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THE GLOBULINS OF COTTON SEEDS

XVIII. THE ACID-LABILE BOND OF SUBUNIT C OF THE 11S GLOBULIN

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In determining the amino acid composition of subunit C [1], traces of methionine were detected. To determine methionine in the protein we cleaved it with cyanogen bromide. With stirring, 500 mg of cyanogen bromide was added to a solution of 10 mg of the protein in 70% HCOOH, and the mixture was kept at 20°C for 20 h. On a peptide map one additional ninhydrin-positive spot was observed (Fig. 1).

On separating the mixture on a column (2 × 70 cm) of Sephadex G-50 (fine) equilibrated with 50% acetic acid we obtained two fractions (Fig. 2). The high-molecular-weight fraction (1) had the N-terminal amino acid His, and fraction 2 had Pro. Fraction 2 was obtained with a yield of 7%. The low yield could be explained by nonspecific cleavage and by the absence of methionine. In actual fact, an attempt to carry out the cleavage of the polypeptide chain at the methionine with freshly prepared Raney nickel [2] proved unsuccessful. The cleavage of an Asp-Pro bond in proteins under the conditions of cyanogen bromide hydrolysis is known [3]. Subsequently, from the peptides of the chymotryptic hydrolysate we isolated a peptide (XT3) containing such a bond.

We determined the amino-acid composition and N-terminal sequences of both fractions: 1) His-Asn-Gln...; 2) Pro-Gln-Asn-Gln.... Fraction 1 gave a positive reaction for a sugar. The presence of such a bond in the protein and the fact of its cleavage undoubtedly facilitated the reconstruction of the whole polypeptide chain of subunit C. The amino acid composition was determined as described previously [1]. The reaction for sugar was also carried out as described previously [4].

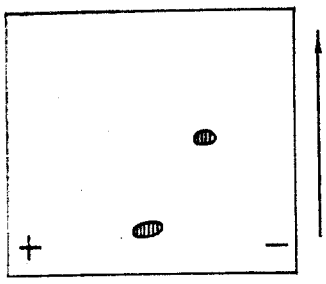

Fig. 1

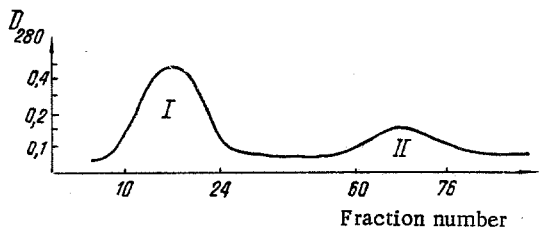

Fig. 2

Fig. 1. Peptide map of the cyanogen bromide hydrolysate of subunit C (conditions described in [1]).

Fig. 2. Chromatography of the cyanogen bromide hydrolysate of subunit C on a column of Sephadex G-50 (5-ml fractions, rate 60 ml/h).

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PREPARATION OF THE [Ala^{16}-B] ANALOG OF BOVINE INSULIN

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In a study of the structural and functional organization of the insulin molecule we have obtained the previously unknown [Ala^{16}-B] analog (I) of bovine insulin.

The preparation of compound (I) was based on combining the natural A-chain of bovine insulin with the synthetic [Ala^{16}]-B chain of bovine insulin [1]. The protected synthetic chain was first demasked by treatment with sodium in liquid ammonia in the presence of sodium amide [2] and was then subjected to oxidative sulfitolysis [3]. This gave the bis-S-sulfonate (II) of the analog of the B-chain, in which the L-tyrosine residue in position B^{16} had been replaced by a L-alanine residue. The amino acid analysis of the bis-S-sulfonate (II) was: Phe 2.8, Val 3.0, Asp 1.0, Glu 3,0, His 2.0, Leu 4.0, Gly 3.0, Ser 0.7, Ala 3.1, Tyr 0.9, Arg. 1.0, Thr 0.8, Pro 0.9, Lys 1.0.

The bis-S-sulfonate (II) was brought into combination with the tetramercapto form of the A-chain of bovine insulin [4] previously prepared by treating the tetra-S-sulfonate of the A-chain of bovine insulin with 2-mercaptoethanol.

The compound (I) obtained was purified by ion-exchange chromatography on a column of CM-Sephadex C-25, using as eluent a 0.04 M acetate buffer solution with pH 4.0, and also by disk electrophoresis in polyacrylamide gel [5]. The purified compound (I) was isolated in the form of hydrochloride.

Compound (I) was homogeneous on paper electrophoresis ("Khromatograficheskaya M" paper, pH 2.6, 720 V, 10 mA) and in its electrophoretic mobility it did not differ from authentic natural bovine insulin. The homogeneity of the compound (I) was also confirmed on its disk electrophoresis in polyacrylamide gel (pH 8.3, concentration of separating gel 7.5%).

Amino acid analysis of compound (I): Gly 4.4, Ile 1.0, Val 4.7, Glu 7.3, Ala 4.0, Ser 3.0, Leu 6.0, Tyr 3.1, Asp 3.0, Phe 2.8, His 1.8, Arg 1.1, Thr 1.0, Pro 0.9, Lys 1.0.

The biological activity of compound (I) on testing for its convulsive effect in mice [6] was 50% (as compared with the activity of the international standard for insulin).

The results obtained show that the replacement in the molecule of bovine insulin of the invariant L-tyrosine residue in the B^{16} position by a L-alanine residue does not lead to the complete disappearance of the specific hormonal activity in the [Ala^{16}-B] analog of bovine insulin.

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