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

Sh. S. Azimova and P. Kh. Yuldashev

UDC 577.15.02

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.

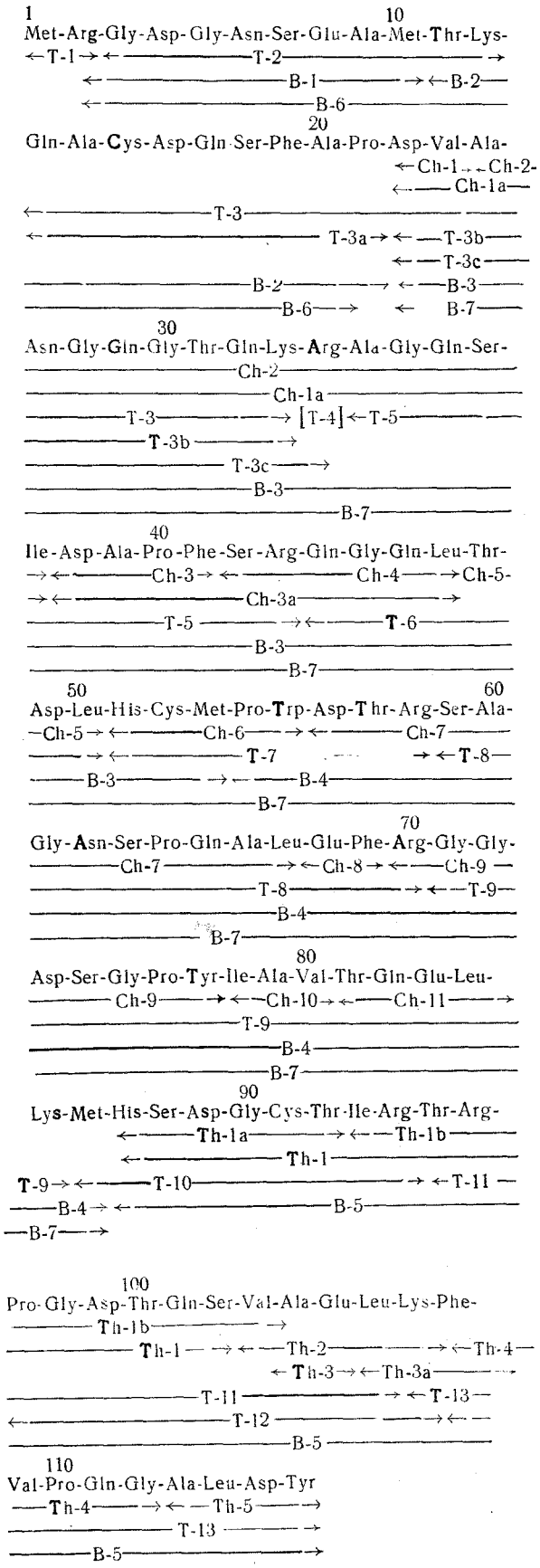
The results of analyses of the large fragments from cyanogen bromide cleavage and tryptic hydrolysis enabled the architecture of the molecule as a whole to be given.

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.

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent.

Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 870-872, November-December, 1977. Original article submitted October 7, 1977.

0009-3130/77/1306-0739$07.50



The amino-acid sequences of the peptides were studied both by Edman's direct method [3] and also in combination with dansylation [4] and by Chen's method [5].

The analysis of the structures of the peptides isolated enabled the polypeptide chain of the protein to be reconstructed.

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