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PREPARATION OF THE [Ala-B^{15}, Ala-B^{16}] and [Phe-B^{16}] ANALOGS OF BOVINE INSULIN

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In connection with the study of the influence of changes in the structure of insulin on its biological activity, we have obtained previously unknown semisynthetic analogs of bovine insulin — [Ala-B^{15}, Ala-B^{16}] insulin (I) and [Phe-B^{16}] insulin (II) — by combining the A-chain isolated from natural bovine insulin with the corresponding synthetic analogs of the B chain of insulin [1]. The protected chains were demasked by sodium in liquid ammonia in the presence of sodium amide [2], and were then subjected to oxidative sulfitolysis [3], as a result of which the following bis-S-sulfonate analogs of the B chain were obtained: (III), in which the leucine-B^{15} and tyrosine-B^{16} residues have been replaced by two alanine residues, and (IV), in which the tyrosine-B^{16} has been replaced by a phenyl alanine residue.

Amino-acid analyses of the bis-S-sulfonates: (III) Lis 0.9, His 1.6, Arg 1.1, Asp 0.7, Thr 0.6, Ser 0.6, Glu 3.1, Pro 0.8, Gly 3.0, Ala 4.1, Val 3.0, Leu 2.7, Tyr 0.8, Phe 2.8; (IV) Lis 1.2, His 1.6, Arg 1.1, Asp 1.0, Thr 0.9, Ser 0.4, Glu 2.8, Pro 1.0, Gly 3.0, Ala 2.0, Val 2.8, Leu 3.7, Tyr 1.0, Phe 4.0.

The bis-S-sulfornate of (III) was subjected to ion-exchange chromatography on SP-Sephadex C-25. After this, the bis-S-sulfornate of (III) [(IV) without preliminary purification] was made to recombine with a four-fold excess of the tetramercapto form of the A-chain of bovine insulin previously prepared by treating the tetra-S-sulfonate of the A-chain of bovine insulin with 2-mercaptoethanol. The combination of the corresponding chains and the isolation of the insulin analogs was carried out by the method of Katsoyannis [4]. Compound (II) was purified by ion-exchange chromatography on CM-Sephadex C-25.

The electrophoretic mobilities of the compounds obtained were compared with the mobilities of natural bovine insulin (electrophoresis on "Khromatograficheskaya M" paper, pH 2.6; 720 V, 10 mA; standard — bovine insulin).

According to the results of disk electrophoresis in polyacrylamide gel, compound (I) was homogeneous, and compound (II) contained impurities, and therefore compound (II) was purified by disk electrophoresis in polyacrylamide gel. This process was carried out by Davis's method [5] at pH 8.3 with a concentration of the separating gel of 7.5%. After electrophoresis, the appropriate sections of the gel were comminuted and eluted with 7% aqueous acetic acid for 16 h. Compound (II) was isolated from the eluate in the form of the picrate, which was then converted into the hydrochloride.

Amino-acid analyses: (I) His 2.0, Lis 1.2, Arg 1.3, Asp 2.5, Thr 1.3, Ser 2.6, Glu 6.5, Pro 0.9, Gly 4.5, Ala 5.4, Val 4.5, Ile 0.8, Leu 5.0, Tyr 2.6, Phe 3.4; (II) His 1.6, Lis 1.1, Arg 1.2, Asp 2.7, Thr 1.2, Ser 2.6, Glu 6.6, Pro 1.0, Gly 4.4, Ala 2.7, Val 4.5, Ile 0.7, Leu 6.0, Tyr 2.7, Phe 4.4.

The biological activity in testing for convulsive effects in mice, in comparison with the activity of international standard insulin, was 5.5% for (I) and 33% for (II). The prep-

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aration of semisynthetic bovine insulin obtained by combining the natural A chain and the synthetic B chain after purification by ion-exchange chromatography and disk electrophoresis possessed the full biological activity.

The facts obtained show that the replacement of the invariant amino-acid residues in the insulin molecule that have been mentioned does not lead to the disappearance of its specific hormonal activity in the insulin analogs formed.

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THE AMINO-ACID SEQUENCE OF TRIACETINASE — A COTTONSEED ESTERASE

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In the course of an investigation of triacetinase — a cottonseed esterase [1] — it has been shown that it is an oligomeric protein consisting of two subunits each of which, in turn, consists of two protomers connected with one another by lipids. It has been established that at acid pH values and high salt concentrations the native enzyme is capable of dissociating reversibly into its component subunits. At the same time, under the action of detergents or organic solvents (chloroform—ethanol, 2:1) a subunit breaks down into two protomers with liberation of the phospholipids. The identity of all the polypeptide chains composing triacetinase has been shown by the peptide map method [2].

The present paper gives the results of an investigation of the amino-acid sequence of triacetinase.

On the basis of the characteristics of the amino-acid composition of triacetinase [2], in order to establish its primary structure we used the classical methods of protein chemistry — cleavage of the reduced and carboxymethylated protein with cyanogen bromide and hydrolysis with trypsin.

The cleavage of CM-triacetinase with cyanogen bromide yielded seven fragments, four of which were formed by cleavage of bonds with methionine, two were the products of incomplete hydrolysis at Met—Thr and Met—Pro bonds, and one fragment was formed by the hydrolysis of an acid-labile Pro—Asp bond.

Tryptic hydrolysis yielded fifteen fragments, some of which were also products of nonspecific hydrolysis.

The results of analyses of the large fragments from cyanogen bromide cleavage and tryptic hydrolysis enabled the architecture of the molecule as a whole to be given.

To determine the complete amino-acid sequence of the protein, the large fragments from cyanogen bromide cleavage were subjected to hydrolysis with chymotrypsin or thermolysin. The peptides from cyanogen bromide cleavage were separated by gel filtration on Sephadex G-25 (superfine) and by paper chromatography, and the peptide from the tryptic hydrolyzate by ion-exchange chromatography on Aminex Q-150 S cation-exchange resin followed by chromatography of the separated fractions on Dowex 1X4, polyamide, and Aminex-MS. The peptides from chymotryptic and thermolytic hydrolysis were isolated directly from the peptide maps.

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