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THE ALTERATION OF SOME AROMATIC AMINO ACIDS AND POLYHYDRIC PHENOLS BY CLAY MINERALS

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Abstract—These studies concern the catalytic activity of clays on amino acids, particularly tyrosine. Polyhydric phenols were included to help understand the tyrosine reactions.

Below pH 3, tyrosine is adsorbed on clay minerals by cation exchange. Above pH 3, oxidative degradation of tyrosine occurs, the *L*-isomer altering more rapidly. The rate of alteration depends upon the particular clay mineral, surface modifications such as polyphosphate treatment, heating, and the presence of copper, aluminium, and mercury. A free radical mechanism is proposed for the alteration.

INTRODUCTION

THE EFFECT of clay minerals on amino acids is of importance not only because these organic compounds are used to date sediments, but also because of the possible role of fine-grained silicates in the prebiotic synthesis of polypeptides. Both areas of study depend upon the catalytic activity of clay minerals. Age dating, as indicated by the loss of optical activity or racemization, (Bada *et al.*, 1970; Kvenvolden and Peterson, 1970) is considered to be a first order reaction, but independent of any specific influence of the fine-grained sediments. The development of polypeptides from amino acids by 'template catalysis' on clay mineral surfaces has been studied by Degens, Mathejar and Jackson (1970) and Jackson (1971). Their findings were of exceptional interest; the *L*-isomer, which predominates in nature, showed an increased adsorption and polymerization rate over that of the *D*-isomer.

These investigations prompted the study of the catalytic activity of clays on some optically active amino acids and polyhydric phenols. The following compounds were selected: *D*- and *L*-tyrosine, *D*- and *L*-phenylalanine, pyrogallol, pyrocatechol, resorcinol and hydroquinone. These particular amino acids were chosen because they absorb light in the u.v. a property which makes possible the quantitative determination of concentration and structural changes.

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EXPERIMENTAL

Materials

A Wyoming montmorillonite (Volclay) supplied by the American Colloid Company was used in the magnesium saturated form. A < 1 μm fraction was employed.

Two Texas bentonites 'Gelwhite L' and an acid activated bentonite, were obtained from Georgia Kaolin Company. The Gelwhite L sample was copper (II) saturated and fractionated to recover the < 1 μm material.

A sample of kaolinite (Georgia Kaolin Co., catalog number 24 UF), selected because of a larger than normal ratio of edge to basal surface area, was used as supplied.

L- and *D*-isomers of tyrosine and phenylalanine were obtained from Nutritional Biochemical Corporation. Pyrogallol, pyrocatechol, resorcinol and hydroquinone were checked, by means of u.v. spectroscopy, for purity. No impurities were detected.

Methods

To 10 ml of 20 mM aqueous solutions of the various polyhydric phenols, 10 ml of clay suspension (10 g 110°C dry clay/l) and 10 ml of deionized water were added to give a final volume of 30 ml and an equilibrium pH of $6{\cdot}7 \pm 0{\cdot}1$.

After 18 hr, the samples were diluted to mark in 100 ml volumetric flasks. The clay was sedimented by a Sorvall SS-1 angle super-centrifuge. Aliquots of 5 ml of the supernatant solutions were diluted to the mark in 50 ml volumetric flasks with HCl–KCl

buffer at pH 2. Blanks containing only the various polyhydric phenols and deionized water were treated in the same fashion. A Beckman DK-2A spectrophotometer was used to record the u.v. spectra between 3600 and 2200 Å for the various samples and blanks.

To 10 ml of 1 mM aqueous solutions of *L*- and *D*-tyrosine or *L*- and *D*-phenylalanine, various amounts of HCl or NaOH and of clay suspensions (about 20 g 110°C dry clay/1) were added to give a volume of 20 ml at various pH values from 2 to 8. After a chosen time, ranging from 1–15 days, the clay was sedimented by centrifugation and aliquots of the supernatant solution were diluted with an equal amount of 1N HCl. Blanks containing only the amino acids and deionized water, but otherwise made under identical conditions, were prepared and the u.v. spectra of the samples and blanks were recorded over the same wavelength range as the phenolic compounds. The pH values of the solutions were determined with a Beckman Expandomatic pH meter, both before and after runs.

Additional experiments involving *L*- and *D*-tyrosine were performed in order to obtain a better understanding of the mechanisms by which clays and amino acids interact. Kaolinite samples were heated at various temperatures from 445 to 600°C. and then dispersed under sterile conditions in sterilized deionized water and the tyrosine alteration at different pH values was determined. Also, as a check on possible bacterial contamination resulting from the presence of clays, water from clay suspensions was used to make tyrosine solutions and these were measured over periods of one to fifteen days.

In several experiments, Mg^{+2}-saturated montmorillonite and kaolinite were pre-treated with 0·1 M sodium hexamethaphosphate solution for 24 hr and then washed with deionized water to remove free metaphosphate. The mechanism of phosphate fixation is not clearly understood (Grim, 1968, pp. 228–230), but sorption probably occurs on the edge surface of the clay particles. Further, some sodium ion was exchanged for magnesium ion. This experiment should illustrate the influence of the edge surface of clay minerals on the reactions of amino acids and fine-grained silicates.

Additional experiments were run using the various clay minerals, *L*- and *D*-tyrosine, and different additives in order to determine the function of the clay surface and the presence of oxygen on the alteration of the amino acids. With acid activated bentonite and *L*-tyrosine under a nitrogen atmosphere at pH 6, the importance of oxygen on the alteration reaction was evaluated. Further experiments involved the addition of mercuric acetate, an alkyl–mercuric acetate (CMP Acetate, Troy Chemical Corporation), or various amounts of aluminum chloride to a mixture of *L*- or *D*-tyrosine and one of the clay minerals. These mixtures were then adjusted to a equilibrium pH of 6 by the addition of sodium hydroxide. This set of experiments should determine the importance of surface impurities on the alteration of tyrosine.

*Most of the experiments were run in triplicate and were found to be reproducible.

RESULTS

The spectral changes of the various polyhydric phenols, which result from the interaction with montmorillonite, are shown in Fig. 1. Also shown are the spectra for the organic reactants and the respective oxidized products. Such products were formed either by adding an oxidizing agent, e.g. $AgNO_3$, or by raising the pH to 10 and introducing oxygen to the blank samples. Figure 2 illustrates the spectral changes of *L*-tyrosine solutions caused by interaction with Mg-saturated montmorillonite over a period of 6 days at pH 4. Figure 3 demonstrates the similarities in the u.v. spectra of the polyhydric phenols and the aromatic amino acids after interaction with clay minerals or oxidation.

The results of the interaction of the *L*- and *D*-optical isomers of tyrosine and phenylalanine with the various clay minerals, over a pH range of 2–8, are shown in Fig. 4.* The percentage decrease in the absorption band at 2750 Å and the increase in absorption at 3000 Å are plotted against equilibrium pH. Two reaction times were considered: 18 hr and 3 days. Similar plots were made for reaction time at pH 4 (Fig. 5), the phosphate treated samples (Fig. 6), and the heated kaolinite samples (Fig. 7). Figure 8 shows the increase in optical density at 3000 Å for *L*- and *D*- tyrosine in association with an acid activated bentonite treated with various surface additives or protected by a nitrogen atmosphere.

DISCUSSION

Discussion of the oxidation of the phenolic compounds is pertinent. The oxidation of pyrogallol has been studied extensively because of its use for determination of oxygen concentration. Nierenstein (1915) and Campbell and Coppinger (1951) have shown that under alkaline conditions pyrogallol is oxidized by molecular oxygen to a polymerized product. Similar behavior is observed for alkaline

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solutions of the other polyhydric phenols. All of these products have an absorption band in the region of 3000 Å.

Oxidation of polyhydric phenols by electron transfer reactions can result in different products. Kaiser and Weidman (1964) examined the oxidation of hydroquinone by an electron transfer process and found as product a monomeric compound, *p*-benzoquinone, with an intense absorption band at 2420 Å. The periodate oxidation of pyrocatechol (Weidman and Kaiser, 1966) results in the formation of a dimeric compound which does not have a definitive absorption band. The oxidation of pyrogallol by electron transfer results in a product like that formed by O_2 oxidation. The amount of oxidized material can be estimated by the increase in optical density in the region of 3000 Å. Resorcinol is the least reactive of the polyhydric phenols and as a consequence only minor alterations in its u.v. spectrum are observed. Figure 1 shows the spectral changes in the phenolic compounds as a result of oxidation.

Therefore, depending upon the mechanism and the phenolic compound being oxidized, two materials may be formed which absorb light either at a longer or a shorter wavelength than the starting compound. The spectral changes can be explained by the basic principles of u.v. spectrophotometry

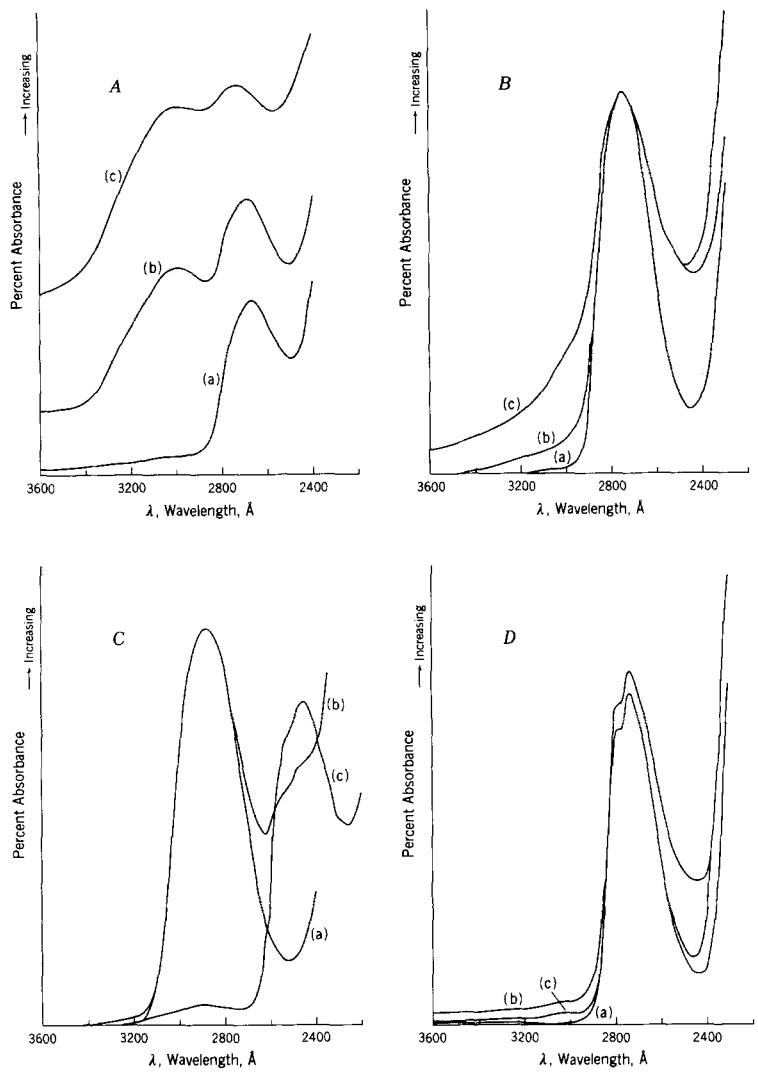

Fig. 1. U.V. spectra of polyhydric phenols. (A) Pyrogallol (B) Pyrocatechol (C) Hydroquinone (D) Resorcinol (a) unoxidized (b) after interaction with clay (c) oxidized product.

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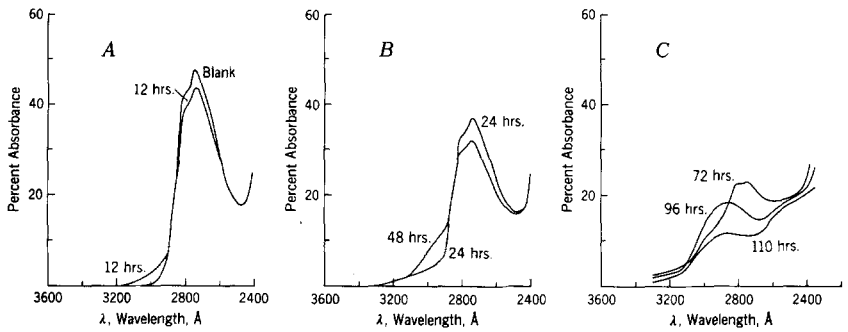

Fig. 2. U.V. Spectra of *L*-Tyrosine after interaction with Mg–montmorillonite as a function of time; (The spectrum after a reaction time of 72 hr is similar to the spectrum of Bityrosine).

(Rao, 1961). Two main factors affect the positions of absorption bands of aromatic compounds: (1) the substituents on the benzene ring; and (2) the extent of conjugation of the electrons, or resonance stabilization energy, which is much less for *p*-benzoquinone than for the oxidation product of pyrogallol. The compound with the greater resonance stabilization energy is expected to absorb radiant energy of longer wavelengths than will compounds with less conjugation of the electrons. On this basis, u.v. spectroscopy can be used to determine alterations in phenolic and other aromatic compounds in the presence of clay minerals has been studied in more detail by Thompson and Moll (1973).

Of great importance to the argument is how these spectral changes, which accompany the oxidation of phenolic compounds, relate to the spectral changes of tyrosine and phenylalanine brought about by contact with clays, deionized water and air. Figure 2 shows that the spectrum of *L*-tyrosine, when in contact with Mg–montmorillonite, changes progressively as a result of chemical alterations in the amino acid. The effect of Mg^{2+} and Cu(II)–montmorillonite on the u.v. spectrum of tyrosine, and of kaolinite on the u.v. spectrum of phenylalanine, are compared in Fig. 3 to the spectral changes of pyrogallol and hydroquinone. It is apparent that the observed spectral changes of the aromatic amino acids can be a result of oxidation. The interaction of *L*-tyrosine with Cu(II)–montmorillonite produces a compound which absorbs light of a shorter wavelength. The reaction probably involves electron transfer to the Cu(II) ion and the formation of a monomeric compound similar to oxidized hydroquinone. The action of Mg–montmorillonite on *L*-tyrosine yields a product which absorbs light of a longer wavelength, similar to oxidized pyrogallol, and therefore is probably polymerized material. The interaction of phenylalanine with kaolinite indicates that this amino acid, while less reactive than tyrosine, is altered, with an increased absorption at 3000 Å. This lower reactivity is not surprising since phenylalanine is not a phenolic compound.

ALTERATION OF TYROSINE AND PHENYLALANINE IN RELATION TO pH

Figure 4 shows the percentage decrease in the optical density of the parent absorption bands of

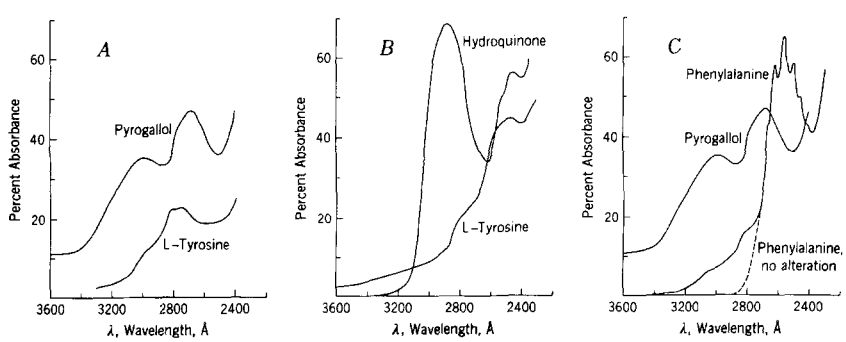

Fig. 3. Comparison of the u.v. spectral changes of some oxidized *L*-amino acids with those of polyhydric phenols. (A) Comparison of *L*-tyrosine and pyrogallol. (B) Comparison of *L*-tyrosine and hydroquinone. (C) Comparison of *L*-tyrosine and pyrogallol.

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tyrosine and phenylalanine after reaction times of 18 hr and of 3 days as a function of equilibrium pH. The increase in optical density at 3000 Å for the various compounds is also shown. From these data plots, the following points have to be considered. A decrease in the intensity of the absorption band of the amino acid without a corresponding increase in the optical density at 3000 Å indicates three possible mechanisms. First, the clay has sorbed the amino acid or intermediates or products formed from the amino acids. Second, products formed from the amino acids are not sorbed by the clay, but have no absorption band at 3000 Å. Third, bacterial contamination has removed the amino acid.

An increase in optical density in the region of 3000 Å, with a corresponding decrease in the intensity of the parent band, was observed for the following clay samples and amino acids: *L*- and *D*-tyrosine on the acid activated clay with 18 hr reaction time; *L*-tyrosine on kaolinite, 18 hr reaction time; and *L*-phenylalanine on kaolinite

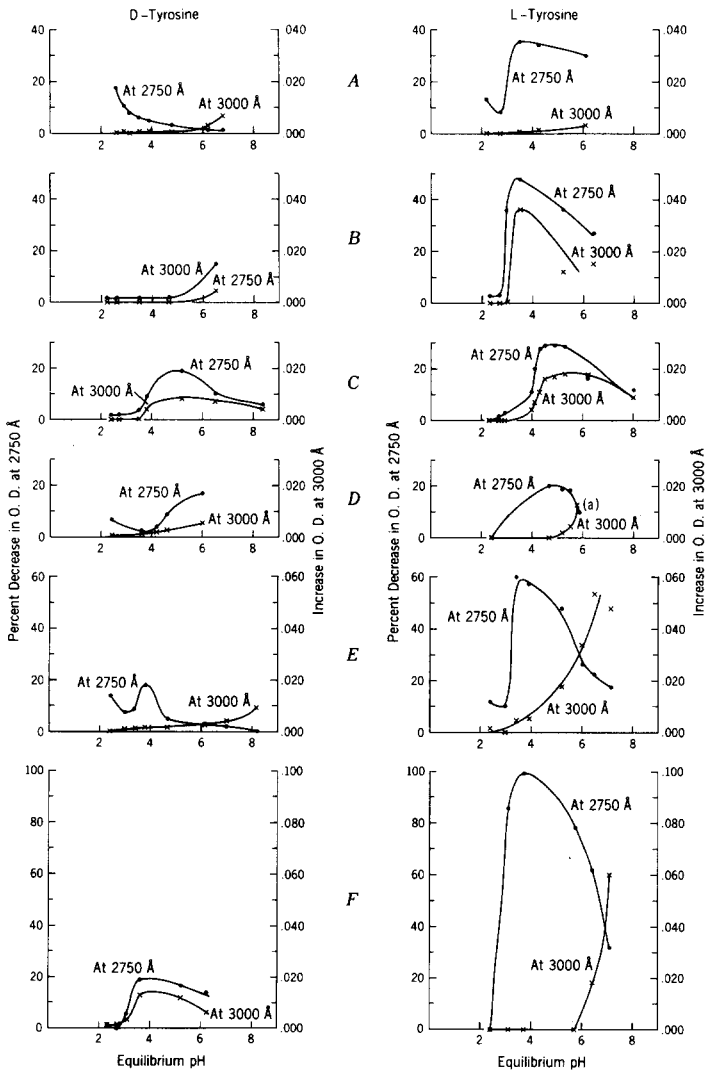

Fig. 4. The alteration of *L*- and *D*-amino acids by clay minerals as a function of pH; (percentage decrease in optical density at 2750 Å; *x* increase in optical density at 3000 Å). (A) Mg–Montmorillonite and *L*- and *D*-tyrosine (18 hr reaction time). (B) Kaolinite and *L*- and *D*-tyrosine (18 hr reaction time). (C) Acid activated bentonite (3 day reaction time). (D) Kaolinite and *L*- and *D*-phenylalanine (3 day reaction time). (E) Mg–montmorillonite and *L*- and *D*-tyrosine (3 day reaction time). (F) Kaolinite and *L*- and *D*-tyrosine (3 day reaction time).

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with 3 day reaction time. This behavior is most likely the result of oxidative degradation of the amino acid. This concept stems from work of Weil *et al.* (1951) and Weil (1965) on the oxidation of tyrosine and other aromatic amino acids by oxygen in the presence of a dye (methylene blue) and light. The changes in the u.v. spectrum of *L*-tyrosine produced by the photochemical action of methylene blue are the same as the spectral changes of *L*-tyrosine after contact with montmorillonite (Fig. 2). Dye activation studies have shown that the oxidation of tyrosine is pH dependent and is related to the ionization of the phenolic group. In the case of photo-oxidation, a sharp increase in the oxidation rate of *L*-tyrosine occurs at pH 7·0, which corresponds to the ionization of the phenolic group. If clay is present instead of methylene blue, a similar increase apparently occurs, but near pH 4. Appreciable oxidation at a lower pH in the presence of clay is probably caused by enhanced dissociation of the phenolic group by the clay.

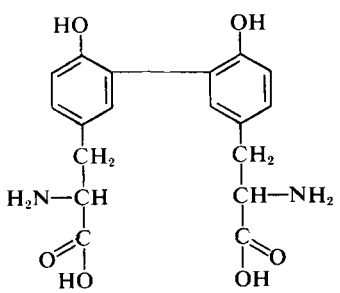
The reactions of *L*- and *D*-tyrosine in the presence of Mg–montmorillonite, for periods of 18 hr and 3 days, respectively, are similar in that the intensity at 2750 Å decreases at pH values less than 5. Sorption of the organic material would provide a possible explanation. The organic material could be any one of the following species, the amino acid, the activated intermediate (e.g. semi-quinone) or the oxidized material. For montmorillonite, the amino acid is most likely the material sorbed at values below pH 3. The amino acid ($pK_a = 2{\cdot}2$) is protonated under these conditions and therefore can be sorbed by cation exchange. This mechanism is supported by the fact that if the pH is raised above 3 under these conditions, some of the tyrosine is released from the clay. The montmorillonite is rapidly removed by centrifugation after the adjustment of pH. At pH > 3, a complex might form between the amino acid, or more likely an activated intermediate, and montmorillonite or kaolinite. This complex probably does not involve one of the observed oxidized products, since observation of a product and adsorption as postulated would be mutually exclusive. Another possible explanation is oxidative degradation to a product which does not absorb u.v. radiation. This point will be discussed in more detail in the next section because the most probable explanation will depend upon the reaction time and pH at which the behavior was observed. Under nitrogen (Fig. 8) the optical density at 3000 Å is about the same as that under aerobic conditions. However, substantial reaction does occur, which suggests that atmospheric oxygen is not essential for the oxidation of tyrosine.

EFFECT OF INTERACTION TIME OF *L*-TYROSINE

Figure 5 gives the spectral data for *L*-tyrosine solution after contact with Mg–montmorillonite or kaolinite for various periods of time, from 6 hr to 6 days, as well as for *L*-tyrosine in water removed from the Mg–montmorillonite suspension. Figure 2 shows the actual spectra for the *L*-tyrosine after association with Mg–montmorillonite. For the process illustrated by Figs. 2 and 5, the initial pH of 3·6 slowly increased to 4·2 during the course of the reaction.

It is seen that both Mg–montmorillonite and kaolinite cause an immediate reduction in intensity of the 2750 Å band. The optical density of 3000 Å increases immediately for the kaolinite-treated sample, which indicates the rapid formation of an altered product. There is approximately a 2 day lag before a similar increase begins to appear for the Mg–montmorillonite. After a one day interaction time for kaolinite and a 4 day interaction time for montmorillonite, the optical density at 3000 Å reaches a maximum and then begins to decrease. This decrease, as well as the decrease in the band at 2750 Å, is most likely a result of a second stage of oxidation brought about by the presence of the clay. The time study of *L*-tyrosine associated with clay suggests that there may be at least two, if not more, steps involved in the alteration and that the mechanism is dependent upon the clay mineral present.

The products formed as a result of the photo-oxidation of *L*-tyrosine have been studied by Lehrer and Fasman (1967). The initial product appeared to be bityrosine, which gives a u.v. spectrum almost identical with that observed in the present work (Fig. 2). The dimeric products are produced as a result of the combination of two phenoxy radicals. Further, the irradiation effect



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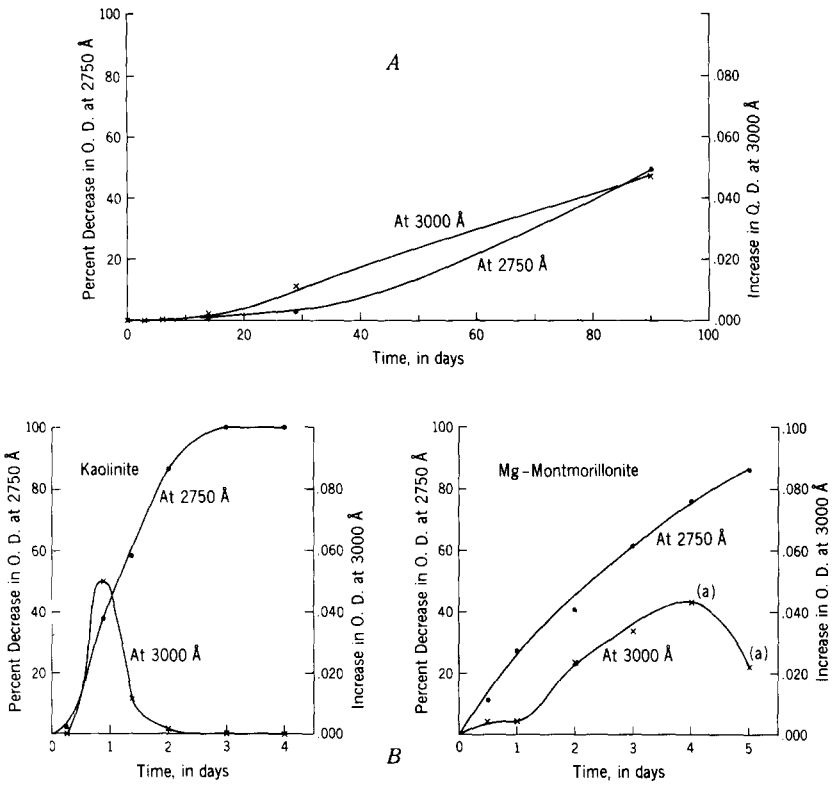

Fig. 5. The alteration of *L*-tyrosine as a function of time (·percentage decrease in optical density at 2750 Å; *x* increase in optical density at 3000 Å). (A) U.V. spectral changes of *L*-tyrosine in deionized water removed from a montmorillonite suspension as a function of time. (B) U.V. spectral changes of *L*-tyrosine by Mg-montmorillonite and kaolinite as a function of time at equilibrium pH 4.

did not depend upon the presence of oxygen. However, oxygen did cause additional oxidation of the monomer and dimer.

Weil's work with methylene blue, mentioned earlier, showed that u.v. light, under aerobic conditions, promotes the dye to an activated state. This activated dye then reacts with tyrosine to produce a dye radical to regenerate the dye and with the tyrosine radical to form an oxidized product. Weil's results correspond closely to those of this investigation.

Comparison of the same reaction times for clay-free tyrosine solution (Fig. 5a) and for solutions containing clay (Fig. 5b) shows little or no alteration of *L*-tyrosine in the blank relative to that which occurs when clay is present. Note that tyrosine alone is altered to some extent after very long reaction times. The rate and mechanism of alteration of *L*-tyrosine in water varies with the presence or absence of clay, and also differs between kaolinite and montmorillonite. If the alteration of *L*-tyrosine by kaolinite or montmorillonite were due only to bacterial contamination, the two curves would nearly be the same, and the sample of *L*-tyrosine solution separated from the clay suspension would have been altered at a rate similar to the rate observed when clay was present. Since there was a rapid but different rate with the two clay samples, and the solution separated from the clay was slow to alter, bacterial contamination seems remote.

The observed differences between the behavior of *L*-tyrosine on montmorillonite and kaolinite may be due to sorption and stabilization of the activated intermediate (a radical or radical ion) by montmorillonite, but not by kaolinite. With kaolinite, a dimer may be formed which can then be oxidized to a material which does not absorb u.v. light. That the montmorillonite surface appears to interact strongly with the radical is indicated by the decrease in optical density at 2750 Å with no large increase in optical density at 3000 Å for the first 1-1/2 to 2

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days. This interaction slows down the formation of the dimer and its subsequent oxidation. Such behavior of a radical, as indicated above, would explain the differences in the data shown in Fig. 5b for the kaolinite and Mg–montmorillonite samples.

EFFECT OF SODIUM HEXAMETAPHOSPHATE TREATMENT

Figure 6 shows the effect of phosphate-treated montmorillonite and kaolinite on *L*-tyrosine. Comparison of Figs. 6 and 4 shows that the extensive sodium phosphate treatment has altered the behavior of the clays when associated with *L*-tyrosine. In the case of montmorillonite the per cent decrease in optical density at 2750 Å at pH values below 3 has increased with no change in optical density at 3000 Å. This observation may be explained by the conversion of the Mg–montmorillonite to the sodium form, which then undergoes cation exchange reactions more readily than does the magnesium form. The per cent decrease in optical density at 2750 Å above pH 3, after 3 days, is less with the phosphate-treated montmorillonite than with the untreated one, but the increase at 3000 Å is greater. In the phosphate-treated kaolinite samples the decrease in intensity at 2750 Å is less, but the oxidation reaction does occur. Most likely, the sorption and alteration of tyrosine by clay minerals is closely related to the mechanism by which polyphosphates are sorbed and retained by the various layer silicates. These observations also indicate that while the clay surfaces do have a strong effect on amino acids, the alteration of tyrosine is not solely a function of the crystal edges. However, at pH > 4, tyrosine sorption on montmorillonite does most likely involve the crystal edges. The differences in the behavior of montmorillonite and kaolinite indicate that bacterial contamination cannot be the primary explanation of the alteration of tyrosine.

EFFECT OF PRE-HEATING THE CLAY

In Fig. 7 are shown the results of experiments in which sterilized *L*-tyrosine solutions were put in contact with kaolinite that had been pre-heated overnight at 445, 550 and 600°C. This heat treatment converted the kaolinite to dehydroxylated metakaolin which retains a layer structure, but in a highly disordered form. The alteration of *L*-tyrosine is reduced by the heating, which would kill any organisms, but is not stopped. This reduction in the oxidizing power of the clay, as a function of temperature, can be related to a reduction in the surface reactivity of the mineral. Alteration of tyrosine by clay under sterile conditions and no tyrosine alteration in the blanks, are additional evidence that a surface and not a biological reaction is operative.

EFFECTS OF ADDITIVES ON THE REACTIVITY OF CLAY MINERALS

The initial variant studied was that of nitrogen replacing oxygen in the system. In the case of acid-activated bentonite, alteration of tyrosine occurred both in nitrogen atmosphere and oxidizing atmospheres, as was found to be the case in the photo-oxidation of tyrosine (Lehrer and Fasman, 1967). The addition of an alkyl–mercury compound, a

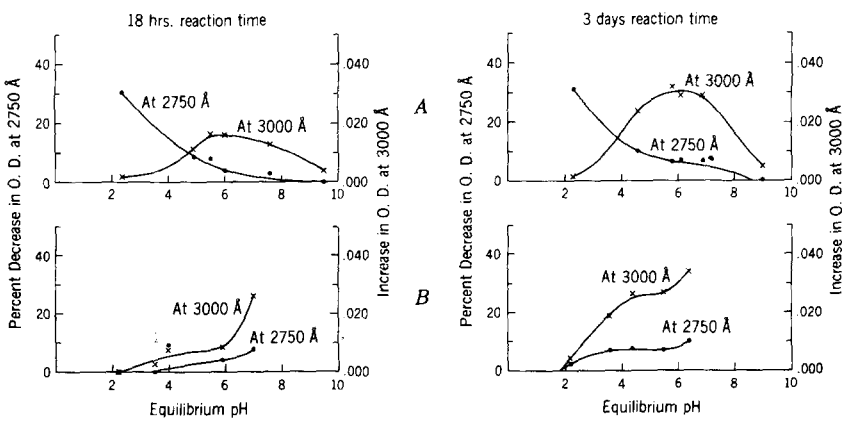

Fig. 6. The alteration of *L*-tyrosine by clay minerals treated with calgon as a function of pH. (·Percentage decrease in optical density at 2750 Å; *x* increase in optical density at 3000 Å) (A) U.V. spectral changes of *L*-tyrosine by Mg–montmorillonite treated with calgon as a function pH. (18 hr and 3 days reaction times). (B) U.V. spectral changes of *L*-tyrosine by kaolinite treated with calgon as a function pH (18 hr and 3 days reaction times).

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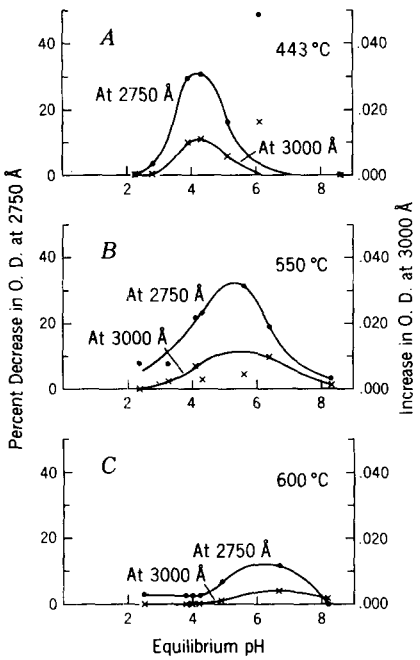

Fig. 7. The alteration of *L*-tyrosine by kaolinite, heated to various temperatures, as a function of pH. (A) Heated to a temperature of 443°C. (B) Heated to a temperature of 550°C. (C) Heated to a temperature of 600°C.

bactericide (CMP–Acetate), to the various clay *L*-tyrosine mixtures did not prevent the alteration of the amino acid. In fact, a sample of acid-activated bentonite and *L*-tyrosine with CMP–Acetate was altered to a greater extent within one day than was the same clay and *L*-tyrosine mixture without the mercuric compound. The fact that a bactericide did not prevent, but actually accelerated, the alteration of tyrosine provides additional proof that the amino acids were not being altered by bacterial action but by the clay surfaces.

This influence of the clay surfaces was examined further by precipitating either mercuric hydroxide or various amounts of aluminum hydroxide on the clays in the presence of *L*- and *D*-tyrosine. Figure 8 shows the results of the various surface treatments by plotting the increase in optical density at 3000 Å for *D*- and *L*-tyrosine in association with an acid-activated bentonite. Treatment with mercuric acetate plus sodium hydroxide completely degraded the tyrosine as indicated by the disappearance of the 2750 Å band. This band disappearance is probably due to an electron transfer mechanism similar to that observed with the Cu(II)-saturated montmorillonite. The effect of precipitated aluminum hydroxide is of extreme interest because such a coating should not be a bactericide nor become involved in electron transfer reactions. After three days the apparent alteration of tyrosine decreases in the order *L*-tyrosine + clay, *D*-tyrosine + clay, *L*-tyrosine + clay + 4% $Al(OH)_3$, and *L*-tyrosine + clay 20% $Al(OH)_3$. Thus, the presence of $Al(OH)_3$ inhibits the reaction, a result which again supports the conclusion that the alteration of tyrosine is a surface reaction and is not due to a bacterial contamination.

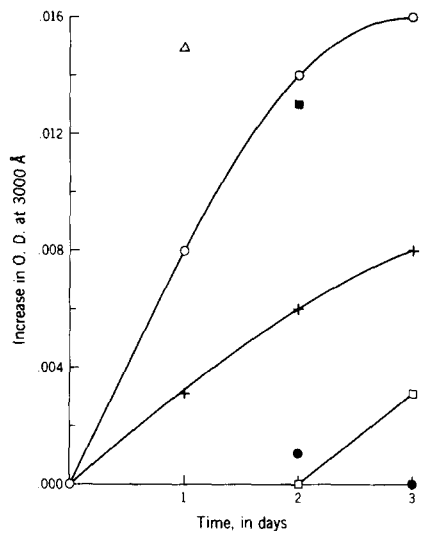

Fig. 8. The influence of additives on the surface acidity of an acid activated bentonite: ○ *L*-Tyrosine + Clay × *D*-Tyrosine + Clay △ *L*-Tyrosine + Clay + CMP–Acetate ● *L*-Tyrosine + Clay + 20% $Al(OH)_3$, □ *L*-Tyrosine + Clay + 4% $Al(OH)_3$, ■ *L*-Tyrosine + Clay + Nitrogen.

CONCLUSIONS

The present experiments were carried out to determine if differences exist between the adsorption or alternation of *L*- and *D*- optical isomers of an amino acid by interaction with clay mineral surfaces in aqueous solutions. Tyrosine was selected because the concentration could easily be measured by u.v. spectrophotometry, and because of its ability to undergo oxidative degradation.

The results show, in all the experiments conducted, that the *L*-isomer is altered at a faster rate than the corresponding *D*-isomer if clay and water are present (Figs. 4 and 8). Montmorillonite at pH < 3 sorbs tyrosine by a cation exchange mechanism, as shown by the release of the tyrosine by treatment with HCl solution. This observation agrees with the behavior of other protonated nitrogenous compounds (Thompson and Brindley, 1969). Tyrosine is oxidized in aqueous solutions (pH 4–8) if clay is present, with the *L*-isomer being altered at

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a greater rate. The altered products have u.v. spectra which closely resemble those of oxidized polyhydric phenols and also the spectrum of bityrosine. Treatments designed to modify the clay surface, high temperature, and the precipitation of $Al(OH)_3$ also modify the reactivity to varying degrees.

The possible existence of bacterial degradation is of importance to the conclusions. Any of the experiments, when taken alone, do not give conclusive evidence, but together the results almost certainly rule out bacterial contamination. The observation that an alkyl mercury compound does not halt the clay-induced degradation of tyrosine indicates that bacterial contamination is not operative. The reduction in reactivity caused by precipitation of $Al(OH)_3$ on the clay surface also is contrary to bacterial degradation as the operative mechanism. Finally, since tyrosine alteration by montmorillonite is different from that followed by kaolinite, a mechanism based on bacterial degradation is untenable.

The following reactions are proposed for the oxidation of tyrosine, catalyzed by clay minerals in the presence and in the absence of oxygen.

In the presence of oxygen.

$$\text{Clay} + O_2 \rightleftharpoons \text{Clay } (O_2)^*$$
$$\text{Clay } (O_2)^* + \text{Tyrosine} \rightleftharpoons \text{Clay } 2(O^{\cdot -1}) + \text{Tyrosine} + 2H^+$$
$$\text{Clay } 2(O.^{-1}) + \text{Tyrosine.} + 2H^+ \rightleftharpoons \text{Tyrosine–Tyrosine} + \text{Clay } 2(O^{\cdot -1})2H^+$$
$$\text{Tyrosine–Tyrosine} + \text{Clay } 2(O^{\cdot -1})2H^+ \rightleftharpoons \text{Tyrosine–Tyrosine (oxidized)} + \text{Clay } (H_2O_2)$$

(Tyrosine–Tyrosine (oxidized) does not absorb in the u.v. range)

In the presence of nitrogen

$$\text{Clay} + 2\ \text{Tyrosine} \rightleftharpoons \text{Clay } 2(H^+) + 2\ \text{Tyrosine}$$
$$\text{Clay } 2(H^+) + 2\ \text{Tyrosine}^- \rightleftharpoons \text{Clay } 2(H^{\cdot}) + 2\ \text{Tyrosine}$$
$$\text{Clay } 2(H^{\cdot}) + 2\ \text{Tyrosine}^{\cdot} \rightleftharpoons \text{Clay} + H_2 + \text{Tyrosine–Tyrosine}$$

In conclusion, clay minerals sorb either the *L*- or *D*-optical isomer, but neither preferentially. However, clay minerals do catalyze reactions of tyrosine, and the *L*-isomer is the more rapidly altered.

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Résumé – Ces recherches portent sur l'activité catalytique des argiles vis-á-vis des amino-acides, la tyrosine en particulier. Des polyphénols ont été également étudiés afin de mieux comprendre les réactions concernant la tyrosine.

En dessous de pH 3, la tyrosine est adsorbée sur les minéraux argileux par échange cationique. Au-dessus de pH 3, la dégradation par oxydation de la tyrosine survient, l'isomère L s'altérant plus rapidement. La vitesse d'altération dépend de la nature du minéral argileux, des modifications de surface telles que le traitement aux polyphosphates, du chauffage et de la présence de cuivre, d'aluminium et de mercure. Un mécanisme par radicaux libres est proposé pour expliquer l'altération.

Kurzreferat – Die Untersuchungen betreffen die katalytische Wirkung von Tonmineralen auf Aminosäuren, insbesondere Tyrosin. Mehrwertige Phenole wurden in die Untersuchungen einbezogen, um zum Verständnis der Tyrosinreaktion beizutragen.

Unterhalb pH 3 wird Tyrosin durch Kationenaustausch an Tonmineralen adsorbiert, oberhalb pH 3 findet ein oxidativer Abbau des Tyrosins statt, wobei das L-Isomer schneller umgesetzt wird. Die Abbaurate hängt von der Art des Tonminerals und von Oberflächenmodifikationen ab, wie sie durch Behandlung mit Polyphosphaten, durch Erhitzen, sowie in Gegenwart von Kupfer, Aluminium und Quecksilber entstehen. Für die Umwandlung wird ein Mechanismus vorgeschlagen, der auf der Beteiligung freier Radikale beruht.

Резюме — Эти исследования относятся к каталитической активности глин на аминокислоты, особенно на тирозин. Для лучшего понимания реакции тирозина включили многоатомные фенолы.

Ниже pH 3, тирозин катионообменом адсорбируется глинистыми минералами. Выше pH 3, происходит окислительная деградация тирозина, при чем скорее всего изменяется L-изомер. Степень изменения зависит от отдельного используемого глинистого минерала, от поверхностных изменений вследствие обработки полифосфатом, от нагрева, от присутствия меди, алюминия и ртути. Полагают, что в изменениях играет роль механизм свободных радикалов.