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Age-dependent changes in the racemisation ratio of aspartic acid in human alveolar bone

Susumu Ohtani [a,*], Toshiharu Yamamoto [b], Iwao Abe [c], Yukihiko Kinoshita [a]

[a] Institute for Frontier Oral Science, Kanagawa Dental College, 82 Inaoka-cho, Yokosuka, Kanagawa 238-8580, Japan
[b] Department of Human Biology, Kanagawa Dental College, 82 Inaoka-cho, Yokosuka, Kanagawa 238-8580, Japan
[c] Department of Applied Chemistry, College of Engineering, Osaka Prefecture University, Gakuen-cho, Sakai, Osaka 599-8531 Japan

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ABSTRACT

We investigated the racemisation ratio of aspartic acid (Asp) in alveolar bone. In addition, we designed and created a new column to detect Asp in a short period of time, which allowed us to detect D-Asp and L-Asp separately from each other within 5 min. Comparing identical ages, the racemisation ratio of alveolar bone was generally lower than that of other bones reported so far. This result suggests that alveolar bone is metabolically more active than other bones, as expected. The rate constant for the racemisation reaction ($k(y)$) of alveolar bone was calculated to be 0.000338 in males and 0.000084 in females. The rate constants in males and females were each similar to the respective ratios of the femur. This result suggests that the age-dependent reduction in metabolic turnover in alveolar bone proceeds similarly to that in the femur, although those changes proceed more slowly in females than in males. The correlation coefficient between the racemisation ratio of alveolar bone and chronological age was 0.660. It was high in males ($r = 0.912$) and low in females ($r = 0.527$), and this gender difference was statistically significant (P: 0.01–0.001), as in the femur.

1. Introduction

Aspartic acid (Asp) shows the largest rate constant for the racemisation reaction among amino acids, and its D enantiomer accumulates with age in metabolically inactive tissues such as the tooth.[1] The D enantiomer has been converted from the L enantiomer by racemisation, and increases almost linearly in dentin with age.[2–7] We previously studied the racemisation ratio of Asp in bone (including the femur), [8–11] and reported that the ratio increased with age, showing a high correlation ($r = 0.947$) with chronological age in males, but a lower correlation in females ($r = 0.663$).[8,9] Alveolar bone is exposed to very severe conditions that no other bones are exposed to, because alveolar bone connects the body of the mandible with the teeth and is repetitively exposed to occlusal pressure over the long term. Thus, it is generally supposed that formation and resorption of alveolar bone is more active than those of other bones.[12–14] We were unable to find any reports of the racemisation ratio of alveolar bone, however. Accordingly, we studied to what extent the racemisation ratio of Asp in human alveolar bone increased with age and whether the ratio correlated with chronological age. In particular, we compared the results with those obtained in the human femur, a bone in which the phenomenon of racemisation is relatively well-studied. In addition, we evaluated a new fused silica capillary column to be exclusively used for Asp.

* *Corresponding author at*: Institute for Frontier Oral Science, Kanagawa Dental College, 82 Inaoka-cho, Yokosuka, Kanagawa 238-8580, Japan. Tel.: +81 46 822 8863; fax: +81 46 822 8801.
E-mail address: ohtanisu@kdcnet.ac.jp (S. Ohtani).
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2. Materials and methods

Alveolar bone removed from 47 patients (26 male, 21 female) during extraction of impacted mandibular third molars were used as specimens. The alveolar bone was cleared by polishing on a whetstone under a stereomicroscope, placed sequentially in ultrasonic baths of distilled water, ethanol and ethyl ether for 5 min each, and 2–10 mg of each specimen was used for experiments after air seasoning.

The racemisation ratio refers to the ratio of D-Asp/L-Asp. After these were measured by the conventional method using a gas chromatograph (Shimadzu GC-17A), the ratio was calculated using the conversion formula $\ln[(1 + D/L)/(1 - D/L)]$.[8] For measurements we used a self-produced fused silica capillary column (length: 12 m, inner diameter: 0.25 mm), coated with L-t-Leo-L-α-naphthylethylamide-polydimethylsiloxane (1:7) as the fixing agent.[15–17] The column temperature started at 110 °C and increased continually to 180 °C by 2 °C per minute. Alter that, the column temperature was maintained at 180 °C.

3. Results and discussion

When the new column was applied to the separation of D-Asp and L-Asp, we were able to separate D-Asp from L-Asp with a retention time of less than 5 min (Fig. 1). This could be achieved due to the high enantioselectivity and thermostability of the column.[15–17] In most previous reports of the analysis of Asp, the retention time ranged from about 8 min (high pressure liquid chromatography)[18] to about 20 min (gas chromatography).[19] Thus, we suggest that this will prove a very useful column for the detection of Asp.

We used 2–10 mg of each specimen, because in our experience the racemisation ratio was not affected by variance within this range.[11] The racemisation ratio of Asp in the alveolar bone (Table 1 and Fig. 2) was considerably lower (in total, 0.0332 ± 0.0066; in males, 0.0341 ± 0.0079; in females, 0.0321 ± 0.0046) than that in the femur (in total, 0.0740 ± 0.0088; in males, 0.0741 ± 0.0111; in females, 0.0739 ± 0.0054).[8–11] Furthermore, alveolar bone exhibited lower racemisation ratios than other bones of the identical age except the sternum and lumbar spine.[10] This seems to indicate that the metabolic rate in the alveolar bone is higher than in the femur. The alveolar bone contains the roots of the teeth in the sockets, and, in cooperation with the periodontal membrane, cementum and gingiva, plays a very important role in maintaining the teeth against incessantly acting occlusal pressure. The properties of the material constituting the alveolar bone do not differ from bones in other parts of the body, but there is a structural feature unique to alveolar bone. While the alveolar bone withstands loading, it requires formation of new bone material by osteoblasts and resorption of bone by osteoclasts. Changes in bone structure proceed by a delicate combination of bone formation and resorption. It is generally supposed that the changes are more active compared with other bones and in young individuals.[12,13] The present study supports this hypothesis based on the racemisation rate. It has been reported that the sternum and lumber spine show similar racemisation ratios at young ages to those of alveolar bone. This suggests that these exceptional bones are under similar load to the alveolar bone. In older specimens, racemisation ratios in females tended to be lower than those in males (Table 1 and Fig. 2). A large number of women suffer from metabolic bone diseases including osteoporosis. It was pointed out that after climacterium the metabolic turnover of

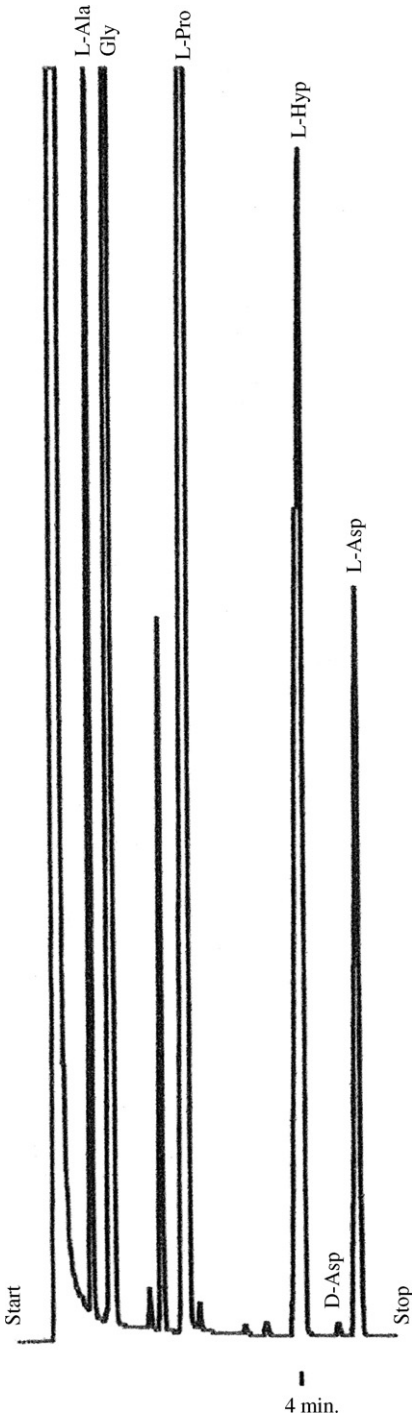

Fig. 1 – Gas chromatogram of amino acids of the alveolar bone using our new column. L-Aspartic acid (L-Asp) was clearly separated from D-aspartic acid (D-Asp) after a retention time of about 5 min. Abbreviations: Gly, glycine; L-Ala, L-alanine; L-Hyp, L-hydroxyproline; L-Pro, L-proline.