A STUDY OF THE STRUCTURE OF THE LACTOSOMATOTROPIC HORMONE

I. ISOLATION AND CHARACTERIZATION OF THE FRAGMENTS OF CYANOGEN CLEAVAGE

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Lactosomatotropic hormone (LSTH), which possesses growth and lactogenic activities has been isolated from cattle hypophyses. A considerable similarity in the amino-acid composition and physicochemical properties of LSTH and of the bovine lactogenic hormone (B-LTH) and of the ovine hormone (O-LTH) has been established [1]. In view of this, the study of the primary structure of LSTH and its comparison with the known structure of O-LTH [2] is a matter of interest.

To obtain peptide fragments, the protein was cleaved with cyanogen bromide [3] at the peptide bonds formed by methionine residues. The cyanogen bromide fragments were separated and purified by gel chromatography on Sephadex G-25, precipitation at the isoelectric point, and preparative electrophoresis on paper. The homogeneity of the fragments was checked by chromatoelectrophoresis in a thin layer of silica gel and also by a determination of the N-terminal amino acids. The amino-acid composition was determined on an automatic amino-acid analyzer. Each fragment was previously checked for its tryptophan content by means of Ehrlich's reagent, and then the tryptophan content of the fragments giving a positive reaction was determined [4, 5]. The N-terminal amino acids were determined by Edman's method in Sjöquist's modification [6] with identification of the phenylthiohydantoin derivatives of the amino acids and also by reaction with dansyl chloride (DNS–Cl) followed by the chromatography of the NDS-derivatives in a thin layer of silica gel [7, 8].

We obtained the following results. The two N-terminal amino acids of fragment B containing four cysteine residues apparently correspond to the N-terminal amino acids of two fragments connected by a disulfide bond. After oxidation with performic acid, by means of gel chromatography on Sephadex G-25 we isolated the fragments B-1 (67 amino acids) and B-2 (28 amino acids) containing isoleucine and alanine, respectively, at the N ends.

The available information enables the amino-acid composition of the cyanogen bromide fragments of LSTH to be compared with sections of known structure of O-LTH. According to the parameters studied, the fragments GD-2 (24 amino acids), E-1 (12 amino acids), D-1 (23 amino acids), and Zh (2 amino acids) are identical with the cyanogen bromide fragments of O-LTH – sections 1-24, 25-36, 82-104, and 130-131 of the structure, respectively. Fragment E-2 is characterized by a smaller number of phenylalanine residues in comparison with section 37-53. Fragment G-1 apparently differs from section 105-129 by the replacement of valine by alanine. In fragment B (95 amino acids) there are differences in the amounts of serine, glutamic acid, glycine, isoleucine, leucine, and histidine, as compared with the fragment $CB-H_1$ of O-LTH.

Thus, from the products of the cyanogen bromide cleavage of LSTH we have isolated eight fragments, as was to be expected from the methionine content of the LSTH molecule. The total amino-acid composition coincides with the amino-acid composition of native LSTH. The results of the investigation performed confirm the considerable similarities of the structures of LSTH and O-LTH.

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