Demonstration of Aminopeptidase B in Human Periodontal Tissues

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Due to the fact that rat erythrocytes contain aminopeptidase B,[1] this enzyme is widely distributed in rat tissues, although certain of them are unable to synthetize it. Human red blood cells have not been tested for aminopeptidase B. It is possible, however, that the enzyme also occurs in several human tissue preparations. Its presence in human foetal liver [2] and in adult dental pulp tissue [3] has already been reported. The aim of this communication is to describe results which demonstrate the presence of aminopeptidase B in human gingiva and in the periodontal ligament which fastens the teeth to the bone. Evidence is given suggesting that most aminopeptidase B present in these tissues is derived from blood.

Human gingiva samples were obtained from 10 to 50 years old persons with or without inflammatory changes in the tissue. The samples, which also contained the gingival epithelium, were carefully washed with cold (+4°C) 0.01 M tris-HCl buffer (pH 7.1). They weighed from 50 to 700 mg (appr. dimensions from 2 mm × 2 mm to 5 mm × 10 mm) and each was analyzed and treated separately. Altogether 40 gingival samples were used. They were stored immediately after the operation for 2 h at +4°C, after which they were homogenized for 3 min in a cooled mortar with the aid of glass wool in a small amount of cold (+4°C) 0.01 M tris-HCl buffer, pH 7.1 (2.0 ml per 100 mg of tissue, wet weight), as described earlier for pulp tissue.[3] The mixture was centrifuged for 10 min at 23 500 *g* at +4°C. The supernatant fluid was analyzed for protein and aminopeptidase activity. An aliquot was chromatographed on Sephadex G-200 columns. The protein concentration of the samples measured by the Folin-Ciocalteu method varied from 1.8 to 3.3 mg/ml. The samples of human periodontal ligaments were collected and handled as described earlier.[4] All other methods and reagents were the same as mentioned elsewhere.[2,5]

The most important results are presented here. In Table 1 the ability of unfractionated human gingiva preparations to hydrolyze various *N*-L-aminoacyl-2-naphthylamines in the presence and absence of 0.2 M NaCl is demonstrated (appr. this NaCl-concentration is known to produce maximal activation of aminopeptidase B [6]). The results showed that only the rate of the hydrolysis of the aminopeptidase B substrates (*N*-L-arginyl- and *N*-L-lysyl-2-naphthylamine) was noticeably increased in the presence of 0.2 M NaCl.

Table 1. Hydrolysis of some *N*-L-aminoacyl-2-naphthylamines (2-NA) by a human gingival enzyme preparation in the presence of 0.2 M NaCl and without added NaCl. The reactions were performed in 0.025 M phosphate buffer, pH 7.2. The gingival enzyme preparation was diluted with water in the ratio of 1:99 just before use. The results are expressed in μmoles of 2-naphthylamine liberated per min and per mg protein ($\times 10^3$). The results from 15 separate activity assays are shown (as a mean). With *N*-L-isoleucyl- and *N*-L-propyl-2-naphthylamine, only a slow rate of hydrolysis was observed with some gingiva preparations.

Substrate	0.2 M NaCl	Without added NaCl
N-L-Alanyl-2-NA	1.35	1.40
N-L-Arginyl-2-NA	1.63	0.61
N-L-Leucyl-2-NA	0.71	0.63
N-L-Lysyl-2-NA	1.24	0.62
N-L-Methionyl-2-NA	1.06	0.96
N-L-Phenylalanyl-2-NA	0.35	0.37

Fig. 1. shows molecular exclusion chromatography of human gingival arylaminopeptidases on Sephadex® G-200. All gingival and periodontal ligament samples gave approximately the same elution pattern. Occasionally a third peak was dem-

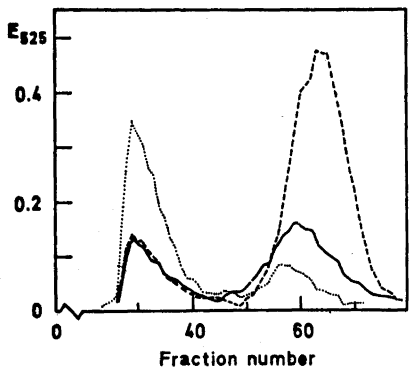

Fig. 1. Molecular exclusion chromatography of human gingival arylaminopeptidases on Sephadex G-200. Column: 83 × 1.1 cm; elution buffer: 0.01 M tris-HCl buffer, pH 7.1; sample: 1.7 ml of a centrifuged gingival enzyme preparation; hydrostatic pressure: 15 cm; temperature: +2°C; flow rate: 0.46 ml/min. —, *N*-L-arginyl-2-naphthylamine, reaction without added NaCl; — — —, *N*-L-arginyl-2-naphthylamine, tested in the presence of 0.2 M NaCl; ···, *N*-L-methionyl-2-naphthylamine, reaction without added NaCl.

onstrated between the two shown in Fig. 1 (using *N*-L-methionyl- and *N*-L-arginyl-2-naphthylamine, when 0.2 M NaCl very slightly increased the rate of the hydrolysis of the latter substrate). This peak was found to have been caused due to microbial contamination of the gingival samples. The result of Fig. 1 resembled that earlier obtained from dental pulp.[3] The elution pattern also resembled that obtained earlier for rat skin aminopeptidase B [7] and also other rat and human tissue aminopeptidase B preparations. Other experiments [3] (less fully described here) showed that the last peak in Fig. 1, revealed by NaCl, actually represents aminopeptidase B. Any tissue specific aminopeptidase B is evidently not involved, but this enzyme is present in gingiva, periodontal ligament and pulp due to the presence of red blood cells in the tissues mentioned. The molecular exclusion chromatography also revealed the following results: (a) the mutual ratio of the hydrolysis of *N*-L-methionyl- and *N*-L-arginyl-2-naphthylamines was almost opposite in the two peaks; (b) only the use of NaCl in the reaction mixtures demonstrates the presence of aminopeptidase B; the curve obtained without added salt is clearly located to the left of that obtained in the presence of the salt; (c) sodium chloride caused little, if any, increase in the rate of the hydrolysis of *N*-L-arginyl-2-naphthylamine by the first peak. Consequently, the activation caused by NaCl could be said to be specific for aminopeptidase B, as was also suggested earlier.[6,8,9] The presence of aminopeptidase B in gingiva and in periodontal ligament may be important in regard to periodontal disease, because the enzyme may contribute to inflammatory and wound healing phenomena.[10]

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