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ENZYMATIC-CHEMICAL TRANSFORMATION OF PORCINE INSULIN INTO HUMAN INSULIN USING THE TRYPTIC TRANSAMIDATION REACTION

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We have performed the enzymatic-chemical conversion of porcine insulin into human insulin by a two-stage scheme involving the use of the enzymatic transamidation reaction.

The first stage of the process consists in the trypsin-catalyzed transamidation of the porcine insulin [I, R = de-Ala^{B30}-(porcine insulin)], which takes place when the latter reacts with a L-threonine ester (II, R' = Alk, ArAlk) in an aqueous organic medium (water–ethanol or water–dimethylformamide) at 25°C and pH 6.3. Under these conditions the enzymatic transamidation reaction takes place only at the Lys^{B29} residue and the undesirable side reaction at the Arg^{B22} residue does not take place.

I. R — Ala — OH III. R — Thr — OR′
II. H — Thr — OR IV. R — Thr — OH

The second stage of the process consists in the chemical demasking of the insulin ester (III) formed in the first stage and has the aim of the exhaustive elimination of the C-protective groupings from the Thr^{B30} residue. The ester derivative (III) was first purified by ion-exchange chromatography on QAE-Sephadex A-25. In the case of the tert-butyl ester of insulin (III, R' = Bu^t), demasking was carried out by treating it with trifluoroacetic acid at 20°C in the presence of anisole as protector. The human insulin formed after acidolysis [IV, R = de-Thr^{B30}-(human insulin)] was isolated from the reaction mixture with the aid of gel filtration of Sephadex G-25 fine. The course and degree of the transformation was monitored by TLC on silica gel, electrophoresis on cellulose, and disk electrophoresis in polyacrylamide gel.

After lyophilization of the eluate, we obtained human insulin (IV) in analytically pure form.

The electrophoretic mobility of compound (IV) was 1.35 (electrophoresis on Whatman No. 1 paper, pH 1.9, 450 V, 7 mA, staining by the Pauli reagent, reference standard the bis-S-sulfonate of the B-chain of human insulin). Amino acid analysis: Asp 3.00 [3], Thr 2.85 [3], Ser 3.20 [3], Glu 7.10 [7], Pro 1.20 [1], Gly 3.80 [4], Ala 0.98 [1], Cys 5.15 [6], Val 4.10 [4], Ile 1.85 [2], Leu 6.00 [6], Tyr 3.35 [4], Phe 2.85 [3], His 1.92 [2], Lys 0.95 [1], Arg 0.98 [1]. The results of the determination of the C-terminal amino acids: Thr 0.98 [1], Asn 0.83 [1].

The proposed method for the enzymatic-chemical transformation of porcine insulin can be extended to the insulins of other biological species and permits the production not only of human insulin but also of various structural analogs of this hormone containing other amino acids in place of the Thr^{B30} residue and also fragments of their various derivatives including peptides.

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