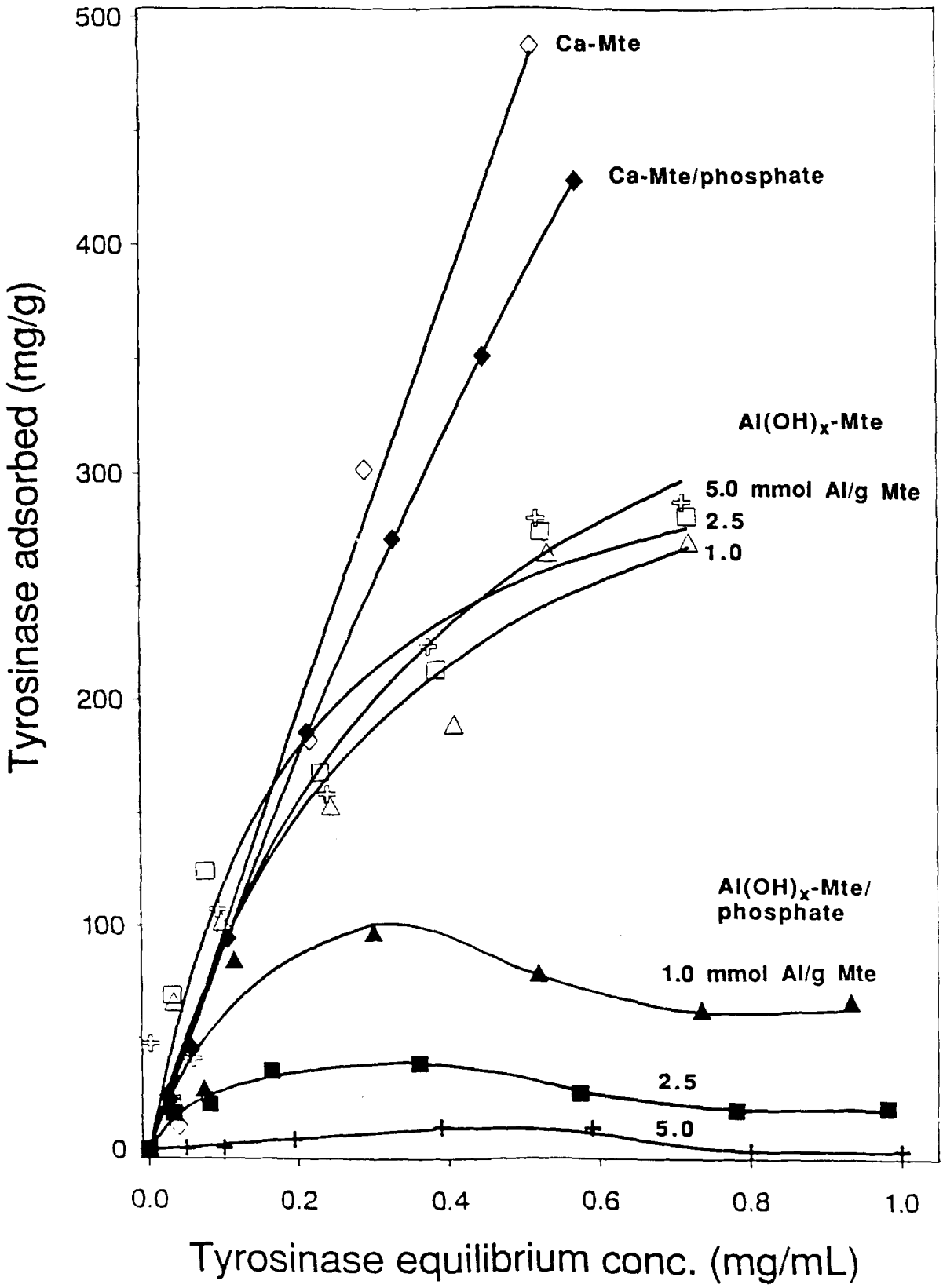
Hydroxy-Al coatings onto Mte decreased its ability to retain tyrosinase (Figure 2). The adsorption isotherm of "clean" Mte in the absence of buffer (type C) indicated a continuous filling of the Mte surfaces by the tyrosinase molecules. The adsorption isotherms of coated Mte (type L) showed a limited and low adsorption capacity for tyrosinase, resulting from the intercalation of $Al(OH)_x$ polymers and their retention at the external surfaces of Mte.

In the presence of phosphate, adsorption isotherms of adsorbed tyrosinase onto $Al(OH)_x$-Mte complexes belonged to Lmx subgroup (Langmuir type with a maximum) (Figure 2). The adsorptive capacity of $Al(OH)_x$-Mte complexes for tyrosinase strongly decreased from 151 to 15 mg/g clay (Table 2). The maximum amount of the enzyme adsorbed was obtained around an equilibrium concentration of tyrosinase of 0.4 mg/mL (Figure 2) and depended on the level of the hydroxyaluminum coatings. The higher the level of the coatings, the lower was the sorptive capacity of the coated clay. Evidently, the adsorption of $H_2PO_4^-$ and HPO_4^{2-} anions at pH 6.5, which have a high affinity toward the positively charged hydroxyaluminum surfaces and the ability to form precipitation products (Violante *et al* 1991; Ferreiro *et al* 1992), may compete with tyrosi-

→

Figure 2. Adsorption isotherms of tyrosinase on Ca-Mte and hydroxy-Al-Mte complexes ($Al(OH)_x$-Mte) at the levels of Al coatings of 1.0, 2.5 and 5.0 mmol Al/g clay, at 25°C and pH 6.5, both in the absence (open symbols) and the presence (solid symbols) of phosphate.

https://doi.org/10.1346/CCMN.1995.0430601 Published online by Cambridge University Press



https://doi.org/10.1346/CCMN.1995.0430601 Published online by Cambridge University Press

Table 2. Adsorption capacity (X_m) and the adsorption coefficient (K) of tyrosinase on Ca-montmorillonite and hydroxy-Al-montmorillonite complexes both in the absence and the presence of phosphate.

Adsorbent[1]	In the absence of phosphate			In the presence of phosphate		
	X_m (mg/g)	K (ml/mg)	r^2[2] —	X_m (mg/g)	K (ml/mg)	r^2 —
Ca-Mte	[3]—	[3]—	0.97	2080	0.5	0.99
$Al(OH)_x$-Mte_1	390	3.1	0.97	151	6.7	0.88
$Al(OH)_x$-$Mte_{2.5}$	354	5.0	0.96	48	12.9	0.98
$Al(OH)_x$-Mte_5	429	3.0	0.95	15	2.8	0.90

[1] See Table 1.
[2] Correlation coefficient of the least square regression with p values < 0.01.
[3] Not applicable, the adsorption isotherm of tyrosinase on Ca-montmorillonite in the absence of phosphate was better fitted in a linear model.

nase molecules for many adsorptive sites. This reduces the amounts of the enzyme molecules adsorbed onto the clay minerals. This finding clearly showed that the nature and the concentration of a buffer solution must be considered when adsorption of proteins onto clays is studied.

X-ray diffraction

The gradual shift to higher d-spacing values of tyrosinase-Ca-Mte complex with increasing amounts of tyrosinase adsorbed in the absence of the phosphate buffer (Figure 3) indicated that tyrosinase molecules with a molecular weight (MW) of 122,000 and an isoelectric point (pi) of 6.1 (Alikhan 1976) were intercalated into the inter-lamellar space of montmorillonite. When the pH is near the pi, which is the case in this study (pH 6.5), the net charge of the enzyme is low. Therefore, the electrostatic interactions with the mineral surface are weak (Quiquampoix 1987). Also, the cation exchange mechanism that is possible when the pH is far below the pi seems precluded in the present study. In addition to the attractive van der Waals interactions, it is likely that tyrosinase was intercalated into the montmorillonite through hydrogen bonding between carboxyl and amino groups of the polypeptide chain of the protein and the surface tetrahedral silicate sheet. No further increase in the d-spacing of the complex (19.5 Å) was observed when the amount of tyrosinase adsorbed was higher than 255 mg/g (not shown). This is evidently due to saturation of the internal surfaces. Also, the d_{001} spacings of 9.8, 6.3, 4.9, 4.0 and 3.2 Å were observed for the tyrosinase-Ca-Mte complex, which virtually corresponded to the integral higher orders (002), (003), (004), (005) and (006). Hence the expansion ($\Delta d = d_{001} - 9.6$ Å) of the aluminosilicate layers due to the intercalation of tyrosinase was 9.9 Å (19.5–9.6 Å). Although Talibudeen (1955) found a good agreement between Δd and van der Waals molecular thickness of polypeptide chain for some proteins adsorbed onto Na-Mte, adsorption and intercalation could affect the conformation of the molecules. After heating to 500°C, the d-spacing of Ca-Mte collapsed to 9.6 Å. Conversely, the d-spacing of the tyrosinase-Ca-Mte complex decreased to 12.6 Å (Δd = 3.0 Å) (Figure 1). This suggests the presence of some material in the interlayers of montmorillonite. This is substantiated by the exotherms at 360 and 465°C (Figure 4), which is attributed to the decomposition of the intercalated tyrosinase molecules. The other very broad exotherms centered at about 650 and 710°C (Figure 4) were probably due to the combustion of carbonious residues of intercalated tyrosinase molecules at the higher temperatures. A similar thermogram was obtained with albumin adsorbed onto montmorillonite (A. Violante unpublished data). The DTA of tyrosinase-$Al(OH)_x$-Mte complex (data not shown) did not exhibit exotherms probably because of the low amount of tyrosinase adsorbed onto the coated montmorillonite.

The coatings of montmorillonite surfaces by hydroxyaluminum modified the adsorption properties of montmorillonite for tyrosinase (Figure 1). At the lower level of coatings (1.0 mmol Al/g clay), the d-spacing of tyrosinase-$Al(OH)_x$-Mte complex remained higher than that of $Al(OH)_x$-Mte complex (Figure 1) even after the 500°C heat treatment. This substantiates the intercalation of tyrosinase when the montmorillonite interlayers are partially occupied by hydroxy-Al ions. When 2.5 to 5.0 mmol Al/g were present on the Mte surfaces, there was no significant change in the d-spacing of $Al(OH)_x$-Mte complexes after adsorption of tyrosinase (Figure 1). This indicated that at such high coatings of hydroxy-Al, it is difficult for tyrosinase to be intercalated. There was low intercalation because of the steric hindrance and the enzyme molecules were adsorbed at the external surfaces and the edges of the $Al(OH)_x$-Mte complexes.

At a coating level higher than 1.0 mmol Al/g clay, hydroxyaluminum ions form a stable "blocking material" occupying the interstitial spaces in the montmorillonite layers (Barnhisel and Bertsch 1989; Hsu

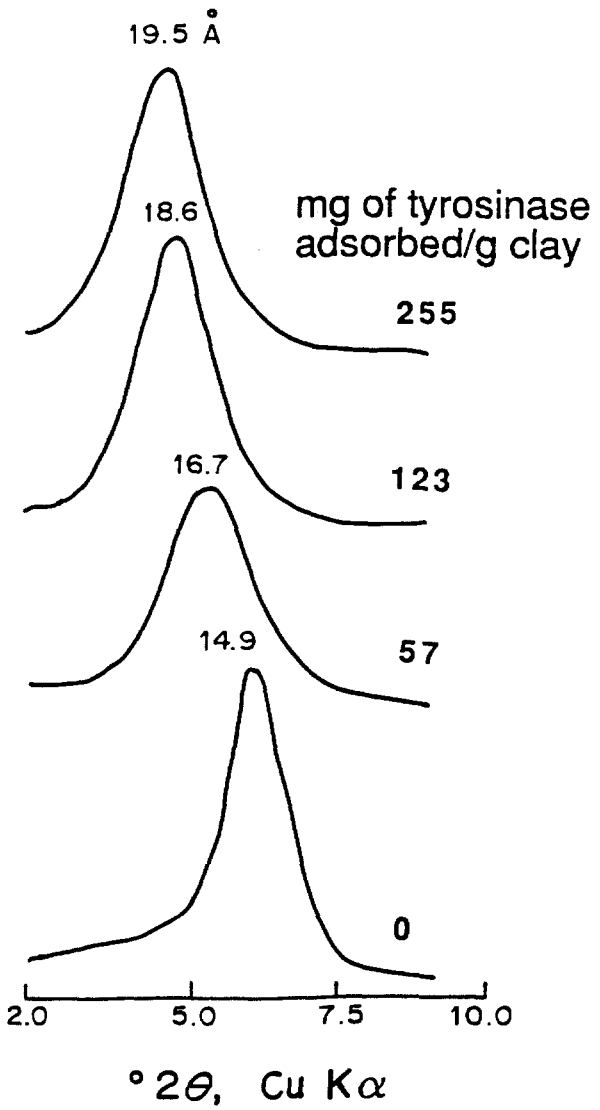


Figure 3. X-ray diffraction patterns of the tyrosinase-Ca-Mte complex at different amounts of tyrosinase adsorbed (mg/g) to the clay in the absence of phosphate at 25°C and pH 6.5.

1992). Similarly β-lactoglobuline was unable to penetrate the interlayer space of montmorillonite coated with polymeric (MW > 100,000) iron oxyhydroxide and was only adsorbed onto the external surfaces (Fusi *et al* 1989). The presence of phosphate retarded the adsorption of tyrosinase through occupying some adsorption sites on whether "clean" or coated montmorillonite (Figure 2). Moreover, the high affinity of positively charged hydroxyaluminum surfaces to phosphate anions (Violante *et al* 1991; Ferreiro *et al* 1992) resulted in substantial suppression of protein molecules adsorption when montmorillonite surfaces were coated with hydroxyaluminum ions (Figure 2).

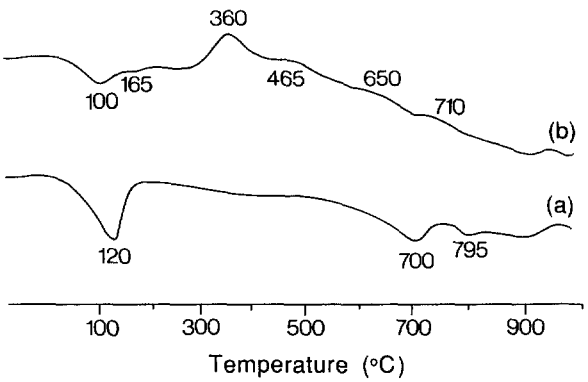


Figure 4. Differential thermograms of (a) Ca-Mte, and (b) the tyrosinase-Ca-Mte complex in the absence of phosphate.

Infrared spectroscopy

FTIR spectra of tyrosinase, tyrosinase-Ca-Mte and tyrosinase-Al(OH)$_x$-Mte complex (2.5 mmol Al/g clay) are presented in Figure 5. The band of amide I (C=O stretching vibration) around 1650 cm^{-1} and that of amide II (N-H deformation) around 1550 cm^{-1} are most useful to estimate the conformation (secondary structure i.e., α helix and β-sheet) of polypeptide backbone chain $-$CO-NH$-$ in naturally occurring or artificial proteins (Susi *et al* 1967; Elliot 1969; Susi and Byler 1983; Mantsh *et al* 1986; Tu 1986). After adsorption of tyrosinase onto Ca-Mte, there is no significant change in the amide II band at 1540 cm^{-1} of the protein. The amide I band at 1654 cm^{-1} shifted to 1671 cm^{-1} (Figure 5a and 5c). This indicates a slight perturbation of the protein structure without a significant change in its cross sectional area (Fusi *et al* 1989). Timasheff *et al* (1967) attributed the shift of amide I band toward a higher frequency to a distortion of the structure due to the steric hindrance and strain. The spectrum of adsorbed tyrosinase onto Al(OH)$_x$ coated Mte (Figure 5e) showed a noticeable decrease in the intensity of amide I and amide II bands, due to the lower adsorption onto the coated clay surfaces as shown in Figure 2. Moreover, the amide II band shifted from 1540 to 1530 cm^{-1}. This may indicate a higher strain exerted by hydroxyaluminum surfaces on the protein structure causing a substantial conformational deformation of the Ca-Mte surfaces. This reasoning is substantiated by the absence of the band at 3073 cm^{-1} representing amide B (ν_2 N-H) band and the broadening of the band at 3300 cm^{-1} (before adsorption) representing amide A (ν_1 N-H) in the differential spectrum of tyrosinase-Al(OH)$_x$-Mte complex and Al(OH)$_x$-Mte complex (Figure 5e). Adsorption of catalase (MW ~ 238,000, pI = 5.7) onto iron oxyhydroxy coated montmorillonite also caused a similar shift in amide I and amide II bands (Fusi *et al* 1989). Conversely, adsorption of pepsin (MW = 35,000, pI = 5.7) onto crystalline

https://doi.org/10.1346/CCMN.1995.0430601 Published online by Cambridge University Press

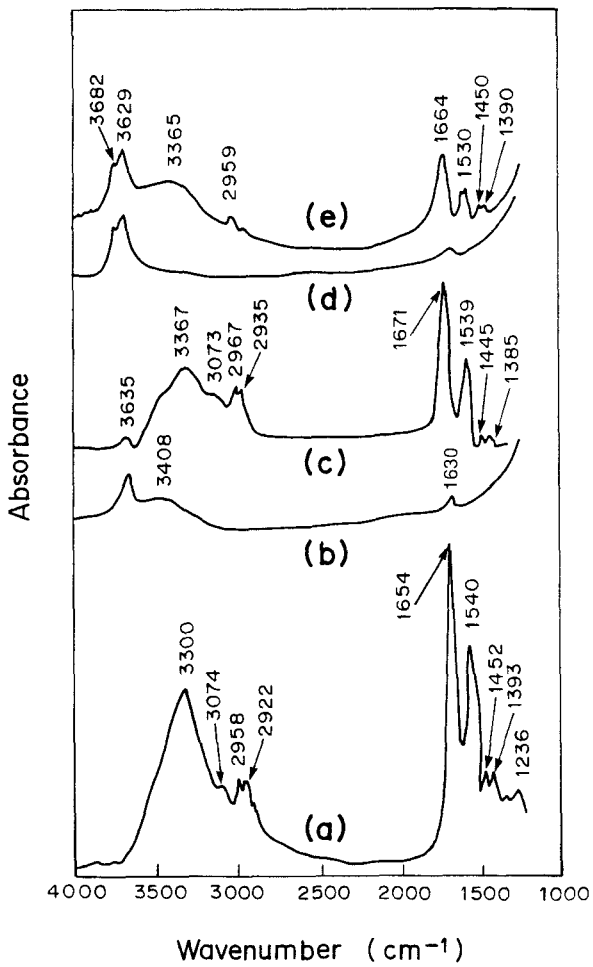


Figure 5. Fourier Transform Infrared spectra of tyrosinase-Ca-Mte and tyrosinase-Al(OH)$_x$-Mte complex at the level of Al coatings of 2.5 mmol Al/g clay, in the absence of phosphate. (a) tyrosinase, (b) Ca-Mte, (c) differential spectrum of tyrosinase-Ca-Mte complex and Ca-Mte, (d) Al(OH)$_x$-Mte complex, and (e) differential spectrum of tyrosinase-Al(OH)$_x$-Mte complex and Al(OH)$_x$-Mte complex.

aluminum hydroxide (boehmite) did not affect amide II band (Sepelyak *et al* 1984).

CONCLUSIONS

Tyrosinase (MW = 128,000, pl = 6.1) was adsorbed onto internal (intercalated) and external surfaces of Ca-montmorillonite at pH 6.5.

Hydroxyaluminum coatings resulted in a substantial decrease in the adsorption of tyrosinase due to the preoccupation of the interlayer space by hydroxy-Al ions and/or the resulting steric hindrance. The adsorbed tyrosinase onto hydroxy-Al coated montmorillonite was more subject to the alteration than that of Ca-montmorillonite. Possibly, the high charge of Al(OH)$_x$ surfaces and the restricted interlayer space caused a higher conformational deformation of the protein structure.

The presence of phosphate slightly reduced the adsorption of tyrosinase onto Ca-montmorillonite by occupying some adsorption sites. The adsorption of tyrosinase onto Al(OH)$_x$ coated montmorillonite was drastically reduced by the presence of phosphate. This was because of the high affinity of aluminous surfaces to phosphate and also the consequent repulsion of the protein molecules from the coated clay surfaces by the negative charge developed on phosphate adsorption.

In view of the effect of hydroxy-Al coatings on the adsorption of tyrosinase by montmorillonite, the activity of the coated clay-adsorbed enzyme warrants in-depth research.

ACKNOWLEDGMENTS

This study was supported by Grant GP 2383-Huang of the Natural Sciences and Engineering Research Council of Canada.

REFERENCES

Alexiades, C. A., and M. L. Jackson. 1965. Quantitative determination of vermiculite. *Soil Sci. Soc. Amer. J.* **29**: 522–529.

Alikhan, M. A. 1976. The tyrosinase system in the terrestrial isopod, *Porcellio laevis* Latreille (*Porcellionidae, Isopoda*). *Comp. Biochem. Physiol.* **54B**: 37–42.

Barnhisel, R. I., and P. M. Bertsch. 1989. Chlorites and hydroxy-interlayered vermiculite and smectite. In *Minerals in Soil Environments,* 2nd ed. J. B. Dixon and S. B. Weed, eds. Madison, Wisconsin: Soil Science Society of America, 729–788.

Boyd, S. A., and M. M. Mortland. 1990. Enzyme interactions with clays and clay-organic matter complexes. In *Soil Biochemistry.* **6**, J. M. Bollag and G. Stotzky, eds. New York: Marcel Dekker, 1–28.

Brunauer, S., P. H. Emmett, and E. Teller. 1938. Adsorption of gases in multimolecular layers. *J. Amer. Chem. Soc.* **60**: 309–319.

Burns, R. G. 1978. Enzyme activity in soil: Some theoretical and practical considerations. In *Soil Enzymes.* R. G. Burns ed. London: Academic Press, 295–399.

Burns, R. G. 1990. Microorganisms, enzymes and soil colloids surfaces. In *Soil Colloids and their Associations in Aggregates.* M. F. De Boot, M. H. B. Hayes, and A. Herbillon, eds. New York: Plenum Press, 337–361.

Chen, J. S., C. Wei, R. S. Rolle, W. S. Otwell, M. O. Balaban, and M. R. Marshall. 1991. Inhibitory effect of kojic acid on some plant and crustacean polyphenoloxidases. *J. Agric. Food Chem.* **39**: 1396–1401.

Elliot, A. 1969. *Infra-red Spectra and Structure of Organic Long-chain Polymers.* London: Edward Arnold Ltd.

Eltantawy, I. M., and P. W. Arnold. 1973. Reappraisal of ethylene glycol mono-ethyl ether (EGME) method for surface area estimations of clays. *J. Soil Sci.* **24**: 232–238.

Ferreiro, E. A., S. G. de Busseti, and A. K. Helmy. 1992. Effect of montmorillonite on phosphate sorption by hydrous Al-oxides. *Geoderma.* **55**: 111–118.

Fusi, P., L. Ristori, L. Calamai, and G. Stotzky. 1989. Adsorption and binding of protein on "Clean" (homoionic) and "Dirty" (coated with Fe oxyhydroxides) montmorillonite, illite and kaolinite. *Soil Biol. Biochem.* **21**: 911–920.

Garwood, G. A., M. M. Mortland, and T. J. Pinnavaia. 1983.

Immobilization of glucose oxidase on montmorillonite clay and ionic modes of binding. *J. Mol. Cat.* **22**: 153–163.

Gianfreda, L., M. A. Rao, and A. Violante. 1991. Invertase (β-fructosidase): Effect of montmorillonite, Al-hydroxide and Al(OH)$_x$-montmorillonite complex on activity and kinetic properties. *Soil Biol. Biochem.* **23**: 581–587.

Gianfreda, L., M. A. Rao, and A. Violante. 1992. Adsorption, activity and kinetic properties of urease on montmorillonite, aluminum hydroxide and Al(OH)$_x$-montmorillonite complexes. *Soil Biol. Biochem.* **24**: 51–58.

Giles, C. H., T. H. McEwan, S. N. Nakhwa, and D. Smith. 1960. Studies in adsorption. XI. A system with classification of solution adsorption isotherms, and its use in diagnosis of adsorption mechanisms and in measurement of specific surface area of solids. *J. Chem. Soc.* **786**: 3973–3993.

Hsu, P. H. 1992. Reaction of OH-Al polymers with smectites and vermiculites. *Clays & Clay Miner.* **40**: 300–305.

Janovitz-Klapp, A. H., F. C. Richard, P. M. Goupy, and J. J. Nicolas. 1990. Inhibition studies on apple polyphenol oxidase. *J. Agric. Food. Chem.* **38**: 926–931.

Kiss, S., M. Dragan-Bularda, and D. Radulescu. 1975. Biological significance of enzymes accumulated in soil. *Adv. in Agron.* **27**: 25–87.

Lowell, S., and J. E. Shields. 1991. *Powder Surface Area and Porosity.* 3rd ed. New York, NY: Chapman and Hall.

Mantsh, H. H., H. L. Casal, and R. N. Jones. 1986. Resolution enhancement of infrared spectra of biological systems. In *Advances in Spectroscopy* **13**. R. J. H. Clark and R. E. Hester, eds. New York: Wiley and Sons, 1–46.

McLaren, A. D., G. H. Peterson, and I. Barshad. 1958. The adsorption and reactions of enzymes and proteins on clay minerals: IV. Kaolinite and Montmorillonite. *Soil Sci. Soc. Am. Proc.* **22**: 239–244.

Morgan, H. W., and C. T. Corke. 1976. Adsorption, desorption, and activity of glucose oxidase on selected clay species. *Can. J. Microbiol.* **22**: 684–693.

Naidja, A., and P. M. Huang. 1994. Aspartic acid interaction with Ca-montmorillonite: Adsorption, desorption and thermal stability. *Appl. Clay Sci.* **9**: 265–281.

Quiquampoix, H. 1987. A stepwise approach to the understanding of extracellular enzyme activity in soil I. Effect of electrostatic interactions on the conformation of a β-D glucosidase adsorbed on different mineral surfaces. *Biochimie* **69**: 753–763.

Sepelyak, R. J., J. R. Feldkamo, T. E. Moody, J. L. White, and S. Hem. 1984. Adsorption of pepsin by aluminum hydroxide I: Adsorption mechanism. *J. Pharmaceutical Sci.* **73**: 1514–1517.

Sjoblad, R. D., and J. M. Bollag. 1981. Oxidative coupling of aromatic compounds by enzymes from soil microorganisms. In *Soil Biochemistry.* **5**, E. A. Paul and J. N. Ladd, eds. New York: Marcel Dekker, 113–152.

Skujins, J. 1978. History of abiontic soil enzyme research. In *Soil Enzymes.* R. G. Burns, ed. London: Academic Press, 1–49.

Susi, H., S. N. Timasheff, and L. Stevens. 1967. Infrared spectra and protein conformation in aqueous solutions. The amide I band in H_2O and D_2O solutions. *J. Biol. Chem.* **242**: 5460–5466.

Susi, H. and M. Byler. 1983. Protein structure by Fourier Transform infrared spectroscopy: Second derivative spectra. *Biochem. Biophys. Res. Comm.* **115**: 391–397.

Talibudeen, O. 1955. Complex formation between montmorillonoid clays and amino-acids and proteins. *Trans. Farad. Soc.* **51**: 582–590.

Timasheff, S. N., H. Susi, and L. Stevens. 1967. Infrared spectra and protein conformation in aqueous solutions. II. Survey of globular proteins. *J. Biol. Chem.* **242**: 5467–5473.

Tu, A. T. 1986. Peptide backbone conformation and microenvironment of protein side chains. In *Advances in Spectroscopy.* **13**, R. J. H. Clark and R. E. Hester, eds. New York: Wiley, 1–46.

Violante, A., C. Colombo, and A. Buondonno. 1991. Competitive adsorption of phosphate and oxalate by aluminum oxides. *Soil Sci. Soc. Am. J.* **55**: 65–70.

Violante, A. Dipartmento di Scienze Chimico-Agrarie, Università di Napoli "Frederico II," Via Università 100, 80055 Portici, Napoli, Italy.

Weast, R. C. 1974. *Handbook of Chemistry and Physics.* 55th ed. Cleveland, Ohio: CRC Press.

(Received 3 November 1994; accepted 7 March 1995; Ms. 2591)