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ENZYMATIC-CHEMICAL CONVERSION OF PORCINE INSULIN INTO RABBIT INSULIN

Yu. P. Shvachkin, A. M. Nikitina,
S. P. Krasnoshchekova, E. N. Voluiskaya,
V. P. Fedotov, and A. I. Ivanova

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In connection with a study of ways and means for converging animal insulins, we have performed the enzymatic-chemical conversion of porcine insulin into rabbit insulin.

The first stage of the process consisted in the trypsin-catalyzed transamination of porcine insulin (I, R = de-Ala^{B30}-(porcine insulin)), which took place on the interaction of the latter with the tert-butyl ester of O-tert-butyl-L-serine (II) in an aqueous organic medium (water–dimethylformamide) at 24°C and pH 6.3. Under these conditions, the enzymatic transamination reaction took place only at the LysB^{29} residue, and there was no undesirable side reaction at the Arg^{B22} residue.

I. R—Ala—OH; III. R—Ser(Bu^t)—OBu^t;
II. H—Ser(Bu^t)—OBu^t; IV. R—Ser—OH.

The second stage of the process consisted in the chemical demasking of the ester derivative of insulin (II) from the first stage and had the aim of completely eliminating the protective tert-butyl groups from the Ser^{B30} residues. The ester derivative (II) was first purified by ion-exchange chromatography on DEAE-Sephadex A-25. Demasking was carried out by treating the ester derivative with trifluoroacetic acid at 20°C in the presence of anisole as protector.

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

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

Rabbit Insulin (IV). R_f 0.50 ($C_5H_5N-CH_3CO_2H-H_2O$(10:15:3:12)); 0.48 (iso-C_3H_7OH–25% NH_4OH (7:4)), 0.90 (iso-C_3H_7CH–25% NH_4OH –H_2O (7:4:6)) (TLC, Silufol UV-254 plates; Pauly chromogenic reagent [2]). 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 porcine insulin. Amino acid analysis: Asp 3.00 (3), Thr 1.80 (2), Ser 3.80 (4), Glu 7.06 (7), Pro 1.00 (1), Gly 4.00 (4), Ala 1.02 (1), Cys 4.60 (6), Val 3.60 (4), Ile 1.80 (2), Leu 6.00 (6), Tyr 3.50 (4), Phe 2.90 (3), His 2.00 (2), Lys 1.00 (1), Arg 0.95 (1). The results of a determination of the C-terminal amino acids: Ser 0.98 (1), Asn 0.97 (1).

On testing for its convulsive effect on mice [3], the biological activity of the rabbit insulin obtained amounted to 100% (in comparison with the activity of the international standard).

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ENZYMATIC SYNTHESIS OF NUCLEOPEPTIDES

G. A. Korshunova, I. M. Dobkina,
N. V. Sumbatyan, and Yu. P. Shvachkin

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In order to determine the possibility of the enzymatic synthesis of stereoregular nucleopeptides [1] including residues of natural nucleo amino acids and some protein amino acids, we have investigated the reaction of N^{α}-acyl-DL-nucleo amino acids (I-V) with the amides of L-leucine and L-methionine in the presence of chymotrypsin or carboxypeptidase Y.

Information on the constants and yields of compounds obtained previously (I-V) is given below (TLC on Silufol UV-254 plates; systems: 1) iso-C_3H_7OH–25% NH_4OH–H_2O (14:1:5); and 2) n-C_4H_9OH–CH_3CO_2H–H_2O (4:1:1)).

Compound	mp, °C	R_f system 1	R_f system 2	Yield, %
Ac-*DL*-Aal-OMe (I)	190	0 66	0,15	73
Ac-*DL*-Ual-OMe (II)	219	0,60	0,20	82
Ac-*DL*-Tal-OMe (III)	230	0,73	0.44	70
Boc-*DL*-Aal-OMe (IV)	187	0,88	0,73	60
Boc-*DL*-Ual-OMe (V)	197	0,80	0,70	70

After a series of experiments, we found that the incubation of the esters (I-V) with the amine of L-leucine or L-methionine and chymotrypsin in 0.2 M carbonate buffer (pH 10.0) containing 20% of a 1:1 mixture of dimethylformamide (DNFA) and dimethyl sulfoxide led to the enzymatic synthesis of stereoregular nucleopeptides. At 20°C, the reaction was complete in 8 h and the yield of nucleopeptides amounted to 60-90%. In experiments with carboxypeptidase Y, the yields of nucleopeptides were low.

As a result of the experiments performed we achieved the enzymatic synthesis of the stereoregular nucleopeptides (VI-XII):

VI. Ac-*L*-Ual-L-*L*eu-NH_2
VII. Ac-*L*-Aal-L-*L*eu-NH_2
VIII. Ac-*L*-Tal-L-Leu-NH_2
IX. Boc-*L*-Aal-*L*-Leu-NH_2
X. Boc-*L*-Aal-*L*-Met-NH_2
XI. Boc-*L*-Ual-*L*-Leu-NH_2
XII. Boc-*L*-Ual-*L*-Met-NH_2

For subsequent use in nucleopeptide synthesis, compounds (IX-XII), each containing a N-protective Boc group readily eliminated on acidolysis, are particularly promising. Information on the constants of these compounds is given below (system 3: $CHCl_3$–CH_3OH (1:1); electrophoretic mobility in electrophoresis on LKB-3276 paper (240 V, 2 h) in systems 1) 1 N CH_3CO_2H (pH 2.5), and 2) 0.05 M aqueous $(C_2H_5)_3NH_2CO_3$ (pH 8.7); reference standard: H-Aal-OH in the case of compounds (IX) and (X), and H-Ual-OH in the case of compounds (XI) and (XII):

Compound	mp, °C	R_f in system 1	R_f in system 2	R_f in system 3	EM in system 1	EM in system 2	$[\alpha]_D^{20}$, deg (in DMFA)
IX	177	0 75	0 70	0,34	1,03	0,25	—30 (*c* 1,0)
X	—	0,76	0,62	0,30	0,73	0,30	—24 (*c* 0,95)
XI	172	0,87	0 77	0,60	0,75	0 32	—52 (*c* 0,1)
XII	157	0,85	0,73	0,58	1,09	0,40	—55 (*c* 0,25)

The structures of the compounds obtained were confirmed by the results of acid hydrolysis and UV and mass spectra, and also by independent chemical synthesis. The optical purity of

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