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On the Localization of Aminopeptidase B and Separation of its two Molecular Forms by Automated Recycling Chromatography

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Earlier studies [1,2] have shown that rat liver contains two molecular forms of aminopeptidase B, one of which is evidently derived from red blood cells present in livers which are used to purify aminopeptidase B. The purpose of this communication is to present a detailed method for preparing the two molecular forms by recycling chromatography on Sephadex® G-200, and to provide evidence that the actual liver enzyme occurs in the soluble phase of the cell.

All reagents and enzyme assay methods have been published earlier.[3,4] The automated recycling chromatography was performed with the LKB ReCyChrom system, which was equipped with the LKB 4938 Programmer (LKB Produkter, Bromma, Sweden).

Wistar albino rats, 10 months old and of both sexes, were used. The livers were removed after the animals were killed by a blow on the neck. A single liver was put for 10 min in 20–30 ml of cold (+4°C) 0.25 M sucrose solution, after which the tissue was cut into fine particles. From this crude homogenate a 10 % suspension (containing appr. 1 g of fresh tissue) was made in cold 0.25 M sucrose. The crude homogenate was treated in 20 ml portions (containing appr. 2 g of fresh tissue) with a Thomas (B) hand homogenizer equipped with a teflon pestle. Two downward strokes were sufficient. The resultant homogenate was filtered through a double-layered lace cloth and the filtrate was centrifuged for 5 min at 600 *g* to remove nuclei, whole cells, larger remnants of tissue, *etc.* The resulting centrifugate was again centrifuged for 20 min at 10 000 *g* to remove lysosomes and mitochondria. The pH of the supernatant fluid was adjusted to pH 5.0 with 0.1 N HCl at room temperature while mixing with a magnetic stirrer. The acid treatment was performed to remove microsomes.[5,6] The suspension was allowed to stand for 60 min at +4°C, after which it was centrifuged for 30 min at 78 000 *g*. The sediment was discarded. The pH of the supernatant fluid was adjusted to pH 7.0 with cold 0.1 N NaOH. No visible precipitate was observed. Ammonium sulphate was added to the supernatant fluid to achieve a final saturation of 80 %. The mixture was centrifuged 60 min later for 15 min at 78 000 *g*. The sediment was dissolved in 0.1 M tris-HCl buffer, pH 7.15, producing a final volume of 2.0 ml (per 2 g fresh tissue).

A typical result from the automated recycling chromatography of the two molecular forms of aminopeptidase B is shown in Fig. 1, and Table 1 shows the time schedule for the separation of the two molecular forms. The third cycle revealed the two molecular forms, but

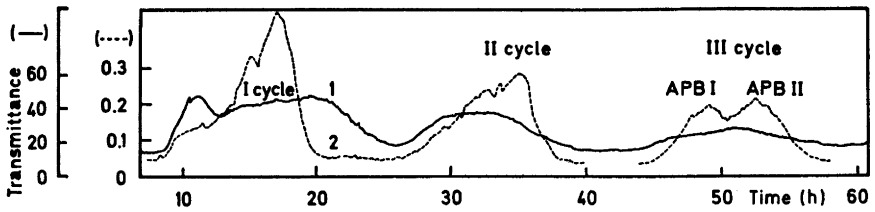

Fig. 1. ReCyChrom separation on Sephadex G-200 of two molecular forms of rat liver aminopeptidase B. Column tube: 3.2 × 100 cm; sample: 3 ml of crude aminopeptidase B solution (see text); elution buffer: 0.1 M tris-HCl buffer, pH 7.15; flow rate: 26 ml/h; fraction volume: 5.2 ml (12-min intervals); chart speed: 10 mm/h; Uvicord cell: 3 mm; bed volume: 803 ml. Curve 1 = transmittance (%); curve 2 = enzyme activity on *N*-L-arginyl-2-naphthylamine (E_{535}).[3]

Table 1. Time schedule for the ReCyChrom separation of two molecular forms of aminopeptidase B from rat liver. Figures in parentheses give elapsed time from start.

Day	Hours	Switching to bleeding	Switching to recycling
1	14.00 (0)	Start 14.00 (0)	
			04.00 (14 h)
2		09.00 (19 h)	
			20.42 (30 h 42 min)
3		03.42 (37 h 42 min)	
4	09.42 (67 h 42 min)	Stop	

the fourth cycle did not improve the separation. The two enzymes were shown to represent aminopeptidase B, since all of the enzymic properties studied were the same with both forms as earlier obtained with their mixtures. The most important of these properties will be published elsewhere.

The localization of the actual liver enzyme was also established in this study: after removal of all cell organelles from the homogenate, the aminopeptidase B activity was encountered only in the soluble phase of the cells. Hopsu, Kantonen and Glenner [7] also came to the same conclusion, but on the basis of the method used by them, such a conclusion would appear to be invalid, since the homogenizations were performed in distilled water. This results in the rupture of a large part of various cell organelles which liberate their enzyme content into the environment. Another source of faults can be found in the homogenization itself; in this study, it was found to be extremely important to perform it with utmost care with a Potter type homogenizer.

The results showed that aminopeptidase B could not be detected in nuclei, mitochondria, lysosomes and microsomes. The enzymes acting in these particles on *N*-L-arginyl-2-naphthylamine did not resemble aminopeptidase B in the elution pattern on Sephadex G-200 and in all enzymic properties investigated (detailed description of these studies will be published later).

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