ISOLATION AND CHARACTERISTICS OF THE PROTAMINE FROM ACIPENSER STELLATUS

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The protamines of the sturgeons are among the least studied compounds of this class of proteins. Only sturin from Acipenser sturio has been studied in some detail, by Kossel and Staudt in 1927 [1] and by Felix in the fifties [2]. Sturgeon protamines belong to the class of triprotamines because they contain all three dibasic amino acids.

This paper gives the results of the isolation and study of a protamine from the sperm of Acipenser stellatus. Using the nomenclature for protamines introduced by Kossel, we have adopted for it the name of stellin. This protamine was obtained previously by Kuraev [3] and also by Lisitsyn and Aleksandrovskaya [4], but they characterized it only by its elementary composition.

Like all the protein studied hitherto, stellin consists of a heterogeneous mixture of peptides. On paper electrophoresis under various conditions (phosphate buffer, pH 5.8, 6.5 and 6.7 at 500 and 1000 V) it gives a broad diffuse band without appreciable separation. The UV spectra of stellin (Fig. 1) confirm the absence from it of tyrosine and nucleic acids, since they give the absorption curve without a maximum in the 260–280 mμ region that is nonspecific for proteins. Its amino acid composition is characteristic for the protamines (Table 1). It must be mentioned that this composition varies somewhat for different preparations. This probably depends on the different state of ripeness of the sperm. Nevertheless, a considerable similarity can be seen between the composition of stellin, particularly in the basic amino acid fraction, and the composition of sturin (Table 2). A similarity also appears in respect of the terminal amino acids: at the N-end of stellin we have found only alanine, while in sturin Felix showed the presence of alanine and a small amount of glutamic acid. Thus, the sturgeon protamines have characteristic differences from the protamines of the bony fishes (Salmonidae, herrings), in which proline predominates at the N-end.

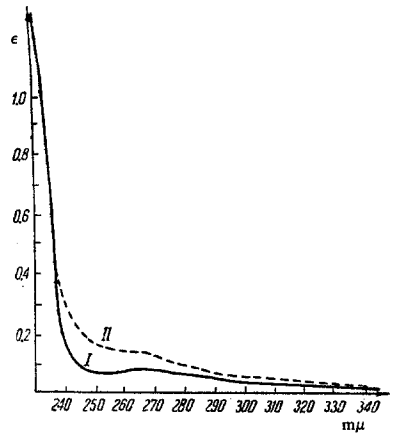

Fig. 1. UV spectra of stellin after separation on Sephadex G-25: I) Main fraction, stellin-1; II) auxiliary fraction desorbed from the column with 0.005 N HCl (spectra of 0.1% solutions of stellin in water).

The mean molecular weight of stellin calculated from the amount of N-terminal alanine was 5300. It is likely that this figure is somewhat high because of the usual incompleteness of the dinitrophenylation reaction. A colorimetric determination with ninhydrin before and after hydrolysis showed that the molecular weights of the fractions (1–5) isolated by chromatography on CM-cellulose are different, those of the last three fractions (3–5) being close to the molecular weight of stellin while the first two fractions contain shorter peptides.

Thus, the heterogeneity of stellin is caused both by differences in the lengths of the peptides composing it and also by their different aminoacid compositions (see Table 1).

Table 1. Amino Acid Composition of Stellin and Its Fractions

Amino acid	Stellin-1		Fraction 1		Fraction 2		Fraction 3		Fraction 4		Fraction 5	
	a*	b	a	b	a	b	a	b	a	b	a	b
Arginine	60.8	395	43.1	276	65.4	42.9	51.6	334	46.4	301	67.2	430
Histidine	9.6	70	6.8	49.4	5.1	36.5	9.3	66	12.5	91	5.6	41
Lysine	13.2	103	11.25	87.8	7.7	60.1	22.2	173	21.5	168	4.7	31
Aspartic acid	0.3	2.9	1.06	9.2	0.76	6.6	0.52	4.5	0.09	0.8	0.6	5.2
Threonine	2.26	20.3	6.16	55.5	0.54	4.86	1.21	10.9	3.98	35.9	1.10	9.9
Serine	3.9	44.8	7.84	90.1	4.85	55.7	5.16	59.3	5.66	65.1	6.21	71.3
Glutamic acid	1.62	12.5	2.66	20.6	4.54	35.2	1.09	8.45	0.22	2.8	4.51	34.9
Proline	0.73	7.5	—	—	4.6	47.4	0.85	8.76	0.38	3.9	2.99	38.8
Glycine	3.21	56.3	10.4	183.0	1.26	22.1	3.83	67.2	2.65	46.5	3.83	67.7
Alanine	2.35	33.1	8.6	121.0	3.41	48.0	1.61	22.6	4.07	57.3	2.21	31.4
Leucine	1.96	17.3	1.68	14.9	1.92	17.0	2.62	23.2	2.49	22.0	0.81	7.2
Mean mol wt	<5300		~2000		~1400		~4300		~5500		~5300	

a*) Percentage of the amino acid; b) number of residues per 100 000 g of stellin.

EXPERIMENTAL

To isolate the protamine we used the method of Callanan et al. [5], which permits the protamines to be separated from the nucleic acids under very mild conditions. Milt of fresh-frozen sevruga was carefully freed from fat and connective tissue, comminuted in a mincing machine, and washed alternately five times with water and 0.1 N NaCl in the cold, and it was then treated three times with 50% ethanol and twice with 96% ethanol, and finally, with a mixture of ether and ethanol (1 : 1). The air-dry mass of sperm was ground in a ball mill, and the powder was defatted in a Soxhlet apparatus. To cleave the nucleoprotamine, the dry mass of sperm was treated with 1.5 volumes of 1.5 M NaCl in 50% ethanol. After careful stirring in a mixer, the mixture was centrifuged, and the residue was subjected to the same treatment five more times. The collected extracts were dialyzed against distilled water. Cellophane that had been heated at 105° C for 20 hr to decrease its porosity was used for the dialysis. The dialyzed solution was freeze-dried. Because of this dialysis through compacted Cellophane, it was possible to raise the yield of stellin from 10 to 14–15 g per 100 g of the mass of sperm.

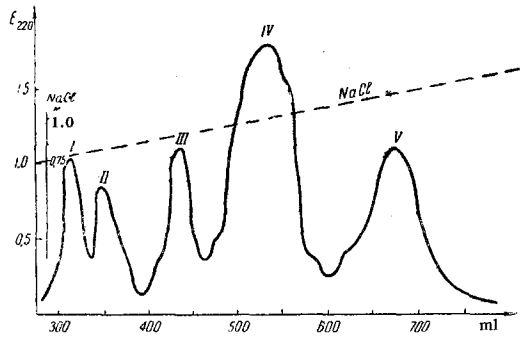

Fig. 2. Separation of stellin into fractions during gradient chromatography on CM-cellulose. I–V) fractions of stellin.

The stellin hydrochloride obtained had the form of a white hygroscopic powder. Its elementary composition was, %: C 37.65; H 7.12; N 23.04; P 0.0023. The content of phosphorus calculated as nucleic acids [6] showed 0.023–0.026% of impurity. Determination of the nucleic acids by Spirin's method [7] gave a similar result, 0.025–0.027%.

When an aqueous solution of stellin hydrochloride was passed through a column of Sephadex G-25, the bulk of the protein issued in the form of a single peak (stellin-I) with a yield of 93%, and a small fraction (stellin-II) was obtained by subsequent elution with 0.005 N HCl. The amino acid composition of this second fraction differed little from that of the main fraction. Nevertheless, the fact that the absorption in the 260–280 mμ region was higher than that of stellin-I showed the presence of impurities. Only the main fraction of stellin was investigated subsequently.

Table 2. Comparison of the Amino Acid Compositions of Stellin and Sturin (Number of Residues for a mol. wt. of 5000)

Amino acids	Stellin	Sturin [2]
Arginine	20	19.5
Histidine	3.5	3.85
Lysine	5	5
Aspartic acid	Traces	none
Threonine	1	0.55
Serine	2.2	1.65
Glutamic acid	0.6	0.55
Proline	0.4	none
Glycine	2.8	1.1
Alanine	1.6	2.75
Leucine	0.8	1.2
N-Terminal amino acids	Alanine	Alanine and traces of glutamic acid
Mean mol. wt.	5000	

In the determination of the N-terminal amino acids by the dinitrophenyl method [8], the spot of dinitrophenylalanine was found on the two-dimensional paper chromatograms. After the extraction of the spot and its hydrolysis, alanine was found on a chromatogram. There were no other N-terminal amino acids.

Fractionation of stellin-I. As follows from Fig. 2, when stellin-I was subjected to gradient chromatography on a column of CM-cellulose in the Na^+ form with an NaCl gradient from 0.72- to 1.8-molar, the substance was separated into five clearly heterogeneous peaks. Since separation into these fractions was fairly satisfactorily reproducible, we studied their amino acid composition in somewhat more detail and determined the mean lengths of their peptide chains. The amino acid analysis was carried out on an automatic amino acid analyzer (Czechoslovakia). The mean chain lengths of stellin and its fractions were calculated on the basis of chemical analyses: from the content of N-terminal alanine after dinitrophenylation and from the increase in the intensity of the coloration with ninhydrin after the acid hydrolysis of the stellin fractions.

CONCLUSIONS

A protamine, which has been named stellin, has been isolated from the mass of sperm of the sevruga (Acipenser stellatus).

Stellin is heterogeneous and consists of a mixture of peptides differing in amino acid composition and in chain length. There is great similarity between stellin and sturin both with respect to amino acid composition and with respect to N-terminal amino acids.

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