Intraspecific myosin light chain polymorphism in the white muscle of herring (*Clupea harengus harengus*, L.)

I. Martinez, R. Ofstad and R.L. Olsen

Institute of Fisheries Technology Research, PO Box 677, 9001-Tromsø, Norway

Received 4 April 1990

The myosin contained in white and red muscles of herring (*Clupea harengus harengus*) was purified, and its subunit composition analyzed by electrophoretic techniques. The only myosin isoform present in red muscles was made up of one type of heavy chain and two types of light chain. The native myosin from white muscles migrated as one wide band. Analysis of the extracts by SDS/glycerol/PAGE from white muscles revealed one main type of heavy chain. Light chains were identified by SDS-PAGE analysis of electrophoretically purified myosin, and two-dimensional electrophoresis of the extracts demonstrated differences in the light chain composition of white and red muscles. Using this methodology, light chain polymorphism was detected in white muscles among members of the same species.

Myosin; Gel electrophoresis; Subunit composition; *Clupea harengus harengus*

1. INTRODUCTION

Previous studies have shown that the myosin isoforms from red and white muscles of fishes resemble that of mammalian, avian, or amphibian species [1-5] in their subunit composition. They contain at least one type of heavy chain, different in red and white muscles [6-8], two types of light chain in red muscles, and three types of light chain in white muscles [6-10]. Homo- and heterodimers of these light chains give rise to three of the myosin isoforms found in the white muscles.

The existence of intraspecific polymorphism in myosin light chains of avian and mammalian slow muscles has already been shown by Carraro et al. [11]. In white muscles from trout (*Salmo trutta fario*), such polymorphism has been suggested [12], although one study on a closely related species (*Salmo gairdneri*) attributed it to contamination by the light chains from red muscle [10]. Our results support the presence of this type of polymorphism in white muscles of fish.

2. MATERIALS AND METHODS

2.1. *Animal samples and myosin extraction*

Red and white muscles of herring ($n = 10$, *Clupea harengus harengus* L., family *Clupeidae*) were used for this work. The samples were taken from 5 fish in pre-rigor. The other five arrived in rigor to our laboratory and the samples were taken immediately after the resolution of rigor mortis. Myosin extracted from the soleus muscle and diaphragm from a 300 g Wistar rat were used as standards for comparison.

Correspondence address: I. Martinez, Institute of Fisheries Technology Research, PO Box 677, 9001-Tromsø, Norway

Myosin extraction was carried out on ice according to [13] as described in [7]. The protein content in the extracts was determined by the Bradford method [14].

2.2. *Gel electrophoresis under non-dissociating conditions*

Native gel electrophoresis under non-dissociating conditions was performed essentially according to [2,15]. About 7 μg of protein were loaded on each tube. The area occupied by the myosin from white muscles was divided into three parts to study the light chain content.

2.3. *Analysis of the myosin light chains*

Myosin light chains were analyzed by two-dimensional electrophoresis [16], as described in [7]. The second dimension was performed in 0.75-mm-thick slab gels containing 15% acrylamide and 0.087% bisacrylamide [17]. The gels were silver stained [18].

Half of a slice from each of the native myosin isoforms isolated from white muscles by native electrophoresis was loaded in the wells of 0.75-mm-thick, 7 × 8 cm slab gels described above, run at 175 V for 1 h, and silver stained.

2.4. *Analysis of the myosin heavy chains and peptide mapping*

Analysis of the heavy chains was performed by SDS-PAGE as described [7] in gels, 7 × 8 cm, 0.5-mm-thick containing 36% glycerol. Electrophoresis took place for 20 min at 100 V followed by 3½ h at 120 V. The gels were silver stained. To perform peptide mapping, the heavy chains were isolated in 6% polyacrylamide gels containing 36% glycerol according to [19] and run under the same conditions as above.

Peptide mapping of the myosin heavy chain was performed according to [20,21] by adding 2 μl of a buffer containing 40 ng of V8 protease from *Staphylococcus aureus* on top of the gel slice containing the purified heavy chain from 1 μg of protein extract. The stacking gel contained 3% acrylamide, 0.08% bisacrylamide and the separating gel 15% acrylamide and 0.087% bisacrylamide [17].

3. RESULTS AND DISCUSSION

The only isoform found in red muscles (Fig. 1A-b) was made up of one type of heavy chain (Fig. 2A) and

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50

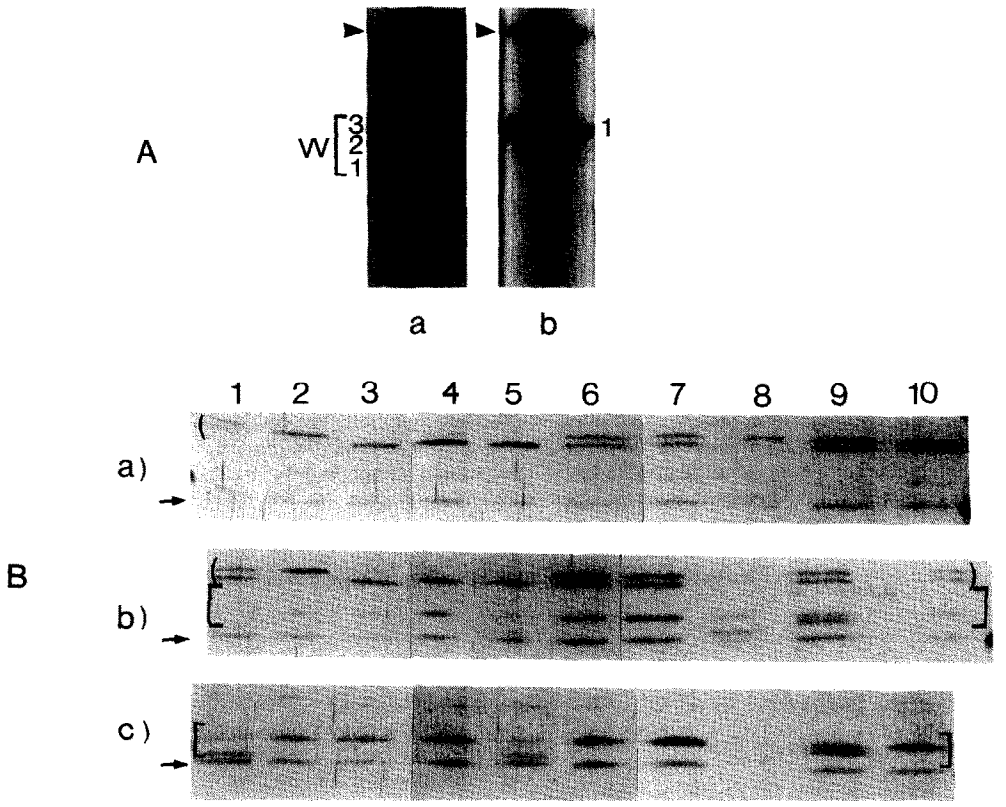

Fig. 1.(A) Electrophoresis under non-dissociating conditions of the myosins from (a) white, and (b) red muscles of herring. W1, W2, and W3 are the areas in which the band was subdivided to analyze their light chain content. Arrowheads, myosin from rat soleus. (B) SDS-PAGE of the slices containing the native myosin isoforms (W1, W2, and W3) in a 15% polyacrylamide gel. Only the area of the light chains is shown. Nos 1 to 10 refer to the individual herrings. Light chains contained in (a) W3, (b) W2, and (c) W1. Round brackets, LC1f; arrows, LC2f; square brackets, LC3f.

two types of light chain (Fig. 3a). Although the heavy chain from red and the main heavy chain from white muscles comigrated (Fig. 2A), peptide mapping (Fig. 2B) showed, as expected [10], that they differ in their amino acid sequence.

The myosin isoforms from white muscles were not resolved in the native system, probably due to the existence of a mixture of myosins differing very slightly in their subunit composition. The area occupied by the myosin was divided into three parts named W1 to W3 (Fig. 1A-a). W1 comprised the main band. The light chain pattern of each of these bands in each herring (nos. 1 to 10) is shown in Fig. 1B. The slow migrating area (W3) seems to be occupied by LC1f homodimers,

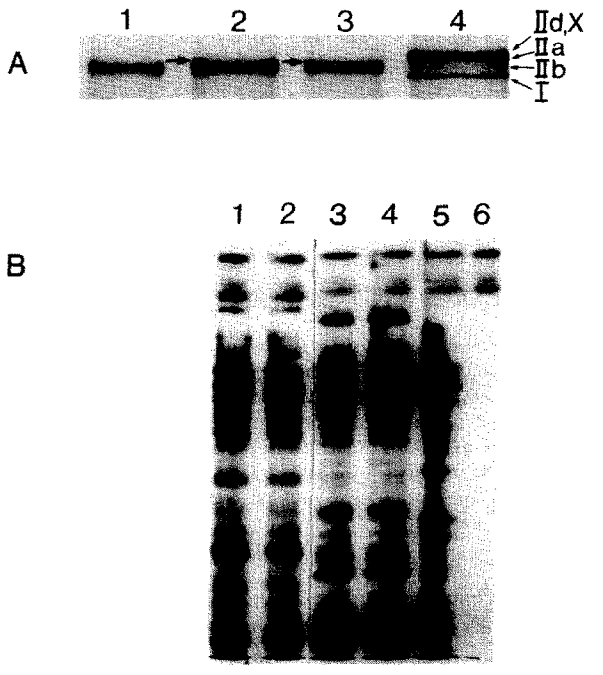

Fig. 2. (A) Heavy chains of myosin from (1) red, (2) comigration of red and white, and (3) white muscles of herring. Arrows, heavy chain present in minor amount. Lane 4 rat diaphragm: IId,X, IIa, IIb, I, types of myosin heavy chain from rat diaphragm. (B) Peptide maps of the myosin heavy chains from red (lanes 1 and 2) and white (lanes 3 and 4) muscles of herring. Lane 5, peptide map of the heavy chains type IId,X and IIa from rat diaphragm myosin. Lane 6, V8 protease of *Staphylococcus aureus*.

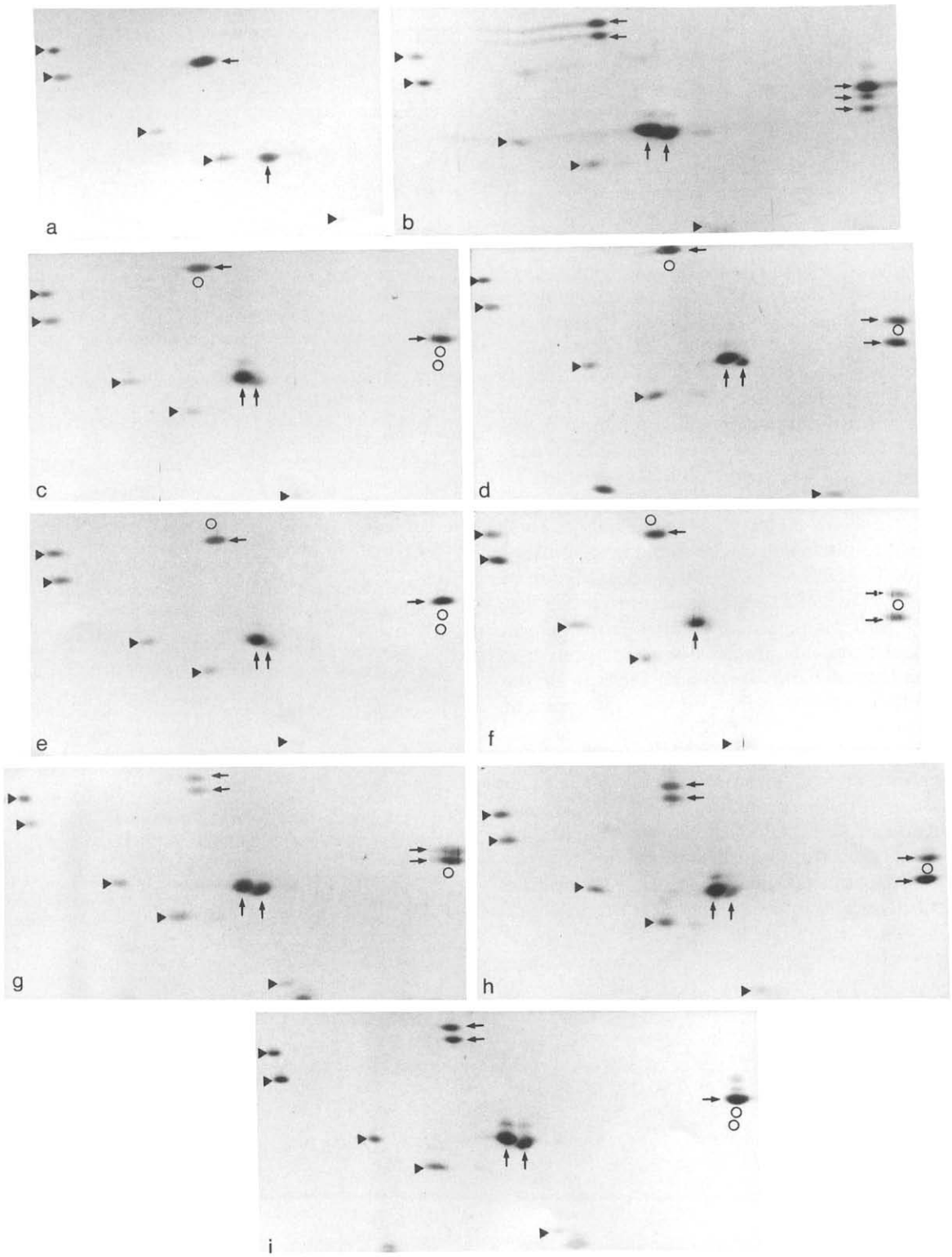

Fig. 3. Two-dimensional electrophoresis of the myosin light chains from (a) red and (b) white muscles of herring. (b) is a comigration of 5 individuals. (c–i) myosin light chains from white muscles of seven individual herrings showing all the combinations found; (○)light chain forms absent in the particular fish. ←, LC1; ↑, LC2; →, LC3. First dimension (isoelectric focusing) from left (basic) to right (acidic), second dimension (15% SDS-PAGE) from top to bottom. The light chains of myosin from rat diaphragm (▶) are, from top to bottom, LC1s, LC1f, LC2s, LC2f, LC3f.

the intermediate (W2) by LC1f-LC3f heterodimers, and the fastest migrating area (W1) by LC3f homodimers.

Two-dimensional analysis of white muscle extracts (Fig. 3b–i) gave 7 spots identified as two types of LC1f, two of LC2f, and three of LC3f. The LC1f forms, as well as the LC3f forms, differed mainly in their M_r values, and very slightly in the p*I* values. The LC2f gave two spots in two dimensional gels differing slightly in both p*I* and M_r values. These two spots may be the LC2f and its phosphorylated form, which, for some unknown reason, may migrate to a slightly different position in the second dimension, or a different type of LC2f, unnoticed in the analysis of the light chain content of individual isoforms due to the very small difference. Fig. 3b shows the pattern after comigration of 5 herrings (individuals 6 to 10) containing all types of light chains. As shown in Fig. 1B and Fig. 3c–i, the individual herrings displayed all possible combinations of LC1f and LC3f forms, except that none of the animals had all three types of LC3f, and the LC3f of highest M_r was present in all of them.

None of the light chains from white muscle comigrated with any of the light chains found in red muscles (not shown). They did not originate by degradation of the peptide with higher M_r value, since the pattern was individual-specific, independent of whether the myosin was extracted from the muscles of pre- or post-rigor animals, and we did not observe an increase of the faster migrating forms concomitant with a decrease of the forms with higher M_r.

In summary, the results presented here agree with the general pattern of myosin isoforms found in red and white muscles of other fish species [6–10], and support the existence of light chain polymorphism in fish muscles, as suggested by Perzanowska et al. [12]. According to Johnston [22], this type of polymorphism would reflect variations in the contractile properties of the isomyosins.

Acknowledgements: We are grateful to Hans Dahlberg and Bjørn Kåre Iversen for taking the photographs. This work was supported by an Utdanningstipend from the Norwegian Fisheries Research Council to I.M.

REFERENCES

[1] D'Albis, A., Janmot, C. and Béchét, J.-J. (1986) Eur. J. Biochem. 156, 291–296.
[2] Hoh, J.F.Y., McGrath, P.A. and White, R.I. (1986) Biochem. J. 157, 87–95.
[3] Kordylewski, L., Faber, J., Görlich, A. and Kilarski, W. (1989) J. Muscle Res. Cell Motility 10, 290–296.
[4] Pliszka, B., Strazelecka-Golaszewska, H., Pantaloni, C. and D'Albis, A. (1981) Eur. J. Cell Biol. 25, 144–149.
[5] Swynghedauw, B. (1986) Physiol. Rev. 66, 710–771.
[6] Karasinski, J. and Kilarski, W. (1989) Comp. Biochem. Physiol. 92B, 727–731.
[7] Martinez, I., Olsen, R.L., Ofstad, R., Janmot, C. and D'Albis, A. (1989) FEBS Lett. 252, 17–28.
[8] Scapolo, P.A. and Rowlerson, A. (1987) Experientia 43, 384–386.
[9] Martinez, I., Ofstad, R. and Olsen, R.L. (1990) Comp. Biochem. Physiol. PtB, in press.
[10] Rowlerson, A., Scapolo, P.A., Mascarello, F., Carpéné, E. and Veggetti, A. (1985) J. Muscle Res. Cell Motility 6, 601–640.
[11] Carraro, U., Dalla-Libera, L. and Catani, C. (1981) J. Muscle Res. Cell Motility 2, 335–342.
[12] Perzanoska, A., Gerday, C. and Focant, B. (1978) Comp. Biochem. Physiol. 60B, 295–231.
[13] D'Albis, A., Janmot, C. and Béchét, J.-J. (1985) Biochem. Biophys. Res. Commun. 128, 94–100.
[14] Bradford, M.M. (1976) Anal. Biochem. 72, 248–254.
[15] D'Albis, A., Pantaloni, C. and Béchét, J.-J. (1979) Eur. J. Biochem. 99, 261–272.
[16] O'Farrell, P.H. (1975) J. Biol. Chem. 250, 4007–4021.
[17] Anderson, C.W., Baum, P.R. and Gesteland, R.F. (1973) J. Virol. 12, 241–252.
[18] Ansorge, W. (1983) in: Electrophoresis '82 (Stathakos, ed.) pp. 235–242, De Gruyter, Berlin.
[19] Laemmli, U.K. (1970) Nature 227, 680–685.
[20] Carraro, U. and Catani, C. (1983) Biochem. Biophys. Res. Commun. 116, 792–802.
[21] Cleveland, D.W., Fischer, S.G., Kirschener, M.W. and Laemmli, U.K. (1977) J. Biol. Chem. 252, 1102–1106.
[22] Johnston, I.A. (1982) Mol. Physiol. 2, 15–29.