Fractionation and characterization of cystine aminopeptidase (oxytocinase) and arylamidase of human serum during pregnancy

Saara Lampelo and T. Vanha-Perttula

Department of Anatomy, University of Kuopio, P.O. Box 138, 70101 Kuopio 10, Finland

Summary. Cystine aminopeptidase and arylamidase activities in human serum were determined by enzymic hydrolysis of L-cystine-di- β -naphthylamide (CysNA) and L-leucine- β -naphthylamide (LeuNA), respectively. The activities of both enzymes increased during pregnancy, cystine aminopeptidase 12.5-fold and arylamidase 8.3-fold.

Serum CysNA and LeuNA hydrolysing aminopeptidases were separated by gel filtration on Sepharose 6B. Serum from non-pregnant women (control) contained arylamidase (I_c), which hydrolysed LeuNA and (weakly) CysNA, and cystine aminopeptidase II, hydrolysing only CysNA. During pregnancy a new enzyme appeared in maternal serum and showed cystine aminopeptidase and arylamidase activity (I_m). Maternal serum Enzyme(s) I had higher pH optima (6.5 with CysNA; 7.5 with LeuNA) and higher molecular weights (309 000) than arylamidase I_c (pH optima at 5.52–5.5 with CysNA and 7.0 with LeuNA; mol.wt ~130 000). Arylamidase I_c was more sensitive to L-methionine, but more resistant to heat than maternal serum Enzyme(s) I. Both control and maternal serum Enzyme(s) I were inhibited by EDTA, but were re-activated by Zn^{2+} and Co^{2+} with LeuNA and CysNA as substrates and by Ni^{2+} with CysNA. Cystine aminopeptidase I_m and arylamidase I_m may be a single enzyme although differences were obtained in pH optima and re-activation by Ni^{2+} after EDTA treatment. Since maternal serum Enzyme(s) I had biochemical characteristics similar to those of placental aminopeptidase(s) I, it is suggested that the activities are of placental origin. Cystine aminopeptidase II appeared in all sera. It differed clearly from both maternal and control serum Enzyme(s) I: it had the lowest molecular weight (76 000), a different pH optimum (6.0), and was resistant to EDTA and L-methionine. It was not as effectively inhibited by Ni^{2+} as was Enzyme(s) I.

Introduction

During pregnancy an aminopeptidase that is able to inactivate oxytocin and vasopressin appears in the maternal serum (von Fekete, 1930; Stoklaska & Wintersberger, 1959). The enzyme, originally named oxytocinase, disappears rapidly after parturition and has been suggested to play an important role in the maintenance and termination of a normal pregnancy (Gazarek *et al.*, 1976). However, the physiological importance of serum oxytocinase has been questioned because oxytocin is rapidly removed from the circulation by the liver and kidneys.

Oxytocinase and cystine aminopeptidase (EC 3.4.11.3) are considered to be the same enzyme (Tuppy & Nesvadba, 1957; Melander, 1965). Cystine aminopeptidase levels show a progressive rise throughout normal pregnancy, reaching a maximum in the last trimester (Babuna & Yenen, 1966; Gazarek *et al.*, 1976). The enzyme is present in high concentration in

the placental tissue (Lampelo & Vanha-Perttula, 1979) and the cystine aminopeptidase (oxytocinase) activity determined in the serum of pregnant women with oxytocin or various synthetic substrates (among these L-cystine-di- β -naphthylamide and L-leucine- β -naphthylamide) may be of placental origin (Oya, Yoshino, Mizutani & Wakabayashi, 1974). The enzyme concentration in maternal serum is supposed to reflect the increased activity of the placental tissue (Spellacy, Usategui-Gomez & Fernandez-deCastro, 1977).

Normal serum contains several aminopeptidases (Kulling & Yman, 1970). The activity of an arylamidase which hydrolyses L-leucine- β -naphthylamide has been known to increase during pregnancy and has been considered to be at least partly due to the appearance of cystine aminopeptidase (oxytocinase), which shows a broad substrate specificity (Kulling & Yman, 1970).

This report is concerned with the fractionation of sera from pregnant and non-pregnant women by gel filtration and the biochemical characterization of the enzyme activities with L-cystine-di- β -naphthylamide and L-leucine- β -naphthylamide as substrates.

Materials and Methods

Blood samples

Maternal blood samples were taken from the brachial vein at 38–41 weeks of pregnancy. The women were healthy and had no complications of pregnancy and in each case there was normal fetal growth. Serum samples from non-pregnant women were used as controls. The women had not used any oral contraceptives. The sera were separated after clotting by centrifugation at 600 g for 10 min, at +4°C.

Reagents

All reagents were obtained as indicated by Lampelo & Vanha-Perttula (1979).

Gel filtration

Gel filtration by Sepharose 6B was carried out as described earlier (Lampelo & Vanha-Perttula, 1979). The gel bed was equilibrated and the serum samples of 5 ml were eluted with 0.02 M-Tris-HCl buffer, pH 7.0. The buffer contained 0.15 M-NaCl. Fractions of 10 ml were collected at +4°C.

Measurement of the enzyme activity

The hydrolysis of L-cystine-di- β -naphthylamide (CysNA) and L-leucine- β -naphthylamide (LeuNA) was determined as described by Lampelo & Vanha-Perttula (1979). The incubation medium contained 0.3 ml 0.1 M-Tris-HCl buffer, pH 7.0 (unless otherwise indicated), 0.1 ml enzyme solution and 0.1 ml substrate solution at a final concentration of 0.25 mM. After incubation at 37°C the liberated β -naphthylamine was coupled with 0.2 ml of Fast Garnet GBC in 1 M-acetate buffer, pH 4.2, to which 10% Tween was added to disperse the colour product. The absorbancies were read spectrophotometrically at a wavelength of 525 nm. The aminopeptidase activities of total sera (unfractionated sera) are given as $\mu\text{mol } \beta\text{-naphthylamine liberated/min/mg protein}$. All the assays were in duplicate or triplicate and in the studies of enzyme characteristics a duplicate control from the same sample was always included. The protein concentrations of total serum samples were estimated according to Lowry, Rosebrough, Farr & Randall (1951). The protein concentration of the chromatographic fractions was monitored by their absorbance at 280 nm.

Studies on enzyme characteristics

The pH optima, thermal sensitivities and molecular weights were determined for the pooled enzyme preparations and the effects of various modifiers were tested as described by Lampelo & Vanha-Perttula (1979). The pH optima for the different enzymes separated by Sepharose 6B gel filtration were determined in a series of phosphate buffers (1/15 M) having a pH range between 5 and 8. Various modifier substances were dissolved in 0.1 M-Tris-HCl buffer, pH 7.0, and these solutions were used as reagent controls in the assays. The effect of different buffers on the response to EDTA (0.75 mM) was studied in Tris-HCl, phosphate and dimethyl glutaric acid buffers with rising molarities at pH 7.0. The molecular weights were measured according to Whitaker's principle (1963), in which the reference proteins were γ -globulin (mol.wt 165 000), bovine serum albumin (mol.wt 67 000) and cytochrome c (mol.wt 12 400).

Statistical analysis

Means and standard errors (s.e.m.) were calculated and the significance of the differences was determined by Student's *t* test.

Results

Aminopeptidase activities of total serum

Total cystine aminopeptidase and arylamidase activities were significantly higher in maternal serum than in control serum ($P < 0.001$) (Table 1). In both sera CysNA was hydrolysed more slowly than LeuNA. However, during pregnancy cystine aminopeptidase activity increased 12.5-fold and arylamidase activity 8.3-fold compared to the activities in control sera (Table 1).

Table 1. Total cystine aminopeptidase (CysNA) and arylamidase (LeuNA) activities of control and maternal sera

Sample	Cystine aminopeptidase	Arylamidase
Control sera (C)	0.02 $\pm$ 0.001 (7)	0.46 $\pm$ 0.06 (7)
Maternal sera (M)	0.25 $\pm$ 0.01 (8)	3.82 $\pm$ 0.25 (8)
M:C	12.5	8.3

Values are mean $\pm$ s.e.m. for the no. of triplicate experiments indicated in parentheses, and are given as μ mol β -naphthylamine liberated/min/mg protein.

Gel filtration

One enzyme activity peak hydrolysing LeuNA and eluting in Fractions 29–31 was separated from control serum by Sepharose 6B fractionation (Text-fig. 1). This enzyme also hydrolysed CysNA weakly and was called control serum arylamidase I (arylamidase I_c). With CysNA as substrate, a second enzyme activity was separated, eluting in Fractions 33–37. This activity peak, hydrolysing only CysNA, was called cystine aminopeptidase II (Text-fig. 1).

Fractionation of the maternal serum on Sepharose 6B showed that a new enzyme appeared in the serum during pregnancy. It was detected with both substrates in Fractions 23–28 and was called maternal serum arylamidase (arylamidase I_m) when LeuNA was used as substrate and maternal serum cystine aminopeptidase I (cystine aminopeptidase I_m) with CysNA as substrate. The last fractions of this first activity peak of maternal serum were overlapped by the weaker activity of arylamidase I. This was more apparent with LeuNA as substrate and gave a tailing to the peak. The maternal serum also had a separate activity, cystine aminopeptidase II,

Text-fig. 1. Elution patterns of enzymes hydrolysing CysNA (○) and LeuNA (▲) after Sepharose 6B gel filtration of (a) control serum and (b) maternal serum of women. Incubation time was 1 h (control serum) or 30 min (maternal serum) with LeuNA and 7 h (control and maternal serum) with CysNA. The protein content of the 10-ml fractions is given as the absorbance at 280 nm (---).

hydrolysing only CysNA. This eluted in coincident fractions with a similar activity of the control serum.

Studies on enzyme characteristics

Three pooled enzyme preparations were utilized: Fractions 23–28 and 33–37 of the gel filtration of maternal serum formed enzyme samples I_m and II, respectively. Enzyme sample I_c was obtained by pooling Fractions 29–31 after gel filtration of control serum.

Text-fig. 2. Effect of pH on the hydrolysis of CysNA and LeuNA by the pooled samples of (a) control and (b) maternal serum enzymes separated by gel filtration. Five separate analyses were carried out and the tracings give representative patterns for arylamidase I_c with LeuNA (△) and with CysNA (○); cystine aminopeptidase I_m (●); cystine aminopeptidase II (□); and arylamidase I_m (▲).

Enzyme II separated from control and maternal sera had similar properties and only the results with maternal serum preparation II are indicated in the tables and text-figures.

Optimal pH. The pooled preparation I_c hydrolysed LeuNA optimally at about 7.0 and CysNA at pH 5.25–5.5 (Text-fig. 2a). Enzyme sample I_m had an optimum at about pH 6.5 for CysNA and at about pH 7.5 for LeuNA (Text-fig. 2b). Enzyme preparation II separated from maternal (Text-fig. 2b) and control sera hydrolysed CysNA optimally at pH 6.0.

The effect of modifiers. Cystine aminopeptidase I_m , arylamidase I_m and arylamidase I_c were nearly totally inhibited by as low a concentration of the chelating agent EDTA as 0.001 mM, whereas cystine aminopeptidase II was not inhibited (Text-fig. 3). Tests with other chelating agents, 2,2'-dipyridyl (Text-fig. 3), EGTA and 1,10-phenanthroline chloride, showed no differences in activity with arylamidase I_c , cystine aminopeptidase I_m , arylamidase I_m and cystine aminopeptidase II.

Text-fig. 3. Effect of various EDTA and 2,2'-dipyridyl concentrations on the hydrolysis of CysNA and LeuNA by the enzyme preparations, cystine aminopeptidase I_m (●); cystine aminopeptidase II (□) and arylamidase I_m (▲). The results of 5 duplicate experiments are given as percentages ($\pm$ s.e.m.) of the appropriate duplicate controls (100%). Values marked with an asterisk differ significantly ($P < 0.001$) from those of the other 2 enzymes.

The effect of the preincubation time on the enzyme activities was studied with EDTA at 0.75 mM concentration (Table 2). The inhibition of cystine aminopeptidase I_m , arylamidase I_m and arylamidase I_c (data not shown) took place gradually at the same pace, but the activity of cystine aminopeptidase II remained stable, even with prolonged preincubation.

Cystine aminopeptidase II was inhibited by EDTA when phosphate buffer was used, noticeably at the high buffer molarities, but not by EDTA (0.75 mM) in Tris-HCl or dimethyl glutaric acid buffer (Text-fig. 4). Cystine aminopeptidase I_m (Text-fig. 4), arylamidase I_m and arylamidase I_c were strongly inhibited by EDTA in all buffers used.

When the effect of various concentrations of divalent metal ions on the enzymes after EDTA pretreatment was studied (Text-fig. 5), Co^{2+} and Zn^{2+} re-activated cystine aminopeptidase I_m , arylamidase I_m and arylamidase I_c (data not shown) at concentrations equimolar with EDTA. At higher concentrations Zn^{2+} was inhibitory, whereas Co^{2+} retained the enzyme activities. Cystine aminopeptidase I_m and arylamidase I_c were also re-activated by Ni^{2+} , but only when CysNA was used as substrate. Mn^{2+} gave partial re-activation (about 40%), but Ca^{2+} and Mg^{2+}

Table 2. The effect of preincubation time with EDTA (0.75 mM) on the hydrolysis rate of LeuNA by the pooled arylamidase I_m and of CysNA by the pooled cystine aminopeptidase I_m and cystine aminopeptidase II

Preincubation time (min)	LeuNA I _m	CysNA I _m	CysNA II
15	35 ± 6.8	28 ± 6.5	85 ± 10.2
30	31 ± 6.9	22 ± 4.2	88 ± 9.8
45	25 ± 5.2	17 ± 3.0	94 ± 8.8
60	23 ± 4.2	15 ± 2.0	96 ± 13.1
75	19 ± 3.3	12 ± 2.2	94 ± 12.5
90	13 ± 4.9	11 ± 1.8	91 ± 10.7
105	10 ± 4.6	8 ± 1.0	92 ± 12.0
120	10 ± 4.1	8 ± 1.0	91 ± 12.5

The values are mean ± s.e.m. percentages of the appropriate duplicate controls (100%) for 3 duplicate experiments.

Text-fig. 4. Effect of 0.75 mM-EDTA on the hydrolysis of CysNA by cystine aminopeptidase I_m (—) and II (---) from maternal serum. The molarities of the buffers (Tris-HCl, □; phosphate, ○; dimethyl glutaric acid, △) are indicated as the initial concentrations. The results of 3 duplicate experiments are given as percentages (±s.e.m.) of the appropriate duplicate controls (100%).

Text-fig. 5. Effect of various concentrations of (a) Co²⁺, (b) Zn²⁺ and (c) Ni²⁺ after a 90-min pretreatment with 0.75 mM-EDTA on the hydrolysis of CysNA and LeuNA by the enzyme preparations, cystine aminopeptidase I_m (●), cystine aminopeptidase II (□) and arylamidase I_m (▲). The results of 5–6 duplicate experiments are given as percentages (±s.e.m.) of the appropriate duplicate controls (100%).

had no effect. Cystine aminopeptidase II did not respond at all to Ca^{2+} , Co^{2+} , Mg^{2+} , Mn^{2+} or Ni^{2+} but Zn^{2+} caused a marked inhibition of this enzyme at higher concentrations.

The effects of the various ions directly on the enzyme activities are shown in Table 3. Ni^{2+} had less inhibitory effect on cystine aminopeptidase II than on the other enzymes, but Zn^{2+} was a clear inhibitor of all of the enzymes. None of the other metal ions tested (Ca^{2+} , Co^{2+} , Mg^{2+} , Mn^{2+}) had such an obvious effect on the enzyme activities and no other ions except Ni^{2+} produced significant differences between the enzyme responses.

Table 3. Effect of some divalent metal ions on the activity of arylamidase I_c (LeuNA), cystine aminopeptidase I_m and II (CysNA) and arylamidase I_m (LeuNA) separated by gel filtration

Ions	Conc. (mM)	LeuNA I_c	CysNA I_m	CysNA II	LeuNA I_m
Co^{2+}	0.2	103 $\pm$ 5.4	93 $\pm$ 8.1	104 $\pm$ 6.4	94 $\pm$ 11.9
	0.6	108 $\pm$ 2.0	81 $\pm$ 19.6	95 $\pm$ 7.7	86 $\pm$ 8.5
	1.0	107 $\pm$ 1.0	100 $\pm$ 16.3	94 $\pm$ 11.0	82 $\pm$ 8.3
Ni^{2+}	0.2	67 $\pm$ 6.1	85 $\pm$ 4.9	110 $\pm$ 3.9*	53 $\pm$ 7.3
	0.6	48 $\pm$ 5.1	46 $\pm$ 5.0	98 $\pm$ 11.9†	19 $\pm$ 4.8
	1.0	31 $\pm$ 5.0	29 $\pm$ 4.6	73 $\pm$ 1.0†	14 $\pm$ 2.1
Zn^{2+}	0.2	52 $\pm$ 2.4	58 $\pm$ 25.2	35 $\pm$ 7.1	41 $\pm$ 8.7
	0.6	25 $\pm$ 7.2	28 $\pm$ 13.5	16 $\pm$ 4.7	14 $\pm$ 4.6
	1.0	14 $\pm$ 7.4	16 $\pm$ 7.3	16 $\pm$ 5.3	8 $\pm$ 3.1

The values are mean $\pm$ s.e.m. percentages of the hydrolysis rates obtained without added ions (100% duplicate controls in every duplicate determination) for 3–6 duplicate experiments.

* Significantly different from the effect on the other enzymes at this concentration, $P < 0.01$.

† Significantly different from the effect on the other enzymes at this concentration, $P < 0.001$.

The enzymes had also clear differences in their response to L-methionine (Text-fig. 6a). Cystine aminopeptidase II was not inhibited but cystine aminopeptidase I_m and arylamidase I_m did show gradual inhibition, and arylamidase I_c was sensitive at every concentration.

Text-fig. 6. Effect of (a) various L-methionine concentrations and (b) temperature on the hydrolysis of CysNA and LeuNA by the pooled enzyme preparations, cystine aminopeptidase I_m (●); cystine aminopeptidase II (□); arylamidase I_m (▲); arylamidase I_c with LeuNA and CysNA as substrate (Δ). The results are given as percentages ($\pm$ s.e.m.) of the appropriate duplicate controls (100%) for 5 duplicate experiments in (a) and of the uninhibited duplicate control values for 4 duplicate experiments in (b). Values marked with an asterisk are significantly different ($P < 0.005$) from those of the other enzymes at that concentration and temperature. The arylamidase I_c (Δ) activities were significantly different ($P < 0.001$) from those of the other enzymes at all concentrations in (a) and at 60°C in (b).

Arylamidase I_c was the most resistant and cystine aminopeptidase II the most sensitive to thermal treatment (Text-fig. 6b).

Molecular weight. The fractionation on Sepharose 6B gave molecular weights of 130 000 for arylamidase I_c, 309 000 for enzyme activity I_m with CysNA and LeuNA, and 76 000 for cystine aminopeptidase II.

Discussion

In this study fractionation of human maternal serum by gel filtration showed that pregnancy caused the appearance of a new enzyme, which has cystine aminopeptidase (cystine aminopeptidase I_m) and arylamidase (arylamidase I_m) activity. The activities appeared to be identical with those previously characterized from human placenta (Lampelo & Vanha-Perttula, 1979). This supports the previous conclusion that maternal serum cystine aminopeptidase (oxytocinase) is of placental origin (Oya *et al.*, 1974; Small & Watkins, 1975). The present study also showed that serum from non-pregnant women contains two aminopeptidases one of which hydrolysed L-leucine- β -naphthylamide (LeuNA) and weakly L-cystine-di- β -naphthylamide (CysNA) and the other only CysNA. Goebelsmann & Beller (1965) isolated by Sephadex G-200 gel filtration one arylamidase from control serum, and another enzyme, which was poorly separated from control serum arylamidase, from maternal serum. They reported that control serum arylamidase hydrolysed LeuNA 75 times faster than CysNA, and maternal serum cystine aminopeptidase was shown to hydrolyse LeuNA 11 times faster than CysNA. Goebelsmann & Beller (1965) concluded that weak cystine aminopeptidase activity in control serum is due to arylamidase activity. Cystine aminopeptidase II, isolated from maternal and control serum in the present study, has not previously been detected.

Serum cystine aminopeptidase (oxytocinase) activities, measured by a bioassay, are approximately 13 times higher in pregnant than in non-pregnant women while arylamidase activities at term are 3.5 times higher (Tuppy & Nesvadba, 1957; Goebelsmann & Beller, 1965). Goebelsmann & Beller (1965) suggested that the 3.5-fold increase in arylamidase activity is due to the appearance of cystine aminopeptidase, and the broad substrate specificity of the aminopeptidase produces pseudo-cystine aminopeptidase values in control serum. We found that both cystine aminopeptidase and arylamidase activities increased markedly during pregnancy, but the increase was higher with CysNA (12.5-fold) than with LeuNA (8.3-fold).

Ryden (1966) and Christensen (1974) determined the pH optimum for unfractionated control and maternal serum cystine aminopeptidase (oxytocinase). They used CysNA as substrate and demonstrated that the pH optimum increased during pregnancy. We found that the pH optima for maternal serum enzyme preparation I were higher than those for arylamidase I_c. Cystine aminopeptidase II had the same pH optimum in maternal and control serum. It is possible that the previously observed increase in pH optimum of cystine aminopeptidase is due to the new maternal serum enzyme(s) appearing only during pregnancy.

EDTA inhibits maternal serum cystine aminopeptidase (oxytocinase) (Werle & Semm, 1956; Ryden, 1966) and control serum arylamidase (Fleisher, Pankow & Warmka, 1964; McDonald, Reilly & Ellis, 1964). In the present study EDTA strongly inhibited cystine aminopeptidase I_m, arylamidase I_m and arylamidase I_c, even at very low concentrations. This could indicate that maternal and control serum Enzymes I have a metal ion which is readily chelated in their active centre. However, the complete inhibition by EDTA required some time. Tuppy & Wintersberger (1960) have demonstrated with serum oxytocinase that 20% inhibition occurred after 15 min and 90% after 120 min of preincubation with EDTA. The possibility that maternal serum cystine aminopeptidase (oxytocinase) is a metallo-enzyme (Melander, 1965) has not yet been confirmed.

Many investigators (Tuppy & Wintersberger, 1960; Werle & Semm, 1956; Mäkinen &

Raekallio, 1974) have studied the effect of divalent metal ions on maternal serum cystine aminopeptidase (oxytocinase) activity and observed activation, inhibition or no response in the enzyme activity. In the present study, maternal and control serum Enzymes I were more sensitive to Ni^{2+} than was cystine aminopeptidase II, whereas Zn^{2+} was inhibitory to all of these enzyme activities. Even though Zn^{2+} in itself was inhibitory, it, like Co^{2+} , re-activated cystine aminopeptidase I_m , arylamidase I_m and arylamidase I_c after EDTA inhibition. Cystine aminopeptidase I_m and arylamidase I_m differed from each other in their response to Ni^{2+} after EDTA pretreatment and also had different pH optima. These observations do not prove that the co-eluting CysNA- and LeuNA-hydrolysing activities of Peak I_m represent different enzymes. The re-activation of arylamidase I_c by Ni^{2+} when CysNA was used as substrate supports the previous studies by Vescia (1956) and Vanha-Perttula, Hopsu & Glenner (1966), who have shown that an ion-determined specificity explains the different response obtained in the hydrolysis of the various amino acid naphthylamide substrates by a single aminopeptidase. Tiderström & Heinegård (1978) have studied the hydrolysis of L-cystine-bis-4-nitroanilide, L-cystine-di- β -naphthylamide and S-benzyl-L-cysteine-4-nitroanilide by unfractionated pregnant serum cystine aminopeptidase. They showed that Co^{2+} activated the hydrolysis but the activation level was different with each of the substrates. Tiderström & Heinegård (1978) suggested that the differences in the response to Co^{2+} were caused by the chemical and steric properties of each substrate used and were probably not due to different enzymes.

Cystine aminopeptidase II was resistant to EDTA, and its activity was not altered by divalent metal ions after EDTA treatment. However, the rate of hydrolysis by this enzyme was also decreased after EDTA treatment at low molarities of phosphate buffer. It has earlier been shown that serum cystine aminopeptidase activity during liver diseases is higher in Tris than in phosphate buffer (Tiderström & Heinegård, 1978), while normal serum arylamidase activity is decreased (Fleisher *et al.*, 1964). Because it is possible that serum aminopeptidases prefer different buffers and the buffer used influences enzyme characteristics, the buffer dependence should be taken into consideration in the characterization and quantitation of serum aminopeptidase activities.

Fleisher *et al.* (1964) showed that L-methionine inhibits competitively the control serum arylamidase. Oya *et al.* (1974) showed electrophoretically that the enzyme in control serum was inhibited by L-methionine (0.02 M), whereas maternal serum enzyme bands (CAP_1 , CAP_2), representing two conformational forms of a single enzyme, were resistant to the treatment. This differential response has subsequently been suggested as being useful for estimating specifically the possible placental aminopeptidase activity in maternal serum (Mizutani, Yoshino & Oya, 1976). The present study shows that cystine aminopeptidase I_m and arylamidase I_m were only partly inhibited by L-methionine. In contrast, L-methionine markedly inhibited arylamidase I_c at every concentration. Oya *et al.* (1974) have shown that control serum arylamidase activity is heat-stable (60°C, 30 min), whereas maternal serum cystine aminopeptidase (oxytocinase) can be inhibited by this treatment. Our finding of a clear difference between these enzymes at 60°C after 15 min preincubation confirms this. Cystine aminopeptidase II was not inhibited by L-methionine, but it was sensitive to thermal treatment. Cystine aminopeptidase I_m , arylamidase I_c and cystine aminopeptidase II therefore appear to be separate enzymes with different reactive sites.

In the present work cystine aminopeptidase I_m and arylamidase I_m had higher molecular weights (309 000) than control serum arylamidase (130 000). Yman & Sjöholm (1967) estimated the molecular weight of oxytocinase purified from retroplacental serum as 325 000 and Yman (1970) obtained a value of 290 000. Oya, Yoshino & Mizutani (1975) estimated the molecular weight of the maternal serum enzyme as 320 000 and that of the control serum enzyme as approximately 145 000.

In our previous study (Lampelo & Vanha-Perttula, 1979) we characterized some biochemical properties of placental aminopeptidase activities hydrolysing CysNA and LeuNA co-

eluting in the same fractions on gel filtration. The activities have similar biochemical characteristics to cystine aminopeptidase I_m and arylamidase I_m . Based on these observations it is suggested that maternal serum cystine aminopeptidase I_m is of placental origin and that it is perhaps the oxytocinase that is released into the maternal circulation during pregnancy. Serum cystine aminopeptidase II also had properties similar to those of placental cystine aminopeptidase II (Lampelo & Vanha-Perttula, 1979), and this enzyme appears to be distinct from any of the previously characterized serum aminopeptidases. Since maternal and control sera contain this cystine aminopeptidase activity which hydrolyses CysNA, a synthetic substrate for oxytocinase, further studies are necessary to explore its tissue origin and physiological function in serum. It should also be taken into consideration during development of a specific method for oxytocinase for monitoring the placental functions.

This study has been supported by grants from The Sigrid Jusélius Foundation, Helsinki, Finland and from The Research and Science Foundation of Lääke Oy, Turku, Finland.

References

- Babuna, C. & Yenen, E. (1966) Enzymatic determination of placental function. A rapid method. *Am. J. Obstet. Gynec.* **95**, 925–934.
- Christensen, A. (1974) Hormone and enzyme assays in pregnancy. I. Studies on the placental and the tissue cystine-aminopeptidase activity in peripheral plasma from non-pregnant and pregnant women and in plasma from umbilical cord. *Acta endocr., Copenh.* **76**, 189–200.
- Fleisher, G.A., Pankow, M. & Warmka, C. (1964) Leucine aminopeptidase in human serum: Comparison of hydrolysis of L-leucylglycine and L-leucyl- β -naphthylamide. *Clin. chim. Acta* **9**, 259–268.
- Gazarek, F., Pohanka, J., Talas, M., Fingerova, H., Janouskova, M., Krikal, Z. & Hamal, Z. (1976) Plasma oxytocin and oxytocinase levels in third trimester of pregnancy and at labour. *Endocr. exp.* **10**, 283–287.
- Goebelsmann, U. & Beller, F.K. (1965) Separation of cystine-amino-peptidase and leucine-amino-peptidase and their determination in pregnant and non-pregnant women. *Z. klin. Chem.* **3**, 49–54.
- Kulling, B. & Yman, L. (1970) Purification and some properties of leucine aminopeptidases from retro-placental serum. *Acta pharm. suecica* **7**, 65–74.
- Lampelo, S. & Vanha-Perttula, T. (1979) Fractionation and characterization of cystine aminopeptidases (oxytocinase) and arylamidase of the human placenta. *J. Reprod. Fert.* **56**, 285–296.
- Lowry, O.H., Rosebrough, N.J., Farr, A.L. & Randall, R.J. (1951) Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193**, 265–275.
- Mäkinen, P.-L. & Raekallio, J. (1974) Aminopeptidases in the serum of pregnant and nonpregnant women. *Biochem. Med.* **11**, 210–216.
- McDonald, J.K., Reilly, T.J. & Ellis, S. (1964) The hydrolysis of amino acyl- β -naphthylamides by plasma aminopeptidases. *Biochem. Biophys. Res. Commun.* **16**, 135–140.
- Melander, S. (1965) Plasma oxytocinase activity. A methodological and clinical study with special reference to pregnancy. *Acta endocr., Copenh.* **48**, Suppl. **96**, 1–94.
- Mizutani, S., Yoshino, M. & Oya, M. (1976) A comparison of oxytocinase and L-methionine-insensitive leucine aminopeptidase during normal pregnancy. *Clin. Biochem.* **9**, 228.
- Oya, M., Yoshino, M., Mizutani, S. & Wakabayashi, T. (1974) The origin of human pregnancy serum oxytocinase. *Gynecol. Invest.* **5**, 276–283.
- Oya, M., Yoshino, M. & Mizutani, S. (1975) Molecular heterogeneity of human placental aminopeptidase isozymes. *Experientia* **31**, 1019–1020.
- Ryden, G. (1966) Cystine aminopeptidase and oxytocinase activity in pregnancy. A comparative study in human and rat tissue. *Acta obstet. gynec. scand.* **45**, Suppl. **3**, 1–105.
- Small, C.W. & Watkins, W.B. (1975) Oxytocinase-immunohistochemical demonstration in the immature and term human placenta. *Cell Tissue Res.* **162**, 531–539.
- Spellacy, W.N., Usategui-Gomez, M. & Fernandez-deCastro, A. (1977) Plasma human placental lactogen oxytocinase, and placental phosphatase in normal and toxemic pregnancies. *Am. J. Obstet. Gynec.* **127**, 10–16.
- Stoklaska, E. & Wintersberger, E. (1959) Zum Mechanismus des Oxytocin- und Vasopressin-Abbaues durch Schwangerenserum. *Arch. exp. Path. Pharmacol.* **236**, 358–364.
- Tiderström, G. & Heinegård, D. (1978) Determination of cystyl-aminopeptidase. Isoenzymes in seminal plasma and serum of different origin. *Clin. chim. Acta* **88**, 293–304.
- Tuppy, H. & Nesvadba, H. (1957) Über die Aminopeptidaseaktivität des Schwangerensersums und ihre Beziehung zu dessen Vermögen, Oxytocin zu inaktivieren. *Mh. Chem.* **88**, 977–988.
- Tuppy, H. & Wintersberger, E. (1960) Reinigung und Eigenschaften der Serum-Oxytocinase. *Mh. Chem.* **91**, 1001–1010.

- Vanha-Perttula, T., Hopsu, V.K. & Glenner, G.G.** (1966) Enzymes in hog kidney hydrolyzing amino acid naphthylamides. *J. Histochem. Cytochem.* **14**, 314–325.
- Vescia, A.** (1956) Ion-determined specificity of peptidase activity. *Biochim. Biophys. Acta* **19**, 174–175.
- von Fekete, K.** (1930) Beiträge zur Physiologie der Gravidität. *Endokrinologie* **7**, 364–369.
- Werle, E. & Semm, K.** (1956) Über die Oxytocinase des Schwangerenblutes. *Arch. Gynaek.* **187**, 449–457.
- Whitaker, J.R.** (1963) Determination of molecular weights of proteins by gel filtration on Sephadex. *Analyt. Chem.* **35**, 1950–1953.
- Yman, L.** (1970) Composition of oxytocinase (cystine aminopeptidase). *Acta pharm. suecica* **7**, 29–36.
- Yman, L. & Sjöholm, I.** (1967) Some physicochemical properties of oxytocinase (cystine aminopeptidase). *Acta pharm. suecica* **4**, 13–22.

Received 15 May 1979