Two Molecular Species of Oxytocinase (L-Cystine Aminopeptidase) in Human Placenta: Purification and Characterization

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Two different forms of oxytocinase (L-cystine aminopeptidase, CAP; EC 3.4.11.3) were purified from the 9000 *g* and 105000 *g* precipitate fractions of human placenta homogenate by sequential chromatography on columns of hydroxyapatite, DE-32, nickel ion affinity, and Sephadex G-200. One species (CAP-I) purified from the mitochondrial/lysosomal fraction migrated on sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis with an apparent molecular mass of 61 kDa; the other (CAP-II) from the microsomal fraction was composed of two subunits with molecular masses of 56 and 40 kDa. The molecular masses of CAP-I and CAP-II estimated by gel filtration were 64 and 97 kDa, respectively. The specific activities of the two species for *S*-benzyl-L-cysteine *p*-nitroanilide increased by 357- (for CAP-I) and 139-fold (for CAP-II) compared with the starting preparations. The optimal pH values toward the artificial substrate were approx. 7.4–8.0 for CAP-I and 6.8–8.0 for CAP-II. The K_m and V_{max} values toward oxytocin were 5.6 μ M and 23.4 μ mol/h/mg protein for CAP-I, and 38 μ M and 15.6 μ mol/h/mg protein for CAP-II. Both enzymes were inhibited by the metal-chelating agents, EDTA and *o*-phenanthroline, whereas they were specifically activated by addition of Co^{2+} : CAP-I was more sensitive to these reagents than CAP-II. L-Methionine strongly inhibited CAP-I, while CAP-II activity was only slightly affected. CAP-II was more sensitive to amastatin than CAP-I. Thus, the two enzymes are quite distinct in their molecular nature and biochemical properties. They may play a regulatory role in the metabolism of oxytocin and other biologically active peptides in intact placenta.

Key words cystinyl aminopeptidase; oxytocin; vasopressin; purification; human placenta

Oxytocinase (L-cystine aminopeptidase, CAP; EC 3.4.11.3) catalyzes the hydrolysis of oxytocin at the N-terminal between cystine and tyrosine.¹⁾ The CAP activity in serum increases with increasing oxytocin levels during pregnancy²⁾ and disappears rapidly after parturition.^{3,4)} Thus, the serum enzyme, which is derived from placenta,⁵⁾ is thought to play a role in the maintenance of normal pregnancy by inactivating the uterotonic hormone.

The fetus itself synthesizes and secretes oxytocin,⁶⁾ suggesting some role in parturition. The higher concentration of oxytocin in arterial cord blood than in venous cord blood⁶⁾ shows that the secreted oxytocin is partly degraded within the placenta. It is most likely that the CAP present in placenta rather than that in serum plays an important role in the inactivation of fetus-derived oxytocin.

To date, the so-called oxytocinases, which include leucine aminopeptidase, have been extensively purified from human retroplacental serum^{2,7–10)} or from human placenta^{11–15)} and characterized. However, there are only limited reports of the subcellular localization and thorough purification of CAPs present in the placenta.¹²⁾ In this study, we have purified two different molecular species of CAP (CAP-I and CAP-II) from the 9000 *g* and 105000 *g* precipitate fractions of human placenta homogenate in parallel using almost identical methods. The biochemical properties and possible roles in the placenta of the two CAPs are described.

MATERIALS AND METHODS

Materials The following materials were commercially

obtained: *p*-nitroanilide derivatives of amino acids, bestatin and amastatin from Sigma; oxytocin and arginine-vasopressin from Peptide Institute, Osaka; hydroxyapatite from Seikagaku Kogyo, Tokyo; DE-32 from Whatman; and Sephadex G-200 and HiTrap chelating from Pharmacia Biotech. All other reagents were of the highest grade available from Wako Pure Chemical Co., Osaka.

Subcellular Fractionation Human placenta obtained after normal delivery was immediately rinsed with cold saline. The tissues (50 g for CAP-I and 500 g for CAP-II) were cut into pieces and homogenized in 4 vol. 10 mM potassium phosphate buffer (pH 7.5)/0.25 M sucrose. After centrifugation at 600 *g* for 10 min, the precipitate (nuclei and unbroken cells) was removed, and the supernatant was centrifuged at 9000 *g* for 20 min. The resulting precipitate (mitochondrial/lysosomal fraction) was collected and used as the source of CAP-I. The supernatant was further centrifuged at 105000 *g* for 60 min, and the precipitate (microsomal fraction) was collected and used as the source of CAP-II. Each precipitate was then treated at 4 °C for 24 h with 9 vol. 10 mM potassium phosphate buffer (pH 7.5)/0.5% (w/v) sodium deoxycholate. After centrifugation at 105000 *g* for 60 min, the supernatant was collected and dialyzed at 4 °C for 24 h against 10 mM potassium phosphate buffer (pH 6.8). The dialysates were used as starting materials for the purification of CAP-I and CAP-II.

CAP Purification Purification of CAP-I and CAP-II required four chromatographic procedures in the following sequence: hydroxyapatite, DE-32, chelating Sepharose HP and Sephadex G-200. These purification steps and experimental conditions were the same as those described

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by us for the purification of porcine kidney CAP.¹⁶⁾ All procedures were carried out at 4°C, unless otherwise indicated.

Hydroxyapatite Chromatography: The deoxycholate extract (CAP-I or CAP-II) was applied to a column (1.6 × 15 cm) of hydroxyapatite, pre-equilibrated with 10 mM potassium phosphate buffer (pH 6.8)/5 mM 2-mercaptoethanol. The proteins were eluted with a linear gradient of potassium phosphate from 10 to 500 mM at a flow rate of 17 ml/h (fraction volume, 5 ml/tube). The activities of both CAP-I and CAP-II were eluted at 250 mM potassium phosphate.

DE-32 Chromatography: The active fractions obtained from the hydroxyapatite column were dialyzed overnight against 10 mM potassium phosphate buffer (pH 7.5). The dialysate was applied to a column (1.6 × 10 cm) of DE-32, pre-equilibrated with 10 mM potassium phosphate buffer/5 mM 2-mercaptoethanol. The proteins were eluted with a linear gradient of potassium phosphate from 10 to 500 mM at a flow rate of 20 ml/h (fraction volume, 3 ml/tube). The activities of CAP-I and CAP-II were eluted at 250 and 200 mM potassium phosphate, respectively.

Nickel Ion Affinity Chromatography: The active fractions obtained from the DE-32 column were dialyzed overnight against 20 mM potassium phosphate buffer (pH 7.2)/0.15 M NaCl/5 mM 2-mercaptoethanol and concentrated on a PM-10 membrane (Amicon). The concentrate was applied to a column of HiTrap chelating (bed volume, 5 ml) that had been saturated with 0.1 M NiCl₂ and then equilibrated with 20 mM potassium phosphate buffer/0.15 M NaCl/5 mM 2-mercaptoethanol. Both CAP-I and CAP-II activities passed through the column. The absorbed proteins, which were eluted with the column equilibration buffer, containing 50 mM acetic acid, had no CAP activity.

Gel Filtration on Sephadex G-200: The active fractions obtained from the metal chelate column were dialyzed at 4°C overnight against 20 mM potassium phosphate buffer (pH 7.2)/0.2 M NaCl/5 mM 2-mercaptoethanol and concentrated on a PM-10 membrane (Amicon). The concentrate was applied to a column (1.6 × 40 cm) of Sephadex G-200, pre-equilibrated with 20 mM potassium phosphate buffer (pH 7.2)/0.2 M NaCl/5 mM 2-mercaptoethanol. The proteins were eluted with the same buffer at a flow rate of 15 ml/h (fraction volume, 3 ml/tube). The active fractions were collected, concentrated and biochemically characterized.

Determination of Amidolytic Activity All enzyme assays with *p*-nitroanilide derivatives of amino acids were carried out on 96-well plates, and the activity was measured at 25°C with a microplate reader (EIA Reader Model MPR-A4, Toyo Soda, Tokyo). The assay mixture contained the following components in a total volume of 210 µl: 0.48 mM substrate, 5 µl human serum to prevent turbidity and 0.1 M potassium phosphate buffer (pH 7.5)/10% (v/v) methoxyethanol. The assay was started by adding 10 µl enzyme solution, and the absorbance at 405 nm was measured up to 30 min.

Hydrolysis of Biologically Active Peptides The assay mixture contained 10 µl enzyme solution, 0.01–0.5 mM oxytocin or arginine-vasopressin and 50 mM Tris-HCl

buffer (pH 7.0) in a total volume of 70 µl. The enzyme reaction was carried out at 37°C for 1 h and terminated with 10 µl 0.2% (v/v) trifluoroacetic acid (TFA). The hydrolyzed product was analyzed by the following HPLC procedures. The HPLC system consisted of a Model 6000 A solvent delivery system (Waters), a Model S-310 A variable-wavelength detector (Soma, Tokyo) and a Chromatopac C-R2AX chromatographic processor (Shimadzu, Kyoto). A 10-µl portion of the reaction mixture was injected into a Capcell Pak C₁₈ column (4.6 × 250 mm i.d., Shiseido, Tokyo). The substrate and its hydrolyzed product were eluted using a concentration gradient of acetonitrile (20 to 45%) in 0.1% TFA at a flow rate of 1.0 ml/min and detected at 210 nm (retention times: oxytocin and its hydrolyzed product, 32.2 and 31.2 min; arginine-vasopressin and its hydrolyzed product, 30.2 and 29.4 min).

Determination of Protein Protein concentration was determined by the method of Lowry *et al.*,¹⁷⁾ using bovine serum albumin as reference.

Electrophoretic Procedures Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of the purified CAP was performed in 10% (w/v) gel according to the method of Laemmli.¹⁸⁾ The protein bands were visualized by silver staining (Bio Rad). After SDS-PAGE, the purified CAP-II molecule was separated into two bands. To evaluate the enzyme activity of each band, gel from the band region was cut out and placed into a flat-bottom plastic vial containing 1 ml 0.1 M Tris-HCl buffer (pH 7.5)/1% Tween 80. After gently shaking for 30 min at 4°C, the solution was removed and replaced with 200 µl 0.1 M Tris-HCl buffer (pH 7.5)/0.5% Tween 80/50 µM ZnCl₂. The slices were shaken for 3 d to renature CAP-II molecule and to elute the protein. The eluted proteins were assayed for CAP activity.

RESULTS

Purification of the Two CAPs in Human Placenta Prior to purification experiments, the subcellular distribution of CAP within placental cells was examined. Approx. 85% of the total CAP activity in the tissue homogenate was found in the 600 *g* supernatant fraction. We further fractionated the supernatant by centrifugation at 9000 *g* for 20 min. The highest specific activity of CAP (0.95 U/mg of protein) was found in the precipitate fraction (mitochondrial/lysosomal fraction). On the other hand, the specific activity in the 9000 *g* supernatant was low (0.36 U/mg of protein), but the total activity was approx. 5 times higher than that recovered from the precipitate fraction. The microsomal fraction obtained by centrifugation of the supernatant at 105000 *g* for 60 min also exhibited high CAP specific activity (0.8 U/mg of protein). Therefore, we tried to isolate CAP from the 9000 *g* and 105000 *g* precipitate fractions using almost identical procedures as described in Materials and Methods. Table 1 summarizes the results of a typical purification of CAPs from 50 g human placenta tissues (the microsomal fraction was obtained from 500 g tissue). Of the four different chromatography columns, the DE-32 and nickel ion affinity columns increased the degree of purity of the

Table 1. Purification of CAPs from Human Placenta

Step	Total protein (mg)	Total activity (U)	Specific activity (U/mg)	Purification (-fold)	Yield (%)
CAP-I from 9000 g precipitate					
Deoxycholate extract	143	198	1.38	1	100
Hydroxyapatite	41.9	145	3.46	2.5	73.2
DE-32	1.40	56.7	40.5	29.3	28.6
Nickel ion affinity	0.14	49.4	353	256	24.9
Sephadex G-200	0.08	39.4	493	357	19.9
CAP-II from 105000 g precipitate					
Deoxycholate extract	153	130	0.85	1	100
Hydroxyapatite	15.9	40.4	2.54	3.0	31.1
DE-32	0.89	22.4	25.2	29.6	17.2
Nickel ion affinity	0.18	16.3	90.6	107	12.5
Sephadex G-200	0.12	14.2	118	139	10.9

The starting materials were 50 g human placenta tissues for CAP-I and 500 g for CAP-II. The CAP activity at each purification step was determined with *S*-benzyl-L-cysteine *p*-nitroanilide as the substrate as described in Materials and Methods. One unit (U) is defined as 1 μ mol *p*-nitroaniline liberated/min.

Table 2. Comparison of Some Properties of the Purified CAPs

Property	CAP-I	CAP-II
Molecular weight		
Gel filtration on Sephadex G-200	64 kDa	97 kDa
Analysis on SDS-PAGE	61 kDa	56, 40 kDa
Thermal stability		
60 °C, 10 min	80% remaining	25% remaining
60 °C, 30 min	23% remaining	6.3% remaining
K_m value	24 μ M	100 μ M
Optimal pH	7.4–8.0	6.8–8.0

The enzyme activity was determined with *S*-benzyl-L-cysteine *p*-nitroanilide as the substrate.

Fig. 1. Molecular Mass Determination of the Purified CAPs on SDS-PAGE

About 3 μ g of each final preparation was applied to 10% gel as described in Materials and Methods. Molecular mass markers: 97 kDa, phosphorylase b; 66 kDa, bovine serum albumin; 42 kDa, aldolase; 30 kDa, carbonic anhydrase; 20 kDa, soybean trypsin inhibitor. Arrows indicate the subunits of CAP-II.

enzymes. CAP-I and CAP-II were finally purified 357- and 139-fold from each starting material with yields of 19.9 and 10.9%, respectively.

Physicochemical Properties of the Purified CAPs Table 2 summarizes some physicochemical properties of the purified CAPs. The apparent molecular masses of CAP-I and CAP-II were estimated to be 64 and 97 kDa, respec-

Fig. 2. Estimation of Enzymatic Activity of the Purified CAP-II after SDS-PAGE

The gel was cut into pieces, and the corresponding areas were homogenized with phosphate buffer. The enzyme activity in each extract was assayed with *S*-benzyl-L-cystein *p*-nitroanilide as described in Materials and Methods.

tively, by gel filtration on Sephadex G-200. Analyses of the purified CAPs on 10% SDS-PAGE under reducing conditions revealed that CAP-I migrated as a single band with a molecular mass of 61 kDa (Fig. 1A). On the other hand, CAP-II migrated as two proteins of 40 and 56 kDa (Fig. 1B), suggesting either a dimeric structure or an impurity. To characterize these two proteins, they were eluted from gel with cold buffer (see Materials and Methods). As shown in Fig. 2, estimation of the amyolytic activity of the individual and the combined proteins suggested a dimeric form for CAP-II (recovery of the enzyme activity was approx. 12%).

CAP-II was more labile on heating than CAP-I, when both enzymes were incubated at 60 °C for 10 min (75% CAP-II activity was lost, while 80% CAP-I activity remained). The K_m for *S*-benzyl-L-cysteine *p*-nitroanilide was determined and gave values of 24 and 100 μ M for CAP-I and CAP-II, respectively. The influence of pH on the activity of the CAPs was also examined. The enzyme activity was maximal at pH 7.8: more than 80% of the maximal activity was obtained over the pH range 7.4–8.0 (CAP-I) or 6.8–8.0 (CAP-II).

Substrate Specificity and Other Enzymic Properties of the Purified CAPs As shown in Table 3, both CAPs

Table 3. Substrate Specificity of the Purified CAPs toward Amino Acid *p*-Nitroanilides

Substrate (<i>p</i> -nitroanilide)	K_m (μ M)	k_{cat} (s^{-1})	k_{cat}/K_m ($s^{-1} \cdot \mu M^{-1}$)
CAP-I			
<i>S</i> -Benzyl-L-cysteine	24	9.2	0.383
L-Alanine	333	23.6	0.071
L-Phenylalanine	400	50.8	0.127
L-Methionine	83	18.3	0.220
L-Lysine	100	22.9	0.229
L-Leucine	50	13.1	0.262
CAP-II			
<i>S</i> -Benzyl-L-cysteine	100	7.6	0.076
L-Alanine	1900	38.2	0.020
L-Phenylalanine	361	12.7	0.035
L-Methionine	386	26.2	0.068
L-Lysine	369	19.1	0.052
L-Leucine	120	8.6	0.07

The activity of CAPs was assayed at pH 7.5 as described in Materials and Methods. Kinetic parameters were obtained from Lineweaver-Burk plot analyses. K_m and V_{max} were calculated from the plot x and y intercepts, respectively. The catalytic rate constant (k_{cat}) was derived from the expression $V_{max}/[E_0]$, where $[E_0] = 25$ nM CAP.

Table 4. Hydrolysis of Oxytocin and Arginine-Vasopressin by the Purified CAPs

Substrate	K_m (μ M)	V_{max} (μ mol/h/mg protein)	V_{max}/K_m (μ mol/h/mg protein $^{-1} \cdot \mu M^{-1}$)
CAP-I			
Oxytocin	5.6	23.4	4.14
Vasopressin	53.0	5.5	0.10
CAP-II			
Oxytocin	38.0	15.6	0.41
Vasopressin	470	10.8	0.02

The assay was performed as described in Materials and Methods. Kinetic parameters were obtained from Lineweaver-Burk plot analyses.

hydrolyzed various amino acid *p*-nitroanilides. *S*-Benzyl-L-cysteine and L-leucine *p*-nitroanilides are good substrates for both enzymes, as judged by their catalytic efficiencies (k_{cat}/K_m). The catalytic efficiency of CAP-I for *S*-benzyl-L-cysteine *p*-nitroanilide was 5 times higher than that of CAP-II. We also examined the hydrolysis of oxytocin and vasopressin by CAPs (Table 4). Oxytocin was a better substrate for both enzymes, but the ability of CAP-I to hydrolyze this peptide was 10 times greater than that of CAP-II (V_{max}/K_m value: 4.14 for CAP-I; 0.41 for CAP-II).

The effects of various reagents on the amidolytic activities of both CAPs are shown in Table 5. *p*-Chloromercuribenzoate (1 mM) effectively inhibited CAP activities, while iodoacetate and *N*-ethylmaleimide hardly affected them. A requirement of the CAPs for a metallic cation was suggested by the inhibitory effects of EDTA and *o*-phenanthroline. However, the addition of Ca^{2+} or Mg^{2+} (1 mM) had little or no effect on the enzyme activity. Both Zn^{2+} and Ni^{2+} considerably inhibited the enzyme activity. Only Co^{2+} increased the enzyme activity of CAP-I (1.7- to 2-fold) and CAP-II (1.6- to 1.7-fold) over the concentration range 0.5–1.0 mM. The effect of various amino acids on the CAP activities was also ex-

Table 5. Effects of Various Reagents on the Amidolytic Activity of the Purified CAPs

Reagent	Final concentration (mM)	Relative enzyme activity (%)	
		CAP-I	CAP-II
None		100	100
<i>p</i> -Chloromercuribenzoate	1.0	30.4	12.1
Iodoacetate	1.0	117.0	127.7
<i>N</i> -Ethylmaleimide	1.0	88.0	89.0
$CaCl_2$	1.0	98.1	71.3
$MgCl_2$	1.0	113.0	94.7
$CoCl_2$	0.5	167.8	159.1
	1.0	201.7	172.3
$ZnCl_2$	1.0	66.8	68.9
$NiCl_2$	0.5	49.6	44.8
	1.0	31.0	26.9
EDTA	1.0	28.8	66.0
<i>o</i> -Phenanthroline	1.0	0	32.1
Glycine	10.0	96.5	80.4
L-Glutamine	10.0	93.1	87.8
L-Lysine	10.0	92.2	78.3
L-Methionine	10.0	21.9	75.6
L-Phenylalanine	10.0	44.1	53.6
L-Leucine	10.0	70.2	55.6
L-Cystine	10.0	33.3	33.6
Bestatin	0.0005	84.3	78.4
Amastatin	0.0005	43.0	11.4

The enzyme activity was determined with *S*-benzyl-L-cysteine *p*-nitroanilide as the substrate. CAP was preincubated for 3 min with each reagent before addition of substrate.

amined. Glycine, L-glutamine and L-lysine had little or no inhibitory effect on both enzymes at a concentration of 10 mM, whereas L-cystine was a potent inhibitor (approx. 70% inhibition). L-Phenylalanine and L-leucine, having a large hydrophobic side-chain, also inhibited CAP activity. Interestingly, L-methionine had a significant inhibitory effect on the activity of CAP-I (78% inhibition). However, the amino acid was less effective toward CAP-II (24% inhibition). Bestatin, a powerful inhibitor of both arginyl and leucyl aminopeptidases, had a weak inhibitory effect on CAP activity. Amastatin, which inhibits both glutamyl and leucyl aminopeptidases, was a potent inhibitor of both CAPs (especially CAP-II).

DISCUSSION

Two forms of CAP that efficiently hydrolyze *S*-benzyl-L-cysteine *p*-nitroanilide were purified near to homogeneity from human placenta tissues (Table 1). CAP-I purified from the mitochondria/lysosomes-rich fraction was a single protein of 64 kDa on gel filtration and 61 kDa on SDS-PAGE (Fig. 1). CAP-II present in the microsomal fraction had subunit masses of 56 and 40 kDa on SDS-PAGE (Fig. 1), but an apparent molecular mass of 97 kDa on gel filtration. CAP-II required both subunits for the hydrolysis of *S*-benzyl-L-cysteine *p*-nitroanilide (Fig. 2). CAPs with molecular masses of 64 and 97 kDa have never been reported previously. Cornell^{1,2)} has purified two forms of oxytocinase from human placenta, using *S*-benzyl-L-cysteine *p*-nitroanilide as substrate. The purified oxytocinases had molecular masses of 24 and 30 kDa, respectively, and appeared to be isozymic forms differ-

ing only in their carbohydrate content. Hayashi and Oshima¹⁹ have shown that a CAP purified from the 30000 *g* supernatant of monkey placental homogenate is a single protein with a molecular mass of 83 kDa on SDS-PAGE and most active at pH 6.5 toward L-cystine di- β -naphthylamide. However, the optimal pH of CAP-I and CAP-II for *S*-benzyl-L-cysteine *p*-nitroanilide was 7.8. CAP-I was more heat-stable than CAP-II: 80% CAP-I activity remained and 75% CAP-II activity disappeared following incubation at 60 °C for 10 min (Table 2). The thermal instability of CAP-II may be due to its dimeric structure.

Substrate specificities of the purified CAPs were determined with various amino acid *p*-nitroanilides (Table 3). Although there were some differences between the kinetic parameters of CAPs toward the substrates, their substrate specificities were broadly similar. Most aminopeptidases hydrolyze L-alanine β -naphthylamide rapidly,^{13,15,19} whereas the CAPs hardly hydrolyzed this substrate. The highest catalytic efficiencies of CAPs were obtained with *S*-benzyl-L-cysteine *p*-nitroanilide (0.38 for CAP-I and 0.08 for CAP-II), suggesting that both enzymes have a high affinity toward substrates possessing N-terminal cystine residues, such as oxytocin and vasopressin. In fact, both enzymes hydrolyzed the two biologically active peptides (Table 4). Oxytocin was a good substrate for both CAPs, but CAP-I hydrolyzed the substrate 10 times more efficiently than CAP-II ($V_{\max}/K_m$ value: 4.14 for CAP-I; 0.41 for CAP-II). Biologically active peptides other than oxytocin may also be substrates for both CAPs. However, the above findings suggest that the enzymes act as oxytocinase at least in the placenta. It is, at present, not known why the catalytic efficiency of CAP-II toward oxytocin and *S*-benzyl-L-cysteine *p*-nitroanilide is lower than that of CAP-I. The present results suggest that CAP-II is a microsome-bound aminopeptidase and requires the dimeric structure. Its solubilization and purification may have altered the intact subunit structure and activity in the membrane environment.

CAP-I and CAP-II were both inhibited by EDTA and *o*-phenanthroline and activated specifically by the addition of Co^{2+} (Table 5), suggesting they are cobalt metalloproteases. Also, L-methionine was a potent inhibitor of CAP-I, but not of CAP-II. As regards the susceptibility of these CAPs to L-methionine and Co^{2+} , CAP-I was comparable with the aminopeptidase purified from human placental microsomes.¹⁵ On the other hand, the nature of CAP-II (L-methionine-insensitive and Co^{2+} -sensitive) was identical with that of aminopeptidases from porcine kidney¹⁶ and monkey liver,²⁰ but apparently different from that of oxytocinase from monkey placenta.¹⁹

Oxytocinase has been identified in the sera of pregnant women. The major source of oxytocinase is apparently the placental syncytiotrophoblast, as suggested by Page *et al.*⁵ Sjöholm and Yman¹ have estimated the molecular

mass of oxytocinase from retroplacental serum as 325 kDa on gel filtration. Lampelo and Vanha-Perttula²⁾ have previously shown that pregnancy causes the appearance of a new type of CAP in serum. The CAP having a molecular mass of 309 kDa is partially inhibited by L-methionine and not activated by Co^{2+} . Thus, the molecular nature and enzymatic properties of the CAP-I and CAP-II purified by us appear to be quite different from those of the serum CAP.

The present results show that CAP-I and CAP-II are tightly bound to mitochondria (or lysosomes) and microsomes, respectively, and imply that they may be a novel family of cystinyl aminopeptidases and play a role in the breakdown of neurohypophyseal hormones. In addition, the CAPs may play an important role in basic biological events such as tissue growth through the degradation of intracellular proteins. However, the real intracellular functions of CAP-I and CAP-II remain to be fully elucidated.

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