Evidence for Selective Adsorption and Polymerization of the L-Optical Isomers of Amino Acids Relative to the D-Optical Isomers on the Edge Faces of Kaolinite

In a series of experiments I showed that kaolinite K-6 supplied by the Fisher Scientific Co. catalyzed polymerization of the L-optical isomers of aspartic acid and serine preferentially with respect to the D-optical isomers[1,2]. The catalytically active sites on the clay crystals were assumed to be located on the enantiomorphous edge (hk) faces[1]. The present work confirms these results and furnishes information about the kinetics and mechanism of the clay-amino acid interaction.

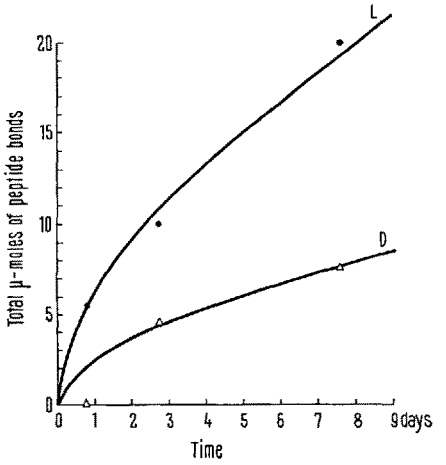
5 ml aliquots of 0.01 M solutions of L- and D-aspartic acid were incubated with 500 mg portions of kaolinite K-6 at 90 °C for varying lengths of time in sealed Pyrex tubes. Aspartic acid solutions without clay served as blanks. After incubation, material adsorbed to the clay was extracted with 2 N NH_3, taken to driness at room temperature, redissolved in water, and analyzed for peptides by the biuret method[3]. Adsorption of L- and D-phenylalanine by the kaolinite under different pH conditions was also investigated. 2.0000 g portions of kaolinite K-6 were suspended in replicate 10 ml aliquots of 0.001 M L- and D-phenylalanine solutions. In one set of runs, the solvent was water, and the initial pH 5.8; in another set, the solvent was 0.01 N HCl, and the initial pH 2.0. All samples, together with blanks, were shaken continuously at room temperature for 24 h, after which the clay was spun down, and the supernates withdrawn for analysis. All solutions were passed through 0.45 μm millipore filters and analyzed by UV-spectrophotometry at a wavelength of 260 nm. The quantities of phenylalanine adsorbed by the kaolinite were computed on the basis of the differences between the absorbances of the sample solutions and the mean absorbances of phenylalanine blanks to which no clay had been added. The significance of the differences between the sample sets was tested statistically at the 5% level by means of the t-test and the Mann-Whitney U-test.

The results of the aspartic acid experiment (Figure) showed that the L-optical isomer was consistently much more highly polymerized than the D-optical isomer. Polymerization proceeded logarithmically in both the L- and D-samples, but the L-isomer polymerized at a much higher rate than the D-isomer. No peptides were detected in aspartic acid solutions heated in the absence of kaolinite, showing that polymerization was catalyzed by the clay.

The phenylalanine data (Table) indicated that the L-optical isomer was more highly adsorbed than the D-optical isomer at pH 5.8, the results being significant at the 4% level. At pH 2.0, the percentage of D-phenylalanine adsorbed was not significantly different than at pH 5.8; however, the percentage of L-phenylalanine adsorbed at pH 2.0 was about half the percentage adsorbed at pH 5.8, the difference being significant below the 1% level. Thus, the addition of 'foreign' H-ions had no effect on the adsorption of D-phenylalanine but markedly inhibited adsorption of L-phenylalanine. This effect is interpreted as follows. The edge faces of the kaolinite crystals preferentially adsorbed the L-optical isomer, whereas the 001 faces adsorbed both optical isomers equally. At pH 2.0 most of the phenylalanine molecules were cationic, and the crystal edges had a net positive charge[4], resulting in mutual repulsion rather than adsorption. This would account for the drop in the percentage of L-phenylalanine adsorbed at the lower pH. Possibly suppression of the ionization of the carboxyl group of phenylalanine also contributed to the inhibition of adsorption on the edge faces of the mineral. The fact that lowering the ambient pH had no detectable effect on the adsorption of D-phenylalanine is readily explained if the D-molecules were almost exclusively adsorbed on the 001 faces, which, in contrast to the edge faces, were presumably insensitive to the pH change[4]. Evidently the edge faces discriminated against the D-optical isomer on the basis of configuration, even under pH conditions favorable for adsorption. This interpretation is consistent with the fact that the edge faces, unlike the 001 faces, are enantiomorphous. The phenylalanine data support the hypothesis that polymerization of aspartic acid was catalyzed by the edge faces of the kaolinite.

Data on the adsorption of L- and D-phenylalanine under different pH conditions

Initial pH	Optical isomer	Mean % adsorbed ± standard error ($t_{0.95}\, s/\sqrt{N}$)	Standard deviation	No. of replicate samples
5.8	L	19.0 ± 3.58	3.87	7
	D	15.0 ± 3.16	3.42	7
2.0	L	9.77 ± 4.30	3.44	5
	D	15.3 ± 7.13	5.70	5



Plot showing rates of peptide bond formation in solutions of L- and D-aspartic acid heated in the presence of kaolinite (data from biuret analyses). ○, L-asp. △, D-asp.

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The results of this study suggest that polymerization of adsorbed amino acids on the edge faces of dioctahedral clay minerals could have played a significant role in the pre-biotic origin of optically active polypeptides, given a range of pH conditions not radically different from that of most contemporary natural waters. The data also suggest that clay minerals, by preferentially adsorbing and reacting with certain organic compounds, or with particular isomers of these compounds, may exert a selective influence in the geochemistry of sedimentary organic matter (cf. [5]).

A more complete manuscript on this work will be published elsewhere[1,6].

Zusammenfassung. Auf Kaolinitkristallen werden die L-optischen Isomeren der Aminosäuren vollständiger adsorbiert und schneller polymerisiert als die D-optischen Isomeren.

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7 September 1970.

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The Redox Cleavage of the Sulfur-Sulfur Bond and Carbon-Sulfur Bond in Organic Disulfides by a Model Coenzyme

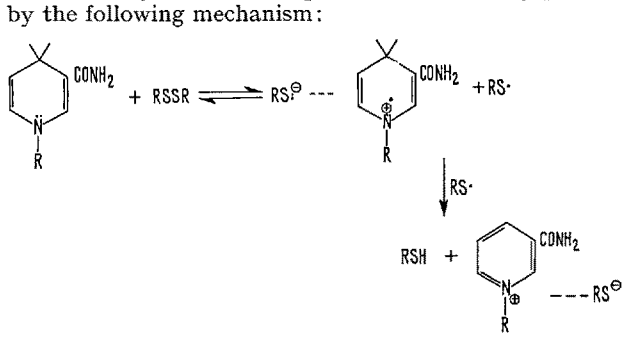
The studies on the oxidation of L-alkyl-1,4-dihydronicotinamide by malachite green[1] and thiobenzophenone[2] as model reactions for the oxidation of the coenzyme nicotinamide-adenine nucleotide, NADH, have been reported to involve the shift of hydride ion. However, these model reactions seem to deviate from the generally accepted one-electron transfer theory in biological oxidation-reductions[3].

As a continuation of our studies on the redox cleavage of sulfur-sulfur bonds in organic disulfides with amines[4],

$$RSSR + R_3N: \longrightarrow RS\cdot + RS:^{\ominus} \text{ ---- } R_3N\cdot^{\oplus}$$

We wish to report briefly the reactions between N-benzyl-1,4-dihydronicotinamide and several disulfides including diphenyl disulfide, α-lipoamide and N,N,N′,N′-tetramethylthiuram disulfide and monosulfide.

Reduction of lipoamide and diphenyl disulfide by N-benzyl-1,4-dihydronicotinamide. Lipoic acid possesses a relatively weak sulfur-sulfur bond which can be cleaved by reducing agents such as ferrocene and N,N-dimethylaniline[4]. However, lipoic acid in ethanol catalyzed the decomposition of the dihydronicotinamide[5]. With lipoamide (1×10^{-4} moles, mp 129–130°[6]) and N-benzyl-1,4-dihydronicotinamide (7.5×10^{-5} moles, mp 115–119°[7]; λ_{max} 350 nm, ε 7220) in absolute ethanol at 25 °C in the dark under nitrogen, the reaction furnished, at the end of 4 weeks, the corresponding pyridinium ion[8] in 30% yield (λ_{max} 265 nm, ε 4250). Under the same conditions diphenyl disulfide and the same dihydronicotinamide furnished the pyridinium ion in 85% yield. By direct analogy to the mechanism proposed for the redox cleavage of sulfur-sulfur bond in diphenyl disulfide with N,N-dimethylaniline[4], the present reaction may proceed by the following mechanism:



This model reaction offers a mechanism which not only concurs with the compulsory one-electron transfer concept in biological oxidation-reductions but also has the advantage that disulfides are better and more attractive oxidants in simulating physiological conditions.

Reduction of N,N,N′,N′-tetramethylthiuram disulfide (TMTD) and monosulfide (TMTM) by N-Benzyl-1,4-dihydronicotinamide[9]. Equal molar quantities of TMTD mp 153–154° lit 145–146°[10], and N-benzyl-1,4-dihydronicotinamide in ethanol at 25 °C in the dark under nitrogen produced, over a period of 4 weeks, 2 products (uv λ_{max} 410 nm and 435 nm respectively). The compound with λ_{max} 410 nm (ε 1700), mp 249–250° (dec.), was isolated in 67% yield.

Analysis calculated for

$C_{16}H_9N_3OS_2$:	C, 57.6;	H, 5.8;	N, 12.6.
Found:	C, 57.4;	H, 5.89;	N, 12.64.

It was identified as N-benzyl-3-carbamylpyridinium dimethyldithiocarbamate.

Attempt to isolate the compound with λ_{max} 435 nm failed because it decomposes on exposure to air.

In a parallel run, N-benzyl-1,4-dihydronicotinamide and N,N,N′,N′-tetramethylthiuram monosulfide (TMTM) (mp 106–108° lit[11] 108–110°) in ethanol under identical conditions as in the previous experiment, the reaction afforded only one product, N-benzyl-3-carbamylpyridinium N,N-dimethyldithiocarbamate, λ_{max} 410 nm, in

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