LITERATURE CITED

1. I. S. Kulaev, in: The Biochemistry of High-Molecular-Weight Phosphates [in Russian], Moscow (1975), p. 247.
2. M. N. Valikhanov and B. O. Beknazarov, Khlopkovodstvo, 1, 36 (1984).
3. M. N. Valikhanov, B. O. Beknazarov, and R. P. Igamnazorov, Fiziol. Rastenii, 27, 296 (1980).
4. B. O. Beknazarov and M. N. Valikhanov, Khim. Prir. Soedin., 674 (1984).
5. H. Weil-Malherbe and R. H. Green, Biochem. J., 49, 286 (1951).
6. O. H. Lowry, N. J. Rosebrough, A. Z. Farr, and R. J. Randall, J. Biol. Chem., 193, 265 (1951).
7. T. Maurer, in: Disk-Elektrophorese, Theorie und Praxis (ed. H. R. Maurer), Walter de Gruyter, Berlin (1968) [Russian translation, Moscow (1971), p. 58].

PREPARATION OF HOMOGENEOUS PROTAMINES BY THE FRACTIONATION OF NUCLEOPROTAMINE

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A new method has been developed for obtaining individual protamines which is based on the chromatographic separation of an ultrasonically treated nucleoprotamine solution. The separation of the nucleoprotamine from the gonads of the Caspian sturgeon Acipenser stellatus on CM-Sephadex G-25 led to the isolation of three fractions. Analysis showed that two fractions contained homogeneous protamine – stellins A and B. The third fraction contained nonprotamines and DNA.

Protamines form a peculiar group of basic nuclear proteins which, in the mature sex cells of a number of organisms (molluscs, amphibia, some reptiles, and also in the majority of fish) are bound into a strong complex with DNA. It is considered that the main function of the protamines is the compactization of the DNA and its protection from various physicochemical actions [1, 2]. Characteristic features of the protamines are their high content of basic amino acids, their limited set of neutral amino acids, and their low molecular weight ($\sim 5 \cdot 10^3$ daltons). As a rule, each species of fish is characterized by its own unique set of protamines consisting of from two to four proteins. The isolation of the individual proteins is one of the important problems arising in the study of the protamines.

The methods for obtaining homogeneous protamines used at the present time generally consist of two stages: 1) the isolation of the total proteins from the nuclear protamines; and 2) the fractionation of the mixture of proteins isolated with the aim of obtaining homogeneous protamines.

Extraction with mineral acids is used most frequently for the isolation of the total protamines [1, 3, 4]. This extremely simple method has a number of disadvantages. In the first place, the destruction of the protamines in the acid medium is possible. In the second place, other basic proteins, and also degradation products of nucleic acids are coextracted with the protamines. In the third place, repeated extraction is used for the complete isolation of the protamines. It is obvious that it would be extremely desirable to eliminate or improve this stage in the isolation of homogeneous protamines.

The second stage – the isolation of individual proteins – is usually performed with the aid of chromatography on cation-exchanging celluloses or Sephadexes [1, 4-6].

We have succeeded in simplifying the method of obtaining homogeneous protamines by excluding the stage of isolating the total proteins.

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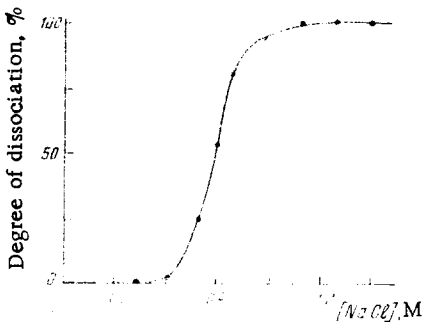

Fig. 1

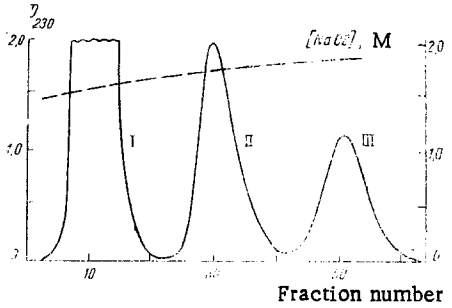

Fig. 2

Fig. 1. Dependence of the degree of dissociation of the nucleostellin on the concentration of sodium chloride.

Fig. 2. Fractionation of ultrasonically treated nucleostellin on CM-Sephadex G-25 in an 0.05 M phosphate buffer, pH 6.0, in the presence of a 1.4 M solution of NaCl.

We had shown previously [6] that in the chromatographic separation of the nuclear proteins from the gonads of the Caspian sturgeon Acipenser stellatus on carboxymethyl-Sephadex G-25 they were eluted at sodium chloride concentration of 1.6-1.9 M. It is known [1] that in solutions with a high ionic strength the nucleoproteins dissociate into protamines and DNA. We have studied the degree of dependence of the degree of dissociation of the nucleoprotamine from the gonads of the sturgeon Acipenser stellatus on the concentration of sodium chloride (Fig. 1). As can be seen from Fig. 1, at sodium chloride concentration above 1.1 the nucleoprotamines are dissociated completely into stellins and DNA. Thus, salt solution of the nucleoprotamine can be used for the chromatographic isolation of the protamines. However, because of the presence of high-molecular-weight DNA, this solution has a high viscosity which creates certain difficulties for ion exchange. To lower the viscosity of the salt solution of nucleoprotamine treatment with ultrasound for several minutes has been used.

Figure 2 shows the elution curve of ultrasonically treated nucleostellin on CM-Sephadex G-25. Three fractions (i-III) were isolated. Fraction I had absorption in the UV spectrum that is characteristic for DNA (absorption maximum at 260 nm). After fraction I had been desalted by dialysis it was found to contain DNA the sedimentation coefficient of which on ultracentrifugation was 8.3 S. Electrophoresis in 15% polyacrylamide gel showed that, together with the DNA, fraction I contained proteins differing considerably from the protamines in their electrophoretic mobility. An amino acid analysis of the composition of the proteins in fraction I that was performed (Table 1) showed a considerable similarity to the amino acid composition of the nonprotamine proteins from nuclei of the gonads of the Russian sturgeon Acipenser güldenstadti [7].

Fractions II and III had the adsorption spectrum in the UV region that is characteristic for protamines. In the amino acid compositions (see Table 1), fractions II and III were identical with stellins A and B, respectively [6, 8]. Electrophoresis in 30% polyacrylamide gel confirmed the result obtained.

Thus, in the present work, using nucleostellin as example, the possibility had been shown of obtaining individual protamines by fractionating an ultrasonically treated solution of a nucleoprotamine, the stage of isolating the total protamines being eliminated. Furthermore, it is an interesting fact that fraction I contained nonprotamine proteins and, therefore, the method described can also be used for the separation of nonprotamine proteins.

EXPERIMENTAL

The nucleoprotamine from the gonads of the Caspian sturgeon Acipenser stellatus, which we isolated by Ando's method [9], was used.

The nucleostellin was fractionated on CM-Sephadex G-25 in 0.05 M phosphate buffer, pH 6.0, containing 1.4 M sodium chloride, using an exponential gradient of sodium chloride. Before deposition on the column, the nucleostellin was homogenized in 0.05 M phosphate buffer, pH 6.0, containing 1.4 M sodium chloride with treatment in a MSE ultrasonic disintegrator for 3 × 2 min at the maximum power and was centrifuged at 12,000 rpm for 20 min.

TABLE 1. Amino Acid Composition on the Proteins from the Gonads of Acipenser stellatus*

Amino acid	Fraction		
	I	II	III
Lysine	5,85	5	—
Histidine	2,80	3	1
Arginine	6,09	12	19
Aspartic acid	12,35	—	—
Threonine	6,99	1	—
Serine	6,83	2	2
Glutamic acid	9,43	—	1
Proline	4,86	—	1
Glycine	8,12	1	2
Alanine	9,55	2	1
Valine	5,05	—	—
Methionine	2,74	—	—
Isoleucine	4,11	—	—
Leucine	6,09	1	—
Tyrosine	5,75	—	—
Phenylalanine	3,31	—	—

*The figures for the amino acid analysis of the proteins of fraction I are given in mole% and for the proteins of fractions II and III in amino acid residues.

The desalting of the protamines and electrophoresis in 30% polyacrylamide gel were carried out by procedures that we have described previously [6].

The influence of sodium chloride on the degree of dissociation of the nucleoprotein was studied in the following way: 2 mg of nucleoprotein (dry weight) was homogenized in 40 ml of 0.05 M phosphate buffer, pH 6.0. Aliquots with volume of 1 ml were taken and were diluted with equal volumes of a solution of sodium chloride of the necessary concentration in the corresponding buffer. The samples were centrifuged at 18,000 rpm for 25 min, and the degrees of dissociation were determined from the optical densities of the supernatant solutions.

Nucelotide compositions were determined as we have described previously [10].

Amino acid compositions were determined after hydrolysis with 5.7 N HCl under standard conditions on an LKB-3201 amino acid analyser (Sweden).

SUMMARY

A new method has been proposed for obtaining homogeneous protamines by fractionating ultrasonically treated protamine. The method can also be used for the isolation of nonprotamine proteins.

LITERATURE CITED

1. T. Ando, M. Yamasaki, and K. Suzuki, Protamines, Springer Verlag, Berlin (1973).
2. R. Balhorn, J. Cell. Biol., 93, 298 (1982).
3. P. Sautiere, G. Briand, M. Gusse, and P. Chevaillier, Eur. J. Biochem., 119, 251 (1981).
4. T. Tobita, M. Nomoto, N. Nakano, and T. Ando, Biochim. Biophys. Acta, 707, 252 (1982).
5. G. Bretzel, Z. Physiol. Chem., 353, 209 (1972).
6. E. P. Yulikova, V. K. Rybin, and A. B. Silaev, Bioorg. Khim., 5, 5 (1979).
7. L. K. Moleva, E. P. Yulikova, and A. B. Silaev, Khim. Prir. Soedin., 507 (1982).
8. E. P. Yulikova, V. K. Rybin, and A. B. Silaev, Khim. Prir. Soedin., 700 (1979).
9. T. Ando, S. Ishii, M. Yamasaki, K. Iwai, C. Hashimoto, and F. Sawada, J. Biochem. (Tokyo), 44, 275 (1957).
10. V. K. Rybin and E. P. Yulikova, Biokhimiya, 45, 733 (1980).