Adsorption of glycine and alanine on montmorillonite with or without coordinated divalent cations

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Adsorption of glycine and alanine on montmorillonite and on Ca$^{2+}$- and Mg$^{2+}$-exchanged montmorillonite clay over a range of pH (4.0-9.0) and temperature (20-35°C) under possible abiotic conditions have been investigated UV-spectrophotometrically. Adsorption of both the amino acids was considerable on all the three adsorbents used. Maximum adsorption was observed at 25°C and neutral pH. Ca$^{2+}$-montmorillonite exhibited relatively better adsorption as compared to Mg$^{2+}$-exchanged form or montmorillonite. The values of $K_L$ and $X_m$ were determined using Langmuir isotherm characteristics.

Ever since Bernal suggested that clay surfaces might have played a significant prebiotic role in selection and concentration through adsorption processes of abiotically formed biomonomers as well as protection against hydrolytic fission in remote prebiotic environments, a new era of investigations opened up in the field of chemical evolution. It has been emphasized that clays and metal ions were most widely spread over the earth's surface, at the site of sea shores and at the bottom of the sea before and during prebiotic and organic evolution, presumably after the formation of early hydrosphere. In early prebiotic times, the earth's hydrocarbon, ammonia and water vapours laden environment was acted upon by violent lightning thunders, electric discharges, ionizing radiations, volcanic heat and vast amount of UV-flux of solar radiations as there was no ozone layer to cut it off. During the period of intense activity of high energy radiations on the heavy blanket of CH$_4$, NH$_3$ and H$_2$O, vapours wrapped the earth, large amount of biomonomers formed might have been trapped by clays, metal ion coordinated clays and silica through adsorption and condensation. Under fluctuating dehydrating periods of wetting and drying cycles they gave rise to large biomolecules like protein and nucleic acid polymers. Clays might have acted as natural ion exchangers and adsorption of amino acids on them led to the formation of amino acid metal complexes. Recently, work of Lawless and Edelson on adsorption and clay initiated synthesis reported by Ferris and co-workers has shown that montmorillonite works as multicaatalytic system in the initiation of reaction of prebiotic significance. Probably clay and mineral sedimentation occurred far and wide across the earth along with adsorption of building blocks of biopolymers.

In our search to retrace the probable abiotic pathways of chemical evolution, occurrence of several amino acids as chemical fossils either of extinct organisms or geochemically formed peptides and proteins in the stromatolites formed from the organic matter of biogenic origin, probably 1350-950 million years ago from now, has been reported. This has provided evidence of existence of ancient sediments possessing adsorbed biomonomers. Kamaluddin and coworkers have reported the adsorption of amino acids and nucleotides on transition metal ferrocyanides as possible adsorbents in primitive lifeless era.

Apart from the efficient adsorption and catalytic capabilities shown by the clays in the process of amino acid and nucleotide oligomerization, a few workers have also proposed that prebiotic reactions might have occurred on the surfaces of water soluble minerals during dehydrating periods of wetting and drying cycles of a fluctuating environment. Bodenheimer and coworkers have investigated the adsorption of amino acids on clays and Cu-ion exchanged clays. Lawless and Levi were able to synthesize peptides by adsorption of monomeric building blocks using clays with divalent coordinated cations. As a remarkable increase in the catalytic activity has been noticed with clays ex-
changed with divalent metal ions, the metal ions with clays have been suggested as natural mineral matrices capable of adsorbing monomeric biomolecules and releasing the resulting products back to aqueous medium in order to preserve their catalytic activity.

Adsorption of amino acids under a range of pH, temperature and concentration on montmorillonite with or without Ca$^{2+}$- and Mg$^{2+}$ cations was investigated using the Langmuir characteristics of glycine and alanine and the results are reported in the present communication.

Materials and Methods

The montmorillonite was from Aldrich Chemical Co. All other chemicals used were of analytical grade. For preparation of solutions/reagents and experimentations, deionised water was used after double distillation in an all glass assembly. All containers, measuring equipments and assemblies were of borosilicate glass. A Jasco, V-550, UV/VIS spectrophotometer was used for determination of absorbance of the amino acids.

Montmorillonite was repeatedly washed with distilled water, its aqueous suspension was centrifuged and the solid material thus isolated was dried in an oven at 35°C. Ca$^{2+}$- and Mg$^{2+}$-exchanged forms of the clay were prepared by saturation of montmorillonite separately with calcium chloride and magnesium chloride and excess of the salts were leached out by washing with distilled water until it was freed of chloride ion. The divalent cation exchanged forms thus obtained were dried at room temperature and kept in vacuum desiccator. Before use, all the glassware after through cleaning, washing and drying was autoclaved along with double distilled water at 20 lbs/sq. inch steam pressure for 20 min. Portions of montmorillonite and its Ca$^{2+}$- and Mg$^{2+}$-exchanged forms (50 mg each) were used in each investigation.

The adsorption of amino acids on clay or cation exchanged clays in aqueous medium was studied as a function of pH and concentration of adsorbate. Therefore, adsorption of glycine and/or alanine in varying concentration (7.0 x 10$^{-5}$ M - 0.5 x 10$^{-5}$ M) over a pH range 3.6-5.5 and 6.8-9.2 was studied in order to obtain saturation point by adding relevant buffer to the amino acid solution (5 ml) containing montmorillonite/Ca or Mg-montmorillonite (50 mg) keeping in mind that the buffer should be a very poor ligand so that stable complex formation with clay could be avoided. Acetate buffer (0.2 N acetic acid and 2 N sodium acetate) and borax buffer (0.2 M boric acid and 0.05 M borax) were used to maintain pH in the range 3.6-5.5 and 6.8-9.2 respectively. This was verified by conductivity measurements as no change in the inflexion point of buffer with or without any type of clay was noticed. The inflexion point was determined by titrating buffer against NaOH. Glycine and alanine concentration in solution was determined UV/VIS spectrophotometrically after adding clay to it.

Buffered solutions of glycine and alanine (5 ml) at different pH were added to montmorillonite, Ca-and Mg-montmorillonite kept in separate conical flasks (50 ml), stirred mechanically for 20 min. and allowed to stand at room temperature for 8 hrs. Similar sets of different concentrations of glycine and alanine with different adsorbents were incubated over a temperature range 20°C to 35°C for varying periods and pH to find out the conditions of maximum adsorption. The study of amino acid adsorption as a function of temperature showed maximum adsorption at 25°C. After about 8 hrs the solutions were centrifuged at 3000 rpm for 15 min. Then supernatant was decanted leaving the clay as residue and the pH of the liquid was found unchanged. The suspensions were used for a separate study on the formation of peptides under wetting and drying cycles for various periods. The concentrations of glycine and alanine in the supernatant were determined by recording their absorbances at $\lambda_{\text{max}}$ ($\lambda_{\text{max}}$ Gly=202 nm and $\lambda_{\text{max}}$ Ala=197.6 nm). The amount of amino acid adsorbed under different conditions was calculated from the difference between the initial amino acid concentration and after adsorption in each case. The equilibrium concentration of amino acids and the amount adsorbed were used to obtain the adsorption isotherms.

Results and Discussion

The adsorption of glycine and alanine on montmorillonite and on Ca$^{2+}$- and Mg$^{2+}$-co-ordinated montmorillonite have been studied as a function of pH (4.0-9.0) and temperature (25°C to 35°C). These data should help to find out the possible catalytic role of alumino-silicate and cation exchanged clays in the abiotic formation, adsorption of amino acids and their subsequent linking to larger biopolymers under conditions comparable to those which might have existed over clay, silica and mineral deposits at the bottom of sea, on sea-shores and under dehydrating-wetting cycles of environment.
Table 1—Percent binding and Langmuir constants of glycine and alanine on montmorillonite clay with or without divalent cation

<table>
<thead>
<tr>
<th>Type of clay</th>
<th>Binding (%)</th>
<th>Langmuir Constants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glycine</td>
<td>Alanine</td>
</tr>
<tr>
<td>Montmorillonite</td>
<td>80.0</td>
<td>42.9</td>
</tr>
<tr>
<td>Ca²⁺- Montmorillonite</td>
<td>95.7</td>
<td>56.3</td>
</tr>
<tr>
<td>Mg²⁺- Montmorillonite</td>
<td>83.3</td>
<td>52.9</td>
</tr>
<tr>
<td></td>
<td>Xₘ (mg g⁻¹)</td>
<td>Kᵢ (L mg⁻¹)</td>
</tr>
<tr>
<td>Glycine</td>
<td>59.9</td>
<td>24.6</td>
</tr>
<tr>
<td>Alanine</td>
<td>90.9</td>
<td>12.5</td>
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<td></td>
<td>73.0</td>
<td>15.2</td>
</tr>
<tr>
<td></td>
<td>54.1</td>
<td>11.9</td>
</tr>
<tr>
<td></td>
<td>69.4</td>
<td>11.8</td>
</tr>
<tr>
<td></td>
<td>66.7</td>
<td>11.3</td>
</tr>
</tbody>
</table>

Fig. 1—Adsorption of glycine on montmorillonite clay (—○—) and montmorillonite with Mg²⁺ (—□—) and with Ca²⁺ (—△—) as a function of pH; temp 25°C.

Fig. 2—Adsorption of alanine on montmorillonite clay (—○—) and montmorillonite with Mg²⁺ (—□—) and with Ca²⁺ (—△—) as a function of pH; temp 25°C.

Our preliminary studies show that the amount of amino acids adsorbed is dependent on the pH of the solution and it was maximum at 25°C and neutral pH in all studies. Subsequent studies were therefore carried out at pH 7.0. Neutral pH is physiologically of great value as biochemical reactions in living systems take place in neutral medium. The variation in the amount of amino acid adsorbed with varying pH on different adsorbents have been illustrated in Figs. 1 and 2. It is seen that glycine and alanine show increase in adsorption at pH 7.0 and uptake decreases with increase of pH of the solution. The isoelectric point of glycine and alanine are 5.97 and 6.1 respectively. Their large adsorption at pH above isoelectric point shows that adsorption of amino acids is in their anionic form. However, interaction of clay and metal ion co-ordinated clays with biomonomers is dependent on other factors also like amino and carboxyl groups as sites for interaction with clay surfaces. In acidic medium glycine and alanine occur as cation and as neutrality approaches they acquire the zwitterion form. Percent binding of glycine and alanine are shown in Table 1. This has been calculated from optical densities of glycine and alanine solution before and after adsorption corresponding to saturation point on the curves. It was found that additional incubation time did not appreciably change the degree of adsorption. The adsorption isotherms (Figs 3 and 4) and Langmuir adsorption isotherms (Figs 5 and 6) show that the amount of amino acids adsorbed increase in their equilibrium concentration in solution up to a certain limit i.e. saturation point. Above this adsorption became independent of glycine or alanine concentration. The asymptotic nature of the curves (Figs 3 and 4) suggest Langmuir type adsorption. Initially the plots show linear relationship between amounts adsorbed and equilibrium concentration of amino acid whereas at higher concentration saturation point occurs and no more amino acid is adsorbed on montmorillonite surface. The adsorption of both the amino acids on clays with or without metal ions follows Langmuir type of adsorption as outlined below:

\[
\frac{C_{eq}}{X_c} = \frac{1}{K_L X_m} + \frac{C_{eq}}{X_m}
\]

Where \(C_{eq}\) is equilibrium concentration of glycine or alanine, \(K_L\) is constant related to energy i.e. enthalpy of adsorption coefficient (\(K_L \approx e^{-\Delta H/RT}\)); \(X_c\) is amount of amino acid (mg) adsorbed per gram of absorbent i.e. montmorillonite or Ca²⁺- and Mg²⁺- exchanged forms; \(X_m\) is the amount of glycine or alanine required for the weight of montmorillonite for forming a complete monolayer on the surface. The adsorption parameters \(X_m\) and \(K_L\) were calculated form the slope
Fig. 3—Adsorption of glycine on montmorillonite clay (---) and montmorillonite with Mg\(^{2+}\) (---) and with Ca\(^{2+}\) (---), pH 7.02; temp 25°C.

Fig. 4—Adsorption of alanine on montmorillonite clay (---) and montmorillonite with Mg\(^{2+}\) (---) and with Ca\(^{2+}\) (---), pH 7.02; temp 25°C.

Fig. 5—Langmuir adsorption for glycine on montmorillonite clay (---) and on montmorillonite with Mg\(^{2+}\) (---) and with Ca\(^{2+}\) (---), pH 7.02; temp 25°C.

Fig. 6—Langmuir adsorption for alanine on montmorillonite clay (---) and on montmorillonite with Mg\(^{2+}\) (---) and with Ca\(^{2+}\) (---), pH 7.02; temp 25°C.

and intercept obtained from the graph of \(C_{eq} / X_e\) versus \(C_{eq}\). These values for glycine and alanine are summarised in Table 1. The value of \(X_m\) can be calculated asymptotically from Figs 3 and 4 on extrapolating the adsorption curve towards Y-axis when saturation occurs. It has been observed that adsorption trend (% of binding, Table 1) of both glycine and alanine on the adsorbants used, though differ in percent binding from each other, was in the following order:

Ca-montmorillonite > Mg-montmorillonite > montmorillonite

\(X_m\) values recorded in Table 1 obtained from Langmuir adsorption isotherms for both amino acids though differ among themselves, show the identical trend as given below:

Ca-montmorillonite > Mg-montmorillonite > montmorillonite

Addition of Ca\(^{2+}\) and Mg\(^{2+}\) increases the adsorption of both the amino acids throughout the entire concentration range of the isotherms. This shows that added divalent metal ion first gets involved in complex formation with glycine/alanine and then amino acids get adsorbed on clay surface or the increase in ionic strength of clay surface lowers the repulsive force between the amino acid and the clay surface or that both these mechanisms may operate. In normal condition in montmorillonite structure \([\text{Al-Mg}_2 \text{OH}_2]^+ [\text{Na-Ca}]_n \text{H}_2\text{O}^+]\), the lattice silica and alumina layers appear to be sandwiched together with some vacant sites. The adsorption of amino acids most likely proceeded via carboxyl and the amino group is either hydrogen bonded to the structural oxygen or as such are tightly fixed with silica structure. The carboxyl groups are generally fixed with positively charged Al-oxy-hydroxy element by means of ionic bridges. The surface of montmorillonite is entirely comprised of silica and in interlayer position where oxygen confront each other, metal cations and polar molecules are located causing lattice expansion. Montmorillonite is thus capable of forming intercala-
tions with amino acid which forms the basis of adsorption property of clays. Earlier Egon T. Degens and Johann Matheja have also drawn similar conclusions relating to montmorillonites and kaolinite's more effective adsorption behaviour. Lawless and Edelson explained the value of $X_m$ as a measure of the accessibility of adsorption sites and $K_1$ as a measure of the affinity of clay for adsorbate. The values of $K_1$ for glycine and alanine on montmorillonite, Ca$^{2+}$- and Mg$^{2+}$-coordinated montmorillonite (Table 1) are related to the enthalpy of adsorption. This observation shows that added metal ion increases the ionic strength of montmorillonite surface and thus lowers the repulsive forces between organic molecule and clay surface so that a lower value of $K_1$ is observed when adsorbent is exchanged with divalent cation.

Conclusion

The adsorption of glycine and alanine on montmorillonite with or without metal ion substitution is maximum at neutral pH and 25°C. These values are of physiological importance as they also correspond to the ideal conditions for various biochemical reactions in living systems.

The adsorption of both the amino acids on all the three forms of montmorillonite follows Langmuir type of adsorption, which clearly indicate monolayer formation of adsorbrates (i.e., glycine and alanine) on clay surface. The percent binding and $X_m$ values for both glycine and alanine on the clays depend on the type of metal ion substitution. Results reported in this communication show that Ca$^{2+}$-exchanged montmorillonite clay has better adsorption properties as compared to Mg$^{2+}$-exchanged form and montmorillonite without cation. Further investigations on adsorption of short peptides of glycine and alanine on montmorillonite clay with or without divalent cations may be considered based on the aforesaid studies.

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