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Age Estimation Using the Racemization of Amino Acid in Human Dentin

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ABSTRACT: This study was made to improve the validity of age estimation from teeth using amino acid racemization. The correlation between actual age and the D/L ratio of aspartic acid was investigated by analyzing not only the total amino acid but also its fractionated substances, insoluble collagen and soluble peptide.

The coefficient values of correlation between the D/L ratio and actual age in adult lower central incisors were 0.996 ($\sigma = \pm 1.0$ year) for the total amino acid, 0.988 ($\sigma = \pm 1.8$ years) for insoluble collagen, and 0.997 ($\sigma = \pm 0.9$ years) for soluble peptide. The corresponding figures in adult first premolars were 0.991 ($\sigma = \pm 1.6$ years), 0.988 ($\sigma = \pm 1.9$ years), and 0.994 ($\sigma = \pm 1.4$ years), respectively. The reactive velocity of aspartic acid racemization was highest for soluble peptide in both the lower central incisors and first premolars and approximately three times as rapid as that for total amino acid. As a result, age estimated from the analysis of soluble peptide was most accurate. The velocity for insoluble collagen was slightly lower than that for total amino acid.

Age estimation was attempted from the teeth of an unknown body. These results suggest that the analyses, not only of total amino acid in dentin but also of its fractionated and extracted substances, can lead to higher reliability in age estimation. Soluble peptide, in particular, has been found to be most effective.

KEYWORDS: odontology, dentition, human identification, age estimation, amino acid racemization, collagen

Most protein components in the body consist of L-amino acid [*1*]. D-amino acid has been found in the bones [*2–5*], teeth [*6–9*], brain [*10*], and crystalline lenses [*11–13*]. Because of its slower metabolic turnover, it is generally believed that D-amino acid decomposes slowly in these organs. Dentin, which is surrounded by enamel and cementum, is best suited for studies of racemization, particularly in age estimation.

Helfman and Bada [*7*] examined aspartic acid (Asp), which has the highest racemization rate of all the amino acid residues. They used the D/L ratio of coronal dentin fragments from 20 subjects to estimate age and obtained very satisfactory results ($r = 0.979$). The authors of this paper, in a previous paper [*8*], cut the dentin transversely into eight blocks and measured the D/L ratio for each block. The D/L ratio showed different changing patterns, depending on the age group. A high coronary D/L ratio was found in the younger age group, while the ratio tended to decrease in the elder group. In view of the fact that its dentin formation is earlier, the crown should have a high D/L ratio. In the

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older group, however, we found a low ln [(1 + D/L)/(1 − D/L)] value, probably because the crown is lower in temperature and more susceptible to environmental changes than the root. Longitudinal sections were cut to isolate the dentin from the tooth. Whole dentin was used to study the correlation between the Asp D/L ratio and actual age. Our results ($r = 0.991$ or higher) were better than those of Helfman and Bada [8]. The Asp D/L ratio has also been tentatively used to estimate the age of unidentified bodies [9]. Utilizing the racemization rate of amino acids, Matsu'ura and Ueta [2] extracted three fractions of amino acids in fossil bones to use as annual rings. All previous age estimations using dentin were made with the total amino acid fraction (TAA).

For a more reliable age estimation, it is important to fractionate TAA into several subfractions and extract each review in correlations between the Asp D/L ratio of each fraction and the actual age. To improve the usefulness of age estimation using amino acid racemization in the teeth, TAA in the dentin was fractionated into an insoluble collagen fraction (IC) and a soluble peptide fraction (SP). Correlations between the Asp D/L ratio of these fractions and the actual age were then made. In addition, this method was applied to age estimations using teeth from actual unidentified bodies.

Materials and Methods

Lower central incisors from 13 persons (37 to 71 years old) and first premolars from 14 persons (20 to 65 years old) were used for this study. These teeth were extracted and preserved in 10% formalin for periods of three months to eight years. The age, sex, and date of extraction were known for each case. Teeth without severe cavities were selected. The periodontal membrane and dental calculus on the teeth were removed using a scaler. The teeth were washed under running water overnight and air-dried.

The teeth were then cut labiolingually or buccolingually through the center using an Isomet cutter (Isomet, Model 11-1180, Buhler). Approximately 1-mm-thick longitudinal sections of the dentin were made. Tissue other than dentin was removed with the cutter. The dentin fragments were washed ultrasonically in 0.2*M* hydrochloric acid, distilled water (three times), ethanol, and ether, in that order, for approximately 5 min each. The fragments were powdered in an agate mortar, and 10 mg was used as a test specimen of TAA.

Fractionation and extraction were originally done according to the method of Matsu'ura and Ueta [2]. One millilitre of 1*M* hydrochloric acid was added to 10 mg of the powder material and centrifuged at 5000 × *g* for an hour at 5°C. The supernatant was used as SP, and the residue was used as IC. The Asp D/L ratio was measured in three fractions: TAA, SP, and IC.

The Asp D/L ratio was quantitatively analyzed by methods previously reported [8,9] using gas chromatography, as shown in Fig. 1.

Results

TAA was detected in the dentin of the longitudinal section by gas chromatography (GC) (see Fig. 2). The largest amount of L-amino acid was detected in glycine (Gly), followed by proline (Pro), alanine (Ala), hydroxyproline (Hyp), and glutamic acid (Glu), in descending order. These results correlated well with the amino acid components of collagen. Asp contained the largest amount of D-amino acid, which suggests that Asp has a high racemization rate. Glu and Ala contained D-amino acid, as well as L-amino acid. However, D-amino acid levels in these substances were small and less reproducible. Therefore, the D/L ratio was studied only in Asp.

When comparing the amount of amino acid in the three fractions, TAA and IC had larger amounts of Gly and Pro than Asp. In the SP fraction, the amount of Asp detected

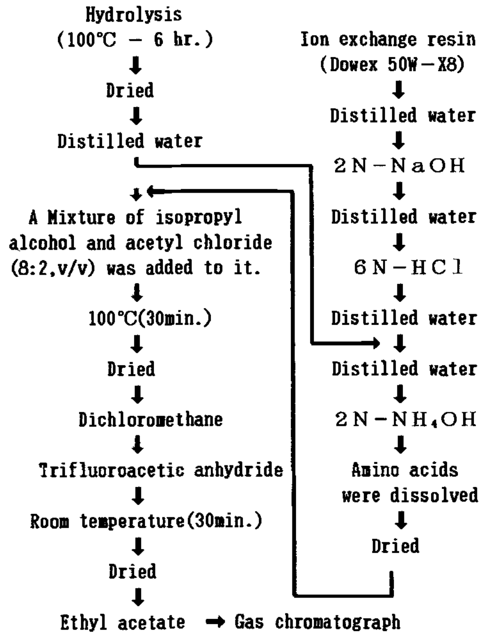

FIG. 1—*Amino acid analysis.*

was greater than that of any other amino acid residue, and larger than Hyp, which characterizes the amino acid component of collagen.

Figure 3 shows the correlation between the Asp D/L ratios of TAA, IC, and actual age. The least square linear regressions for these fractions are expressed by the following equations:

Lower Central Incisor

TAA: $\ln[(1 + D/L)/(1 - D/L)]_t = 0.001\,320\,t + 0.0265$

$r = 0.996 \quad \delta = \pm 1.0$

IC: $\ln[(1 + D/L)/(1 - D/L)]_t = 0.001\,014\,t + 0.0265$

$r = 0.988 \quad \delta = \pm 1.8$

SP: $\ln[(1 + D/L)/(1 - D/L)]_t = 0.003\,753\,t + 0.0260$

$r = 0.997 \quad \delta = \pm 0.9$

First Premolar

TAA: $\ln[(1 + D/L)/(1 - D/L)]_t = 0.001\,281\,t + 0.0238$

$r = 0.991 \quad \delta = \pm 1.6$

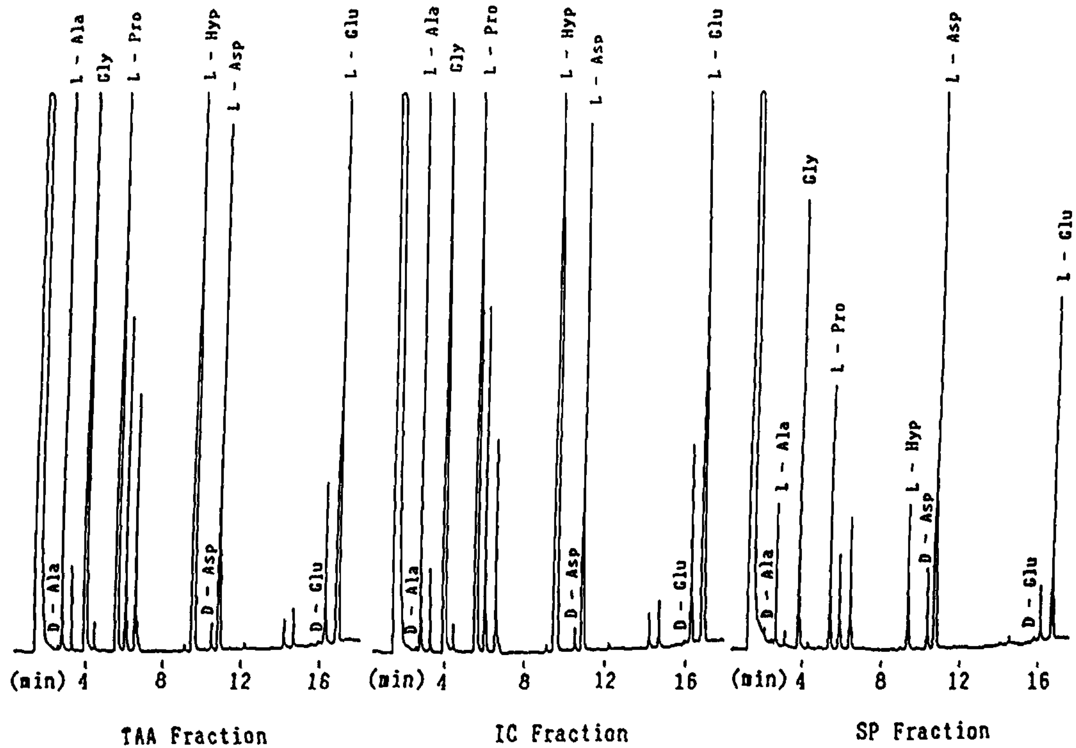

FIG. 2—*Gas chromatogram of* N-*trifluoroacetyl isopropyl esters of amino acid in dentin. Column temperature, 110°C, 4 min hold, and then programed to 180°C at 3°C/min; injection temperature, 220°C; carrier gas, helium; split ratio, 40:1. TAA is the total amino acid fraction, IC is the insoluble collagen fraction, and SP is the soluble peptide fraction.*

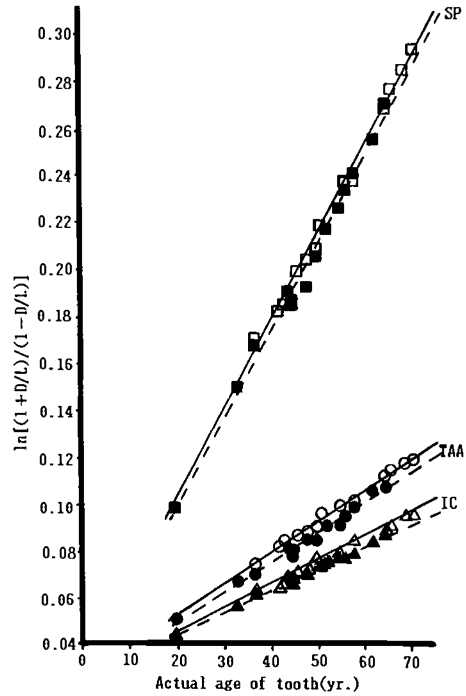

FIG. 3—*Correlation between D/L ratio and actual age:* ———, ○ *(TAA),* △ *(IC), and* □ *(SP) show the lower central incisor;* — — — —, ● *(TAA),* ▲ *(IC), and* ■ *(SP) show the first premolar.*

IC: $\ln[(1 + D/L)/(1 - D/L)]_t = 0.000\,941\,t + 0.0259$

$r = 0.988 \quad \delta = \pm 1.9$

SP: $\ln[(1 + D/L)/(1 - D/L)]_t = 0.003\,725\,t + 0.0241$

$r = 0.994 \quad \delta = \pm 1.4$

where t = actual age, r = coefficient of correlation, and δ = standard deviation.

All three fractions showed high correlation between the D/L ratio and actual age. The maximum variation fell within a range of ±3 years, except for one IC fraction obtained from a first premolar which had a variation of +4 years. The best results were obtained with SP.

The racemization rate constant, k, was analyzed for Asp in viable dentin. The constant was 0.000 660 in TAA from the lower central incisor, 0.000 507 in IC, and 0.001 876 in SP. In the first premolar, the constant was 0.000 641 in TAA, 0.000 471 in IC, and 0.001 863 in SP. This suggested that the racemization rate was slightly lower in IC than in TAA. In addition, racemization occurred three times faster in SP than in TAA.

Applications

Age estimation from teeth using the racemization of Asp was applied to an actual unknown body.

A decomposed male corpse wrapped in a mattress and carpet was found in the woods in Yamakita-cho, Kami-gun, Ashigara, Kanagawa-ken, Japan, in November 1988. At autopsy, the corpse was presumed to be 40 to 50 years old. It was believed to be a 36-year-old man, "S," because the age and physical features were very similar to "S." Eventually, the victim was identified as "S" based on dental findings and matched blood group (Group A).

As shown in Tables 1 and 2, one lower left and one lower right lateral incisor were extracted from the body. By a comparison with five control teeth of the same tooth type, TAA, IC, and SP were analyzed for age estimation. The age was estimated to be 39 years from TAA on each of the two kinds of specimens, 34 and 32 years from IC, and 37 and 36 years from SP. SP provided the closest estimate of the victim's actual age (36 years).

Discussion and Conclusions

There are many reports on age estimation using teeth [*14*]. Most of these reports are based on morphological changes in the teeth.

The method presented is based on chemical reactions in the teeth, using the reversible primary reaction to racemization. Matsu'ura and Ueta [*2*] stated that further study is needed to determine which fractionation method of bone fossil annual rings is most efficient in racemization studies of amino acids.

An analysis of the amino acid components of TAA detected Hyp, which is rarely found in proteins other than collagen. This suggested that most of the detected amino acids may be derived from collagen. Large amounts of Gly, Pro, and Ala, acceptable as characteristics of general collagens, were detected in TAA and IC, while in SP, Asp and Glu were the most common amino acids. This may be because these components are hydrophilic. SP contained only a small amount of free amino acid, most likely because the majority of free amino acid was removed by washing, and amino acids were measured after SP was hydrolyzed. This suggested that SP might be composed of peptides. The racemization rate of TAA is usually faster than that of free amino acids. In the fossil samples, it is generally believed that the racemization rate is faster in free amino acids than in bound amino acids. The racemization of free amino acid is considered to be accelerated by metal ions which coexist in the fossil. In this study, the Asp D/L ratio was higher in SP than in TAA and IC, (SP > TAA > IC). This may be the result of metal ions coexisting in the dentin with amino acids, as shown in the free amino acids found in the fossil. SP displays weak intermolecular bonding and is unstable, which might accelerate racemization. This suggests that the high D/L ratio of SP might affect the washing of materials, and the method of washing might affect the D/L ratio. Therefore, in actual age estimations, samples to be analyzed and control teeth must be washed in the same manner, and powder particles must be as homogenized as possible.

The coefficient of correlation between the D/L ratio and actual age was 0.99 or higher ($\sigma = \pm 0.9, \pm 1.0, \pm 1.4, \pm 1.6$) in TAA and SP for both lower central incisors and first premolars. IC also had a very high coefficient of 0.98 ($\sigma = \pm 1.8, \pm 1.9$). This may be explained by the facts that the same morphological type of tooth was used and the experiments were conducted under uniform conditions. SP provided the best results because of a high racemization rate, which enabled us to measure the D/L ratio relative to age. Slightly better results were obtained with the lower central incisors than with the first premolars. This may be explained by the fact that when longitudinal sections were made, the lower central incisor had a smaller volume than the first premolar, which facilitates the measurement of D/L for the entire tooth.

On the other hand, in actual age determination, it would be best if a formula of actual ages as opposed to D/L ratios of aspartic acid were worked out from data derived from many teeth of known age as controls and if the formula were used instead. However,

TABLE 1—*Age estimation.*

Materials Investigated	Actual Age	TAA Fraction D/L	TAA Fraction Age Estimated	TAA Fraction Differential	IC Fraction D/L	IC Fraction Age Estimated	IC Fraction Differential	SP Fraction D/L	SP Fraction Age Estimated	SP Fraction Differential
⌈2[a]	24	0.0514	25	+1	0.0490	25	+1	0.1080	24	±0
2⌉	46	0.0776	44	−2	0.0704	44	−2	0.1966	44	−2
2⌉	50	0.0840	48	−2	0.0752	48	−2	0.2228	50	±0
2⌉	57	0.0972	57	±0	0.0846	57	±0	0.2494	56	−1
2⌉	63	0.1062	64	+1	0.0928	64	+1	0.2830	64	+1
(2⌉)	36	0.0716	39	+3	0.0596	34	−2	0.1634	37	+1
(⌈2)	36	0.0708	39	+3	0.0568	32	−4	0.1596	36	±0

[a](2⌉) (⌈2), Specimen: (D/L), ln [(1 + D/L)/(1 − D/L)]$_t$. ⌈2 = lower left lateral incisor; 2⌉ = right lateral incisor.

TABLE 2—*Age calculation formula.*[a]

Fraction	Age Calculation Formula		
TAA	$t = [(D/L) - 0.016\,032]/0.001\,401$	$n = 5$	$r = 0.994$
IC	$t = [(D/L) - 0.021\,168]/0.001\,109$	$n = 5$	$r = 0.996$
SP	$t = [(D/L) - 0.001\,064]/0.004\,438$	$n = 5$	$r = 0.998$

[a]n = number of controls; r = coefficient of correlation; t = age estimated; D/L = $\ln[(1 + D/L)/(1 - D/L)]^t$.

the samples in question and more than four controls of the same kind and of the same jaw, whose ages are known, must be used and tested every time under the same conditions to ensure accuracy. The reason is because gas chromatography analyses include setting delicate conditions, such as column stability, and it is difficult to obtain the same formula every time since it depends on changes in the conditions. The variation between the estimated and actual ages in TAA was +3 years for the two types of teeth. In IC, the variation was −2 years for the central incisor and −4 years for the first premolar. SP showed variations of +1 year and ±0 years, respectively. Thus, SP provided the closest estimation of the actual age of the victim.

In conclusion, age estimation from teeth using the racemization of amino acid (Asp) can be very accurate for the following reasons: (1) the correlation between Asp D/L and actual age, ranging from 20 to 71 years old, is expressed by a reversible linear equation for IC and SP, as well as TAA, and (2) the amino acids are analyzed after being divided into several fractions. SP appeared to provide the most reliable age estimation because of a relatively high racemization rate.

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