Volume 282, number 2, 273–276 FEBS 09667 May 1991

ADONIS 001457939100375R

Microheterogeneity and interspecific variability of the nuclear sperm proteins from *Mytilus*

Catherine Mogensen, Steve Carlos and Juan Ausio

Department of Biochemistry and Microbiology, University of Victoria, Victoria, B.C., Canada

Received 30 January 1991

We have used acetic acid-urea-triton (AUT) gel electrophoresis and ionic exchange chromatography in order to analyze the interspecific variability and microheterogeneity pattern of the protamine-like (PL) proteins of the sperm of 4 different species of the bivalve mollusc, *Mytilus*. We have found that based upon these 2 criteria, it is possible to unambiguously distinguish each species from the rest. We have thus been able to corroborate the identity of *M. trossulus*. We have also analyzed the amino acid composition of some of the PL components for each different species. In the case of the PL-II* fraction, we have analyzed the composition of its major protein subcomponents.

Protein microheterogeneity; Nuclear protein; Sperm protein; *Mytilus* sp.

1. INTRODUCTION

The sperm-specific proteins of the bivalve molluscs exhibit an extensive structural variability while maintaining a very similar chemical composition [1]. Because of their composition (R + K > 45%, T + S = 10–25%), these proteins fulfil the protamine definition proposed by Subirana [2]. Therefore they are called protamine-like (PL) proteins. Nevertheless, some of the structural variability among different PL proteins seems to arise from the fact that some of them can also be related to proteins of the histone H1 family [3–5].

Different extents of microheterogeneity have been described both for protamines [6] and for histone H1 proteins [7]. Whereas at the functional level, histone H1 microheterogeneity seems to play a role in maintaining different discrete patterns of chromatin condensation [7], the presence of several subcomponents in protamines does not seem to have any apparent specific physiological advantage [6].

Here we have analyzed the microheterogeneity pattern of the protamine-like components of 4 different species of *Mytilus*. All PL proteins in these organisms exhibit different extents of microheterogeneity. Also the extent of microheterogeneity and amino acid composition for a given PL protein changes among the different species studied.

Correspondence address: J. Ausio, Department of Biochemistry and Microbiology, University of Victoria, P.O. Box 3055, Victoria, B.C., Canada V8W 3P6. Fax: (1) (604) 721885.

2. MATERIALS AND METHODS

2.1. *Living organisms*

Specimens of *Mytilus edulis* Linnaeus, 1758, and *Mytilus galloprovincialis* Lamarck, 1819, were obtained from commercial suppliers in Barcelona (Spain). *Mytilus californianus* Conrad, 1837, were collected at Yaquinna Lighthouse (Newport, OR) and *Mytilus trossulus*, Gould, 1850, were collected at Esquimalt Lagoon (Victoria, BC).

2.2. *Gel electrophoresis*

Acetic acid urea triton (AUT) polyacrylamide gels of different lengths were prepared as described elsewhere [5].

2.3. *Sperm collection and protein isolation*

The sperm was collected, and the sperm nuclei were purified as described elsewhere [1]. Crude HCl protein extracts were purified on CM-Septradex C-25 as described previously [1]. Alternatively, a 10 × 100 mm SP 8HR FPLC column from Waters-Millipore was also used. In this case, the sample was dissolved in 0.7 M NaCl, 50 mM Na-phosphate buffer pH 6.8 and eluted with linear NaCl gradients at a flow rate of 2 ml/min.

2.4. *Purification of the PL-II* subcomponents*

The PL-II* fractions obtained in *2.3* were loaded onto a 1 × 20 cm BioRex 70 column in order to isolate their protein subcomponents. The column and the resin were prepared as described in [8]. Elution was carried out with a 100 ml (Bio-Rad) guanidinium chloride (Schwarz-Mann) linear gradient (12–16%) at a 1 ml/h flow rate.

When necessary, previous to the amino acid analysis, the protein fractions thus obtained were further purified using HPLC on a C_8Vydac column 228TP 104 using an acetonitrile gradient in 0.1% TFA.

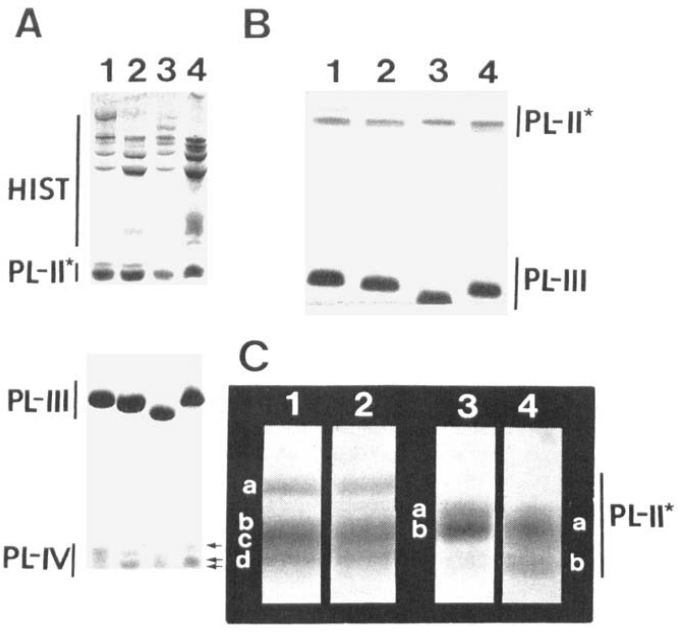

Fig. 1. Acetic acid-urea-triton (AUT) polyacrylamide gel electrophoresis of the sperm-specific nuclear proteins of different species of *Mytilus*: 1, *M. edulis*; 2, *M. galloprovincialis*; 3, *M. californianus*; and 4, *M. trossulus*. The length of the gels was increased from A to C in order to achieve higher levels of resolution.

2.5. *Amino acid analysis*

Amino acid analyses were carried out as described elsewhere [1].

3. RESULTS

Figure 1 shows the electrophoretic analysis of the nuclear sperm-specific proteins from 4 different species of *Mytilus*. As it can be seen there, the protamine-like components PL-II* and PL-IV exhibit different extents of microheterogeneity. At least 3 main different subcomponents can be clearly distinguished in PL-IV (Fig. 1A). Although their relative mobility changes from one species to another, the 3 fractions were observed in all the species analyzed and they seem to be present in the same stoichiometric ratio.

In the case of PL-II* the amount and relative mobility of its subcomponents seem to be more variable. At least 4 proteins components can be distinguished in *M. edulis* and *M. galloprovincialis* and 2 in *M. californianus* and *M. trossulus*.

PL-III exhibits a species dependent electrophoretic mobility in AUT gels and an apparent molecular homogeneity. Nevertheless, when FPLC is used in order to purify PL-III, this protein component also exhibits a certain extent of microheterogeneity (Fig. 2).

Based on the fact that PL-II* might be related to proteins of the histone H1 family [9,10], we have fractionated this protein using the method employed to isolate the different histone H1 components [8] (see Fig. 3). As can be seen in this figure, using this method and very shallow guanidinium chloride gradients, we have been able to isolate each of the major components observed for PL-II* in the AUT gels (Fig. 1C). The amino acid composition for each of these fractions is shown in Table I. As shown in this table, all the amino acid compositions are extremely alike and the differences between each fraction are quite small as it would be expected from microheterogeneity. Table II shows the amino acid compositions from the unfractionated PL-III components. In this case the differences in amino acid compositions are more significant. They clearly reflect the inter-specific differences observed in their electrophoretic mobility (see Fig. 1A, B). Thus, combining the electrophoretic and amino acid analysis of the different PL proteins it is clearly possible to distinguish between the different species of *Mytilus*.

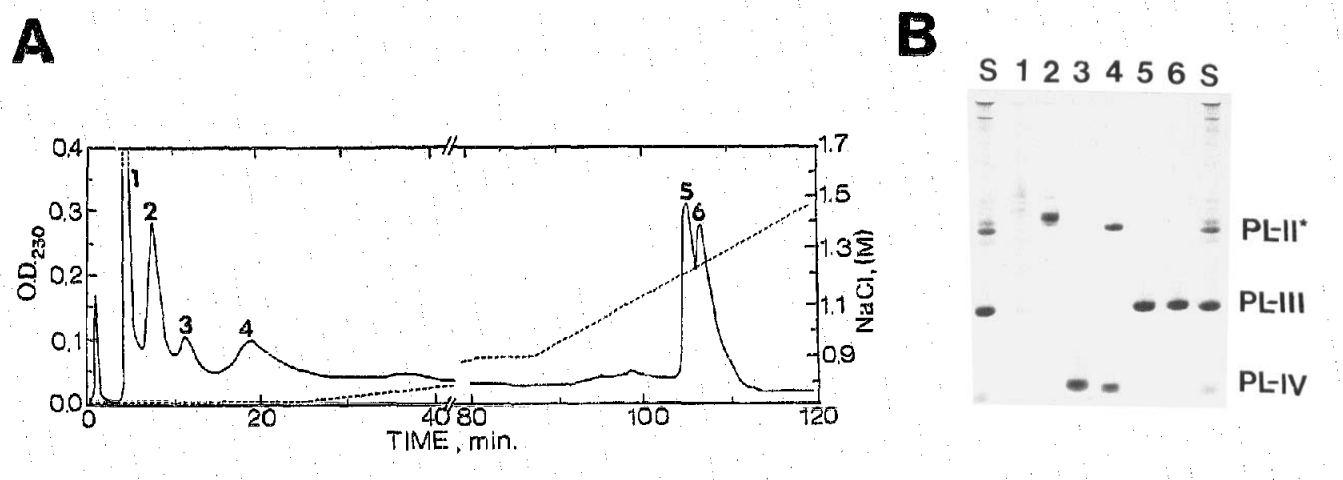

Fig. 2. (A) FPLC fractionation of the protamine-like proteins of *M. galloprovincialis* on an SP 8HR column. (B) Electrophoretic analysis of the chromatographic profile shown in (A). S = starting protein sample.

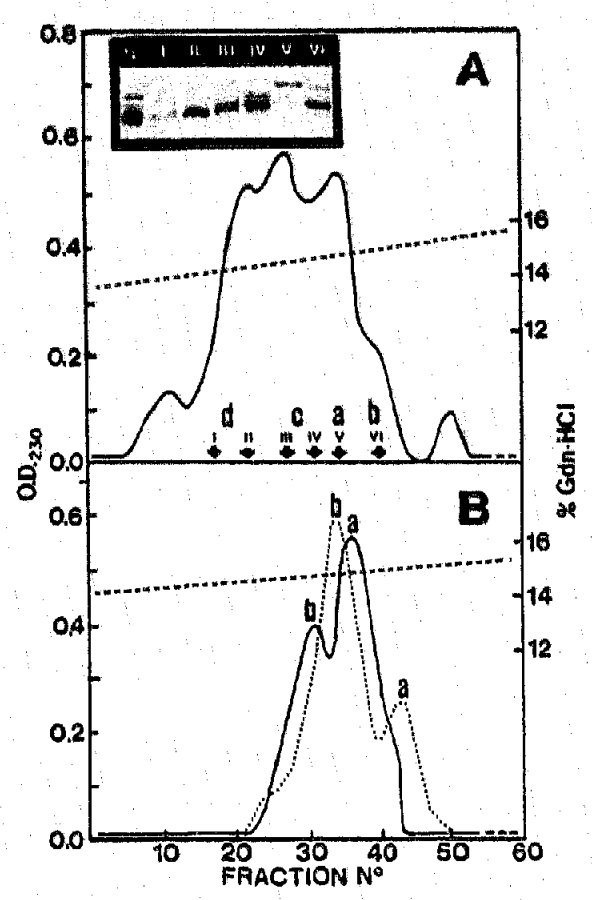

Fig. 3. Ionic exchange chromatography on Bio Rex of the PL-II* protein fraction from *Mytilus*. (A) *M. edulis*. (B) (——) = *M. trossulus*; (·····) = *M. californianus*. Elution was carried out with linear guanidinium chloride gradients in 50 mM sodium phosphate buffer pH 6.8. The inset in (A) shows the electrophoretic analysis of some of the fractions collected. Letters 'a', 'b', 'c' and 'd' have the same meaning as in Fig. 1. S = starting protein sample.

4. DISCUSSION

We have shown that all PL proteins from *Mytilus* exhibit different interspecific microheterogeneity. Although the reason for this variability amongst the different species is unknown at present, it most likely reflects the different evolutionary constraints undergone by each species. This idea is supported by the recent studies carried out by Milyutina and Petrov [11] using DNA-DNA hybridization to study the extent of DNA sequence divergence in different species of *Mytilus*. In their analysis, they were able to show that *M. edulis* and *M. galloprovincialis* diverged very recently compared to other species of *Mytilus*. In our analysis, PL-II* from *M. edulis* and *M. galloprovincialis* exhibits the same qualitative and quantitative microheterogeneity both in gel electrophoresis (see Fig. 1C) and by chromatography (results not shown), whereas all the other PL seem to be variable.

Our results, like those from [11], clearly show the phenotypical differences observed between different 'forms' within the genus *Mytilus* have their counterpart at the molecular level. Whether or not a major taxonomic separation exists between each of these forms, seems however, to be more complicated. Thus, whereas our data show that there are some clear differences in PL-III and PL-IV between *M. edulis* and *M. galloprovincialis*, they do not allow us to settle the controversy [12] initiated by Lubet [13] on whether these 2 forms should be considered as different species or merely as different ecotypes. They provide, however, support to the identification of *M. trossulus* from the Pacific Northwest shores of North America as being different from the Atlantic form of *M. edulis* [14].

Molecular and biochemical studies such as those

Table 1

Amino acid composition (mol %) of the PL-II* subcomponents of some species of *Mytilus*

	M. edulis				*M. californianus*		*M. trossulus*	
	a	b	c	d	a	b	a	b
Lys	16.5	18.2	17.0	18.7	21.6	16.4	17.8	18.0
His	0.6	0.7	0.3	0.2	0.3	0.6	0.5	0.6
Arg	10.1	10.6	10.9	10.4	9.3	10.2	9.7	9.4
Asx	5.9	5.9	5.5	5.2	5.9	6.0	5.4	5.5
Thr	5.4	4.4	4.1	3.7	3.7	4.2	4.0	4.0
Ser	15.4	15.6	14.3	13.8	14.4	17.0	14.8	15.0
Glx	1.9	1.7	1.7	1.8	2.0	1.8	1.1	1.1
Pro	6.4	6.8	7.5	7.5	6.9	7.1	7.7	7.4
Gly	8.5	8.6	8.4	9.1	10.6	8.7	8.5	8.7
Ala	15.4	14.4	15.4	14.3	12.7	13.9	15.0	14.7
1/2 Cys	—	—	—	—	—	—	—	
Val	3.5	3.8	4.3	4.4	3.1	3.5	4.1	4.1
Met	1.7	1.8	1.6	1.3	1.7	1.7	2.0	1.9
Ile	3.2	3.1	2.8	2.8	2.8	3.5	3.2	3.5
Leu	4.1	3.9	4.4	4.6	4.2	4.6	4.3	4.3
Try	0.7	0.7	0.8	0.6	0.8	0.7	0.7	0.8
Phe	1.4	1.4	1.2	1.3	1.3	1.6	1.3	1.3

Table II

Amino acid composition (mol %) of the PL-III proteins from some species of *Mytilus*

	M. edulis	*M. galloprovincialis*	*M. Californianus*	*M. trossulus*
Lys	19.5	20.2	20.5	24.0
His	—	—	—	—
Arg	30.3	26.8	32.1	27.5
Asx	—	—	—	—
Thr	3.6	4.1	2.5	3.7
Ser	18.9	14.7	15.4	17.7
Glx	—	—	—	—
Pro	5.4	9.0	5.1	5.1
Gly	6.7	7.3	7.5	6.9
Ala	14.9	16.5	15.9	14.1
1/2 Cys	—	—	—	—
Val	1.0	1.4	1.1	0.9
Met	—	—	—	—
Ile	—	—	—	—
Leu	—	—	—	—
Tyr	—	—	—	—
Phe	—	—	—	—

presented here are very important for, in conjunction with other genetic hybridization studies, they may be able to provide information on the minimum requisites involved, at the molecular level, in the presence of speciation.

Acknowledgements: We would like to dedicate this work to Prof. Mercè Durfort of the Biochemistry and Physiology Department (Cell Biology Unit) of the Faculty of Biological Sciences at the University of Barcelona for her encouragement and suggestions in pursuing this research. This work was supported by NSERC Grant OGP 004-6399 to Juan Ausio.

REFERENCES

[1] Ausio, J. (1986) Comp. Biochem. Physiol. 85B, 439–449.
[2] Subirana, J.A. (1983) in: The Sperm Cell (André, J. ed.) Martinus Nijhoff, The Hague, pp. 197–213.
[3] Giancotti, V., Russo, E., Gasparini, M., Serrano, D., Del Piero, D., Thorne, A.W., Cary, P.D. and Crane-Robinson, C. (1983) Eur. J. Biochem. 136, 509–516.
[4] Ausio, J., Toumadje, A., McParland, R., Becker, R.R., Johnson Jr., W.C. and van Holde, K.E. (1987) Biochemistry 26, 975–982.
[5] Ausio, J. (1988) J. Biol. Chem. 263, 10141–10150.
[6] Ando, T., Yamasaki, M. and Suzuki, K. (1973) Protamines, Springer, Berlin, 114 pp.
[7] Cole, R.D. (1987) Int. J. Peptide Protein Res. 30, 433–449.
[8] Cole, R.D. (1989) Methods Enzymol. 170, 524–532.
[9] Uschewa, A., Patriotis, C. and Avramova, Z. (1985) Cell Biol. Int. Rep. 9, 253–263.
[10] Jutglar, L., Borrell, I. and Ausio, J. (1991) J. Biol. Chem. (submitted).
[11] Milyutina, I.A. and Petrov, N.B. (1990) Mol. Biol. (USSR) 23, 1091–1098.
[12] Dixon, D.R. and Flavell, N. (1986) J. Mar. Biol. Ass. (UK) 66, 219–228.
[13] Lubet, P. (1959) Revue des travaux de l'Office (scientifique et technique) des peches maritimes 23, 384–548.
[14] McDonald, J.H. and Koehn, R.K. (1988) Marine Biol. 99, 111–118.