A STUDY OF THE STRUCTURE OF LACTOSOMATOTROPIC HORMONE

V. CHARACTERIZATION OF THE PEPTIDES OF THE TRYPTIC HYDROLYSIS OF FRAGMENTS B-1 and B-2 OF LSTH

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Eight fragments of lactosomatotropic hormone (LSTH) obtained after its cleavage with cyanogen bromide at the seven methionine residues have been isolated and characterized previously [1]. The fragments B-1 (67 amino acids) and B-2 (28 amino acids), which are linked in the native molecule by a disulfide bridge, were separated after the oxidation of fragment B (95 amino acids) with performic acid. We have used hydrolysis with trypsin for the further study of fragments B-1 and B-2.

From the products of the tryptic hydrolysis of fragment B-2 which contains one lysine residue in its amino-acid composition, by high-voltage electrophoresis on paper [2] we obtained two peptides, which we denoted by the symbols B2T-1 and B2T-2. In the amino-acid composition (Table 1) of the N-terminal peptide B2T-1 (16 amino acids) we found one semicystine residue participating in the formation of a disulfide bridge with the cyanogen bromide fragment B-1. The N-terminal amino acid of peptide B2T-2 (12 amino acids) is glutamic acid.

To isolate the peptides linked by disulfide bridges, the native fragment B of LSTH was subjected to tryptic hydrolysis. When the products were chromatographed on Sephadex G-25,

TABLE 1. Amino-Acid Compositions and N-Terminal Amino Acids of the Peptides from the Tryptic Hydrolysis of Fragment B of LSTH

Amino acid	BT-1a	BT-1b B2T-1	BT-2	BT-3a	BT-2b	BT-4	BI-5 B2T-2	BT-6	BT-7	BT-8	Fraction IV	Sum*	Fragment B of LSTH
Lys	—	1,0	1,1	—	—	1,0	—	1,1	1,0	—	—	5	5
His	1,8	1,0	—	—	—	—	1,0	—	—	—	—	4	4
Arg	1,0	—	—	0,6	—	—	—	—	—	1,0	1	4	4
Asx	1,0	2,0	—	1,1	2,9	—	—	0,9	1,0	2,0	—	11	11
Thr	—	1,8	2,0	—	—	—	0,9	—	1,0	—	—	6	6
Ser	1,0	3,0	2,0	0,8	—	—	1,1	2,0	—	—	—	10	10
Glx	—	1,1	3,2	—	—	1,2	4,0	—	—	1,2	—	10	10
Pro	—	2,0	2,7	—	—	1,0	—	—	—	—	—	6	6
Gly		—	1,1	0,9	—	2,0	1,1	—	—	—	—	5	5
Ala	1,0	0,9	—	—	—	1,0	1,0	—	—	1,0	—	5	5
Cys	1	1	—	1	1	—	—	—	—	—	—	4	4
Val	—	—	1,0	—	—	0,6	0,9	—	—	—	—	3	3
Met	—	—	—	—	—	—	1	—	—	—	—	1	1
Ile	—	—	—	—	0,6	1,4	—	—	1,0	—	—	4	4
Leu	3,0	2,0	1,9	1,1	—	—	1,0	—	1,0	—	—	10	10
Tyr	0,8	—	1,0	—	1,0	—	—	—	0,9	—	—	4	4
Phe	0,8	—	—	—	—	0,9	—	—	—	—	—	2	2
Trp	—	—	1	—	—	—	—	—	—	—	—	1	1
Sum of the amino-acid residues	12	16	17	6	6	10	12	4	6	5	1	95	95
Number of residues	His	Ala	Glx	Gly	Ile	Ile	Glx	Asx	Ile	Asx	—	—	Ala, Ile

*Total amino-acid composition of the peptides of fragment B of LSTH.

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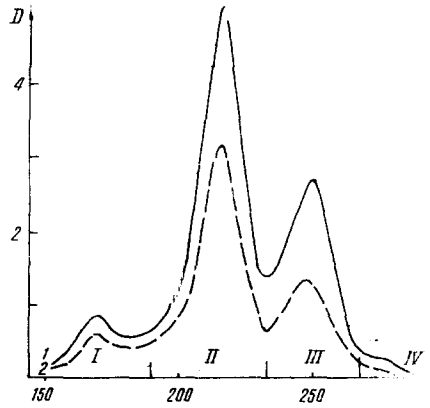

Fig. 1.

Fig. 2

Fig. 1. Results of the gel chromatography of the products of tryptic hydrolysis of fragment B of LSTH on Sephadex G-25 [column 1.8 × 150 cm (380 ml), 0.2 solution of acetic acid; rate of elution 25 ml/h; fraction volume 5 ml]: 1) 278 nm; 2) 253 nm.

Fig. 2. Results of the chromatography of fractions I-IV of the products of the tryptic hydrolysis of fragment B of LSTH.

we obtained four fractions denoted by the symbols I, II, III, and IV, with yields of 26%, 59%, 14%, and 1%, respectively (Fig. 1). After rechromatography under similar conditions, the first three fractions were investigated chromatographically in the butanol—water—pyridine—acetic acid (15:12:10:3) (BWPA) system. As can be seen from Fig. 2, fraction I contains a single component while fraction II contains two peptides and fraction III contains three. The chromato-electrophoresis of the fractions showed no other peptides. In fraction IV we found only free arginine. Since all the peptides of fractions II and III were separated sharply by chromatography, for their isolation we used partition chromatography on a column of cellulose. The BWPA system was used for equilibrating the column and for eluting the peptides. The monitoring of the eluate fractions, permitting the zones of elution of the individual peptides to be revealed, was performed by thin-layer chromatography in the same system. The amino-acid compositions of the N-terminal amino-acid peptides of the tryptic hydrolysis of fragments B-1 and B-2 of LSTH are given in Table 1.

Peptide BT-1 (28 Amino Acids). The danzyl method showed the presence of two N-terminal amino acids, alamine and histidine. Since the amino-acid composition includes two semicystine residues, the amino acids found probably correspond to the N-terminal amino acids of two peptides linked by a disulfide bond. After the treatment of peptide BT-1 with performic acid, high-voltage paper electrophoresis yielded a dodecapeptide BT-1a and a hexadecapeptide BT-1b with histidine and alamine at the N-ends, respectively. Peptide BT-1b had parameters identical with those of the peptide B2T-1 isolated from the tryptic hydrolyzate of the cyanogen bromide fragment B-2. Consequently, the disulfide bond between the cyanogen bromide fragment B-1 and B-2 in the LSTH molecule is due to the semicystine residues of the peptides BT-1a and B2T-1.

Peptide BT-2 (17 Amino Acids). The N-terminal amino acid of the peptide is glutamic acid. We found the single tryptophan residue of fragment B of LSTH in this peptide. Of the products of the prolonged tryptic hydrolysis of peptides BT-2 (24 h), by high-voltage paper electrophoresis we isolated peptides the N- and C-terminal amino acids of which showed their positions in the peptide unambiguously: Glx-(Thr, Ser, Glx, 2Pro, Gly, Val, Leu, Tyr, Trp)-Pro-(Ser, Glx, Leu)-Thr-Lys.

Peptide BT-3 (12 Amino Acids). The two N-terminal amino acids isoleucine and glycine correspond to the N-terminal amino acids of two peptides linked by a disulfide bond. After the oxidation of the disulfide with performic acid, by high-voltage paper electrophoresis we obtained the hexapeptides BT-3a and BT-3b with glycine and isoleucine, respectively, as the N-terminal residues.

Peptide BT-5 (12 Amino Acids). This peptide was identical in its amino-acid composition and N-terminal amino acid with the peptide B2T-2 isolated from the cyanogen bromide fragment B-2.

The free arginine found in the products of the tryptic hydrolysis of the native fragment B of LSTH (fraction IV, Fig. 1) apparently appeared as the result of the hydrolysis of an Arg—Arg fragment of B-1, since the Lys—Arg bond resists the action of trypsin.

Thus, the peptides from the tryptic hydrolysis of the cyanogen bromide fragments B-1 and B-2 of LSTH have been isolated and characterized. The total amino-acid composition of the peptides agrees with that of the native fragment B. No peptides produced by the nonspecific action of trypsin were found. All the peptides contained at the C-end basic amino acids — lysine or arginine — with the exception of peptides BT-5 and BT-3b — the C-terminal peptides of the cyanogen bromide fragments. Since the cyanogen bromide fragment B-1 occupies the C-terminal position in the molecule of LSTH, peptide BT-3b is the C-terminal peptide of the hormone. Peptide BT-3 includes the internal disulfide bridge of fragment B-1, and the second bridge binds the cyanogen bromide fragments B-1 and B-2 in the LSTH molecule.

The lactosomatotropic hormone isolated from bovine hypotheses which possesses lactogenic and growth activities, is close to the lactogenic hormones not only biologically but also in other ways. The results of a comparison of the cyanogen bromide fragments of LSTH and LTH [1, 3] and also of the peptide maps of the products of tryptic hydrolysis [4] showed a considerable similarity of the structures of the two hormones. The total information on the peptides of the tryptic hydrolysis of the cyanogen bromide fragments B-1 and B-2 makes it possible to compare the structures of the cyanogen bromide fragments of LSTH with the corresponding fragments of bovine LTH (O-LTH), the amino-acid sequence of which has been established [3]. Below we give the structure of the cyanogen bromide fragments CB—H_1—O—B (section 54-81 of the structure) and CB—H_1—O—A (section 132-198 of the structure) of the O-LTH molecule. Under the lines of the structure is given information on the peptides of the tryptic hydrolysis of the fragments of LCTH so that the homology of the peptide chains of the two hormones is obvious. As can be seen, the peptides of LSTH basically repeat the sequence of the peptides of O-LTH. Differences in the amino-acid compositions were found in the peptides BT-5, BT-3a, and BT-3b in comparison with the corresponding peptides of O-LTH.

	54 69
B2T-1 or BT-1b	Ala-Leu-Asp-Ser-Cys-His-Thr-Ser-Ser-Leu-Pro-Thr-Pro-Glu-Asp-Lys-Ala-
	[Leu, Asx, Ser, His, Cys, Thr, Ser, Ser, Leu, Pro, Thr, Pro, Glu, Asx]Lys
	70 81
B2T-2or BT-5	Glu-Glu-Ala-Glu-Glu-Thr-His-His-Glu-Val-Leu-Met-
	Glx-Glx-Ala-Gly-Glx-Thr-His-Val-Ser-Glu-Leu-Met
	132 141
BT-4	Ile-Val-Gly-Gln-Phe-Ile-Pro-Gly-Ala-Lys-
	Ile[Val,Gly.Glx,Phe,Ile,Pro,Gly,Ala]Lys
	142 158
BT-2	Glu-Thr-Glu-Pro-Tyr-Pro-Val-Trp-Ser-Gly-Leu-Pro-Ser-Leu-Gln-Thr-Lys-
	Glx[Thr,Glx,Pro,Tyr,Pro,Val,Trp,Ser,Gly,Leu]Pro[Ser,Leu,Glx]Thr-Lys
	159 163
BT-8	Asp-Glu-Asp-Ala-Arg-
	Asp(Glx,Asp,Ala)Arg
	164 176
BT-1a	His-Ser-Ala-Phe-Tyr-Asn-Leu-Leu-His-Cys-Leu-Arg-Arg
	His-Ser[Ala,Phe,Tyr,Asx,Leu,Leu,His,Cys,Leu]Arg,Arg
	177 180
BT-6	Asp-Ser-Ser-Lys-
	Asx-Ser-Ser-Lys
	181 186
BT-7	Ile-Asp-Thr-Tyr-Leu-Lys-
	Ile[Asx,Thr,Tyr,Leu]Lys
	187 191
BT-3a	Leu-Leu- Asn-Cys-Arg-
	Gly-Leu-Ser-Asx-Cys-Arg
	192 198
BT-3b	Ile-Ile-Tyr-Asn-Asn-Asn-Cys-COOH
	Ile -Tyr-Asx-Asx-Asx-Cys-COOH

The structures of these peptides were studied by a combination of Edman's method and dansylation. The amino acids were eliminated successfully from the N-end with phenyl isothiocyanate [5], using trifluoroacetic acid as cyclizing agent [6]; the N-terminal amino acids at each stage of cleavage were identified in an aliquot from the reaction with dansyl chloride [3] followed by two-dimensional chromatography of the dansyl derivatives on polyamide plates [7]. The following amino-acid sequences were found for the two peptides: BT-3a — NH_2-Gly-Leu-Ser-Asx-Cys-Arg-OH; and BT-3b — NH_2-Ile-Tyr-Asx-Asx-Asx-Cys-OH.

The C-terminal sequence of peptide BT-5 was determined by digestion with carboxypeptidase A. The incubation of 0.3 μmole of the peptide with the enzyme for 20 h liberated homoserine, 0.30 μmole of leucine, 0.22 μmole of glutamic acid, 0.13 μmole of serine, and 0.04 μmole of valine. These results and six stages of Edman cleavage suggested the following structure for the peptide BT-5: NH_2-Glx-Glx-Ala-Gly-Glx-Thr-His-Val-Ser-Glu-Leu-Met-OH.

The peptide chains of LSTH, which were identical in their amino-acid compositions with segments of the known structure of O-LTH, can be distinguished from the latter only by a rearrangement of individual amino-acid residues. The amino-acid sequences of the homologous peptides have not been studied.

In an investigation of the structure of bovine LTH (B-LTH), complete identity of peptide chains 70-81 of B-LTH and of O-LTH was found [8]. Consequently, the differences in the amino-acid sequence of LSTH and O-LTH in this region cannot be explained by species differences of the hormones. According to Lewis, in B-LTH, as compared with O-LTH, there is a deletion of histidine in position 164. The structure of LSTH in this section coincides with that of O-LTH, and point 164 corresponds to the N-terminal histidine of peptide BT-1a of LSTH. Consequently, bovine lactosomatotropic and lactogenic hormones, while they have the same number of histidine residues in their amino-acid compositions, differ by their positions in the molecule.

Thus, the characterization of the peptides of the tryptic hydrolysis of the cyanogen bromide fragments B-1 and B-2 of LSTH and the results of a study of the structures of individual peptides have shown differences in the amino-acid sequence of LSTH as compared with those of the lactogenic hormones.

EXPERIMENTAL METHOD

Hydrolysis with Trypsin. The pH of a solution of 300 mg of the cyanogen bromide fragment B of LSTH in 30 ml of water was brought to 8 with 5% ammonia solution, and a 0.1% solution of trypsin treated with carpenter's inhibitor [10] in 10^{-4} N hydrochloric acid was added, and the mixture was incubated in the thermostat at 37°C. During the course of an experiment, the trypsin was added three times to a final substrate—enzyme ratio of 1:30. Hydrolysis was continued for 6 h, and the pH was corrected by means of ammonia solution. The hydrolyzate was lyophilized.

Gel Chromatography on Sephadex G-25. Columns 1.8 × 150 cm (380 ml) and 1.8 × 100 cm (240 ml) were filled with Sephadex G-25 and equlibrated with a 0.2 M solution of acetic acid. A solution of 300 mg of the tryptic hydrolyzate of fragment B of LSTH in 2 ml of acetic-acid solution was deposited on the 1.8 × 150-cm column. When fractions I, II, and III were rechromatographed on the 1.8 × 100-cm column, in each case 50-100 mg of product dissolved in 1 ml of buffer was used. The rate of elution was 25 ml/h, 5-ml fractions being collected and their absorptions being measured at 253 and 278 nm.

Chromatoelectrophoresis. Chromatography was performed in the BWPA system on 20 × 20-cm plates in a layer of MN-300 cellulose (Serva). Electrophoresis in the perpendicular direction was performed in water—pyridine—acetic acid (270:30:1) buffer, pH 6.5, at 900 V for 50 min.

High-voltage electrophoresis for preparative purposes was performed on FN-17 paper in water—pyridine—acetic acid (225:25:1) buffer, pH 6.5, at 3000 V for 1 h.

Detection of the Peptides. A ninhydrin—collidine reagent for showing up the peptides was prepared in the following way: to a 0.14% solution of ninhydrin in ethanol were added collidine (60:1) and, just before the actual use of the mixture, glacial acetic acid (3:1, by volume). Tryptophan-, tyrosine-, histidine-, and cysteine-containing peptides were revealed by using qualitative reactions described in the literature [11].

The amino-acid compositions of the peptides were determined by the method of Moore and Stein [12]. About 0.05 μmole of the sample was hydrolyzed in 1 ml of hydrochloric acid (5.7 N) in sealed tubes at 110°C for 22 h or, when fractional values for the valine, isoleucine, and leucine residues were obtained for 72 h. The cystine was present in the form of cysteinic acid. Methionine was calculated from the amounts of homoserine and homoserine lactone. Tryptophan was determined colorimetrically [13].

Carboxypeptidase A digestion was performed in a 1% solution of sodium bicarbonate, pH 8, at 37°C with a substrate–enzyme ratio of 25:1 and by weight. Samples were taken after 0.5, 3, and 20 h. The free amino acids were detected on an automatic amino-acid analyzer.

The stepwise degradation of the peptides was performed by Edman's method [5]. As the reagent causing cyclization we used trifluoroacetic acid [6].

The N-terminal amino acids were determined by dansylation [3]. The dansylamino acids were identified by two-dimensional chromatography on polyamide plates [7].

SUMMARY

1. Peptides from tryptic hydrolysis of the cyanogen bromide fragments B-1 and B-2 of LSTH have been isolated and characterized.

2. The differences found in the amino-acid sequence of the individual peptides of fragment B of LSTH as compared with the corresponding sections of the O-LTH molecule are not due to species differences of the hormones.

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