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Racemization in enamel among different types of teeth from the same individual

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Abstract We measured the quantity of D-aspartic acid (degree of racemization of aspartic acid) in the enamel of different types of teeth from the same individual. We studied the correlation between the degree of racemization and the time of formation of each particular tooth, as well as the applicability of the degree of racemization to estimation of chronological age. If the environmental condition of the teeth is the same, the degree of racemization is expected to be highest in teeth that completed formation in the earliest period of time. Different degrees of racemization in enamel were found among different types of teeth, even in the same individual. The degree of racemization in enamel was found to be higher in molars than in incisors, and showed a tendency that did not necessarily coincide with the time of formation. This seemed to be due to the fact that the environmental temperature was higher in the molar region located deeper in the oral cavity than the front region, and that enamel was more affected by breathing air than dentin because the D/L ratios in enamel were lower than those in dentin. Using enamel, a better estimation of chronological age was obtained from calculations based on the degree of racemization of each type of tooth than from all the different teeth together. However, these estimated ages were not better than those from dentin.

Keywords Enamel · Aspartic acid racemization · Intraindividual differences · Age estimation

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Introduction

D-amino acids are produced during the course of aging by racemization of L-amino acids [1], and of all the amino acids, aspartic acid (Asp) has the highest racemization reaction rate [2]. D-Asp is accumulated in slowly metabolizing tissues, such as teeth [2–6], bone [7–12], eye lens [13, 14] and brain white matter [15], and increases with aging. In particular, D-Asp increases regularly with age in the dentin of human teeth. Thus racemization rates are used for the estimation of chronological age in addition to the morphological methods [16, 17] using teeth. In forensic medicine this racemization method has been established as a method of age estimation for deceased subjects [18] but for living subjects, other methods should be utilized [19, 20]. However, there are only few reports on D-Asp in enamel. In 1975 Helfman and Bada reported a high correlation between D-Asp in enamel and aging [2]. We have compared the degree of racemization of Asp between enamel and dentin using central incisors from the same individual, and reported that dentin showed a higher degree of racemization and a higher correlation with aging than enamel [21]. We have also compared the degree of racemization and the correlation with age between enamel, dentin and cementum of central incisors from the same individual and reported that the D/L ratio in enamel was lowest among the three tissues [22].

In view of the process of formation of D-Asp, the earlier the formation of a particular tooth, the higher the degree of expected racemization. Racemization is a chemical reaction, however, and is possibly affected by environmental conditions [23]. We compared the degree of racemization of Asp in dentin among different types of teeth from the same individual, and reported that the degree of racemization is different according to the time of tooth formation in middle-aged people, but not in elderly people [24].

Hence, we investigated the correlation between the degree of racemization of Asp in enamel of not only incisors, but also various other types of teeth, and the time of formation in the same individual, and compared how these results were different from those of Asp in dentin. In addition, we examined the differences between the chronological age and the age estimated from the measured values of the degree of racemization of Asp in enamel.

Materials and methods

The material consisted of a total of 49 teeth obtained from 8 donated corpses, ranging from 58 to 88 years old at death (5–7 types of teeth per body, excluding the third molar). Longitudinal sections of ca. 1 mm thickness with no dental caries were prepared as specimens, from which sections containing only enamel were collected as far as possible (3–6 mg) with a cutter. The enamel sections underwent ultrasonic cleaning sequentially in distilled water, ethanol, and ether for 5 min, respectively. After the specimens were dried, they were immediately used for analysis. The quantity of D-Asp was obtained from the degree of racemization after analysis with conventional methods by gas chromatography (GC-17A, Shimadzu, and Kyoto) [4, 18]. A 25 m-long FS capillary column (G. L. Science Co. Tokyo) with an internal diameter of 0.3 mm lined with Chirasil-Val was used [25]. The degree of racemization was determined by substituting D and L in the following equation with the area of D-Asp and L-Asp peaks on the gas chromatogram:

$$\ln[(1 + D/L)/(1 - D/L)]$$

Measurements were repeated 2–4 times, and the mean of the measured values was taken as the degree of racemization. For comparison among different types of teeth, the homonymous upper and lower teeth were regarded as the same type of tooth.

Results and discussion

Racemization is a chemical reaction consisting of a reversible first-order reaction converting L-enantiomers to D-enantiomers, and vice versa. Racemization is more evident in slowly metabolizing tissues than highly metabolizing tissues, and D-amino acids are assumed to increase regularly in a stable environment. However, it has been reported that racemization is strongly affected by the environment, especially by temperature [26].

Enamel is formed earlier than dentin during tooth formation. With respect to the types of teeth it is generally agreed that, with the exception of the third molar of which the formation time varies depending on individuals, the first molar is formed first, followed sequentially by the central incisor, lateral incisor, canine, first premolar, second premolar and second molar [27]. If the oral cavity is a uniform environment, then the degree of racemization of Asp should be highest in the type of tooth that completes its formation in the earliest time period. As we reported previously, the degree of racemization in dentin of middle-aged people was highest in the first molar, consistent with the sequential order of tooth formation. In elderly people, however, the degree of racemization was actually highest in the second molar, which is formed last [24]. This may be due to a higher environmental temperature in the molar region located deep in the oral cavity than in the region of the front teeth [28, 29]. The longer the teeth are located in the oral cavity in the elderly, the more the effects of environmental temperature are realized.

Thus, since enamel is directly exposed to the breathed air, we studied how different the degree of racemization in enamel was from that in dentin, with respect to the same type of tooth from the same individual. The degree of racemization of Asp in enamel was different between the front teeth region and the molar region (Table 1), rather than among types of teeth (t=4.55, P=0.01–0.001). In contrast to dentin, racemization rates tended to be high in the molars and low in the incisors in both middle-aged and elderly people. The degree of racemization of Asp in enamel on the average was ranked in the order of first premolar>second molar>first molar>second premolar>canine>lateral incisor>central incisor, which is not necessarily consistent with the sequential order of tooth formation. This suggests that the degree of racemization of Asp in

Table 1 D/L ratios in the enamel of various teeth

Initials and age (years)	First molar	Central incisor	Lateral incisor	Canine	First premolar	Second premolar	Second molar
K.A. 58	0.0976	0.0806	0.0814	0.0924	0.1008	0.0954	0.0924
S.A. 59	0.1006	0.0828	0.0866	0.0940	0.1010	0.0950	0.1012
U.A. 61	0.1010	0.0894	0.0876	0.0926	0.1022	0.1044	0.1024
T.R. 61		0.0942	0.0874	0.0918	0.1072	0.1046	
N.Y. 62		0.0946	0.0866	0.0936	0.1046	0.1018	
M.A. 79			0.0988	0.1024	0.1164	0.1128	0.1132
Y.Y. 80	0.1148	0.1036	0.1042	0.1130	0.1196	0.1126	0.1176
I.K. 88	0.1196		0.1038	0.1158	0.1194	0.1232	0.1214

enamel of a tooth is more affected by its location in the intraoral environment than by the time of its formation. However, we could not exclude the possibility that other factors such as humidity, pH and protein composition may cause these differences.

On the other hand, the degree of racemization of Asp in dentin is used in forensic medicine for estimation of the chronological age from teeth. We tested linear regression analysis for the relationship between the chronological age and the degree of racemization of Asp in enamel although sample numbers for each type of tooth were not considered to be sufficient. Regression lines were derived by the least squares method with respect to each type of tooth (n=5–8, r=0.840–0.996; Table 2) as well as all the types of teeth (n=49, r=0.796; Table 2). By putting the degrees of racemization of Asp in enamel into this formula, the chronological age was estimated (Table 2). As the results show, using the formula derived from each type of tooth, the estimated age was the same as the chronological age in 4 cases, different by ±1 year from the chronological age in 13 cases, ±2 years in 7 cases, ±3 years in 9 cases, ±4 years in 6 cases, ±5 years in 8 cases, and ±6 or more years in 2 cases, from a total of 49 cases. Using the formula derived from all the types of teeth, in a total of 49 cases the estimated age was the same as the chronological age in 6 cases, different by ±1 year from the chronological age in 4 cases, ±2 years in 3 cases, ±3 years in 5 cases, ±4 years in 3 cases, ±5 years in 3 cases, and ±6 or more years in 25 cases amounting to over half of the total cases. Thus, it was reconfirmed that estimation of the chronological age using the formula derived from each type of tooth yielded better results than the estimation from all the types of teeth, as in the case of age estimation based on the degree of racemization of Asp in dentin [24]. In addition, the correlation of the degree of racemization of Asp in enamel with the chronological age was lower than that in dentin as expected.

We have studied the correlation between the degree of racemization of Asp in enamel, dentin and cementum with aging, and reported that the correlation was highest in dentin [22]. The reason for this is assumed to be due to the fact that dentin is surrounded by enamel and cementum, the water content is kept constant with the aid of dentinal tubules, the individual difference is small, and the environment is maintained nearly constant. It was found that the degree of racemization was highest in cementum and lowest in enamel [22], which seems to be due to differences in environmental temperature. In fact, the surface temperature of the teeth is lower than the surrounding soft tissue temperature [28]. Alternatively, these differences may associate with different protein compositions among enamel, dentin and cementum because it was suggested that racemization rates of amino acids were different depending on their position within a protein [30, 31].

Table 2 Equations deduced by the method of least squares from respective types of teeth using enamel

Type of tooth	n	Regession equation	r	Mean
First molar	5	Y=1406.61X−80.91	0.996	0.1067
Central incisor	6	Y=813.20X−10.39	0.840	0.0909
Lateral incisor	8	Y=1296.06X−50.80	0.968	0.0921
Canine	8	Y=1149.25X−45.78	0.955	0.0994
First premolar	8	Y=1383.61X−82.17	0.965	0.1089
Second premolar	8	Y=1167.84X−55.55	0.945	0.1062
Second molar	6	Y=1123.48X−50.54	0.961	0.1080
Total teeth	49	Y=790.23X−12.07	0.796	

Y, age; X,ln[(1+D/L)/(1−D/L)]
r Coefficient of correlation

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