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John J. McCullough and Richard M. Lemmon

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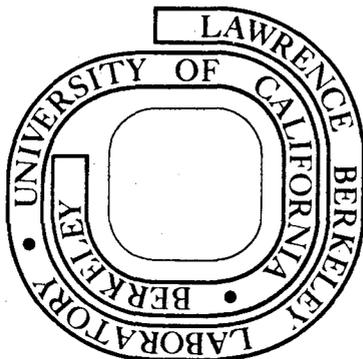
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The Question of the Possible Asymmetric Polymerization of Aspartic
Acid on Kaolinite

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Summary. We have been unable to confirm the recent report that kaolinite catalyzes the polymerization of aqueous D- and L-aspartic acid at different rates. In experiments where DL-asp was used, no induced optical rotation was found in the reaction solution. No evidence for polymer (or other product) formation was found when L-asp-2-¹⁴C was used, and products were searched by paper chromatography and X-ray film autoradiography. Asp is adsorbed by kaolinite, but no selectivity for one or the other enantiomer was observed.

Key-Words: Aspartic Acid - Kaolinite - Optical Activity - Polymerization - Adsorption.

Introduction

It has been reported that, in water solution and in the presence of certain minerals, amino acids will polymerize to form polypeptides (Degens and Matheja, 1968, 1970; Degens, Matheja, and Jackson, 1970). Since there is no satisfactory explanation for the almost exclusive appearance of L-amino acids (rather than the D-isomers) in natural proteins (Calvin, 1969; Harada, 1970; Elias, 1972; and Bernstein, Lemmon and Calvin,

1972), we were particularly interested in the recent report that the presence of kaolinite causes L-aspartic acid (L-asp) to polymerize more rapidly than the D-enantiomer (Degens et al., 1970). The results were obtained by heating L-, DL- and D-asp (0.01 M in water) separately with kaolinite, and the disappearance of asp was observed with an amino-acid analyzer. It was claimed that 7% of L-asp "polymerized" in three days at 90°C, but only 3% of the D-enantiomer had so reacted. In four weeks, 25% of L- but only 5% of D-asp had disappeared. There is no known asymmetry of kaolinite structure (Brindley, 1961; and Grim, 1968) that could give the necessary diastereomeric interaction between the mineral surface and the asp enantiomers (Mislow, 1966). We therefore studied the interaction of asp with kaolinite under the above conditions, using optical rotation measurements and ¹⁴C labeling to monitor optical induction and product formation respectively. The disappearance of the asp was also followed with an amino acid analyzer, and the rates for D- and L-asp were compared.

Experimental

Aspartic acids were from Calbiochem, A grade, and were recrystallized from ethanol-water for some experiments. The results were independent of whether the asp was recrystallized or not. Kaolinite was from Fisher Scientific Co., Lot no. 715655, and had an approximate average particle diameter of 2.5 μ , measured under the microscope.

Attempted Observation of Optical Rotation in Reaction of DL-asp. A solution of DL-asp (50 ml, 0.01 M) and kaolinite (5.0 g) were stirred and heated at 90°. The system was sterilized initially by heating to 100° for 30 min, and protected from bacterial contamination by cotton plugs

and biocide solutions. The reaction flask was fitted with a condenser and a septum. Through the latter, aliquots were removed with a syringe. A control experiment without kaolinite was also done. The pH of the clay mixtures was 3.6. Aliquots (6.0 ml) were withdrawn after reaction times of three days, one week, and four weeks. The clay was removed by centrifugation and/or filtration, and the clear solutions were examined immediately for optical rotation in a Cary Model 60 spectropolarimeter. The instrument was calibrated with 0.01 M solutions of DL-asp, to which accurately measured amounts (10^{-5} - 10^{-3} M) of D-asp were added. Thus, we determined that a 1% excess of D-asp in 0.01 M DL-asp can easily be observed. The rotatory dispersion was scanned from 300-190 nm, and D-asp had $[\phi]_{214 \text{ nm}}^{\text{H}_2\text{O}} = 1240^\circ$, in fair agreement with a recently reported value for L-asp (Jennings, Klyne, and Scopes, 1965). No detectable rotation was found in any of the aliquots, and the dispersion curves of the starting solution (unheated), control solution, and sample from the kaolinite mixture were all identical.

Search for Products in the Asp-kaolinite Reaction Using ^{14}C -asp. A solution of L-asp-2- ^{14}C (20 ml, 0.01 M; specific activity 100 $\mu\text{Ci}/\text{mmole}$) was stirred and heated at 90° with kaolinite as described in the previous experiment. Aliquots were withdrawn, cooled, and filtered, and 0.3 ml samples were spotted on Whatman No. 1 paper. The spots were developed with phenol:water:acetic acid (34:6.4:0.4 by vol.) (A) and with n-butanol:propionic acid:water (37:18:24.5 by vol.) (B) in separate, one-dimensional chromatograms. The paper was dried and examined by X-ray film autoradiography. In both solvent systems, only asp was found on the chromatograms, as evidenced by the single spot at $R_f = 0.25$ in solvent (A), and at 0.30 in (B). (No radioactivity was left at the chromatographic origins, and,

if the polymer were there, we could have detected a one percent yield.) These spots were identified by comparison of the R_f values with authentic L-asp- ^{14}C , and by spraying the paper with ninhydrin. The outlines of the ninhydrin-developed spots were co-incident with the radioactivity blackened areas on the films. The R_f values were also in reasonable agreement with literature values for asp (Heyns and Anders, 1951; Kopple, 1966).

Determination of Asp Concentration by Amino Acid Analysis. Solutions of D- and L-asp were heated with kaolinite in separate experiments as described above. The apparatus (containing the kaolinite) was assembled and sterilized in an autoclave at 115° , and the asp solutions were also sterilized in the same way. The mixtures (50 ml of 0.01 M asp and 5 g kaolinite) were stirred and heated at 90° , and aliquots were withdrawn periodically. The reactions were terminated after 16 days, since this corresponds to about 3 half-lives for the racemization of asp at 90° , which we found to occur at similar rates with or without kaolinite present. This racemization is presumably related to the well-known reversible deamination of asp (Bada and Miller, 1970), and was also observed by Degens (Dr. E. T. Degens, personal communication).

The aliquots were centrifuged, and diluted to give asp solutions in the range $1 \mu\text{m}/\text{ml}$. The asp in each was determined on a Beckman Model 120 amino-acid analyzer; elution was with buffer at pH 3.28, and detection was by the ninhydrin color reaction. The color yield was calibrated with standard solutions of asp. The elution volume of asp was 57 ml, and this was the only component detected in the reaction samples.

Although these determinations were carried out with great care, and several runs were performed, we had difficulty in obtaining consistent results. The results from one run are shown in the figure.

Apparently, the asp concentration does fall by 10-15% of its initial value, remaining constant after 3 days. Within the limits of reliability of the experiment, the behavior of the asp enantiomers are identical.

Asp determination of aliquots after hydrolysis with 6 N HCl at 110° showed no significant increase in asp over the unhydrolyzed samples. This is consistent with the above tracer experiment, in showing that there is little or no peptide formation.

Discussion and Conclusions

The results of the three kinds of experiments form a consistent picture. The amino acid analysis shows that although 10-15% of the 0.01 M asp is removed from the solution in the first three days of heating, no polymer appears to be formed. Thus, the result is consistent with absorption of 10-15% of the asp. Also, the asp enantiomers both behaved in the same way, and the absorption is apparently unselective. The optical rotation measurements are consistent with this conclusion; absorption or reaction of one enantiomer selectively would have produced an excess of the other enantiomer in solution. Had the reported rate differences for D- and L-asp been real, there should have been a 20% excess of D- over L-asp in solution after four weeks of reaction, and this excess would have been observed.

The ¹⁴C labeling experiment corroborates the results of the amino acid analyses in showing that no peptides are formed. Unless the reaction produces a narrow range of polypeptide molecular weights all of which have the same R_f as asp in both solvent systems--an unlikely event--the tracer experiment shows that no peptides are formed in our experiments.

We therefore draw the following conclusions. First, it is very doubtful that kaolinite catalyzes the polymerization of asp in water at 90°; all that seems to occur is absorption of 10-15% of the asp. Second, kaolinite fails to show any stereoselectivity for L- over D-asp. Professor W. A. Bonner and Dr. J. Flores have recently informed us of their independent studies on the aspartic acid-kaolinite system, in which they came to the same conclusions.

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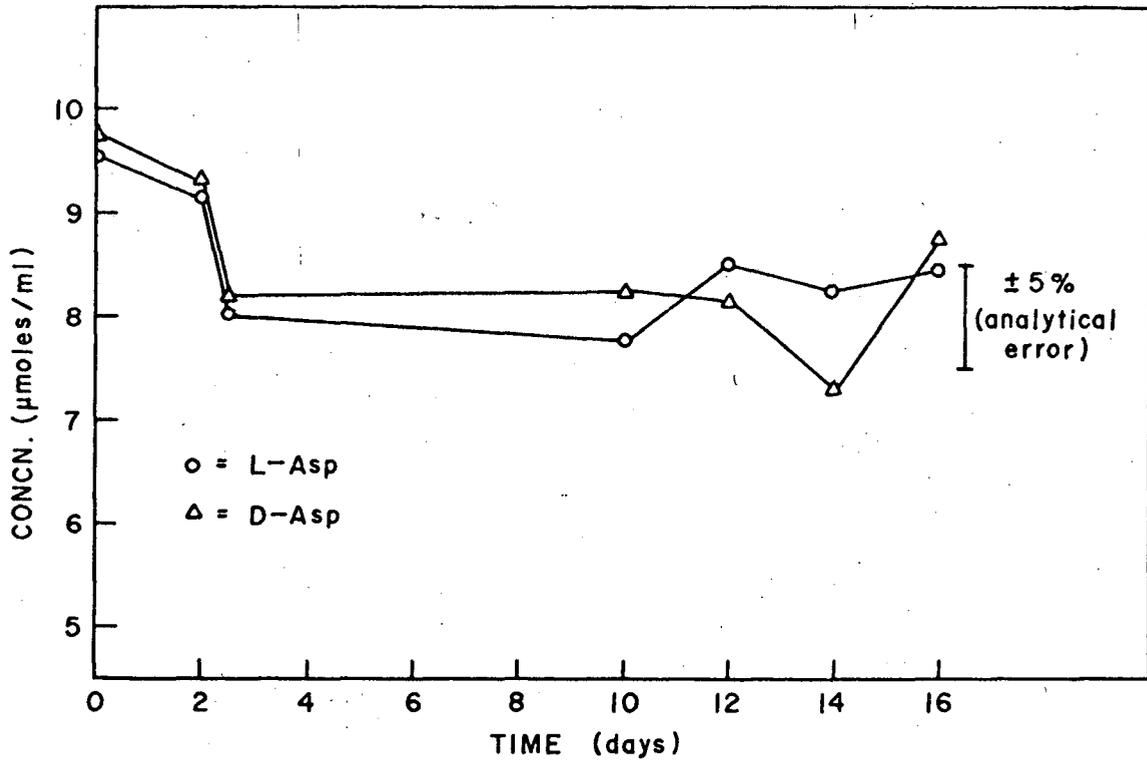
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FIGURE CAPTION

Fig. 1. Change of asp concentration on heating 0.01 M solutions with kaolinite at 90°C. The estimated analytical error is $\pm 5\%$.



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