AN AGE DETERMINATION OF AN ANCIENT BURIAL MOUND MAN BY APPARENT RACEMIZATION REACTION OF ASPARTIC ACID IN TOOTH DENTIN

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An age of a man from an ancient burial mound dated to the seventh century was estimated to be about 50 years old, using an apparent racemization phenomenon of aspartic acid found from the tooth dentin. For this estimate, the D/L ratios of modern teeth and those by a heating experiment were also examined.

Accumulation of D-aspartic acid during human life-time has been reported in tooth enamel¹⁾ and dentin,²⁾ eye lens,³⁾ and brain.⁴⁾ These accumulations were considered to be due to the racemization of proteinaceous L-aspartic acid, and the extents of the racemization were related with ages of individuals. We estimated the age of a man who was buried in a stone tomb of an ancient burial mound dated to the seventh century, by the examination of D/L-aspartic acid ratios from his teeth unearthed in 1983. The D/L ratios of the samples were assumed to be sums of the ratios during his life-time and the ratios during burial preservation. To estimate his age from the ratios during the life-time, the D/L ratios of modern teeth of various ages were examined. The extent of the racemization during the burial preservation was determined by a laboratory heating experiment.

The teeth of the ancient mound man were recovered from Tohkayama-kofun located near Narita, Chiba Prefecture. Some of the man's teeth were still loosely attached to the right lower jaw bone. The canine and second molar of the right lower jaw were used for analysis. The present day tooth samples were extracted recently from individuals of ages ranging from 8 to 83 years old. All of the samples analyzed were dentin portions of tooth roots. After hydrolysis with 6 mol dm^{-3} HCl at 108 °C for 20 h, the residue of the hydrolyzate was treated to yield N-(trifluoroacetyl)amino acid isopropyl esters in the usual manner. These amino aicd esters were analyzed by a gas chromatograph equipped with a Chirasil-Val glass capillary column which separated the D- and L-enantiomers. The ratios of D/L-aspartic acid were determined from the D- and L-peak areas obtained by an integrator. The laboratory heating experiment was carried out at 121, 132, and 138 °C. For each heating temperature, several dentin samples arranged from a single wisdom tooth (third molar) were sealed in glass tubes under reduced pressure, and were heated for 8 to 60 h. After the heating, the tubes were opened and the tooth samples were subjected to the same analytical procedure as the unheated samples.



Fig. 1. Relation between aspartic acid racemization in human dentins and ages of individual at tooth extraction. 1 to 7, permanent teeth; 8 and 9, milk teeth; 10 to 15, wisdom teeth.

The gas chromatographic analysis showed the D-enantiomer peak only for aspartic acid. In Fig. 1 are plotted the D/L ratios of aspartic acid determined for various dentin samples and the ages of individuals at the tooth extraction. In the graph, permanent teeth(excluding wisdom teeth for convenience) make a straight line with respect to the D/L ratios and the ages. The points of the milk teeth appear above the straight line and those of the wisdom teeth scatter below the line. This distribution of the three different kinds of teeth was probably reflected by the order of tooth formation, i.e., first milk teeth, second permanent teeth, and finally wisdom teeth. The straight line indicates that the extent of accumulation of the D-enantiomer are concordant with ages of individuals at the tooth extraction. A similar straight line was reported, in which the accumulation was considered as a result of the racemization.²

Racemization of aspartic acid in a simple system is a reversible first-order reaction and the rate expression for the reaction is

$$-\frac{d[L]}{dt} = k[L] - k[D]$$
(1)

where [L] and [D] represent the concentration of the L- and D-aspartic acid, respectively, and k is the rate constant for the interconversion of the D- and Lenantiomers. Integration of Eq. 1 gives

$$\ln\left(\frac{1 + D/L}{1 - D/L}\right)_{t} = 2kt + \ln\left(\frac{1 + D/L}{1 - D/L}\right)_{t_{o}}$$
(2)

where t is time of the reaction and t_0 stands at t=0. Accordingly, the line in Fig. 1 can be written, using a least-squares fit, as

$$\ln\left(\frac{1 + D/L}{1 - D/L}\right)_{t} = 2(6.06 \pm 0.22) \times 10^{-4} t + 0.073$$
(3)

where t is the age in year(y) of the individual. The constant, 0.073, in Eq. 3

probably due to an induced racemization by the acid hydrolysis. The previous $study^{2}$ used an irreversible first-order reaction, where the two constants were somewhat different from ours but were at the same order of magnitudes. We adapted the reversible reaction for the aspartic acid racemization in dentin, since the D/L ratios were significantly large for the kinetic study.

The rate constants in Eq. 2 at the elevated temperatures were determined by the heating experiment and are listed in Table 1, together with that for the individual life-time being placed at 37 °C(human body temperatures have been known as $36.9 \pm 0.7 \text{ °C}^{(5)}$). From the rate constants at these four temperatures, an Arrhenius equation was determined as

Table 1.Rate constantst/°C
$$k/y^{-1}$$
37(6.06 ± 0.22) x 10^{-4}121(3.12 ± 0.43) x 10132(6.29 ± 0.57) x 10138(1.66 ± 0.18) x 10^2

$$\ln k = - (15621 \pm 250) \frac{1}{T} + 42.9$$
 (4)

From Eq. 4 an activation energy of 31.4 kcal mol^{-1} was obtained for the racemization of aspartic acid in dentin. A relatively similar value(33.4 kcal mol^{-1}) of an activation energy was reported for alanine, glutamic acid, leucine and isoleucine in modern bovine bone.⁶⁾

The rate constants at 121, 132, and 138 °C alone yielded a constant, - 15190 K y^{-1} , for the slope of the Arrhenius plot. The extrapolation from the three points at those elevated temperatures gave a temperature 35 °C, close to 37 °C, for the rate constant, 6.06 x $10^{-4} y^{-1}$, of the individual life-time. This agreement shows that the same apparent racemization reaction took place during the heating experiment and during the individual life-time. Therefore, the extent of the accumulation during the burial preservation of the ancient mound man could be estimated according to the racemization reaction.

The D/L ratios of the two dentin samples of the ancient mound man yielded an average of 0.0790 for the canine and 0.0771 for the second molar. For each sample, the ratio during the man's life-time was calculated by Eq. 2, using the rate constants at the temperatures and time periods for the burial preservation, and the ratios, 0.0790 and 0.0771. These estimates are listed in Table 2. The rate

		D/L		D/L		D/L	
	····	1200 y		1300 y		1400 y	
t/°C	k/y ⁻¹	No. l	No. 2	No. l	No. 2	No. 1	No. 2
11	5.97 x 10^{-6}	0.0720	0.0700	0.0717	0.0694	0.0708	0.0689
12	7.21×10^{-6}	0.0705	0.0686	0.0698	0.0678	0.0690	0.0671
13	8.69×10^{-6}	0.0687	0.0668	0.0679	0.0659	0.0670	0.0651
14	1.06×10^{-5}	0.0664	0.0645	0.0654	0.0635	0.0643	0.0624
15	1.28×10^{-5}	0.0638	0.0619	0.0625	0.0606	0.0613	0.0593

Table 2. Rate constants and life-time D/L ratios of the canine(No. 1) and the second molar(No.2) of the ancient mound man from different burial temperatures and years

1200 y			1300 y		1400 y					
t/°C	No. 1	No. 2	No. 1	No. 2	No. l	No. 2				
11	59	56	58	55	57	54				
12	56	53	55	52	54	51				
13	53	50	52	49	50	47				
14	50	46	48	45	46	43				
15	45	42	43	40	41	38				

Table 3. Age estimated for the ancient mound man under different burial temperatures and years from D/L ratios of canine (No. 1) and second molar(No. 2)

constants at the temperatures of the burial preservation were obtained by Eq. 4 in which the constant for the slope was defined more accurately by the point at 37 °C from the individual life-time. The temperatures for the burial preservation were chosen from 11 to 15 °C, since the recent mean annual temperature has been at about 15 °C in the area where the ancient mound locates. Finally, the man's age was estimated by Eq. 3, using the ratio during his life-time for the canine and the second molar(Table 2), and is listed in Table 3. From these calculations, his age was deduced between 45 and 55 years old.

It is clear, as seen in Table 3, that the age determination by the present method is more sensitive to the burial temperature in comparison to the burial time period. An age estimate of an Alaskan mummy was reported by a D/L ratio of aspartic acid in a dentin sample.⁷⁾ In that study, the extent of the postmortem racemization was not evaluated, since the mummy had been buried in frozen soil. Whereas, our study demonstrates that an age determination can be made also for such tooth samples in that the postmortem racemization actually took place.

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