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Amino acid sequence of phospholipase A from porcine pancreas

Pancreatic phospholipase A (phosphatide acyl-hydrolase, EC 3.1.1.4) arises from an inactive precursor (prophospholipase A) by the tryptic cleavage of the 7th bond (Arg–Ala) in the chain[1]. Mass spectrometry showed the released heptapeptide to have the following formula[2]: Pyroglu–Glu–Gly–Ile–Ser–Ser–Arg, which deviates from the originally proposed structure[1] by the reversed sequence Glu–Gly. It is not yet known whether the N-terminal pyroglutamic acid residue preexists in the molecule or results from an internal cyclization of a glutamic acid or glutamine residue during the purification procedure.

The amino acid composition of the enzyme (molecular weight about 14 000) has been reported to be[1,3]: Ala_8, Arg_4, Asx_{23}, Cys_{14}, Glx_7, Gly_6, His_3, Ile_5, Leu_7, Lys_9, Met_2, Phe_5, Pro_6, Ser_{10}, Thr_7, Trp_2, Tyr_8, Val_2. However, subsequent determinations of tryptophan[4] and of half-cystine content by oxidation to cysteic acid[5] demonstrated the presence of only 1 tryptophan and 12 half-cystine residues in the enzyme molecule.

Application of the Edman degradation technique to the intact protein showed alanine and leucine to be the first and second residues of the chain. A kinetic study of the amino acids liberated from the reduced aminoethylated enzyme by a commercial sample of carboxypeptidase B further suggested that the C-terminal sequence is Lys–Lys–Tyr–Cys.

In order to determine the full sequence of the enzyme, the *S*-sulfo derivative was exhaustively digested with trypsin which cleaved the 9 lysine and 4 arginine bonds of the molecule, as well as 1 asparagine and 2 tyrosine bonds. Accordingly, a total of 17 peptides were obtained containing all the residues of the original protein. After separation of these tryptic peptides on Sephadex G-25 (fine grade) and paper, their sequences were fully or partially determined by Edman degradation and digestion by carboxypeptidases A and B. Hydrolysis of the enzyme by pepsin, chymotrypsin or papain gave rise to rather complex mixtures from which only some short peptides could be purified. The sequence of these confirmed our previous results obtained with the tryptic peptides.

A correct positioning of the tryptic peptides was achieved by preparing large fragments, according to the following methods: a very short attack by trypsin or chymotrypsin; cyanogen bromide cleavage at the level of the two methionines; and finally by tryptic digestion of the *S*-sulfo enzyme after amidination of the lysine residues[6]. The resulting large fragments were separated by chromatography on Sephadex and electrophoresis–chromatography on paper. In most instances, the determination of their amino acid composition and N- and C-terminal residues was not sufficient to ascertain the overlaps between the tryptic units. When this occurred, the fragments were further hydrolyzed by trypsin or chymotrypsin, and the resulting peptides were indentified.

In Fig. 1, the tryptic units are unambiguously assembled from the N-terminal residue until Arg_{105} and from the C-terminal residue until Asn_{106}. No overlap has been obtained thus far between these two residues; therefore, the proposed sequence has to be considered as preliminary. However, it is noteworthy that Table I includes 122

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 8   9  10  11  12  13  14  15  16  17  18  19
Ala-Leu-Trp-Gln-Phe-Arg-Ser-Met-Ile-Lys-Cys-Ala-
20  21  22  23  24  25  26  27  28  29  30  31
Ile-Pro-Gly-Ser-His-Pro-Leu-Met-Asp-Phe-Asn-Asn-
32  33  34  35  36  37  38  39  40  41  42  43
Tyr-Gly-Cys-Tyr-Cys-Gly-Leu-Gly-Gly-Ser-Gly-Thr-
44  45  46  47  48  49  50  51  52  53  54  55
Pro-Val-Asn-Glu-Leu-Asn-Arg-Cys-Glu-His-Thr-Asp-
56  57  58  59  60  61  62  63  64  65  66  67
Asn-Cys-Tyr-Arg-Asp-Ala-Lys-Asn-Leu-Asn-Asp-Ser-
68  69  70  71  72  73  74  75  76  77  78  79
Cys-Lys-Phe-Leu-Val-Asp-Asn-Pro-Tyr-Thr-Glu-Ser-
80  81  82  83  84  85  86  87  88  89  90  91
Tyr-Ser-Tyr-Cys-Ser-Ser-Asn-Thr-Glx-Ile-Thr-Cys-
92  93  94  95  96  97  98  99 100 101 102 103
Asn-Ser-Lys-Asn-Asn-Ala-Cys-Glu-Ala-Phe-Ile-Cys-
104 105 106 107 108 109 110 111 112 113 114 115
Asn-Arg//Asn-Ala-Ala-Ile-Cys-Phe-Ser-Lys-Ala-Pro-
116 117 118 119 120 121 122 123 124 125 126 127
Tyr-Asn-Lys-Glu-His-Lys-Asn-Leu-Asn-Thr-Lys-Lys-
128 129
Tyr-Cys
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Fig. 1 Amino acid sequence of reduced phospholipase A. The numbering of the residues in phospholipase A is the same as in the precursor. Accordingly, the alanine residue which is N-terminal in the enzyme is designated as Ala_8. // the overlap between residues 105 and 106 has not been established

residues, while the amino acid composition suggests the presence of 125 residues in the protein. The agreement between both values is satisfactory.

The pairing of the half-cystine residues is presently under investigation. Among the 6 disulfide bridges present in the enzyme, four have been identified already. They connect residues 18–83, 34–129, 68–98 and 91–103, respectively.

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1 G. H DE HAAS, N. M. POSTEMA, W. NIEUWENHUIZEN AND L. L. M VAN DEENEN, *Biochim Biophys. Acta*, 159 (1968) 118.
2 G. H. DE HAAS, F. FRANČK, B. KEIL, D. W. THOMAS AND E. LEDERER, *Federation European Biochem. Soc Letters*, 4 (1969) 25.
3 G. H. DE HAAS, N. M. POSTEMA, W NIEUWENHUIZEN AND L. L. M VAN DEENEN, *Biochim. Biophys Acta*, 159 (1968) 103
4 J. R. SPIES AND D. C. CHAMBERS, *Anal. Chem.* 21 (1949) 1249
5 S. MOORE, *J. Biol. Chem*, 238 (1963) 235
6 J. H REYNOLDS, *Biochemistry*, 7 (1968) 3131.

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