A MONOMERIC FORM OF PROCARBOXYPEPTIDASE A FROM THE SPINY PACIFIC DOGFISH*

Andras G. Lacko and Hans Neurath
Department of Biochemistry, University of Washington
Seattle, Washington 98105

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INTRODUCTION

Although the activation of trypsinogens and chymotrypsinogens from various species has been extensively studied (Neurath, 1964), only limited information is available on the mechanism of activation of procarboxypeptidases. The activation of bovine procarboxypeptidase A (PCPA) is complicated by the tri- and dimeric aggregates in which this zymogen occurs (Brown et al., 1963). Folk and Schirmer (1965) have suggested that porcine PCPA may occur in the monomeric form but no data have yet been reported on the isolation and purification of such a zymogen.

In this preliminary communication, we should like to report the purification and partial characterization of a monomeric form of PCPA isolated from the Spiny Pacific Dogfish. This tissue is known to contain the same kind of proteolytic enzymes, in the zymogen form, which are characteristic of the bovine pancreas (Prahl and Neurath, 1966 a,b). Apart from possible phylogenetic implications, the isolation of dogfish PCPA provides an excellent system for studying the mechanism of activation of the zymogen precursor of carboxypeptidase A.

EXPERIMENTAL

Dogfish pancreas glands were obtained from commercial fishermen in the Puget Sound area and processed according to Prahl and Neurath (1966 a), to ob-

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tain the pancreatic acetone powder. In a routine preparation 100 gm of acetone powder were extracted in the cold room with 1 liter of distilled water containing 20 mg of soybean trypsin inhibitor. After a four hour extraction period, the particulate matter was removed by centrifugation at 8000 g under refrigeration. The supernatant was then saturated with $(NH_4)_2SO_4$ to precipitate the proteins, leaving most of the nucleic acid material in the supernatant. After two hours in the cold room the precipitate was removed by centrifugation as above and taken up in about 200 ml of 0.01 M sodium phosphate pH 7.0 and dialyzed overnight against two 6 liter portions of the same buffer containing 10^{-4} M DFP. Next the dialyzed extract was pumped onto a DEAE cellulose column (5 x 60 cm) which had previously been equilibrated with 0.01 M sodium phosphate pH 7.0. The chromatogram was developed with a sodium chloride gradient (Fig. 1). Dogfish procarboxypeptidase A was always eluted from DEAE cellulose following procarboxypeptidase B as described by Prahl and Neurath (1966 b). Carboxypeptidase activity of the fractions was monitored by using hippuryl-DL-β-phenyllactic acid (HPLA) after tryptic activation of the appropriate fractions. Activity against acetyl-tyrosine ethyl ester (ATEE) was also followed.

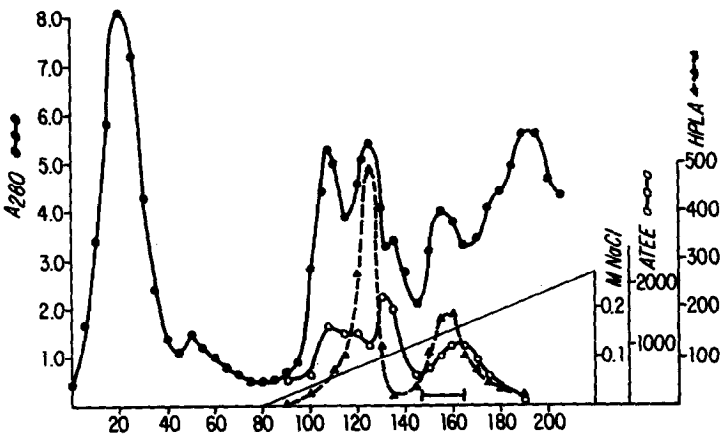

Fig. 1. Elution profile of chromatography on DEAE cellulose of an aqueous extract of acetone powder of dogfish pancreas. Units of activity are μmoles of substrate hydrolyzed per minute per ml of solution. For other details, see the text.

As may be seen in Fig. 1, the fractions containing crude PCPA contained also considerable ATEEase activity, confirming the observations of Prahl and Neurath (1966 b). To obtain pure zymogen, the pooled fractions from DEAE-cellulose columns were subjected to gel filtration by passing them twice through Sephadex G-100. After such treatment, the zymogen appeared to be pure by all criteria tested including disc gel electrophoresis, moving boundary electrophoresis and sedimentation analysis, and exhibited only carboxypeptidase activity upon activation.

Dogfish PCPA sedimented in the ultracentrifuge (Spinco Model E) with a sedimentation coefficient of $s_{20} = 3.9 \pm 0.05$, and by sedimentation equilibrium was found to have a molecular weight of $42,500 \pm 1,000$ (0.1 M Tris-HCl, 0.01 M $CaCl_2$, pH 8.0). These physical properties are similar to those reported by Neurath and Freisheim (1966) for succinyl fraction I, the precursor of carboxypeptidase A isolated from the bovine pancreas. The amino acid composition of the dogfish enzyme also appeared very similar to that of the bovine counterpart, except for the sulfur-containing amino acids. Specifically, dogfish PCPA contained 14 methionine residues as compared to 4 for the bovine and 4 "half cystines" as compared to 2 (determined as cysteic acid and as S-carboxymethyl cysteine).

Activation of the zymogen could be accomplished by both bovine trypsin and Nagarse, while bovine chymotrypsin was much less efficient in this respect (Fig. 2). Optimum conditions for activation required the presence of 0.5 M $CaCl_2$ in the activation mixture. At lower Ca^{++} ion concentrations, the activation rate fell off rapidly.

Dogfish carboxypeptidase (CPA) was isolated by passing the activation mixture through a Sephadex G-50 column and precipitating the enzyme with 21% saturated $(NH_4)_2SO_4$ from the concentrated breakthrough peak fractions. After such treatment the enzyme appeared to be pure upon sedimentation in the ultracentrifuge.

Activation of the zymogen to active enzyme by bovine trypsin was accompan-

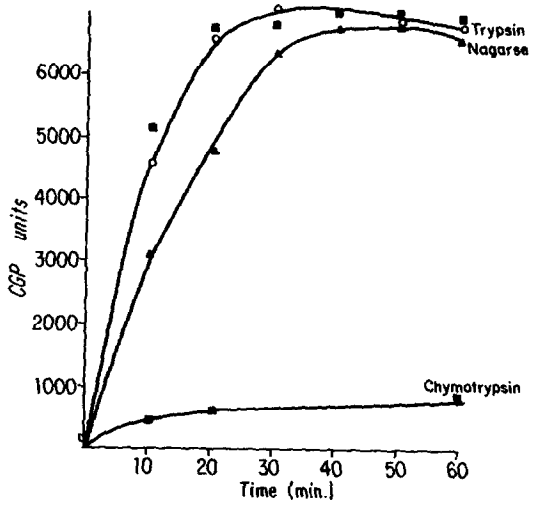

Fig. 2. Activation of dogfish PCPA at 25° by trypsin, Nagarse and chymotrypsin. Zymogen:enzyme ratio 100:1 in all cases. Solvent: 0.1 M TRIS-HCl, 0.5 M $CaCl_2$, pH 7.9. Zymogen concentrations, 1.5 mg/ml. Unit of activity ΔA_{223} per minute per g zymogen. Substrate, 10^{-3} M CGP in 0.005 M TRIS-HCl, 0.1 M NaCl, pH 7.5. Duplicate experiments are illustrated for trypsin.

ied by a change in sedimentation coefficient from 3.9 to 3.2, corresponding to a decrease in molecular weight from 42,500 to 36,500 (sedimentation equilibrium). The active enzyme was found to be much less anionic above pH 7 and less soluble in aqueous buffers than its precursor PCPA. Comparison of the amino acid compositions of PCPA and CPA indicated an excess of about 60 residues per molecule in the zymogen. Of these about twenty-eight are accounted for by glutamic or aspartic acid or their respective amides. If the activation process indeed consists of a single proteolytic split, one would then expect to find a rather large peptide of strongly anionic character in the activation mixture. Isolation of such a peptide is presently being pursued in our laboratory.

In analogy to bovine CPA, it was possible to modulate the activity of the dogfish enzyme towards ester and peptide substrates in a manner similar to that described by Vallee (1964). As may be seen from Fig. 3, upon treatment

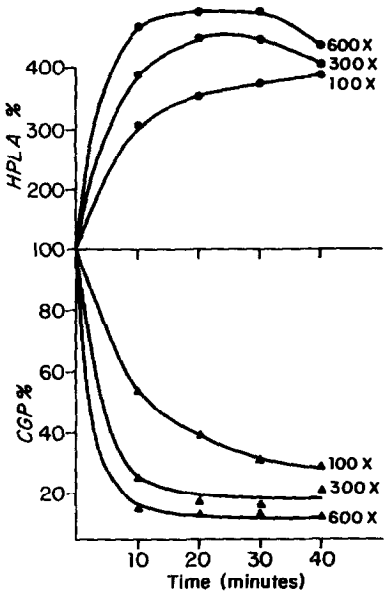

Fig. 3. Effects of acetylation with N-acetyl imidazole on esterase (HPLA) and peptidase (CGP) activities of dogfish CPA. Conditions: 0.005 M sodium phosphate, 0.5 M NaCl, pH 7.5, 25°. Enzyme concentration 4 mg per ml.

with N-acetyl imidazole, the activity against HPLA increases while the activity against carbobenzoxyglycyl-L-phenylalanine (CGP) declines. The magnitude of these effects depends on the molar excess of the reagent used. When the enzyme was dialyzed against 10^{-3} M $CdCl_2$, the activity against HPLA increased twofold while peptidase activity dropped to 3% of the initial value. Upon dialyzing the "cadmium enzyme" against 10^{-3} M Zn^{++}, both activities approached the initial values, thus indicating that the native enzyme probably contains zinc. The fact that dogfish carboxypeptidase A is a metalloenzyme was also demonstrated by the progressive loss of enzyme activity upon dialysis against 10^{-3} M 1,10 phenanthroline.

The above data convincingly show the existence of a monomeric zymogen in dogfish pancreatic tissue that gives rise to CPA activity upon activation.

Detailed investigations of the properties of the zymogen, the enzyme as well as the activation process are now in progress in our laboratory.

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