ENZYMATIC CHEMICAL TRANSFORMATION OF RABBIT INSULIN INTO HUMAN INSULIN

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In connection with a study of the routes of transformation of animal insulins and methods for obtaining human insulin, we have performed the enzymatic-chemical transformation of rabbit insulin into human insulin.

The transformation was based on the trypsin-catalyzed transamidation of rabbit insulin (I, R = de-Ser^{B30} rabbit insulin) taking place on the interaction of the latter with L-threonine tert butyl ester (II) in an aqueous organic (water–dimethylformamide) medium at 24°C and pH 6.3. Under the conditions stated, 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–Ser–OH; III. R–Thr–OBu^t;
II. H–Thr–OBu^t; IV. R–Thr–OH.

For the exhaustive elimination of the protective tert-butyl groups from the Thr^{B30} residue, the insulin ester derivative (III) formed was subjected to chemical demasking. First, compound (III) was purified by ion-exchange chromatography on DEAE-Sephadex A-25. Demasking was performed by treating the ester derivative with trifluoroacetic acid at 20°C using anisole as protector.

The human insulin (IV, R = de-Thr^{B30} human insulin) formed after acidolysis was separated from the reaction mixture with the aid of gel filtration on Sephadex G-25f. The course and degree of purification was monitored by TLC on silica gel, by electrophoresis on cellulose, and by disc electrophoresis in polyacrylamide gel.

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

Human Insulin (IV). R_f 0.52 (C_5H_5N–C_4H_9OH–CH_3CO_2H–H_2O (10:15:3:12)); 0.50 (iso-C_3H_7OH–25% NH_4OH (7:4)); 0.92 (iso-C_3H_7OH–25% (NH_4OH–H_2O (7:4:6)); 0.80 (C_5H_5N–CH_3COCH_3–H_2O (1:1:2)) (TLC on Silufol UV-254 plates with visualization by means of the Pauly reagent [1]). Electrophoretic mobility: 1.35 (electrophoresis on Whatman No. 1, paper, pH 1.9, 450 V, 7 mA; reference standard: the bis-S-sulfonate of the B chain of human insulin). Amino acid analysis: Asp 2.70 (3), Thr 2.80 (3), Ser 2.75 (3), Glt 6.85 (7) Pro 1.03 (1), Gly 4.00 (4), Ala 1.05 (1), Val 3.75 (4), Ile 1.70 (2), Leu 6.00 (6), Tyr 3.55 (4), Phe 2.85 (3), His 1.95 (2), Lys 0.90 (1), Arg 0.95 (1). Results of a determination of the C-terminal amino acids: Asn 0.96 (1), Thr 0.98 (1).

On testing with respect to the spasmodic effect on mice [2], the biological activity of the human insulin obtained amounted to 100% (in comparison with the activity of the international standard).

LITERATURE CITED

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2. K. L. Smith, Methods Horm. Res., 2, 439 (1962).

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