PREPARATION AND PROPERTIES OF PHENYLALANINE-B^{30}-(HUMAN INSULIN)

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In a directed experimental evaluation of the influence of the replacement of an aliphatic amino acid residue in the C-terminal part of the B-chain of insulin by an aromatic amino acid residue, we have prepared the previously unknown phenylalanine-B^{30}-(human insulin) and have investigated the biological activity of this analog, which differs from the natural hormone by the replacement of a L-threonine residue by a L-phenylalanine residue.

The phenylalanine B^{30}-insulin [I, R = de-ThrB^{30}-(human insulin)] was obtained by an enzymatic-chemical method using the tryptic transamidation reaction [1]. This reaction takes place on the interaction of porcine insulin [II, R = de-Ala B^{30}-(porcine insulin)] with L-phenylalanine tert-butyl ester (III) in an aqueous organic medium (water–dimethylformamide) at 25°C and pH 6.3. Under these conditions, the transamidation reaction takes place only at the Lys B^{29} residue, and the undesirable side reaction at the Arg B^{22} residue does not proceed:

I. R–Phe–OH; III. H–Phe–OBu^t;
II. R–Ala–OH; IV. R–Phe–OBu^t.

After the purification of the ester derivative of insulin (IV), formed as an intermediate, by ion-exchange chromatography on DEAE-Sephadex A-25, compound (IV) was subjected to chemical demasking with the aim of the exhaustive elimination of all the C-protective groups from the Phe B^{30} residue. Demasking was effected by treating compound (IV) with trifluoroacetic acid at 20°C in the presence of anisole as protector. The phenylalanine-B^{30}-(human insulin) (I) formed was isolated from the reaction mixture by gel filtration on Sephadex G-25F. The course and degree of purification were monitored by thin-layer chromatography on silica gel, by electrophoresis in cellulose, and by disk electrophoresis in polyacrylamide gel.

After lyophilization of the eluate, phenylalanine-B^{30}-(human insulin) (I) was obtained in the analytically pure state.

Phenylalanine-B^{30}-(Human Insulin) (I). R_f 0.48 (C_5H_5N – C_4H_9OH – CH_3CO_2H H_2O, 10:15:3:12), 0.65 (C_5H_5N – CH_3COCH_3 – H_2O, 1:1:2), 0.34 iso-C_3H_7OH – 25% NH_4OH, 7:4), 0.88 iso-C_3H_7OH – 25% NH_4OH – H_2O, 7:4:6) (TLC on Silufol UV-254 plates; visualization with the Pauly reagent [2]. Electrophoretic mobility: 1.38 (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 2.77 (3), Thr 1.44 (2), Ser 2.36 (3), Glu 7.07 (7), Pro 1.09 (1), Gly 3.76 (4), Ala 1.05 (1). Val 4.02 (4), Ile 1.72 (2), Leu 6.00 (6), Tyr 3.51 (4), Phe 3.94 (4), Lys 1.03 (1), His 1.95 (2), Arg 0.89 (1). Results of a determination of C-terminal amino acids: Asn 0.97 (1), Phe 0.99 (1).

When tested for its convulsive effect in mice [3], the biological activity of compound (I) amounted to 95% (in comparison with the activity of the international standard).

LITERATURE CITED

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