FURTHER CHARACTERIZATION OF TWO CO-LIPASES FROM PORCINE PANCREAS[1]

Bengt Borgström, Charlotte Erlanson and Berit Sternby
Department of Physiological Chemistry, University of Lund, Lund, Sweden

Received June 17,1974

SUMMARY

Two proteins with co-lipase activity can be obtained from porcine pancreas, differing in mobility on disc-electrophoresis at pH 9.3. The experimental evidence presented indicates that the amino acid composition of these two proteins does not differ significantly, the only difference is in the number of free carboxylic acid groups. Co-lipase I and II contain 9 and 10 aspargine + glutamine, respecively, out of a total of 22 aspartic + glutamic acid residues. Co-lipase modified by reaction with glycine methylester after activation with a water-soluble carbodiimide is biologically inactive.

Co-lipase is a polypeptide co-factor for pancreatic lipase which reactivates bile salt inhibited lipase (1). The protein from porcine pancreas was first partly characterized by Maylie et al. (2), who reported a molecular weight by ultracentrifugation of 9400-9900 but only 75-77 amino acid residues corresponding to a weight of around 8000. Erlanson and Borgström (3) later purified porcine co-lipase from the fresh gland and found 95 amino acid residues corresponding to a molecular weight of 10325 with no indication of a non-protein component. Erlanson et al. (4) later reported the presence of two co-lipases in extracts of porcine pancreatic gland and juice. The two species were isolated from the fresh gland by ion exchange chromatography and were separated by disc-electrophoresis at pH 9.3. Amino acid analysis indicated no significant difference in amino acid composition with 102-107 residues and identical N-terminal eight amino acids with N-terminal valine. It was suggested that the co-lipases differed in the number of free glutamyl and/or aspartyl residues.

In the present studies the number of free aspartyl and glutamyl residues of co-lipase have been analysed according to the method described by Hoare and Koshland (5).

[1]Supported by the Swedish Medical Research Council (grant B72-74-13X).

TABLE I

Amino acid composition of co-lipase I

Amino acid	Number of residues				
	Co-lipase I			Co-lipase I: Modified	
	Experimental	Nearest integer		Experimental	Neareast integer
Ala	4.94	5		5.55	6
Arg	6.18	6		5.45	5
Asx	12.78	13		13.17	13
Cys	10.88	11	(9-11)	11.32	11
Glx	9.23	9	(9-10)	10.00	10
Gly	9.22	9	(9-10)	23.41	23
His	2.11	2		2.22	2
Ile	5.99	6		6.00	6
Leu	10.41	10		10.19	10
Lys	4.73	5		4.85	5
Met	0	-		0	-
Phe	2.98	3	(2-3)	2.25	3
Pro	3.39	3		3.02	3
Ser	11.01	11	(10-11)	9.95	11
Thr	5.87	6		5.78	6
Trp	0	-		0	-
Tyr	2.59	3		2.75	3
Val	4.20	4		4.60	4
Total number of residues		106	102-108		106+14Gly
Total weight of residues		11502			
Asx+Glx =		22			
Asp+Glu =		13			
Asn+Gln =		9			

TABLE II

Amino acid composition of co-lipase II

Amino acid	Number of residues				
	Co-lipase II			Co-lipase II: Modified	
	Experimental	Neareast integer		Experimental	Nearest integer
Ala	4.68	5		5.28	5
Arg	4.98	5	(5-6)	5.04	5
Asx	13.08	13	(13-14)	12.58	13
Cys	10.89	11	(9-11)	11.07	11
Glx	9.22	9	(9-10)	9.16	9
Gly	8.62	9		21.84	22
His	2.03	2		2.14	2
Ile	5.39	5	(5-6)	5.27	5
Leu	9.39	9		9.37	9
Lys	4.57	5		4.48	5
Met	0.0	-		0.0	-
Phe	2.06	2		2.01	2
Pro	3.18	3	(3-4)	2.80	3
Ser	10.95	11	(10-11)	9.61	10
Thr	5.43	5	(5-6)	4.95	5
Trp	0.0	-		0.0	-
Tyr	2.82	3		-	3
Val	4.01	4		4.30	4
Total number of residues		101	98-107		100 + 13Gly
Total weight of residues		10872			
Asx+Glx =		22			
Asp+Glu =		12			
Asn+Gln =		10			

Methods: Co-lipase I and II were obtained from extracts of porcine pancreas as described (4). In a typical experiment 1 μmole co-lipase was dissolved into 2 ml of 5 M guanidine hydrochloride that was 1 M with respect to glycine methylester. After adjusting the pH to 4.75, 0.2 mmole of 1-ethyl-3(3-dimethylaminopropyl)carbodiimide (Fluka, Buchs, Switzerland) was added and the pH kept constant by automatic titration with 0.1 M HCl. After two hours of reaction the acid consumption had ceased and the carbodiimide was quenched by the addition of 1 M acetic acid and the product separated by gel chromatography (Sephadex G-25). The modified co-lipases, which no longer displayed any co-lipase activity (1) were analysed with a Jeol amino acid analyser under standard conditions (3) and the composition compared to that of the unmodified compounds.

Results: Tables 1 and 2 give the data from the amino acid analysis of co-lipase I and II and their modified products. The Tables also contain in parentheses highest and lowest figures for the amino acid residues obtained from a number of analysis of preparations of co-lipase I and II. It seems clear that the amino acid composition of the two co-lipases does not differ significantly. The total number of residues in different analysis varies between 102-107. In the actual analysis the sum of aspartic and glutamic acid residues is 22 for both co-lipases. The increase in glycine residues in the modified co-lipases is 13 and 14 in co-lipase I and II indicating 12 and 13 free glutamic and aspartic acid groups (one of the extra glycines is bound to the free carboxyl group of C-terminus).

Co-lipase I and II thus differ only in one acidic residue - co-lipase II being more acidic which is in line with the differences in electrophoretic mobility. The N-terminal sequence of our co-lipase preparation is Val-Pro-Asp-Pro-Arg-Gly-Ile-Ile-Ile- (4).

Maylie et al. (6) have recently given evidence for the occurrence of multiple species of co-lipase in extracts of porcine pancreas. They isolated three proteins with co-lipase activity containing 68-70, 84-85 and 94-95 amino acid residues. All three preparations had a N-terminal glycine and the N-terminal sequence of the proteins with 84-85 and 94-95 residues was Gly-Ile-Ile-Ile-Asn- a sequence also recognized in our co-lipase I and II, and it

is therefore obvious that the two co-lipases of Maylie et al. (6) have lost five N-terminal amino acid residues during the preparation most likely due to tryptic digestion . With an intact N-terminal chain the two co-lipases of Maylie et al. (6) would contain around 100 and 90 amino acids. Therefore, differences most likely are also found in the C-terminal end. The co-lipase first isolated by us with 95 amino acid residues had the N-terminal sequence Ile-Ile-Ile- thus most likely had lost six amino acids from the N-terminal end.

The finding that porcine pancreatic juice contains two co-lipases with electrophoretic mobility identical to our preparations co-lipase I and II would indicate that they represent the products secreted by the pancreas. It seems that a family of co-lipases with shortened N- and/or C-terminal chains are formed during preparation most likely as a result of proteolytic degradation.

The modified co-lipase had no activity when assayed with lipase in bile salt solution (1).

REFERENCES

1. B. Borgström and C. Erlanson (1971) Biochim. Biophys. Acta 242, 509-513.
2. M.F. Maylie, M. Charles, C. Gache and P. Desnuelle (1971) Biochim. Biophys. Acta 229, 286-289.
3. C. Erlanson and B. Borgström (1972) Biochim. Biophys. Acta 271, 400-412.
4. C. Erlanson, P. Fernlund and B. Borgström (1973) Biochim. Biophys. Acta 310, 437-445.
5. D.G. Hoare and D.E. Koshland Jr. (1967) J. Biol. Chem. 242, 2447-2453.
6. M.F. Maylie, M. Charles, M. Astier and P. Desnuelle (1973) Biochem. Biophys. Res. Com. 52, 291-297.