34. *Organogeochemical Studies of a 200-meter Core Sample from Lake Biwa. V*

Racemization of Amino Acids in the Sediments Determined by Gas-liquid Chromatography

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Proteinaceous compounds of contemporary biological materials consist of amino acids which are primarily in the L-stereoisomeric configuration. They convert to amino acids with the D-stereoisomeric configuration with time after they are introduced to lake sediments. Most protein amino acids contain a single asymmetric carbon atom and, thus, the interconversion of the configuration at the asymmetric carbon atom of a given amino acid forms a racemic mixture consisting of equal quantities of the D- and L-enantiomers.

The degree of racemization of amino acids in the sediments has been determined by the measurement of the epimers of L-isoleucine (Bada *et al.*, 1970; Wehmiller and Hare, 1971) in aid of the standard ion-exchange chromatography and of the enantiomers of several amino acids by gas chromatography equipped with a capillary column (Bada *et al.*, 1973; Kvenvolden *et al.*, 1973; Wehmiller, 1977). Two methods may be applicable for the separation of amino acid enantiomers by gas-liquid chromatography. These methods involve the derivatization of D, L-mixtures to the compounds with high vapor pressure and separation by gas-liquid chromatography on optically inactive stationary phases and the direct injection of derivatized enantiomeric mixtures onto optically active dipeptide stationary phases. Bada *et al.* (1973), Kvenvolden *et al.* (1973) and Wehmiller (1977) have reported gas-chromatographic separations of optically active N-trifluoroacetic(+)-2-butyl esters of amino acid enantiomers from the sediments using a capillary column coated with the optically inactive liquid phase.

Recent studies on the optically active stationary phases have greatly advanced our understanding of the nature of asymmetric solute-solvent interactions. It has been shown that the N-trifluoroacetyl (TFA)-L-L-dipeptide cyclohexyl esters as stationary phased produce the optimum gas-liquid chromatographic separation for N-

TFA alkyl ester derivatives of D, L-amino acids (Nakaparksin *et al.*, 1970; Parr and Howard, 1973).

In the present paper, the authors intend to report the application of N-caproyl-L-valyl-L-valine cyclohexyl ester for the separation of N-TFA isobutyl ester derivatives of D, L-amino acids from the hydrolysate of the sedimentary samples of a 200-meter core obtained from Lake Biwa. Chronological implications on the degree of racemization of amino acids in the sediments will be discussed.

Materials and methods. Preparations of amino acids from the sedimentary samples and of their N-TFA isobutyl ester derivatives are reported in detail elsewhere.

A sedimentary sample was treated with 6N HCl in a sealed tube for 24 hr to hydrolyze combined amino acids. After the removal of insoluble materials by filtration, the filtrate was applied to the ion exchange resin column (Cu-Chelex-100) to separate amino acids from inorganic materials. The amino acids were eluted from the column with 6M NH_4OH and evaporated to dryness. The residual materials were treated with HCl-isobutyl alcohol (5% W/W) at 80°C for 3 hr. After the removal of excess reactants by evaporation, the residue was treated by trifluoroacetic anhydride at the room temperature for 1 hr. N-TFA isobutyl ester derivatives of amino acids were extracted with methylene chloride thrice and the combined extracts were washed with distilled water, dried over anhydrous sodium sulfate and evaporated to dryness. The residue was dissolved in methylene chloride, a portion of which was injected into the gas chromatography equipped with a capillary column (0.35 mm $\times$ 30 m) coated with N-caproyl-L-valyl-L-valine cyclohexyl ester (10%).

Authentic specimens of D, L-amino acids were also treated in identical procedures to prepare N-TFA isobutyl ester derivatives, which were used as reference compounds for the identification of D, L-amino acids from the sedimentary samples in the gas-liquid chromatography.

Results and discussion. A gas-liquid chromatogram of the total hydrolysate of the sedimentary sample from the 200-meter core sample is shown in Fig. 1. This method permits the examination of a number of enantiomeric pairs from a single chromatographic analysis, although only D, L-alanine and D, L-valine are identified on the reference of the retention time of the authentic samples at this moment. D, L-alanine and D, L-valine are well separated as observed in Fig. 1 and thus reliable measurement of the D/L ratio of these amino acids is achieved based on their peak heights. Table I lists the D/L ratio of alanine and the fission track age of the 200-meter core sample from Lake Biwa determined by Nishimura and Yokoyama (1973).

Fig. 1. Gas-liquid chromatogram of N-TFA isobutyl ester derivatives of various amino acids from the total hydrolysate of the sediments of the 200-meter core sample. Chromatographic condition: 0.35 mm $\times$ 30 m glass capillary column; 120°C isothermal operation; injection port temperature 200°C; detector port temperature 250°C; carrier gas He at 1.2 kg/cm².

An examination of the data in Table I shows that the D/L ratios of amino acids increase as the depth and the age of the samples increase. Any contamination by L-amino acids from the biosphere during the sample preparation can have an effect of reducing the D/L ratio to a great extent. In spite of these potential effects, a fairly consistent trend in the D/L ratio of alanine was observed. This fact clearly indicates that amino acid analysis is completed practically free of the contamination of L-amino acids.

Table I. D/L ratios of alanine of the total acid hydrolyzed sedimentary samples from various layers of the 200-meter core sample

Depth below top of the core (m)	37.0	62.5	82.7	99.4	110.0	181.1	4.8
Fission track age $\times 10^4$ yr B.P.	8	11	17	18	27	46	1.0
Ratio of alanine C_D/C_L	—	—	—	—	—	—	0.143
Depth below top of the core (m)	26.9	31.5	36.5	62.5	115.9	136.8	176.6
Fission track age $\times 10^4$ yr B.P.	5.8	6.8	7.9	11	29	34	45
Ratio of alanine C_D/C_L	0.239	0.387	0.352	0.401	0.704	0.756	0.957

Amino acids once entered into the sediments undergo a variety of chemical reactions to decrease their concentrations in the sediments with time. These decay reactions as well as the racemization reaction can be analyzed by a first-order reversible kinetic model (Bada *et al.*, 1970; Wehmiller and Hare, 1971; Bada and Schroeder, 1972).

The decay processes of L-amino acids can be described as follows:

where k and g are the first-order rate constants for the interconversion of the L-amino acid and the D-amino acid and degradation in the sediment, respectively. The rates of change in the concentration of D, L-amino acids in the sediments are described as follows:

$$-\frac{dC_L}{dt} = (g+k)C_L - kC_D \quad (2)$$

$$-\frac{dC_D}{dt} = (g+k)C_D - kC_L \quad (3)$$

where C_L and C_D are the concentrations of the L-amino acid and of the D-amino acid. The solution of the equations is

$$\text{Ln} \frac{1+C_D/C_L}{1-C_D/C_L} = 2kt. \quad (4)$$

A detailed derivation of equation (4) from equations (2) and (3) will be given elsewhere.

In this study, the authors intend to determine the value of k by the measurement of the ratio of D- and L-enantiomers of alanine because the fission track age of the sediment has been determined at several layers of the 200-meter core sample by Nishimura and Yokoyama (1973). The linearity between $\text{Ln}(1+C_D/C_L)/(1-C_D/C_L)$ and t was observed to occur evidently in the whole range from 34.2

Fig. 2. Graph of $\ln(1+C_D/C_L)/(1-C_D/C_L)$ vs time.

$\times 10^4$ yr B.P. to the present as shown in Fig. 2. It can be concluded from this fact, that the racemization reaction of alanine can be applied to the determination of the age of the sediment in the time ranging from the present to at least 4×10^5 yr B.P.

k of $2.5 \times 10^{-6} \text{ yr}^{-1}$ was obtained from Fig. 2. Several pieces of information of k for alanine have been available so far. Values ranging from 1.1 to $2.8 \times 10^{-5} \text{ yr}^{-1}$ for k of alanine were obtained by using mollusks from marine deposits in Puget Sound, Washington by Wehmiller (1977). However, very low values of k for alanine ranging from 0.32 to $0.79 \times 10^{-6} \text{ yr}^{-1}$ were measured in the deep-sea sediments from the Caribbean Sea (Kvenvolden *et al.*, 1973), of which bottom water temperature is less than $5\text{--}6^\circ\text{C}$ observed in the bottom water of Lake Biwa all year around. Thus, the difference in k between the samples from Lake Biwa and the Caribbean Sea must be attributed to the fact that the former sample has been exposed to more effective diagenetic temperatures than the latter sample has. The fact that the mollusks collected from a midden at Del Mar, California give higher values of k ranging 5.5 to $11 \times 10^{-5} \text{ yr}^{-1}$ for alanine, can be explained by the same idea because the soil temperature in the burials of the mollusk samples is affected to a great extent by the annual ground temperature at the surface between 13 and 15°C . The effect of temperature of the sediment on the rate of amino acid racemization will be examined in more detail.

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