SIMILARITIES IN PRIMARY STRUCTURES OF COW COLOSTRUM TRYPSIN INHIBITOR AND BOVINE BASIC PANCREATIC TRYPSIN INHIBITOR

D.ČECHOVÁ, V.ŠVESTKOVÁ, B.KEIL and F.ŠORM
Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague

Received 14 July 1969

1. Introduction

During the past few years the primary structure of the basic trypsin inhibitor from bovine pancreas (BPTI) [1–3] and of the kallikrein inhibitor from bovine lungs and parotid glands [4,5] were determined. Notwithstanding the fact that these inhibitors differ in the site of their origin in the organism and also in molecular weight, their primary structures are identical. This brought us to the interesting question as to whether the remaining bovine trypsin inhibitors share at least a part of their structure in common or whether they are derived from the same precursor. The cow colostrum trypsin inhibitor (CTI) is especially interesting from this viewpoint since it differs markedly from BPTI and KI in its isoelectric point (4.2.) [6], low resistance toward pepsin [7], considerable content of a nonprotein component, and amino acid composition. In this study, CTI which had been obtained before only in heterogeneous form [6], was resolved into three electrophoretically homogeneous inhibitors [9]. Of the latter, the one present in the highest amount was investigated in detail.

2. Methods

The disulfide bonds of the native inhibitor were cleaved by oxidative sulfitolysis [10]. The S-sulfonated inhibitor was digested with trypsin (2 hr, 37°C, enzyme to substrate ratio 1:50, by weight) or by chymotrypsin (3 hr, 37°C, enzyme to substrate ratio 1:50, by weight). The peptides from both digests were isolated by high-voltage paper electrophoresis and by descending chromatography [12]. The N-terminal end-group of the protein was determined by dinitrophenylation, the C-terminal end-group by hydrazinolysis. The N-terminal amino acids of peptides were established by the Dansyl technique [11]. The Dansyl-amino acids were identified by thin-layer chromatography on aluminum sheets coated with silicagel ("Silufol", a product of the Institute of Organic Chemistry and Biochemistry of the Czechoslovak Academy of Science, Prague [12]). The Edman degradation procedure was as a rule combined with the Dansyl-technique [15].

3. Results and discussion

As starting material for this study served the trypsin inhibitor which is present in cow colostrum in dominant quantity. This inhibitor was found to contain only one N-terminal amino acid, phenylalanine, and its sole C-terminal amino acid was serine. From the tryptic digest of the S-sulfonated protein we isolated five peptides which account for the total number of amino acid residues present in the molecule of the inhibitor. The amino acid sequence of the peptides was determined mostly by Edman degradation. The results obtained with the chymotryptic digest were used to complete the structure and to determine the order of tryptic fragments.

The data presented in the table show that the primary structure of the dominant cow colostrum trypsin inhibitor resembles the primary structure of the basic trypsin inhibitor from pancreas and thus also the structure of the kallikrein inhibitor. Of the total number of 58 amino acid residues in BPTI and 67

Table 1
Comparison of amino acid sequence of cow colostrum trypsin inhibitor with amino acid sequence of basic pancreatic trypsin inhibitor. Amino acid residues occupying identical positions in both proteins are set in a frame.

Arg-Pro-Asp-Phe-Cys-Leu-Gly-Pro-Pro-Tyr-Thr-Gly-Pro-Cys-Lys-Ala-Arg-Ile -Ile -Arg-	
-Phe-Gln-Thr-Pro-Pro-Asp-Leu-Cys-Gln-Pro-Pro-Gln-Ala-Arg-Gly-Pro-Cys-Lys-Ala-Ala-Leu-Leu-Arg-	
-Tyr-Phe-Tyr-Asn-Ala-Lys-Ala-Gly-Leu-Cys-Gln-Tyr-Phe-Val-Tyr-Gly-Gly-Cys-Arg-Ala-Lys-Arg-Asn-	
-Tyr-Phe-Tyr-Asx-Ser-Thr-Ser-Asn-Ala -Cys-Glu-Pro-Phe-Thr-Tyr-Gly-Gly-Cys(asx,asx,asx,asx,glx,	
-Asn-Phe-Lys-Ser-Ala-Gly-Asp-Cys-Met-Arg-Tyr-Cys-Gly -Gly-Ala	
,glx,glx,thr,thr,gly,met,phe)Cys-Leu-Arg-Ile -Cys-Asx-Pro-Pro-Glx-Glx-Thr-Glx-Lys-Ser	

amino acid residues in CTI the location of at least 21 amino acid residues is identical. The homology becomes especially marked when we regard the number and location of the half-cystine residues in the molecule and the amino acid sequence around lysine residue No. 15 in BPTI which has been known to effect the binding of BPTI to trypsin [14,15] and is thus responsible for the inhibitory activity. Since CTI has been regarded also as a "lysine" inhibitor [16] it can be assumed that it is bound to trypsin by lysine residue No. 18.

Eventhough there is a considerable similarity in the structure of the two proteins, it is obvious that two different genes are possible for their synthesis. Originally, however, there was most likely one gene which controlled the synthesis of trypsin inhibitors. During subsequent evolution this gene become duplicated and the two new genes underwent mutations which led to their differentiation.

References

[1] B.Kassel and M.Laskowski Sr., Biochem. Biophys. Res. Commun. 20 (1965) 463.
[2] V.Dlouhá, D.Pospišilová, B.Meloun and F.Šorm, Coll. Czech. Chem. Commun. 30 (1965) 1311.
[3] J.Chauvet, G.Nouvel and R.Acher, Biochim. Biophys. Acta 92 (1964) 200.
[4] F.A.Anderer, Naturforsch. 20 (1965) 462.
[5] F.A.Anderer and S.Hörnle, J. Biol. Chem. 241 (1966) 1569.
[6] M.Laskowski Jr., P.H.Mars and M.Laskowski, J. Biol. Chem. 198 (1952) 745.
[7] B.Kassell and M.Laskowski Sr., J. Biol. Chem. 219 (1956) 203.
[8] D.Cechová, V.Svestková and F.Sorm, European J. Biochem., in press.
[9] D.Pospisilová-Cechová, V.Dlouhá and F.Sorm. Vth Meeting FEBS Abstr. 1968, No. 971.
[10] F.Franek and O.M.Zorina, Coll. Czech. Chem. Commun. 32 (1967) 3229.
[11] W.R.Gray and B.S.Hartley, Biochem. J. 89 (1963) 59 P.
[12] J.Novotný and F.Franek, Chem. Listy 62 (1968) 995.
[13] W.R.Gray and B.S.Hartley, Biochem. J. 89 (1963) 379.
[14] J.Chauvet and R.Acher, J. Biol. Chem. 242 (1967) 4274.
[15] L.F.Kress and M.Laskowski Sr., J. Biol. Chem. 243 (1968) 3548.
[16] R.Haynes and R.E.Feeney, Biochem. 7 (1968) 2879.