Biochimica et Biophysica Acta, 678 (1981) 168–170
Elsevier/North-Holland Biomedical Press

BBA Report

BBA 61403

AMINOPEPTIDASE A IN HUMAN PLACENTA

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(Received May 12th, 1981)
(Revised manuscript received August 25th, 1981)

Key words: Aminopeptidase A; Leucine aminopeptidase; Angiotensinase; (Human placenta)

Aminopeptidase A (L-α-aspartyl(L-α-glutamyl)-peptide hydrolase, EC 3.4.11.7) was found in human placenta, partially purified from it and briefly characterized in comparison with the placental leucine aminopeptidase. The aminopeptidase A could be separated from leucine aminopeptidase after trypsin digestion followed by Sephacryl S-300 chromatography. The angiotensinase (EC 3.4.99.3) activity of aminopeptidase A in human placenta was confirmed by a biological method.

Glenner et al. [1] demonstrated the existence of an enzyme, which specifically splitted *N*-terminal L-aspartic acid of angiotensin II and referred to it as aminopeptidase A (L-α-aspartyl(L-α-glutamyl)-peptide hydrolase, EC 3.4.11.7). Until now, only three studies on aminopeptidase A purification have been reported. Yman and Kulling [2] and Nagatsu et al. [3] purified aminopeptidase A from human retroplacental serum and normal human serum, respectively, and more recently, Gorvel et al. [4] purified it about 800-fold from rabbit intestinal brush-border. In the present study, we have discovered aminopeptidase A in human placenta, partially purified and characterized it briefly. The data on leucine aminopeptidase from human placenta were also presented for comparison.

Aminopeptidase A and leucine aminopeptidase were assayed with L-aspartyl- and L-leucyl-β-naphthylamide as substrates by the methods of Nagatsu et al. [5] and Takenaka [6] with slight modifications, respectively. Angiotensinase activity was biologically estimated using the blood pressure of a rat as described previously [7,8]. Protein was measured by the method of Lowry et al. [9].

The membrane-bound leucine aminopeptidase (EC 3.4.11.2) was purified from human placenta approx. 137-fold, essentially according to the method of Hiwada et al. [10].

The approximate molecular weight was determined by gel filtration with Sephadex G-200 according to the method of Whitaker [11].

Human placental aminopeptidase A was purified by the following procedures. 600 g placenta were washed twice with 4 vol. cold 0.9% NaCl solution to remove blood, and homogenized with 1 l distilled water in a Waring Blendor. The homogenate was mixed with Triton X-100 to a final concentration of 0.83%, followed by stirring for 24 h. The suspension was centrifuged at 8000 × *g* for 10 min. The supernatant fraction was mixed with zinc sulfate (final concentration 8 mM at pH 6.0), incubated at 40°C for 1 h and centrifuged at 6000 × *g* for 5 min. It was

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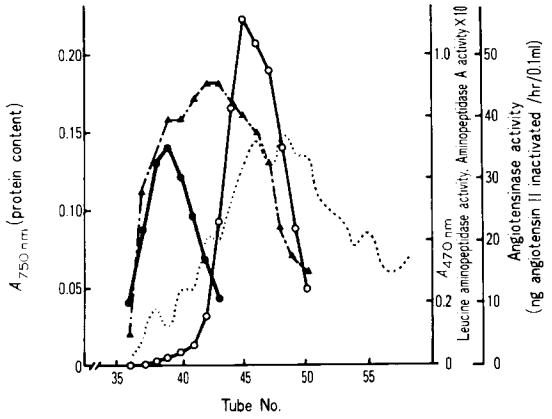

Fig. 1. Elution patterns of aminopeptidase A, leucine aminopeptidase and angiotensinase activities in human placenta by Sephacryl S-300 column chromatography after trypsin digestion. The enzymes were eluted with 20 mM Tris-HCl/malate buffer (pH 7.0)/0.5 M NaCl/0.3% Triton X-100. Each fraction was 2.5 ml. Column size, 2.6 × 40 cm; flow rate, 36 ml/h. •———•, aminopeptidase A activity; ○———○, leucine aminopeptidase activity; ▲· – · – ·▲, angiotensivase activity; - - - - - -, protein.

then subjected to batch-wise DEAE-cellulose chromatography equilibrated with 40 mM phosphate buffer and eluted with 269 ml 40 mM phosphate buffer (pH 7.0)/1 M NaCl/0.2% Triton X-100. The eluted sample was concentrated to 2.0 ml, dialyzed against 50 mM Tris-HCl buffer (pH 8.0), and incubated with trypsin (trypsin : protein = 1 : 10), at 37°C for 30 min. It was applied to a Sephacryl S-300 column (2.6 × 40 cm; flow rate, 36 ml/h) and eluted with 200 ml 20 mM phosphate buffer (pH 7.0)/0.5 M NaCl/0.3% Triton X-100. The active fractions of aminopeptidase A were pooled, concentrated and dialyzed against 50 mM Tris-HCl buffer (pH 8.0).

The enzyme preparation possessed a specific activity of 0.629 units/mg protein, which corresponded to a 77.8-fold purification with a yield of about 30.5% (Table I).

Fig. 1 shows the elution pattern of aminopeptidase A from Sephacryl S-300 after the trypsin digestion. The peak of aminopeptidase A was clearly separated from that of leucine aminopeptidase, showing the existence of aminopeptidase A in human placenta. In the previous work [3] dealing with the purification of aminopeptidase A from human serum, it was only slightly separated from leucine aminopeptidase. Patterson et al. [12] first used trypsin digestion for separating the enzymes, which hydrolyse L-leucine-amide (EC. 3.4.11.1) and L-leucyl-β-naphthylamide (EC 3.4.11.2) of Ehrlich-Lettré hyperdiploid mouse ascites carcinoma.

Some properties of aminopeptidase A in human placenta are presented in Table II in comparison with those of leucine aminopeptidase (EC 3.4.11.2). The results on heat stability and effects of metal ions clearly comfirmed that the present aminopeptidase A is a distinct enzyme from leucine aminopeptidase. Ca^{2+} activation of aminopeptidase A was first reported by Glenner et al. with rat kidney enzyme [1], which is also shown in our present enzyme from human placenta. The molecular weight of aminopeptidase A was much larger than that of leucine aminopeptidase. Since monomeric weights of various aminopeptidases were generally reported to be 80 000–200 000 [10,13], our present enzyme seems to be in a polymeric form.

In this study, we have presented partial purification and a brief characterization of aminopeptidase A

TABLE I

PURIFICATION OF AMINOPEPTIDASE A FROM HUMAN PLACENTA

One unit is defined as the amount of enzyme catalyzing the formation of 1 μmol β-naphthylamine/min at 37°C.

Step	Protein (mg)	Total activity (units)	Yield (%)	Specific activity (units/mg protein)	Purification
1. Triton-extraction	53 528	433	100	0.008	1
2. $ZnSO_4$-treatment	7 590	248	57.3	0.033	4.0
3. DEAE-cellulose chromatography	4 050	180	41.7	0.045	5.5
4. Trypsin treatment and Sephacryl S-300 gel filtration	210	132	30.5	0.629	77.8

TABLE II

PROPERTIES OF HUMAN PLACENTAL AMINOPEPTIDASE A AND LEUCINE AMINOPEPTIDASE

	Aminopeptidase A	Leucine aminopeptidase
Molecular weight	$60 \cdot 10^4$ daltons	$26 \cdot 10^4$ daltons
Optimal pH	pH 7.0	pH 7.2
Heat stability at 60°C, 30 min, pH 6.3 (residual percent activity)	22%	78%
Effect of metal ion (2 mM), % Act.:		
Ca^{2+}	412.9	105.8
Co^{2+}	49.4	141.4
Mg^{2+}	51.9	103.6
Mn^{2+}	83.4	102.7
Zn^{2+}	35.9	117.4

from human placenta. To our knowledge, this is the first report to demonstrate the existence of aminopeptidase A in human placenta.

Fig. 1 also shows the pattern of the angiotensin II-destroying activity. This activity was found to cover both aminopeptidase A and leucine aminopeptidase. It is well established that human serum contains at least two angiotensinases; one is angiotensinase A that is similar to aminopeptidase A as described by Glenner et al. [1] and the other is specific for angiotensin II amide [14]. The angiotensinase activities in pregnancy sera are extremely enhanced probably by the contribution of the placenta [15]. Sjöholm and Yman [16] suggested that angiotensinase activity in pregnancy serum can be ascribed to cystine aminopeptidase (oxytocinase) derived from placenta, which is similar to placental leucine aminopeptidase [17,18]. Our results (Fig. 1), therefore, lend further support to the idea that the increased angiotensinase activity in sera during pregnancy is due to placental aminopeptidase A and/or leucine aminopeptidase.

We wish to express our thanks to Dr. T. Suyama the Director of the research laboratory of the Green Cross Corporation for much assistance and to Dr. O. Suzuki (Hamamatsu University School of Medicine, Dept. Legal Medicine) for reading the manuscript and useful suggestions.

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