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Amino acid sequence of the unique protamine from yellow perch

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By the criteria of gel electrophoresis, ion-exchange chromatography, and reverse-phase HPLC, yellow perch protamine behaves as a single component. This observation was confirmed by automated Edman degradation which gave a single unambiguous amino acid sequence PRRR-RHAARPVRRRRRTRRSSRVHRRRRAVRRRR. Yellow perch protamine has 34 amino acids, including 21 arginines. It has two histidines, neither of which interrupts an arginine tract. It is unusual among fish protamines in not having a serine or threonine N-terminal to the second arginine tract, and is unique in not being a mixture of components.

Spermatogenesis; DNA-binding protein; Amino acid sequence

1. INTRODUCTION

The variety in sperm DNA-binding proteins in animals [1–3] contrasts strongly with the conservation of somatic histone sequences and nucleosome structure [4]. In fish, some species retain histones in their sperm and others replace them completely with protamines. This dichotomy is difficult to rationalise with current phylogenetic schemes. Moreover, extreme variants of these options can also occur [5]. The true protamines of fishes resemble each other in their small size, high content of arginine, the grouping of this amino acid into long tracts, and their limited content of other amino acids among which serine and proline predominate [6]. Within the teleost fishes, protamines are less variable but they are not nearly as well conserved as the histones. Consequently, it has been difficult to define a consensus sequence and mode of interation with DNA. The limited sequence identity between protamines has also made it difficult to isolate protamine genes from unrelated species using protamine gene probes [7]. Recent additions to the list of protamine sequences have generally come from conventional protein sequencing [8–12]. Yellow perch (*Perca flavescens*) is a species which undergoes a complete histone to protamine transition during spermatogenesis [7]. The apparent lack of diversity in perch protamine has facilitated its purification and sequencing.

2. MATERIALS AND METHODS

2.1. *Tissue*

Yellow perch were collected in November from the Bay of Quinte on Lake Ontario near Belleville, Ontario. Testes were removed, frozen on solid CO_2 and stored at −60°C.

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2.2. *Purification of protamine*

Acid-soluble nuclear proteins were prepared from individual and pooled testes by the method of Marushige et al. [13]. Protamine was purified from this protein fraction by ion-exchange chromatography on carboxymethyl-cellulose [14,15] or by reverse-phase HPLC on a Vydac C18 column (10 mm×250 mm) using a gradient of methanol in water with 0.1% (v/v) hepafluorobutyric acid as the counter-ion. Protein purity was checked by gel electrophoresis [16].

2.3. *Amino acid analysis*

Amino acid analysis was done as previously described [11]. The amino acid composition of perch protamine was confirmed by an independent analysis using an Applied Biosystems 420H instrument for in situ hydrolysis, derivitization, and separation of phenylthiocarbonyl amino acids.

2.4. *Protein sequencing*

A preliminary sequence for perch protamine purified by ion-exchange chromatography was determined by automated Edman degradation in a Beckman 890C sequencer using methodology previously described [11]. The assignment of seryl and threonyl residues was clarified by sequencing HPLC-purified protamine using an Applied Biosystems 473A protein sequencer with on-line analysis of phenylthiohydantoin amino acids.

3. RESULTS AND DISCUSSION

3.1. *Perch protamine purification*

Total acid-soluble protein from perch testes nuclei collected in November was predominantly protamine, and the amount of contaminating histone was insufficient to warrant its removal by gel filtration [7]. Polyacrylamide gel electrophoresis in acetic acid/urea failed to fractionate the protamine into more than one component (not shown). This was also true for ion-exchange chromatography on CM-cellulose (Fig. 1) done under conditions in which trout protamines with different

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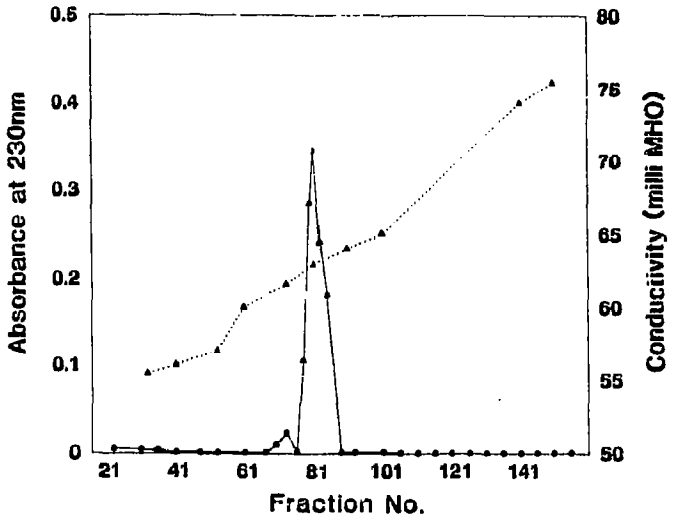

Fig. 1. Carboxymethyl-cellulose chromatography of perch testis acid-soluble nuclear protein. Acid-soluble nuclear protein (15 mg) in 20 ml of 20 mM ammonium acetate (pH 5.5) was applied to a column of Whatman CM52 carboxymethyl cellulose (1 cm×51 cm) equilibrated in the same buffer containing 0.7 M LiCl. The column was developed with a linear gradient of 0.7 to 1.2 M LiCl in 20 mM ammonium acetate (pH 5.5) of total volume 1l at a flow rate of 15 ml/h. Fractions (5 ml) were collected and monitored for absorbance at 230 nm (●—●) and conductivity (▲---▲).

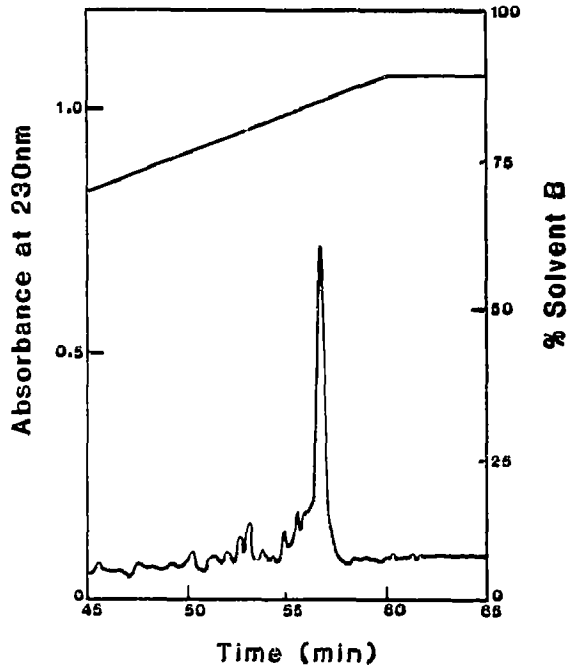

Fig. 2. Reverse-phase HPLC of perch testis acid-soluble nuclear protein. Acid-soluble nuclear protein (1 mg) was applied in solvent A (0.1% heptafluorobutyric acid in 10% methanol) to a semi-preparative Vydac C18 column (10 mm×250 mm) equilibrated in the same solution. The sample was eluted by a linear gradient from 100% solvent A to 90% solvent B (0.1% heptafluorobutyric acid in methanol) over 60 min with a flow rate of 3 ml/min. Eluate was monitored at 230 nm.

charge-to-mass ratios are separable [17]. Since reverse-phase HPLC has been shown to separate protamine components of similar charge-to-mass ratios that differ by a single amino acid replacement [17,18], perch protamine was analysed by this technique for signs of heterogeneity. Total acid-soluble nuclear protein from pooled testes of several fish (Fig. 2) and from an individual testis (not shown) both chromatographed as one major peak with identical retention times.

3.2. *Sequence homogeneity*

Amino acid compositions were determined for perch protamine purified by ion-exchange and reverse-phase chromatography. The two composition sets gave similar ratios for the seven amino acids present (Table I). The initial automated Edman degradation of protamine using a Bechman 890C sequencer in conjunction with HPLC analysis of the phenylthiohydantoin amino acids produced a 34-residue sequence. All but three residues (17, 20 and 21) were unequivocally identified. The difficulty in assigning these three residues was due to an inability to distinguish between threonine and serine using existing methodology. Subsequently, perch protamine was resequenced using the Applied Biosystems sequencer with on-line phenylthiohydantoin amino acid analysis. The same sequence was obtained but with the identification of residue 17 as a threonine and residues 20 and 21 as serines. In each case there was nog sign of a second sequence, or of ambiguities that would indicate the existence of more than one protamine component.

The sequence of protamine from yellow perch is **PRRR-RHAARPVRRRRRTRRSSRVHRRRRAVRRRR.**

3.3. *Protamine structure*

Structure/function relationships for protamines have not yet been worked out. Additional sequences are, therefore, useful in defining a consensus structure. In

Table I

Amino acid composition of perch protamine

Amino acid	A	Ratio	B	Ratio	C
Thr	9.5	1.0	49.4	0.7	1
Ser	19.7	2.1	125.0	1.7	2
Pro	20.6	2.2	176.0	2.4	2
Ala	30.6	3.3	248.4	3.4	3
Val	21.6	2.3	229.3	3.1	3
His	16.5	1.8	153.1	2.1	2
Arg	198.6	21.3	1491.5	20.5	21
Total	317.1		2472.7		34

(A) Composition of protamine from ion-exchange chromatography expressed as nmol recovered in each peak. (B) Composition of protamine from reverse-phase chromatography expressed as pmol recovered in each peak. Values shown are the average of four determinations. (C) Composition derived from the amino acid sequence expressed as number of residues per molecule.

this regard the perch protamine sequence is unique and yet similar in its general characteristics to other teleost protamines [6]. It has four arginine tracts but is unusual in not having a serine or threonine prior to the second of these tracts. The aliphatic hydroxyl groups of these residues are thought to be involved in reversible phosphorylations that may help the ordered binding of protamine to DNA and/or the compaction of the nucleoprotamine [6]. The placement of these side chains further down the molecule is presumed not to affect their function.

We have previously commented on the fact that other basic amino acids do not substitute for arginine within the tracts [11]. This is also true for the two histidines in perch protamine, which are both located beside arginine tracts.

The most unusual feature of perch protamine is its homogeneity. Typically, teleosts produce several closely related protamines [6]. In rainbow trout, six distinct protamine components have been recovered and sequenced from an individual testis [17]. It is estimated that the protamines in this species are encoded by a gene family with 15–20 members [6]. Although perch protamine could in theory be the product of a single gene it could also be encoded by a multigene family that has undergone recent expansion or convergence through gene conversion. The homogeneity of perch protamine could greatly facilitate the analysis of protamine/DNA binding.

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