ISOLATION OF INDIVIDUAL PROTAMINES BY HIGH-PERFORMANCE LIOUID CHROMATOGRAPHY

V. K. Rybin, N. N. Yaglitskaya, and N. V. Makarov

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Using as an example the protamine from the gonads of the sturgeon Acipenser stellatus, a procedure has been developed for isolating homogeneous protamines (stellins) by high-performance liquid chromatography which, as compared with the ion-exchange chromatography used for these purposes at the present time [1, 2], permits a considerable shortening of the time of isolation and the elimination of the procedure for desalting the proteins obtained, which frequently leads to their destruction.

Chromatography was performed with the aid of a model 8800 liquid chromatograph (USA) fitted with a UV-spectrophotometric detector with a flowthrough cell (wavelength 220 nm; rate of flow 1.0 ml/min). Zorbax ODS column (4.6 × 250 nm) at a column temperature of 35°C. The proteins were eluted with aqueous ethanol, using a concentration gradient of ethanol of from 0 to 30%. To prevent the irreversible binding to the sorbent that is characteristic for basic proteins [3], trifluoroacetic acid was added to the mobile phase in a concentration of 0.1-0.2%.

Figure 1 shows a chromatogram of stellin. Two components (I and II) were isolated. With respect to their amino acid compositions, these proteins proved to be identical with stellins B and A [4]. The less hydrophobic stellin B was eluted first [5].

When from 5 to 100 μg of an individual protamine was deposited on the column, the area of the chromatographic peaks depended linearly on the amount of protein added. The correlation coefficient calculated by the method of least squares on a Spectra-Physics SP-4100 computer (USA) was 0.95-0.97 for both proteins.

Thus, the possibility has been shown of the successful use of high-performance liquid chromatography for separating protamines.

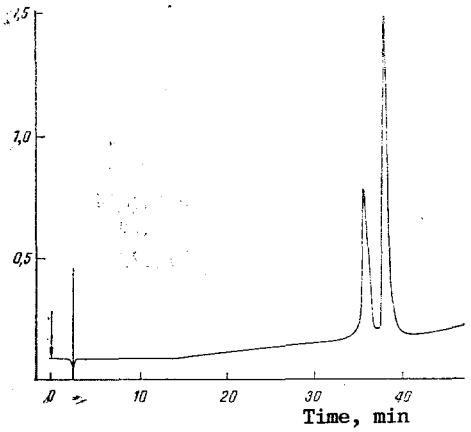

Fig. 1. Chromatogram of the total stellin from the gonads of the sturgeon Acipenser stellatus on a Zorbax ODS column. Amount of protein deposited, 25 μg; concentration of trifluoroacetic acid, 0.15%; for other conditions, see text.

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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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