3. J. Tomko and A. Vassovà, Pharmazie, 20, 385 (1965).
4. J. Tomko and A. Vassovà, Chem. Zvesti, 18, 266 (1964).

PRIMARY STRUCTURE OF SUBUNIT B OF THE 11S GLOBULIN OF COTTON SEEDS OF VARIETY 108-F. V. SOME TYPES OF CHEMICAL CLEAVAGE OF SUBUNIT B

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In the determination of the amino acid composition of subunit B, 1-2 methionine residues were found. In order to determine the methionine in the protein and to obtain the large fragments necessary for reconstructing the molecule, we have performed its cleavage with cyanogen bromide. With stirring, a 100-fold excess of cyanogen bromide was added to a solution of 10 mg of the protein in 70% HCOOH. Then the mixture was left at room temperature for 23 h. On a peptide map, one additional spot that had given a ninhydrin-positive reaction was observed (Fig. 1).

When the hydrolysate was separated on a column (1.1 × 70 cm) of Sephadex G-50 fine equilibrated with 30% HCOOH, two fractions were obtained (Fig. 2). The fraction of higher molecular mass, 1, had Val as the N-terminal amino acid and its composition was similar to that of the initial subunit B with the only difference that it contained no Met. Fraction 2 proved to be a homogeneous peptide with His as the N-terminal amino acid. Peptide 2 was obtained with a yield of 10%. Its amino acid sequence (His-Phe-Arg) was determined by the manual Edman method in the modification of Gray and Hartley [1]. Since the N-terminal sequence of subunit B itself coincides with the sequence of peptide 2, the Met residue is present in the fourth position from the N-end of the molecule. Cleavage at Met gave little information for the reconstruction of the molecule, and therefore to obtain large fragments we performed cleavage at Trp residues with N-bromosuccinimide. The qualitative reaction with the aid of the Ehrlich reagent on the peptide map revealed 1-2 Trp.

The protein (5 mg) was dissolved in a solution of 0.2 M ammonium acetate buffer, pH 4.0, in 6 M urea and the solution was left overnight for denaturation. Then a tenfold excess of N-bromosuccinimide was added and the mixture was stirred at room temperature of 2.5 h. After the end of the reaction, the mixture was desorbed on a column of Sephadex G-25 in 30% CH_3COOH. The degree of cleavage was monitored by the TLC method and by determining N-terminal amino acids (Fig. 3).

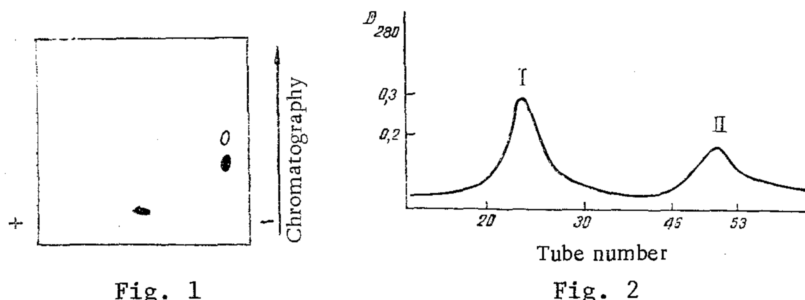

Fig. 1 Fig. 2

Fig. 1. Peptide map of the cyanogen cleavage of subunit B.

Fig. 2. Gel chromatography of the products of the cyanogen bromide cleavage of subunit B.

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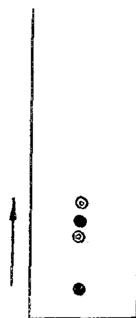

Fig. 3. TLC of the products of the bromosuccinimide cleavage of subunit B.

The hydrolysate was separated on a column (1.1 × 70 cm) of Sephadex G-50 fine equilibrated with 30% CH_3COOH. As in the case of cyanogen bromide cleavage a peptide of low molecular mass was obtained for which the N-terminal amino acid was found to be Val and the amino acid composition: Val_1, Asp_2, Ser_1, Glu_3, Gly_1, Als_1, Leu_1, Phe_1, Arg_1. The yield of peptide was 15%. The compositions of the peptides were determined after hydrolysis in 5.7 N HCl at 110°C for 24 h on a LKB 4101 analyser.

Thus, subunit B contains one Met and one Trp residue. Chemical cleavage at these residues did not lead to the formation of relatively large fragments, since Met and Trp are present in the terminal sections of the polypeptide chain of subunit B.

LITERATURE CITED

1. W. R. Gray, Methods Enzymol., 25, 333 (1972).

PREPARATION AND PROPERTIES OF LEUCINE-B^{30}-INSULIN

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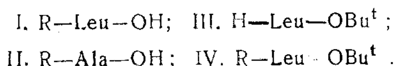
In connection with an investigation of the laws of the structural-functional organization of the insulin molecule, we have performed the synthesis of a previously unknown analog of human insulin differing from the natural hormone by the replacement of the L-threonine residue in the B^{30} position by a L-leucine residue.

Leucine-B^{30}-insulin (I) was obtained by an enzymatic-chemical method using a two-stage scheme involving the use of tryptic transamination [1].

The first stage of the process consisted in the trypsin-catalyzed transamination of porcine insulin (II, R = de-Ala^{B30}-[porcine insulin]) which took place when the latter was treated with L-leucine tert-butyl ester (III) in an aqueous organic medium (water–dimethylformamide) at 24°C and pH 6.3. Under these conditions the enzymatic transamination of the fraction takes place only at the Lys^{B29} residue and the undesirable side reaction at the Arg^{B22} residue does not occur.



I. R—Leu—OH; III. H—Leu—OBu^t;
II. R—Ala—OH; IV. R—Leu—OBu^t.

The second stage of the process consisted in the chemical demasking of the ester derivative of insulin formed (IV) and had the aim of the exhaustive elimination of the C-protective grouping from the Leu^{B30} residue. The ester derivative (IV) was first purified by ion-exchange chromatography on DEAE-Sephadex A-25. Demasking was carried out by treating compound

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