

Comparison of protamines from freshwater and marine bivalve molluscs: evolutionary implications

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We characterized for the first time a protamine from the spermatozoa of a freshwater bivalve, *Dreissena polymorpha*. We found that it contains a protamine very similar in composition to those found in marine bivalves. We concluded that freshwater does not impose any restriction on the composition of sperm basic proteins. We compared our results with those recently published by other authors and proposed an evolutionary pathway for bivalve sperm proteins as originating from histone H1.

Protamine; Histone; Protein evolution; (Mollusc)

1. INTRODUCTION

Bivalve molluscs are abundant in marine environments, but are also common in freshwater. Since in both cases they have free swimming spermatozoa, it is of interest to compare the nuclear proteins associated with DNA in the sperm heads. The different ionic conditions might influence protein-DNA interactions and result in changes in the protein composition. To our knowledge, no studies of this type have been undertaken on freshwater molluscs, whereas the nuclear proteins from several marine bivalves have been studied [1-10]. With this purpose, we have characterized the acid extracted nuclear proteins from a freshwater bivalve, *Dreissena polymorpha*, which is wide spread in Europe. We find that the sperm proteins are very similar to those found in other marine bivalves. We also discuss the phylogenetic and evolutionary significance of bivalve sperm

proteins and suggest an evolutionary pathway for these proteins.

2. EXPERIMENTAL

Mytilus edulis was from Spanish commercial sources and *D. polymorpha* was collected by Professor Franzén in Lake Malaren (Sweden). Ripe testes were used as a source of spermatozoa. Protamines were extracted from ripe spermatozoa with 0.25 N HCl and precipitated with 6 vols acetone. In order to purify the protamine from *D. polymorpha*, the spermatozoa were first extracted with 35% acetic acid and the extract, containing histones, was discarded. The sediment was again extracted with 0.25 N HCl and the extract was precipitated with 3 vols acetone. In this way pure protamine was recovered as shown in fig.1. Electrophoresis was carried out in 15% acrylamide, containing 6.25 M urea [11]. The aggregate which appears at the origin is most likely due to the protamine, as it is found in other high molecular mass protamines [8]. The molecular mass was estimated from the electrophoretic mobility of the protamine [3]. Amino acid analyses have been obtained in a Biotronic 6000 analyzer. The value for serine has

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been estimated by extrapolation after 24, 48 and 72 h of hydrolysis in 6 N HCl at 110°C. Tryptophan was absent as deduced from the UV spectrum.

3. RESULTS AND DISCUSSION

As shown in fig.1, the spermatozoa of *D. polymorpha* contain histones plus a slowly moving protein, with a size of about 250 amino acids, the composition of which is given in table 1. This size and composition are very similar to those found in many marine bivalves [1-9], characterized by high

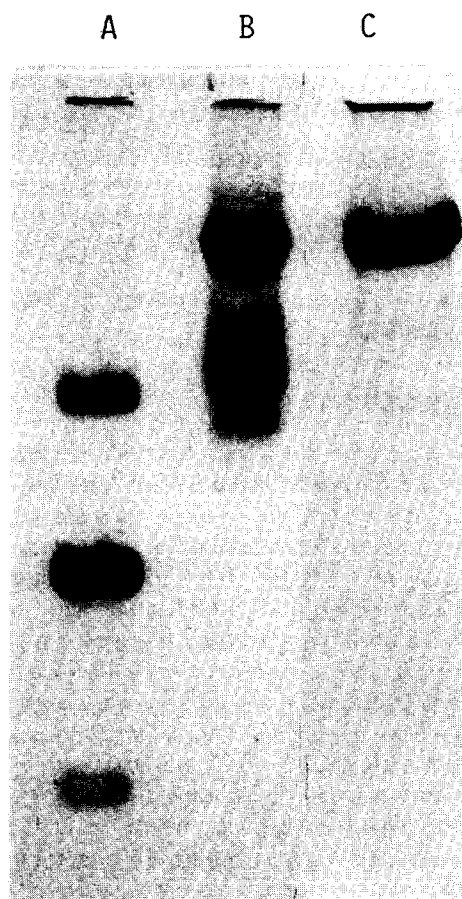


Fig.1. Gel electrophoretic pattern of basic sperm proteins from (A) *Mytilus edulis*, (B) *Dreissena polymorpha* and (C) purified protamine from *D. polymorpha*. The origin is at the top. *M. edulis* shows three bands [1,22]: a slowly moving H2b-like histone, a protamine with the composition given in table 1 and a fast moving lysine-rich protein.

Table 1

Amino acid composition of some bivalve protamines (%)

Amino acid	<i>M. edulis</i>	<i>S. solidissima</i>	<i>D. polymorpha</i>
Gly	6.3	3.0	3.2
Ala	13.6	14.2	7.7
Val	1.0	2.3	1.9
Leu	—	1.7	0.8
Ile	—	0.5	0.5
Pro	6.3	2.4	1.5
Ser	16.9	21.7	23.9
Thr	3.5	4.3	1.5
Asp	—	0.6	0.8
Glu	—	0.6	0.5
Cys	—	—	—
Met	—	0.4	1.1
Lys	21.7	24.8	27.0
Arg	28.7	23.1	27.5
His	—	—	1.0
Phe	—	0.3	0.8
Tyr	—	0.3	0.6
Try	—	0.3	—
Number of residues	100	297	250 (approx.)

The analyses of *M. edulis* $\phi 1$ and *S. solidissima* have been taken from [21,8], respectively

lysine, arginine and serine percentages, followed by alanine as the fourth major amino acid. In the table a comparison is made with the composition of the analogous proteins from *Spisula solidissima*, which has a slightly larger molecular size [8], and from *M. edulis*, which has a much smaller size. In conclusion, it appears that freshwater does not impose any selective pressure on the protein composition of spermatozoa. This is also true in fish. It has been argued [12] that histones are favoured in the spermatozoa of freshwater fish species, but in fact either histones [13,14] or protamines [15] may be found in both marine and freshwater environments.

The result we have obtained is also of phylogenetic interest. The bivalve molluscs show a radial pattern of evolution and their classification presents considerable difficulties [16,17]. In fact *D. polymorpha* had been classified in the Mytilaceae [18], which have quite different sperm

proteins ([1,7,10] and fig.1). Although recent classification schemes [17] have corrected this assignment, our results show that sperm proteins may be of interest in order to establish phylogenetic relationships. Indeed Ausió [19] has shown that different bivalve families contain characteristic sets of protamines. He has also shown that protamine characterization may be also helpful in systematic studies at the species level.

Given the diversity in size, number of components and, in some cases, amino acid composition of bivalve protamines, it appears of interest to try to establish an evolutionary pathway for these proteins. In fact the recent work of Ausió et al. [20] confirms that the high molecular mass proteins found in many bivalves are related to histone H1. An analysis of the number of components present in spermatozoa and of their amino acid composition suggests an evolutionary scheme which is presented in table 2. Thus *Crasostrea gigas* [4], *Swiftopecten swifti* [2] and *Chlamis islandicus* [21] have a basic sperm protein with a composition similar to somatic histone H1, although significantly more basic due to an increased arginine content. This would be the first detectable

change in sperm histone H1. In other bivalves [1,3,8,9] a characteristic sperm protein with a composition similar to those shown in table 1 is found and we consider them to be more evolved. The arginine and serine content is now higher. In these proteins it appears that the globular part is still preserved [20], although significantly modified from that found in somatic histone 1.

A further step in the evolution of these proteins appears to be a breakdown into several components, as found in *Donax trunculus* [3] and in *Ensis minor* [5]. The largest component may still contain a globular part, whereas the smaller components appear as typical protamines, derived from the basic N-terminal and C-terminal regions of the high molecular mass sperm histones [20]. An even more evolved case is found in the Mytilaceae, where the somatic-type histones are reduced to a single H2b-like component [22] and two different protamines are found [1,7,10,21]. It is worth noting that in *S. solidissima* the amino acids Tyr, Trp, Thr and Met are found in the globular part of the protein [20]. These amino acids are absent in the $\phi 1$ protamine from *M. edulis*, as shown in table 1, thus supporting the view that protamines are derived from the histone H1 basic tails.

The proposed pattern of evolution presented here is in line with our suggestion [1] that protamines have evolved from histones. The bivalve molluscs with their radial pattern of adaptation offer examples with different degrees of change and are thus a class of animals which represent a privileged object of study in order to unravel the evolutionary origin and biological function of sperm nuclear proteins. At the same time sperm proteins may help to clarify the complex phylogeny of bivalves [17].

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Table 2

Histone H1 evolution in bivalve spermatozoa

	Representative species
Somatic histone H1	
(↓)	
histone H1 (high Arg)	<i>Swiftopecten swifti</i> [2] <i>Crasostrea gigas</i> [4] <i>Chlamis islandicus</i> [21]
(↓)	
High molecular mass histone H1 (high Arg; high Ser)	<i>Spisula solidissima</i> [20] <i>Dreissena polymorpha</i> (this work)
(↓)	
Several sperm proteins with similar composition	<i>Donax trunculus</i> [3] <i>Ensis minor</i> [5]
(↓)	
Several sperm proteins with different compositions	<i>Mytilus edulis</i> [1] <i>Aulacomya ater</i> [7] <i>Crenomytilus grayanus</i> [21]

Note: In the first three stages core histones are present in the spermatozoa. In the last stage only an H2b-like histone is found [22]

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