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ROLE OF SMECTITES AND Al-SUBSTITUTED GOETHITES IN THE CATALYTIC CONDENSATION OF ARGININE AND GLUCOSE

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Abstract—The polyphenol theory of humic-substance formation has been studied extensively; however, an alternative theory, that humic substances are formed through the condensation of amino acids and reducing sugars (Maillard reaction), has not been explored to the same extent. The general objectives of this study were to determine whether smectites and goethites catalyze the abiotic polymerization of arginine and glucose to form humic-like compounds. The effects of smectite type, saturating cation, and the degree of Al substitution in goethites on the polymerization reaction were also studied. Four cation-saturated smectites and four Al-substituted goethites were incubated abiotically with solutions containing a mixture of arginine + glucose for 21 days at 37ºC. After the incubations, total C recovered ranged from 80.6 to 123.8% and from 100.5 to 105.1% for the smectite and goethite systems, respectively. At the end of the incubations, 21.4–50.3% of the added C and 16.5–90% of the added N were sorbed on the various smectites, and 6.2–9.0% of the added C and 2.3–4.6% of added N was sorbed on the goethites in a form that could not be desorbed by washing with 100 mM $CaCl_2$. X-ray diffraction analysis indicated that some of the sorbed C was intercalated in the smectites and FTIR analysis provided evidence of new absorption bands at 1650 and 1668 cm^{-1}, which are consistent with Maillard reaction products. Thus, it is concluded that smectites catalyze the condensation of arginine and glucose to form humic-like products. Goethites, however, have little or no ability to catalyze this reaction.

Key Words— Arginine, Catalysis, Condensation, Glucose, Goethites, Humic Substances, Maillard Reaction, Smectites

INTRODUCTION

The polyphenol theory of humic-substance formation, the abiotic copolymerization of phenols and amino acids, has been studied extensively using clay minerals and various oxides as catalysts (Lehmann *et al.*, 1987; Wang and Huang, 1989). An alternative theory for the formation of humic-like materials, the Maillard reaction, has received less attention. The Maillard reaction is the non-enzymatic condensation of amino acids and reducing sugars to form melanoidins, insoluble brown nitrogenous-containing compounds (Stevenson and Cole, 1999).

Maillard reactions have been investigated extensively from the geological and food sciences point of view. After a 5 day incubation of glucose + glycine at 60ºC, 25% of the glucose was transformed to form melanoidins (Reyes *et al.*, 1982). Furthermore, it has been reported that basic amino acids are more reactive with reducing sugars than are neutral or acidic amino acids (Hedges, 1978; Yamamoto and Ishiwatari, 1989). Smectites and illites have been shown to catalyze Maillard reactions when samples are subjected to wetting-drying cycles at relatively high temperatures (70–100ºC), mimicking pre-biotic conditions on Earth (Arfaioli *et al.*, 1999; Bosetto *et al.*, 2002). Although there is a large amount of information on the role of clay minerals in catalyzing the condensation of melanoidins from the geological point of view, there is little information on the ability of smectites to abiotically catalyze the condensation of amino acids and reducing sugars under conditions found in soil environments.

Stevenson and Cole (1999) reported that 65–80% of the total N in soils is acid-hydrolyzable N. Using a size-fractionation approach, Laird *et al.* (2001) found that 76–97% of the N in clay-associated organic matter was in amino acids and 4–6% in amino sugars, respectively. Arginine, a simple amino acid, was the most abundant amino acid (50–66% of the total N) in the clay fractions of the soils studied by these researchers. A total of only 30–52% of the total C in the different clay-size fractions was extracted as monosaccharides, amino sugars, amino acids and fatty acids (Laird *et al.*, 2001).

Smectites, considered both Lewis and Brönsted acids, can catalyze some organic reactions (Balogh and Laszlo, 1993). Thus, it is hypothesized that smectites catalyze the condensation of amino acids and sugars under conditions similar to those found in soil environments. On the other hand, there is a lack of information on the role of Fe oxides in the catalytic polymerization of amino acids.

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The specific objectives of this study were: (1) to determine whether different types of smectites and their associated saturating metal cations catalyze the abiotic co-polymerization of arginine and glucose to form humic-like compounds; and (2) to determine if synthetic Al-substituted goethites can catalyze the abiotic co-polymerization of arginine and glucose to form humic-like compounds.

MATERIALS AND METHODS

Only a brief description of sample preparation, incubation and analyses will be presented. More details can been found in Gonzalez (2002).

Sample preparation

The clay fraction (<2 µm) of four reference smectites, Panther Creek beidellite (Panther), Otay white montmorillonite (Otay), IMV saponite (Saponite), and ferruginous smectite (SWa) was separated from bulk mineral samples by sedimentation. The clay samples were saturated with Na, Ca, Cu and Al by washing with the appropriate metal salts. A portion of each Na-saturated clay was coated with $Fe(OH)_3$ polymers made from a dialyzed $Fe(NO_3)_3$ solution (Rengasamy and Oades, 1977). Excess salt was removed by dialyzing the cation-saturated clays against distilled water. The cation-saturated clay samples were freeze dried and saved for later use. Four Al-substituted goethites with different Al/(Fe+Al) mol/mol ratios were synthesized, washed with distilled water, and freeze dried (Schwertmann and Cornell, 2000).

Incubation

About 0.25 g of M^{n+}-saturated clays or 0.50 g of Al-substituted goethites were placed in 15 mL amber vials with open-top phenolic screw caps. The vials were capped loosely using PTFE/silicone septa and sterilized in an autoclave. Arginine and glucose were filter-sterilized, and added to the cooled-vials containing the sterilized clay or Fe oxide under sterile conditions. The final concentration of arginine and glucose was ~0.5 mmol g^{-1} clay or Fe oxide each, or a combined total of 1.0 mmol g^{-1} clay or Fe oxide. The vials containing the M^{n+}-saturated clay or Al-substituted goethite + arginine + glucose systems were capped tightly, vortex-mixed, and incubated in the dark for 21 days at 37±0.5ºC. Three replicates were run for each treatment.

Analysis

After incubation, the evolved-CO_2 concentration was measured with a CO_2 infrared gas analyzer. Then, 5 mL of 100 mM filter-sterilized $CaCl_2$ solution were added to the vials under sterile conditions, mixed, and centrifuged for 10 min at $4500 \times g$. The supernatant liquid was analyzed for arginine, glucose, total organic C and pH.

Arginine and glucose were analyzed by reverse phase-HPLC using pre-column derivatization with a chromophore. DABS-Cl [4-(dimethylamino)azobenzene-4-sulfonyl chloride] solution was used for arginine and DABS-hydrazine was used for glucose. Calibration was performed using external standards.

Soluble organic carbon in the supernatant liquid was analyzed by the rapid dichromate oxidation technique (Yeomans and Bremner, 1988). The sediment, rinsed with water and freeze dried, was analyzed by dry combustion (for total C and total N), X-ray diffraction (XRD) using glass slides prepared by the paste method, and infrared (IR) spectroscopy using the diffuse reflectance Fourier transform infrared (DRIFT) technique.

RESULTS

Characterization of the smectites and Al-substituted goethites

The smectites and goethites used in this study were described elsewhere (Gonzalez, 2002). In summary, typical XRD patterns for smectites were observed. In addition to smectite peaks, the XRD patterns for the saponite showed small illite and quartz peaks. The main differences between the clay minerals were the chemical composition and the relative magnitude of layer charge in the tetrahedral and octahedral sheets. The Al-substituted goethites were named Goe-00, Goe-20, Goe-51, and Goe-94 after their levels of Al substitution (0, 2.0, 5.1 and 9.4% mol/mol Al/(Al+Fe), respectively). The total C and N in all untreated samples (smectites and Al-substituted goethites) ranged from 0 to 0.4% and 0 to 0.2%, respectively.

C and N analysis

Carbon and nitrogen recoveries for the M^{n+}-clays + arginine + glucose systems are presented in Tables 1 and 2. Evolved CO_2-C was relatively small for all clays (0 to 2.5%). Total C recovered ranged from 81.4 to 123.8% (Table 1a). The most C sorbed was found in the Cu-clays and the least C sorbed was found for the Ca-clays (Table 1b). Greater arginine-C solution recoveries were observed for the Fe-coated clays (13.0 to 22.5%) and arginine-C solution recoveries were less for the Cu-saturated clays (0.9 to 2.0%). The sum of the glucose C and arginine C in solution was lower than the soluble organic C by 7.1 to 54.4%. Glucose C recoveries for the Cu-clays were not used for the statistical analysis because of hydrazine oxidation by Cu^{2+} (Gonzalez, 2002). From 55.8 to 93.4% of the total added N was recovered from the clays (arginine-N + NH_4^+-N + retained N) (Table 2a), whereas 16.5 to 90.0% of the total N added was retained by clays (Table 2b). In general, NH_4^+-N content increased as sorbed N decreased.

Carbon and N mass balances for the Al-substituted goethite + arginine + glucose systems are presented in

Table 1a. Total C (%) recovered from the clay + arginine + glucose systems at the end of the incubations, relative to the amount of C added at the beginning of the incubations.[†]

	Clay mineral			
Cation	SWa	Panther	Saponite	Otay
Na	n.d.	n.d.	106.4 a	89.8 a
Ca	90.9 A[§] b[¶]	99.6 A a	99.2 A a	91.1 A a
Cu	111.9 C c	106.6 B a	107.3 B a	104.0 A b
Al	108.6 C c	82.4 A a	101.1 B a	106.4 C b
Fe	81.4 A a	90.0 A a	115.1 B b	123.8 B c

Table 1b. Carbon sorbed (% recovered) on the clays in clay + arginine + glucose systems at the end of the incubations, relative to the amount of C added at the beginning of the incubations.

	Clay mineral			
Cation	SWa	Panther	Saponite	Otay
Na	n.d.	n.d.	38.8 d	25.2 b
Ca	21.4 B[§] a[¶]	24.5 C a	21.7 B a	15.5 A a
Cu	50.3 D d	45.0 B b	47.7 C e	43.3 A c
Al	24.6 A b	27.9 A a	26.1 A b	33.0 B b
Fe	26.8 A c	26.6 A a	34.3 A c	42.6 B c

n.d. – not determined

[†] Sum of total C in solution + sorbed C + CO_2 C (data not shown)

[§] Within each cation, means followed by the same uppercase letter are not significantly different, by the Student-Newman-Keuls test ($P<0.05$)

[¶] Within each clay mineral, means followed by the same lowercase letter are not significantly different, by the Student-Newman-Keuls test ($P<0.05$)

Table 3. Total C recoveries for the Al-substituted goethite + arginine + glucose systems ranged from 100.5 to 105.1%. Evolved CO_2-C ranged from 0 to 0.3% indicating negligible microbial activity during the incubations. Total C in solution ranged from 91.4 to 98.3%, arginine C in solution ranged from 28.8 to 30.1%, and glucose C in solution ranged from 37.5 to 41.6%. Unidentified soluble organic C (total soluble C - arginine C - glucose C) ranged from 19.6 to 29.5%. From 67.5 to 71.1% of the total N added was recovered (arginine N + NH_4^+ N + sorbed N). Arginine N in solution ranged from 58.2 to 59.9% and NH_4^+ N in solution ranged from 7.2 to 7.7%.

XRD analysis of samples

The XRD analyses (Table 4) were performed for the untreated and treated (arginine + glucose) samples that were equilibrated at 54% relative humidity (RH) from the use of a saturated $Mg(NO_3)_2$ solution. The *d* spacings for all of the treated clays, except the Fe-coated clays, were restricted to a relatively narrow range (1.34–1.41 nm). The Fe-coated SWa had a *d* spacing of 1.38 nm; however, *d* spacings for the other Fe-coated clays were between 1.50 and 1.56 nm. By contrast, the untreated clays had a much wider range of *d* spacings (1.22–1.59 nm).

FTIR

The FTIR spectra for all M^{n+}-Otay + arginine + glucose systems, untreated Ca-Otay, glucose, and arginine (crystalline form) are shown in Figure 1. The arginine spectrum was complex with 11 distinct peaks in the 1200–1800 cm^{-1} range. The glucose spectrum shows overlapping bands from ~1300 to 1460 cm^{-1} corresponding to CH_2-, CH- and OH-bending modes (Vazko *et al.*, 1972). The spectra for Na-, Ca-, Al- and Fe-clays + arginine + glucose systems in the 1300–1550 cm^{-1} range have some similarities with the arginine spectrum. Peaks near 1475 and 1423 cm^{-1} in the spectra for the treated clays are attributable to the arginine δCH_2 and ν_s COO^-, respectively. The ν C=O band (1720 cm^{-1}) of arginine was not evident in the spectra for the treated clays and the ν_{as} C=N and ν_{as} COO^- bands were not resolved because of overlap with the δHOH of clays. At least two new peaks, 1650 and 1668 cm^{-1}, are evident in the spectra of the treated clays but not in the untreated clay or arginine spectra. The spectra for the Cu-saturated clays + arginine + glucose systems were different in the 1550–1300 cm^{-1} range from the other spectra in that the ν_s COO^- band was shifted to a lower frequency (1388 cm^{-1}).

DISCUSSION

The amount of C released as CO_2 during the incubations ranged from 0 to 2.5% of added C indicating that there was little or no microbial activity in the systems. Total C recoveries for the clay + arginine + glucose systems ranged from 81.4 to 123.8% of added C.

Table 2a. Total N recovery (%) from the clay + arginine + glucose systems at the end of the incubations relative to the amount of N added at the beginning of the incubations[†].

	Clay mineral			
Cation	SWa	Panther	Saponite	Otay
Na	n.d.	n.d.	55.8 A a	67.3 B b
Ca	60.5 A[‡] a[¶]	71.8 B a	62.8 A b	58.6 A a
Cu	93.4 B b	88.3 A b	90.3 AB e	91.6 AB c
Al	70.6 A a	73.9 B a	81.9 C d	89.5 D c
Fe	66.2 A a	70.0 A a	71.6 A c	76.9 A d

Table 2b. Nitrogen (% recovered) sorbed on the clays in clay + arginine + glucose systems at the end of the incubations, relative to the amount of N added at the beginning of the incubations.

	Clay mineral			
Cation	SWa	Panther	Saponite	Otay
Na	n.d.	n.d.	16.5 A a	40.6 B b
Ca	30.2 C[‡] b[¶]	35.7 D b	26.4 A c	27.8 B a
Cu	90.0 C d	84.0 A d	86.9 B e	89.4 C d
Al	36.8 A c	51.2 C c	46.3 B d	63.5 D c
Fe	20.8 A a	21.4 A a	22.4 A b	29.3 B a

n.d. – not determined

[†] Sum of solution arginine-N + solution-NH_4^+-N + sorbed N

[‡] Within each cation, means followed by the same uppercase letter are not significantly different, by the Student-Newman-Keuls test ($P<0.05$)

[¶] Within each clay mineral, means followed by the same lowercase letter are not significantly different, by the Student-Newman-Keuls test ($P<0.05$)

Total C recoveries for the Fe-SWa and Al-Panther systems were <90% and total C recovery for the Fe-Otay system was 123.8%; however, C recoveries in the other 15 systems, which had complete data sets, were between 90 and 110%. Thus, in general, we were able to account for most of the added C. The reasons for the two abnormally low and one abnormally high C recoveries are not clear. By contrast, total N recoveries averaged only 74.5% and ranged from 55.8 to 93.4% of added N. Nitrogen recoveries tended to be higher for the Cu^{2+} systems and lower for the Na^+ and Ca^{2+} systems. Thus, we were not able to account for all of the added N. Volatilization loss of NH_3 gas, for systems with pH > 7.0, and loss of adsorbed arginine and NH_4^+ ions, when the clays were washed with water, are the most likely explanations for the incomplete N recoveries.

Table 3. Recoveries (%) of total C and N and sorbed C and N from the goethite + arginine + glucose systems at the end of the incubations, relative to the amounts of C and N added at the beginning of the incubations[†].

	Sample			
	Goe-00	Goe-20	Goe-51	Goe-94
Sorbed C	6.2 a[‡]	8.1 a	8.8 a	9.0 a
Total C[†]	105.1 a	100.5 a	104.7 a	103.8 a
Sorbed N	2.3 a	3.5 b	3.4 b	4.6 c
Total N[§]	67.5 a	70.6 a	69.1 a	71.1 a

[†] Sum of total C in solution + sorbed C + CO_2-C

[‡] Within each C- or N-fraction, means followed by the same letter are not significantly different, by the Student-Newman-Keuls test ($P<0.05$)

[§] Sum of solution arginine-N + solution NH_4^+-N + sorbed N

For the clay systems (except the Cu-clays, which will be discussed separately), recoveries of soluble organic C from the solutions ranged from 42.1 to 90.0% of the total added C. The arginine C + glucose C in the solutions at the end of the incubations ranged from 28.8 to 64.4% of the total added C. Therefore, the data indicate that 7.1–33.7% of the added C was abiotically transformed to soluble compounds other than glucose and arginine during the incubations. The glucose C to arginine C ratio of the starting solutions was 1.00. By contrast, the glucose C to arginine C ratio of the solutions at the end of the incubations averaged 2.30 and ranged from 1.07 to 3.35. The glucose C to arginine C ratios of the Fe-treated clay solutions were significantly lower than the ratios for the other cation treatments. These results indicate that arginine was either selectively sorbed on the clays and/or selectively decomposed in the solutions relative to glucose. High sorption of arginine on smectites has been reported (Hedges and Hare, 1987).

At the end of the 21 day incubation, between 14.9 and 50.3% of added C and between 16.5 and 90.0% of added N was sorbed on the clays in a form that could not be readily removed with 100 mM $CaCl_2$ or by washing with distilled water. The mass C:N ratios of the adsorbed compounds generally ranged from 1.33 to 5.21, which is

Table 4. Basal spacings (nm) of the smectites determined by XRD after incubation with arginine + glucose (treated) and of controls (untreated).

	Treated†			Cation‡		
Clay		Na	Ca	Cu	Al	Fe
SWa	No	1.27	1.34	1.31	1.49	1.32
	Yes	1.36	1.40	1.36	1.34	1.38
Panther Creek	No	1.22	1.51	1.30	1.56	1.44
	Yes	1.35	1.38	1.36	1.39	1.50
Saponite	No	1.50	1.45	1.34	1.34	1.55
	Yes	1.39	1.41	1.37	1.40	1.56
Otay	No	1.28	1.54	1.29	1.58	1.30
	Yes	1.38	1.41	1.39	1.40	1.52

† Clays treated with arginine and glucose
‡ Saturating cation

slightly to substantially higher than the 1.29 C:N ratio of arginine (Table 5). However, the mass C:N ratios of Al-Saponite, Cu-Saponite, and Cu-Otay were 1.16, 1.23 and 1.27, respectively. The mass C:N ratios for the sorbed compounds were significantly different among the cation treatments (Fe > Ca > Cu = Al), but no significant differences were observed for the various clays. The relatively high C:N ratios for the sorbed compounds indicate that some of the C originally in glucose was co-sorbed with the arginine C on the clays. By contrast, analysis of glucose only (no arginine) interactions with the same clays under identical conditions (Gonzalez, 2002) indicated that little or no glucose-derived C was sorbed on the clays.

The FTIR spectra of the clay systems showed evidence that arginine and/or arginine + glucose

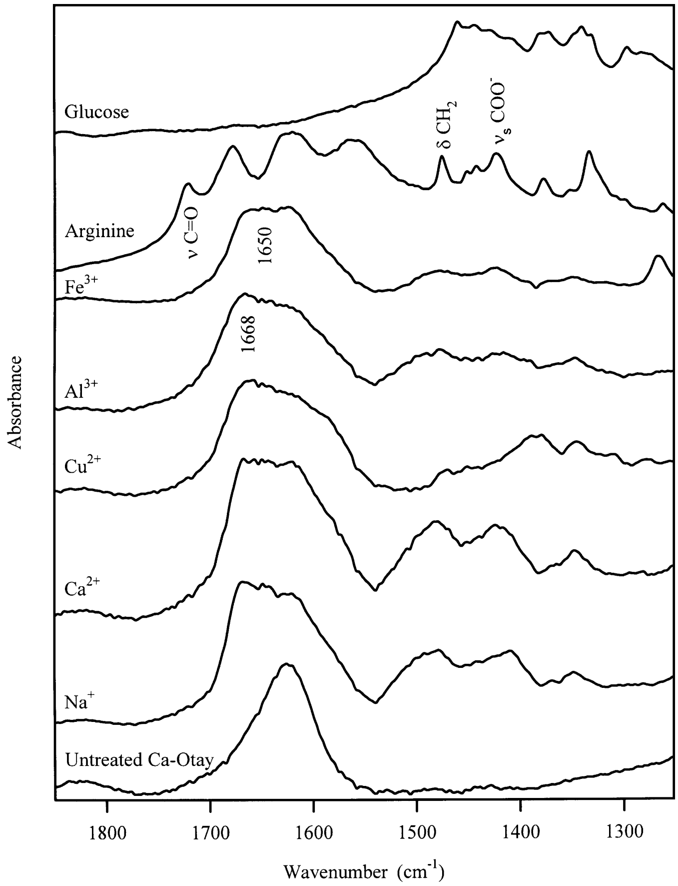

Figure 1. FTIR spectra of glucose, arginine and M^{n+}-Otay untreated and treated with arginine + glucose.

condensation products interacted with the clay surfaces. The 1650 and 1668 cm^{-1} bands, which were only observed for the clay systems (Figure 1), can be assigned as stretching modes of amide groups, quinones or C=O groups of H-bound conjugated ketones. Such functional groups are consistent with Maillard reaction products from clay + amino acids + glucose systems (Arfaioli *et al.*, 1999; Bosetto *et al.*, 2002). X-ray diffraction analysis (Table 4) indicated that the arginine + glucose-treated clays had relatively consistent and stable *d* spacings of ~1.4 nm (except the Fe-treated clays). A *d* spacing of 1.4 nm is consistent with a monolayer of organic molecules in the interlayers. Intercalation of arginine and other amino acids into the inter-lamellar spaces of montmorillonite (*d* spacing of ~1.4 nm) have been reported (Dashman and Stotzky, 1985; Naidja and Siffert, 1989). The *d* spacing of the glucose + arginine-treated Fe-clays were more variable probably due to the effects of inorganic Fe$_x$(OH)$_y$ interlayer polymers.

High arginine N sorption on Cu-clays (84.0 to 90.0%) was observed and can be explained by the fact that amino acids form coordination complexes with Cu^{2+} ions (de Farias *et al.*, 2002). The FTIR spectra of Cu-clays treated with arginine + glucose (Cu-Otay shown in Figure 1) showed evidence of ligand exchange, the ν_sCOO$^-$ band shifted from 1423 to 1388 cm^{-1} as reported for aqueous Cu^{2+}-arginine (Phan *et al.*, 1975) and solid Cu^{2+}-arginine complexes (de Farias *et al.*, 2002). Several other observations further support the formation of strong arginine-Cu-clay complexes; (1) NH$_4^+$ N from arginine derivation with the chromophore was small, 0.3–0.4%; (2) high sorbed C and N concentrations were observed; (3) the C:N ratio of the sorbed compounds, 1.3 to 1.4, was similar to that of arginine; and (4) only small amounts of arginine were detected in the solutions after the incubation. Moreover, the very high recovery of sorbed N for the Cu-clays (Table 2a) indicates that little of the sorbed arginine was desorbed with 100 mM CaCl$_2$ or distilled water washes.

Table 5. Total amount (mg g^{-1} clay) of C and N sorbed on the clays, and C:N ratios of the sorbed organic materials after the incubations.

Sample	Sorbed C		Sorbed N		C:N	
Na						
SWa	n.d.		n.d.		n.d.	
Panther	n.d.		n.d.		n.d.	
Saponite	24.0	[§]	4.6		5.21	
Otay	18.3		11.4		1.61	
Ca						
SWa	13.6	a[†]	8.6	b	1.58	b
Panther	16.9	b	10.2	c	1.66	b
Saponite	12.0	a	7.5	a	1.60	b
Otay	11.2	a	7.8	a	1.44	a
Cu						
SWa	34.4	c	25.6	b	1.34	a
Panther	31.2	ab	23.5	a	1.33	a
Saponite	30.1	a	24.6	b	1.23	a
Otay	32.1	b	25.3	b	1.27	a
Al						
SWa	16.0	b	10.5	a	1.53	c
Panther	19.1	c	14.4	c	1.33	b
Saponite	15.2	a	13.1	b	1.16	a
Otay	23.8	d	17.7	d	1.34	b
Fe						
SWa	17.4	a	6.0	a	2.63	a
Panther	18.8	a	6.2	a	3.02	a
Saponite	21.1	b	6.3	a	3.35	a
Otay	33.7	c	9.0	b	3.75	a

n.d. – not determined

[†] Within each cation and within each sorbed fraction, means followed by the same letter are not significantly different, by the Student-Newman-Keuls test (P<0.05)

[§] No statistical analysis was done for the Na-clays because of missing data

The results of this study do not provide enough evidence to elucidate the effect of the nature of the layer charge on the formation of the Maillard reaction products. However, it has been observed that the cation exchange capacity (CEC) is more important than the type of cation (K$^+$, Ca^{2+}, Al^{3+}) for the formation of humic-like substances during incubations of clay (kaolinite, montmorillonite or quartz)-glycine-glucose systems at 70ºC for 30 days (Bosetto *et al.*, 2002). In a different study, Arfaioli *et al.* (1999) found no substantial effect of the type of cation (Ca^{2+} and Al^{3+}) except for Cu^{2+} on the amount of humic-like substances formed and that free cations are more efficient than adsorbed cations at humifying N in clay (kaolinite, montmorillonite or quartz)-tyrosine-glucose systems at 70ºC for 30 days.

The goethites, by contrast with the smectites, sorbed relatively little glucose and arginine and were substantially less effective at catalyzing the transformation of glucose and arginine in the aqueous phase. Only a relatively small amount of C (6.2 to 9.0% of total added C) and even less N (2.3 to 4.6% of total added N) was sorbed on the goethites after the 21 day incubation. The ratio of glucose C to arginine C recovered in the aqueous solutions after the incubations ranged from 1.30 to 1.38, which is substantially lower than the glucose C to arginine C ratios for all of the smectite systems except the Fe-Panther. The results also indicate that the goethites were much less selective in the transformation and/or sorption of arginine relative to glucose than the smectites. There were no clear trends in the effects of degree of Al substitution in the goethites on sorbed C or solution levels of glucose, arginine or NH$_4^+$. Minor differences in levels of sorbed N and total solution C were not consistent with the levels of Al substitution.

Solution pH for the clay and goethite + arginine + glucose systems ranged from 4.3 to 8.9. Arginine is a basic amino acid with three pK_a values; 2.01(COOH), 9.04 (NH$_2$) and 12.28 (guanidinium group). Under the conditions of this study, arginine is a zwitterion and sorption is not expected to be affected by solution pH. The Fe oxides, however, have a pH-dependent surface charge with a point of zero charge (PZC) of ~8.3 (Herbillon, 1988). Because the pH of the goethite systems (8.5 to 8.6) was near the PZC, relatively low sorption of arginine was anticipated.

Glucose is dehydrated under acidic conditions to form furfural compounds, whereas under alkaline conditions glucose is oxidized to form saccharinic acids (Theander and Nelson, 1978). Solution pH for the clay + arginine + glucose ranged from 4.3 to 8.9. Thus, it is possible that both reactions may have occurred in the clay systems. Previous results indicate that clays catalyze the dehydration of glucose to form 5-hydroxymethyl-2-furfural (HMF) and levulinic acid under the conditions of this study (Gonzalez, 2002). The HMF and levulinic acid are highly reactive compounds capable of polymerizing or co-polymerizing with amino acids (Maillard reactions) (Gogus *et al.*, 1998). The ratio of NH_4^+-N relative to arginine-N in solution for all but three of the clay systems was higher (0.09 to 0.56) than the same ratio for the aqueous control (0.08). The high NH_4^+-N content relative to the control suggests that some arginine was degraded during the incubation. Catalytic deamination and decarboxylation of amino acids by smectites (Naidja and Siffert, 1989) and by aldehydes and metal salts (Ikawa and Snell, 1954) have been reported. New C and N are added to soil primarily as proteins and carbohydrates. During microbial degradation of proteins and carbohydrates, amino acids and monosaccharides are released to the soil solution. Although most of these labile compounds will be consumed by microbes, some will reach the surfaces of soil clays. The results of this study provide evidence that amino acids and monosaccharides are abiotically transformed by smectites. Furfural compounds and levulinic acid are the likely degradation products of monosaccharides. These compounds are known to be highly reactive, and thus are likely to be polymerized or co-polymerized with amino acids (Maillard reaction). Accordingly, the catalytic transformation of amino acids and monosaccharides by smectite is suggested as one of the pathways for the incorporation of new C and N into soil organic matter and for the formation of new humic substances. Iron oxides are less effective than smectites for catalyzing these reactions. Areas needing further investigation are the effects of pH, type of amino acid, and whether oligosaccharides and peptides are also catalytically transformed by smectites.

CONCLUSIONS

The polyphenol theory for the formation of humic substances in soils has received considerable attention, whereas the theory that humic substances are formed through Maillard reactions has received comparatively little attention. The results of this study provide evidence that smectites are capable of abiotically catalyzing the condensation of arginine and glucose to form humic-like substances under conditions similar to those found in soils. On the other hand, goethites were largely ineffective in catalyzing the reaction. The transformation of biological materials into humic substances undoubtedly involves many different biological and abiotic processes and reactions. This study demonstrates that smectite-catalyzed Maillard reactions are one possible mechanism for the formation of new humic substances in smectitic soils.

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