

ent yellowish syrupy mass had formed. The same operation was repeated once more after the addition of 25 ml of water.

The "syrup" was placed in the refrigerator for two days for complete crystallization, which takes place with the evolution of heat. Where necessary it is possible to initiate crystallization by the addition of a seed of (II). When working with large amounts of reactants [for example, with an increase in the amount of (III) used in the reaction to 200 g, with a corresponding increase in the amount of (I) and (IV)], it is more convenient to carry out crystallization in a polyethylene packet, which facilitates the subsequent extraction of the crystalline (III). The crystals obtained were dried in the air to constant weight. Yield 90-95%. mp 130-131°C (according to the literature, 127.5-128°C [5], 136-137°C [6]). pH of a 1% solution 2.85-2.90. R_f values on Silufol plates - 0.35 in the methyl ethyl ketone-acetic acid-pyridine-water (32:4:2:6) system, and 0.54 in the methanol-chloroform (3:7) system.

After additional purification of the (II) with the aid of reprecipitation by ethyl acetate, a sample of (II) containing, according to the results of amino acid analysis, 0.2-0.3% of free (III) was obtained. $[\alpha]_D^{20} +22.22^\circ$ (c 1; 15 N, HCOOH). Found, %: C 37.07; H 4.13; N 8.49. $C_5H_7O_5N$. Calculated, %: C 37.27; H 4.37; N 8.69.

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ISOLATION OF A THIRD COMPONENT OF STELLIN - A PROTAMINE FROM THE GONADS OF *Acipenser stellatus*

V. K. Rybin, L. P. Revina, L. A. Baratova,
and N. V. Makarov

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We have previously reported the isolation and the determination of the structures of two protamines from the gonads of *Acipenser stellatus* - stellins A and B [1, 2]. By a combination of ion-exchange chromatography on CM-Sephadex G-25 and reversed-phase HPLC on a Zorbax C-8 column we have succeeded in isolating a third component of stellin - stellin C, which, from the results of amino acid analysis according to Kossel's classification [3] is a triprotamine. To determine the structure of the protein we used thermolysin hydrolysis and Edman degradation. Analysis of the amino acid sequence by Edman's method was performed on a solid-phase sequenator by the method described previously [4]. To separate the thermolysin peptides we used reversed-phase HPLC on a Zorbax ODS column. Eight peptides were isolated and their structures were established. The results obtained, taken together, enabled the complete amino acid sequence of stellin C to be established:

A comparison of the amino acid sequence of stellin C with that of stellin A showed that they practically coincide. The difference consists in the fact that stellin A contains an additional alanine as the N terminal amino acid residue. It is not yet clear whether the observed difference is connected with the functioning of two different genes or is due to

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features of the posttranslational modification of the protamines. An interesting feature of stellin C is that its molecule begins directly from an arginine block. In known fish protamines the N-terminal arginine block is, as a rule, preceded either by proline or by alanine [5, 6]. Recently, a protamine - illexin I₂₋₁ - the molecule of which, like that of stellin C, begins from a block of five arginine residues, was isolated from squid spermatozoa [7]. Protamines of this type apparently play a completely definite role in the compactization of the chromatin of the sex cells.

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