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Partial Structural Revision of Porcine Pancreatic Colipase[1)]

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Two sequence deletions noted in the structure of porcine pancreatic colipase (corresponding to positions 37 and 50 of equine colipase) were investigated and both positions were found to be occupied by Ser residues.

Keywords——porcine colipase; equine colipase; pancreatic insulin fraction; clostripain digestion; Edman degradation

Introduction

Colipase is a protein which is assumed to act as a cofactor by anchoring lipase to emulsify insoluble triglyceride substrates. From porcine pancreas, several active forms of colipase, formed by partial enzymatic hydrolysis of the N- and C-terminal parts of the precursor,[2)] have been isolated, *i.e.*, a 84-residue peptide by Charles *et al.*[3)] in 1974, a 93-residue peptide (colipase-A) by Canioni *et al.*[4)] in 1980 and others.[5)] When the structure of equine colipase was elucidated by Bonicel *et al.*[6)] in 1981, sequence deletions at two positions in porcine colipase A (corresponding to positions 37 and 50 of equine colipase) were noted. Soon after, Rathelot *et al.*[7)] placed Ala and Ser residues at these two positions, respectively, as shown in Fig. 1. In 1984, when the structure of human colipase was determined, Sternby *et al.*[8)] inserted Leu and Ser at the above deleted positions of porcine colipase, respectively. However, in both cases, no experimental evidence was offered. In the course of fractionation studies on porcine pancreatic proteins, a fraction which we isolated in a relatively high yield was found to have an amino acid composition nearly identical with that of colipase A. Thus, we decided to examine the areas in which the above sequence deletions were recorded.

Results and Discussion

A crude insulin fraction, prepared according to Davoren,[9)] was fractionated (Chart 1) by gel-filtration on Sephadex G-50 (Fig. 2-1), followed by successive ion-exchange chromatog-

H–Val–Pro–Asp–Pro–Arg–Gly–Ile–Ile–Ile–Asn–Leu–Asp–Glu–Gly–Glu–Leu–Cys–Leu–Asn–Ser–
Ala–Gln–Cys–Lys–Ser–Asn–Cys–Cys–Gln–His–Asp–Thr–Ile–Leu–Ser–Leu– 37 –Arg–Cys–Ala–
Ala[7)]
Leu[8)]
Leu–Lys–Ala–Arg–Glu–Asn–Ser–Glu–Cys– 50 –Ala–Phe–Thr–Leu–Tyr–Gly–Val–Tyr–Tyr–Lys–
Ser[7,8)]
Cys–Pro–Cys–Glu–Arg–Gly–Leu–Thr–Cys–Glu–Gly–Asp–Lys–Ser–Leu–Val–Gly–Ser–Ile–Thr–
Asn–Thr–Asn–Phe–Gly–Ile–Cys–His–Asn–Val–Gly–Arg–Ser–Asp–Ser–OH

Fig. 1. Proposed Amino Acid Sequence of Porcine Collipase[4,6)]

defatted porcine pancreas powder
↓ 1. extraction with 0.17 N HCl/75% EtOH
↓ 2. adjusted to pH 8.3 with conc. NH_4OH
filtrate
↓ 1. adjusted to pH 5.3 with 6 N HCl
↓ 2. addition of EtOH–ether (2–4 vol)
precipitate
↓ 1. suspended in H_2O
↓ 2. adjusted to pH 2.5 with 2 N HCl
↓ 3. addition of 30% NaCl (1 vol)
precipitate (crude insulin-fraction)
↓ 1. gel-filtration on Sephadex G-50
eluted with 1 M AcOH (Fig. 2—1)
↓ 2. DEAE-Sepharose
gradient with 0.4 M NH_4HCO_3, pH 8.4 (2—2)
↓ 3. 2nd DEAE-Sepharose
gradient with 0.3 M NH_4HCO_3, pH 8.1 (2—3)
↓ 4. SP-Sephadex
gradient with 0.2 M $AcONH_4$, pH 4.8 (2—4)
purified colipase

Chart 1. Isolation of Porcine Colipase

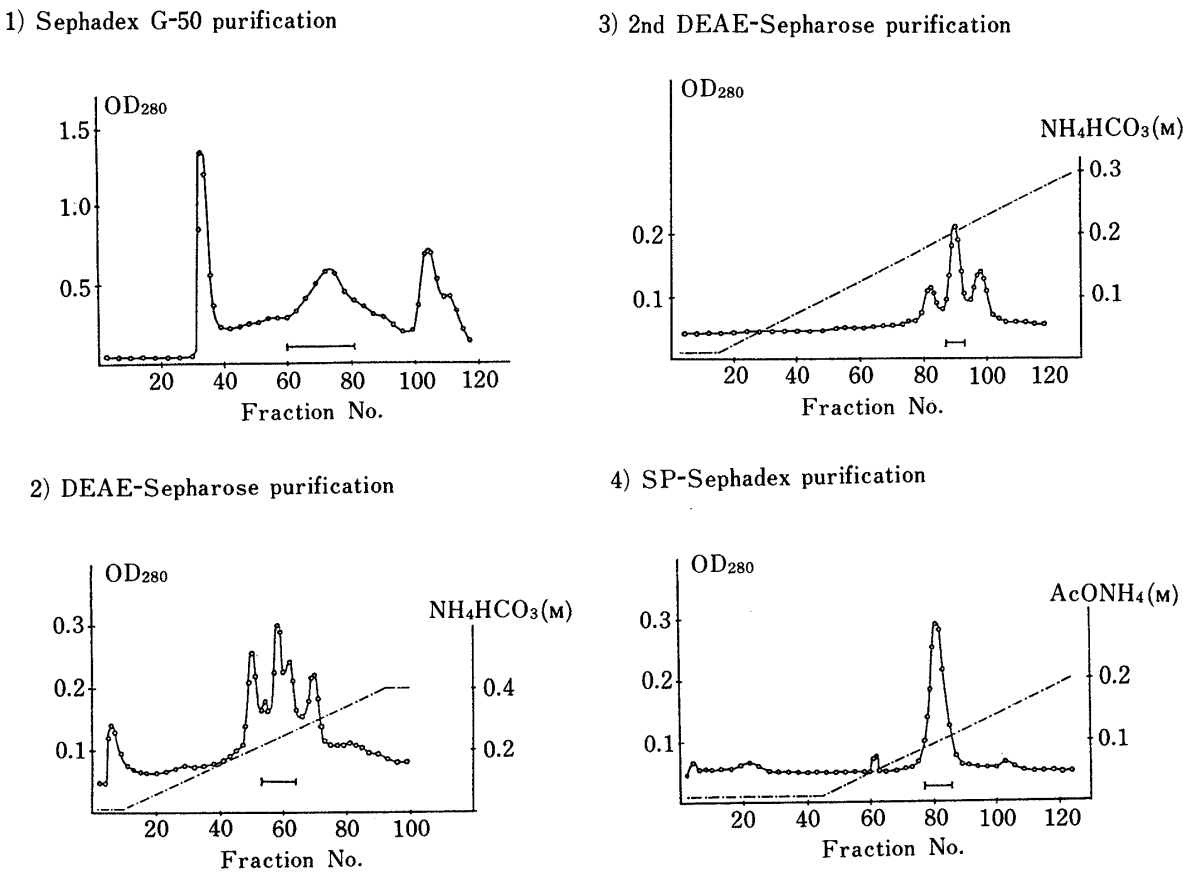


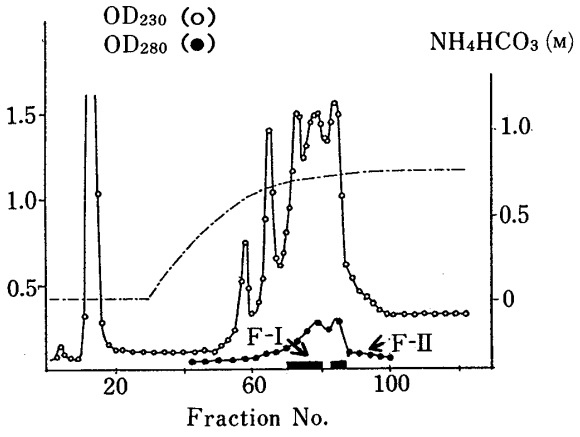
Fig. 2. Chromatographic Purification of Porcine Colipase

Fractions underlined by bars were collected.
Conditions employed: 1) Column 3.2 × 130 cm, fractions of 9 ml each, sample 860 mg, yield = 98 mg. 2) Column 1.8 × 14 cm, fractions of 6 ml each, sample 95 mg, yield = 23 mg. 3) Column 1.8 × 12 cm, fractions of 4 ml each, sample 17 mg, yield = 6 mg. 4) Column 1.2 × 17 cm, fractions of 4 ml each, sample 29 mg, yield = 14 mg.

TABLE I. Amino Acid Composition of Isolated Porcine Colipase

	6 N HCl hydrolysates			Residues nearest No.	Colipase A (lit. 4)
	24 h	48 h	72 h		
Asp	10.46	10.44	10.54	11—12	12
Thr	4.16	4.04	3.96	4— 5	5
Ser	7.72	7.10	6.84	8— 9—10	8
Glu	7.38	7.36	7.46	8	8
Pro	3.30	3.24	3.26	3	3
Gly	7.92	7.82	8.02	8	8
Ala	4.30	4.06	4.14	4	4
Cys(1/2)	7.32	6.04	4.64	9—10	10
Val	3.74	3.70	3.76	4	4
Ile	4.74	6.16	5.42	6	6
Leu	8.52	8.50	8.58	9	9
Tyr	2.26	2.12	2.08	3	3
Phe	2.00	2.00	2.00	2	2
Lys	3.84	3.80	3.86	4	4
His	1.94	1.96	2.04	2	2
Arg	4.52	4.40	4.52	5	5
Total				94—95	93

a) fractionation of the clostripain digest of CM-colipase on DEAE-Sepharose



b) amino acid composition of the Tyr-containing fragment (F-II)

		experimental nearest No.
Asp	1.24	1
Thr	0.99	1
Ser	2.04	2
Glu	2.98	3
Pro	1.00	1
Gly	1.19	1
Ala	1.04	1
Cys(CM)	3.46	3
Val	0.77	1
Ile	0.11	0
Leu	1.14	1
Tyr	2.36	2—3
Phe	0.94	1
Lys	1.00	1
His	0.02	0
Arg	0.90	1
Total		20—21

c) amino acid sequence of the Tyr-containing fragment (F-II)

H–Glu–Asn–Ser–Glu–Cys(CM)–Ser–Ala–Phe–Thr–Leu–Tyr–Gly–Val–Tyr–Tyr–
(50)
Cys(CM)–Pro–Cys(CM)–Glu–Arg–OH (positions 45—65)

Fig. 3. Isolation and Sequence of a Clostripain-Fragment Containing the Tyr Residues

CM-colipase (50 mg) was digested with clostripain (E/S = 1 : 100 w/w). Column 1.1 × 35 cm, fractions of 4 ml each.

raphies on DEAE-Sepharose CL-6B and SP-Sephadex C-25 (Figs. 2-2, 3, 4). The main component thus isolated exhibited a single band (pH 5.2) in isoelectrofocusing on a polyacrylamide-gel plate containing Ampholine (pH 3.5—9.5). The yield of this main component was 12.4 mg from 40 g of a defatted porcine pancreatic powder. The integrated amino acid ratios in the 6 N HCl hydrolysates (for 24, 28 and 72 h) of this component are close to those of colipase A (Table I). After oxidation with performic acid,[10] the N-terminal sequence of the oxidized product was determined by manual Edman degradation[11] and found to be H–Val–Pro–Asp–Pro–Arg–Gly–Ile–Ile–Ile–Asn–Leu–Asp–Glu–Gly–Glu–Leu–. The C-terminal sequence was determined by the carboxypeptidase technique.[12] Digestions of the native component by carboxypeptidase A, followed by carboxypeptidase B, revealed the C-terminal sequence, –Arg–(Ser, Asp)–Ser–OH. From these results, we concluded that the component we isolated is a protein corresponding to colipase A named by Canioni *et al.*[4]

In order to examine the residue at position 50, we digested the S-carboxymethylated-protein[13] with clostripain[14] (E/S = 1 : 100 w/w) in a pH 7.6 buffer (100 mM K_2HPO_4, 10 mM DTT, 1 mM $CaCl_2$) at 37 °C for 16 h and looked for a Tyr-containing fragment, since three Tyr residues of colipase A are located near position 50. The resulting fragments were fractionated by column chromatography on DEAE-Sepharose CL-6B and as shown in Fig. 3-a, the desired fragment, named F-II, was easily isolated when the eluates were monitored by ultraviolet absorption (UV) measurement at 280 nm. By Edman degradation, the sequence of the N-terminal portion (up to 14 residues) was determined (Fig. 3-c). From these date and the amino acid composition after acid hydrolysis, we concluded that the fragment we isolated is a heneicosapeptide corresponding to positions 45 to 65 of colipase A, and the Ser residue has to be placed next to Cys (49).

Next, in order to identify the residue at position 37, we examined each DEAE-fraction of the above clostripain digests by acid hydrolysis and judged that the amino acid ratios of a fraction, named F-I, are very close to those of the desired fragment (positions 6—38). This fraction was re-chromatographed on DEAE-Sepharose CL-6B (Fig. 4-a). The main component (F-I-2), obtained in an apparently homogeneous state, was digested by trypsin (E/S = 1 : 50 w/w) into two fragments (T-1 and T-2). The amino acid composition of fragment T-1 matched very well with those of the nonadecapeptide portion (positions 6 to 24) of colipase A. Fragment T-2 consisted of 14 amino acids. It should correspond to positions 25 to 38 of colipase A. No Ala was found, but one extra residue of Ser (total 3, instead of 2) was

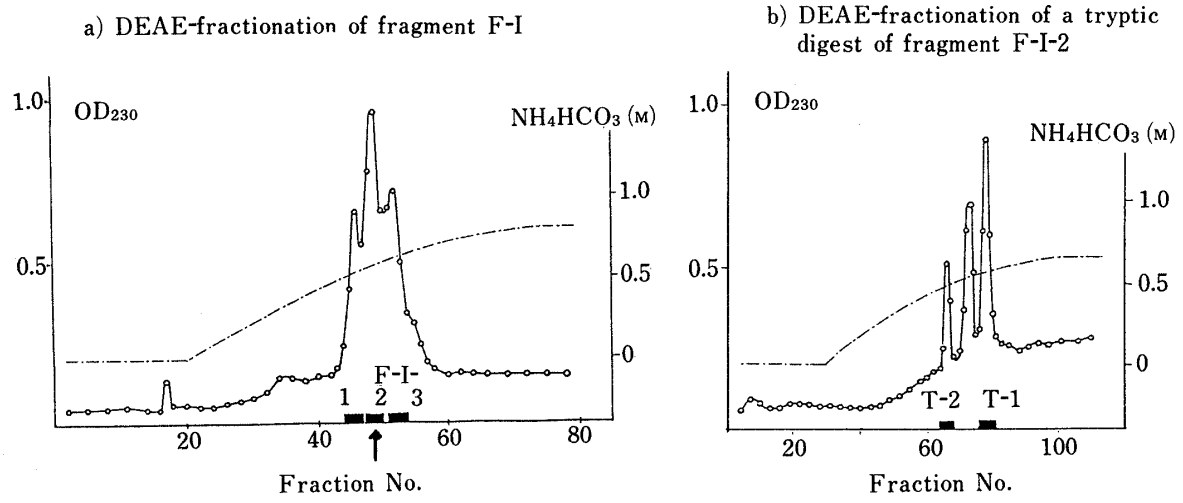


Fig. 4. Isolation of a Fragment (F-I-2, Positions 6—38) and Its Tryptic Digestion

Conditions employed: a) Column 1.1 × 35 cm, fractions of 4 ml each, sample 13 mg. b) Fragment F-I-2 (3.5 mg) was digested with trypsin (E/S = 1 : 50 w/w). Column 1.1 × 12 cm, fractions of 2 ml each.

TABLE II. Amino Acid Compositions of Clostripain Fragment (F-I-2) and Its Tryptic Fragments (T-1 and T-2)

	F-I-2	Reported[4]	Tryptic fragments		Reported[4]
			T-1	T-2	
Asp	5.43 (5)	5	2.81 (3)	2.18 (2)	2
Thr	0.85 (1)	1		0.93 (1)	1
Ser	3.85 (4)	3	1.16 (1)	3.50 (3)	2
Glu	4.53 (4)	4	2.74 (3)	1.10 (1)	1
Gly	2.45 (2)	2	1.88 (2)	0.61 (0)	0
Ala	1.09 (1)	1	0.94 (1)	0	0
Cys(CM)	3.51 (4)	4	1.70 (2)	1.49 (2)	2
Ile	1.78[a]	4	1.31[a]	0.67 (1)	1
Leu	5.00 (5)	5	2.80 (3)	1.84 (2)	2
Lys	1.22 (1)	1	1.00 (1)	0	0
His	0.89 (1)	1		1.00 (1)	1
Arg	0.62 (1)	1		0.94 (1)	1
Total	33	32	19	14	13

a) 24 h hydrolysis gave a low recovery of Ile.
Sequence of T-2 (positions 25—38):
H–Ser–Asn–Cys(CM)–Cys(CM)–Gln–His–Asp–Thr–Ile–Leu–Ser–Leu–Ser–Arg–OH
(37)

found in the acid hydrolysate. After sequence studies, we concluded that the Ser residue has to be placed next to Leu (36) as indicated in Table II, instead of the Ala or Leu residue assigned by Rathelot *et al.*[7] or Sternby *et al.*[8]

It seems rational to conclude that the total numbers of Ala and Leu residues of porcine colipase A are 4 and 9, respectively, as originally reported by Charles *et al.*[3] and found by us (Table I). Concerning the Ser residue at position 50, our result is consistent with those assigned by Rathelot *et al.*[7] and Sternby *et al.*[8] Ser residues in proteins are known to be decomposed considerably by heating with 6 N HCl and this often makes it difficult to estimate the correct number of Ser residues in proteins. This may be responsible for the minor discrepancy reported here.

References and Notes

1) An amino acid analyzer (Hitachi 835-01) was used. The following abbreviations are used: DEAE = diethylaminoethyl, SP = sulfopropyl, CM = S-carboxymethyl, DTT = dithiothreitol.
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