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SEQUENTIAL HOMOLOGIES BETWEEN PROCARBOXYPEPTIDASES A AND B FROM PORCINE PANCREAS

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Automated Edman degradation of monomeric procarboxypeptidases A and B from porcine pancreas shows that their N-terminal regions (from residue 1 to 34-37) present a high degree of sequential homology to each other as well as to other related procarboxypeptidases. Conformational predictions based on these sequences confirm their structural homology and indicate the probable existence of two β-turns, one β-chain and a long α-helix in them. On the other hand, tryptic peptide maps on a reverse-phase column indicate great sequential similarities (if not identity) between monomeric procarboxypeptidase A and the procarboxypeptidase A subunit isolated from its binary complex with proproteinase E.

Procarboxypeptidases are the inactive precursors of carboxypeptidases, a class of pancreatic exopeptidases which degrade polypeptides from their COOH terminus. On the basis of the different specificity shown by the active (mature) enzymes on peptidic substrates, procarboxypeptidases are classified in A and B forms (1). Moreover, the A form shows subforms due to its occurrence as a monomer and as oligomeric complexes with other proproteinases (1,3), or due to sequential allelomorphism which is known to occur in the C-terminal region of the proenzyme (4). On the other hand, the B form apparently occurs only as a monomer (5,6). It is interesting to note that the activation of pancreatic procarboxypeptidases is accompanied by the proteolysis of a large activation segment (approx. 100 residues long) (6,7), located in the N-terminal region. In the case of procarboxypeptidase A the activation segment constitutes a structural globular domain of the protein and specifically inhibits its activity, as we reported recently (7,8). Both facts are probably also applicable to the homologous procarboxypeptidase B.

In order to progress towards the elucidation of the conformation, genetic evolution and inhibitory properties of the activation segment of

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these proenzymes we have analysed the primary structure of their N-terminal regions. It is shown that the N-terminal regions of monomeric procarboxypeptidases A and B from porcine pancreas exhibit extensive sequence and conformation homologies to each other, as well as to other related procarboxypeptidases. In addition, a comparison by peptide maps indicates a great sequence similarity between the two protomeric procarboxypeptidases A from porcine pancreas: the natural monomer and the subunit isolated from its natural binary complex with proproteinase E (2,9).

MATERIALS AND METHODS

Protein purification. The monomeric and the binary complex forms of procarboxypeptidase A and monomeric procarboxypeptidase B were isolated from defatted acetone powders from porcine pancreas.The isolation method involves a fractionation of an aqueous extract of porcine pancreas powder by ammonium sulphate precipitation, followed by chromatography on DEAE-Sepharose at pH 8.0 and a second chromatography on DEAE-Sepharose at pH 5.7, which resolves both procarboxypeptidases A. Monomeric procarboxypeptidase B was isolated from a fraction in this chromatography at pH 5.7 which showed a high potential carboxypeptidase B activity. In this fraction procarboxypeptidase B appears to be contaminated with other proteins which were subsequently removed by chromatofocusing on a column of PBE-94 resin (Pharmacia) from pH 5.5 to pH 4.0, using elution with polybuffer PE-74 (Pharmacia). Following the above method, pure procarboxypeptidase B devoid of activity against the substrate benzoyl-glycyl-L-arginine was obtained. Protomeric procarboxypeptidase A was isolated from its natural binary complex with proproteinase E by chromatography on DEAE-Sepharose in the presence of 7M urea as reported elsewhere (9).

Amino acid sequence. The N-terminal aminoacid sequence of carboxymethylated monomeric procarboxypeptidases A and B were obtained by automated Edman degradation on a Beckman sequencer, model 890-D with the Beckman 1M Quadrol program. The detached PTH were analysed by thin-layer chromatography (10) and amino acid analysis after back hydrolysis with HCl (11). Details of the materials, protein treatment, degradation program used in the sequencer and analytical procedure were as in (10,11).

Peptide maps. Tryptic fragmentation of carboxymethylated procarboxypeptidases A and B was carried out by two succesive additions of bovine trypsin (TPCK-treated, Worthington) at ratio 50/1 (w/w) in 20mM ammonium bicarbonate (pH 8.5), at 37°C, for two hours each. Peptide maps were obtained by dissolving the tryptic digests in 5M guanidinium chloride, 0.1% trifluoroacetic acid, and submitting them to high pressure liquid chromatography (HPLC) on a C-18 μ-Bondapak reverse phase column. The chromatography was followed by UV absortion at 214 and 295nm. The last wavelength was used to detect specifically tryptophan-containing peptides. The isolated fractions were subsequently spotted on different thin-layer silica gel plates (Merck) and analysed for histidine and tyrosine containing peptides by the Pauly and α-nitroso-β-naphtol stainings (12).

Secondary structure. Predictions of secondary structure have been performed according to Chou and Fasman (16).

RESULTS AND DISCUSSION

The primary structure of the first 34 residues from the N-terminal region of natural monomeric procarboxypeptidase A from porcine pancreas has

been analysed by automated Edman degradation (Figure 1). The sequence of the first seven residues is in total agreement with that reported by Kobayashi et al. (2) from the same protein isolated from porcine pancreatic secretion. On the other hand, the entire sequence of 34 residues analysed here also shows a strong homology with the sequence of the equivalent region in rat procarboxypeptidase A deduced by Quinto et al. from the sequence of DNA bases (13) (Fig. 1). The strong homology found, up to 82%, seems also to be present between the same regions of rat and bovine procarboxypeptidase A, as indicated by Quinto et al. in their report. It seems reasonable to think that such a high percentage of sequence conservation could have important structural and/or functional reasons.

In Figure 1 the primary structure of the first 37 N-terminal residues of procarboxypeptidase B from porcine pancreas is also presented. If a three residue shift is made and a few deletions are considered, an evident homology can be observed between this sequence and those from rat and porcine procarboxypeptidases A. Thus if the presence of only 16 identical residues among the first 37 residues is considered, the homology is not more than 43%, but if conservative substitutions (13 in total) are taken into account, the homology rises to 79%. It may be added that the strict sequential homologies between bovine carboxypeptidase A and bovine carboxypeptidase B comprise 49% of their primary structure (4).

Native porcine pancreatic procarboxypeptidases A and B, isolated and sequenced in this work, when analyzed on SDS-polyacrylamide gels yield apparent Mr's of 46,200 and 48,900 in the non-reduced state and apparent Mr's of 44,300 and 47,000 in the reduced and carboxymethylated state. Since the active porcine carboxypeptidase B possesses a molecular weight very similar to active porcine carboxypeptidase A, Mr's 34,300 and 34,800 (14,15), our measurements of molecular weigth indicate that porcine procarboxypeptidase B could possess an activation region longer by 15-25 residues than the corresponding one in porcine procarboxypeptidase A. In that case these extra residues would not be located in the N-terminal end of the activation segment, according to the results shown in Fig. 1.

It is interesting to note that, according to Quinto et al. (13), the first 37 residues of rat procarboxypeptidase A are coded by the same exon. The sequential comparisons discussed here seem to indicate that this

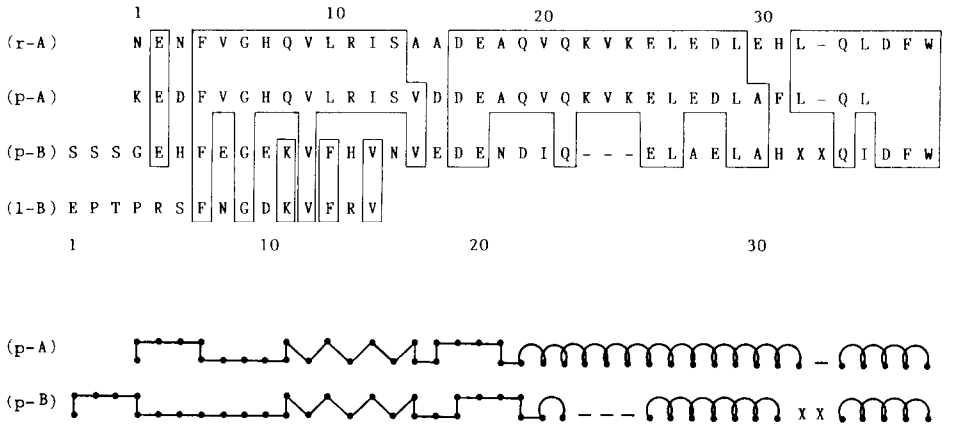

Figure 1. N-terminal sequence comparison between pig monomeric procarboxypeptidases A and B (p-A and p-B), rat procarboxypeptidase-A (r-A) and lungfish procarboxypeptidase-B (1-B). Common sequences are indicated in boxes. X, unidentified residues. The predicted secondary structures of pig procarboxypeptidases A and B, based on the displayed sequences are shown below: the drawing refers to regions with β-turns ( ), β-chains ( ) and α-helix ( ). Probability potentials for these regions according to Chou and Fasman (16) are indicated in text.

genetic unit is preserved without important changes in sequence; this can explain the small variation observed between the rat, ox and pig proteins. The information available regarding the corresponding regions in procarboxypeptidase B for the time being suggest that this conservation is also present in procarboxypeptidase B, particularly if the conservative substitutions are taken into account. Thus the sequence of 10 residues known for the N-terminal end of lungfish procarboxypeptidase B (6) shows important homologies with the corresponding region of porcine procarboxypeptidase B, as shown in Fig. 1.

Conformational predictions elaborated from the sequences in Fig. 1, according to the Chou and Fasman method (16) (with boundary analysis) indicate the probable existence of a β-chain in residues 8-14 and an α-helix in residues 19-34 for both porcine procarboxypeptidase A ($\langle P\beta \rangle$ = 1.30, $\langle P\alpha \rangle$ = 1.20, respectively) and rat procarboxypeptidase A ($\langle P\beta \rangle$ = 1.17, $\langle P\alpha \rangle$ = 1.20, respectively) (See Fig. 1, bottom). The application of the same predictive method to porcine procarboxypeptidase B indicates a very similar structural disposition, providing that the above mentioned three residue shift is made. Thus a high probability is found for a β-chain in residues 11-17 and for an α-helix in residues 23-35 ($\langle P\beta \rangle$ = 1.28, $\langle P\alpha \rangle$ = 1.24, respectively).

On the other hand, one β-turn can be predicted in the N-terminal end of all the proteins shown in Fig. 1, if we also take into account the high probability of existence of this β-turn in rat procarboxypeptidase A ($\langle Pt \rangle$= 1.2 x 10-4), porcine procarboxypeptidase B ($\langle Pt \rangle$ = 3.2 x 10-4), and lungfish procarboxypeptidase B ($\langle Pt \rangle$= 0.75 x 10-4). A similar assumption of conformational homology also suggests the existence of another β-turn located between the β-chain and the α-helix in both porcine procarboxypeptidases A and B, the intermediate probability of existence of this β-turn in the former protein ($\langle Pt \rangle$= 0.7 X 10-4) being reinforced by the high probability of its existence in the latter ($\langle Pt \rangle$= 1.4 x 10-4). It must be added that all the above conformational predictions are fully supported when the hydrophobicity profiles of the same sequences are analysed according to the method of Cid et al. (17).

Monomeric procarboxypeptidase A from porcine pancreas and the protomeric procarboxypeptidase A isolated from its binary complex with

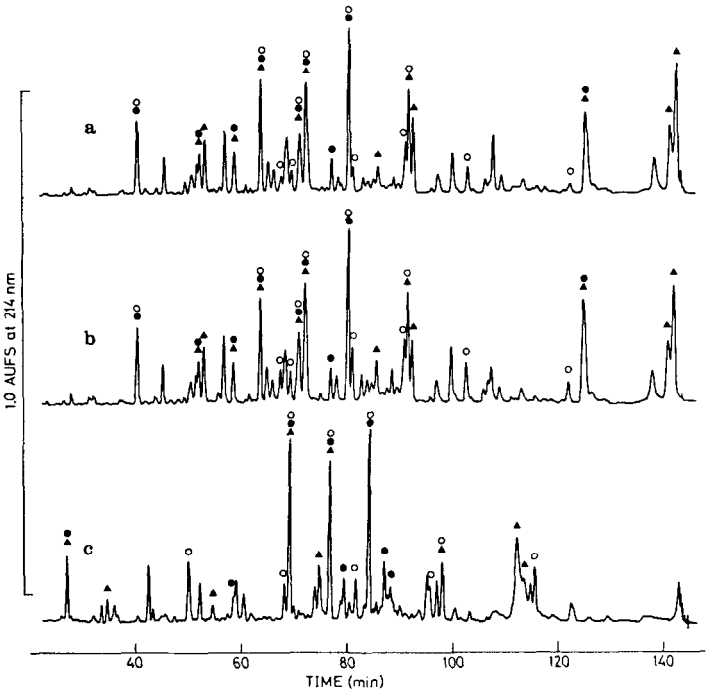

Figure 2. Comparative high pressure liquid chromatography tryptic peptide maps of monomeric procarboxypeptidase-A (a), procarboxypeptidase-A protomer isolated from its binary complex with proproteinase-E (b), and monomeric procarboxypeptidase-B (c), all from pig pancreas. Chromatographies were carried out on a C-18 μ-Bondapak reverse phase column, the peptides being eluted with a linear gradient from 0% to 80% acetonitrile, in 0,1% trifluoroacetic acid, in 140 minutes, and monitored at 214nm (——). Histidine (▲), tyrosine (●), and tryptophan (O) containing peptides were specifically detected as described in Methods.

proproteinase E found in the same tissue, seem to present similar amino acid compositions, as shown by Kobayashi et al. (2), and similar functional properties, as we previously reported (9). A question arises, however, about their possible identity in primary structure. To answer this question, tryptic peptide maps of both proteins were obtained by high pressure liquid chromatography (Fig 2). The chromatograms in the Figure show a nearly complete identity of homologous peaks. Moreover, an absolute identity is observed for peptides eluting in the same positions and containing His and Tyr (important residues for carboxypeptidase activity) and Trp residues. These results make very unlikely the existence of crossing-over in the positions of peaks between both chromatograms. Summarizing, natural monomeric and protomeric procarboxypeptidase A from the binary complex possess very similar sequences, probably identical with respect to the functionally important regions, although their absolute sequential identity can only be established by determination of their primary structure. On the contrary the peptide map of porcine procarboxypeptidase B (Fig. 2) is clearly different from those of procarboxypeptidases A, despite the sequential homologies mentioned above.

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