LIGHT CHAINS OF CARP AND PIKE SKELETAL MUSCLE MYOSINS. ISOLATION AND CHARACTERIZATION OF THE MOST ANODIC LIGHT CHAIN ON ALKALINE pH ELECTROPHORESIS

B. FOCANT* and F. HURIAUX

Laboratory of General Biology, Institut Van Beneden, University of Liège, quai Van Beneden, 22, B-4020 Liege, Belgium

Received 30 January 1976

1. Introduction

It is now well accepted that mammalian white skeletal muscle myosin is made of two large subunits of 200 000 daltons (heavy chains) and four smaller ones (light chains). These light chains are divided in two classes: two chemically related 'alkali' light chains of a molecular weight of 25 000 (Alk_1) and 16 000 (Alk_2) daltons determined on SDS gels, and two apparently identical DTNB light chains of 18 000 daltons [1]. These last ones can be removed without loss of myosin ATPase activity and phosphorylated [1,2].

Myosin from fish white skeletal muscle has a macromolecular structure similar to those of warm-blooded vertebrates but is less stable [3,4]. A few papers have also made clear that, as with mammals, the myosin of fish red muscle differs from that of white muscle, chiefly in ATPase activity [3,5]. In preliminary notes, we have shown that the light chains differ in the white (fast), red (slow) and cardiac muscles of carp; they correspond thus to three different types of myosin [6,7]. But these light subunits have not been further investigated so far.

In this work, we describe the light chain patterns of the white skeletal muscle myosin from two cold fresh water fishes: carp (*Cyprinus carpio,* L.) and pike (*Esox lucius,* L.), as well as the first isolation of a light chain of each fish species, the fast moving component in alkaline urea gel, and their characterization by mol. wt. determination, ultraviolet absorption and amino acid analysis.

2. Materials and methods

2.1. *Isolation of the light chains*

Skeletal white muscle myosins were prepared from fresh carp and frozen pike muscles by neutral ammonium sulphate fractionation of actomyosins in the presence of 0.4% ATP and 0.02 M $MgSO_4$ between 35 and 60% of saturation for carp and 40–60% for pike. After dissociation by 8 M urea at neutral pH, the heavy chains were precipitated by dilution and the supernatants containing the light chains were concentrated by absorption on a DEAE-cellulose column at pH 8.0. The fast moving light chain from both fishes was isolated by chromatography on a 65 × 2.2 cm column of ion exchange DEAE-cellulose (Whatman DE-52, microgranular, pre-swollen) equilibrated at 4°C with 0.05 M potassium phosphate, pH 5.9 and 2 mM betamercaptoethanol according to Weeds and Lowey [1]. Elution was carried out with a linear phosphate gradient. The myosin DTNB treatment was made as described by Gazith et al. [8]. The reaction mixture contained 10 mg/ml myosin, 0.5 M KCl, 10 mM EDTA and 10 mM DTNB at pH 8.5. After 10 min at 0°C, the reaction was stopped by addition of 10 vol of cold water which precipitate the myosin. The DTNB was eliminated by extensive dialysis against beta-mercaptoethanol.

*Chercheur qualifié of the Fonds National de la Recherche Scientifique.

Abbreviations: DTNB, 5-5'-dithiobis (2-nitrobenzoic acid); SDS, sodium dodecyl sulphate.

North-Holland Publishing Company – Amsterdam

2.2. *Physico-chemical characterization*

Vertical polyacrylamide slab gel electrophoreses were carried out at alkaline pH in 8 M urea [9] and in the presence of SDS [10], as described in legends. Protein concentrations were determined by the biuret procedure [11]. An Hitachi-Perkin Elmer model 124 spectrophotometer was used for the ultraviolet absorption determinations. Amino acid compositions were obtained in duplicate on a Beckman 120 B amino acid analyser by the method of Benson and Patterson [12]. The performic acid oxidized proteins [13] were hydrolysed at 110°C during 24, 48 and 72 h in constant boiling 6 N HCl. Analyses were carried out on two different light chain preparations from each species.

3. Results and discussion

Ammonium sulphate fractionation of dissociated actomyosin enables to obtain a myosin almost devoid of actin, tropomyosin and associated proteins (with a yield of 1.6 g of myosin/100 g of fresh muscle for carp and 0.8 g/100 g of frozen muscle for pike).

Fig.1a shows typical electrophoretic diagrams of fish myosin in the presence of dissociating 8 M urea on a polyacrylamide gel which allows only tropomyosin and light chains to enter the gel. Carp and pike myosins possess a fast moving component (LC_3), a slow moving one (LC_1, slower than tropomyosin in carp myosin) and a double-banded component of intermediate mobility (LC_2). Their mol. wts. have been determined by SDS-gel electrophoresis (fig.1b): the figures obtained are of 25 000 (LC_1), 20 500 (LC_3) and 17 500 (LC_2) in the case of the carp myosin and 27 700 (LC_1), 17 900 (LC_2) and 16 300 (LC_3) daltons in that of the pike myosin.

After treatment of both myosins with DTNB, the supernatant containing the DTNB-removed light chain and the precipitated myosin were analysed on urea and SDS-polyacrylamide gels. The LC_2 (mol. wt. of about 18 000) was removed by DTNB to an extent of about 75% from carp and 65% from pike myosin.

On the other hand, carp myosin or its light chain fraction were incubated in the presence of [γ-^{32}P]ATP and rabbit myosin phosphorylating enzyme, ATP: myosin light chain phosphotransferase or LCK, according to Pires et al. [14]. Urea gel of the incubated

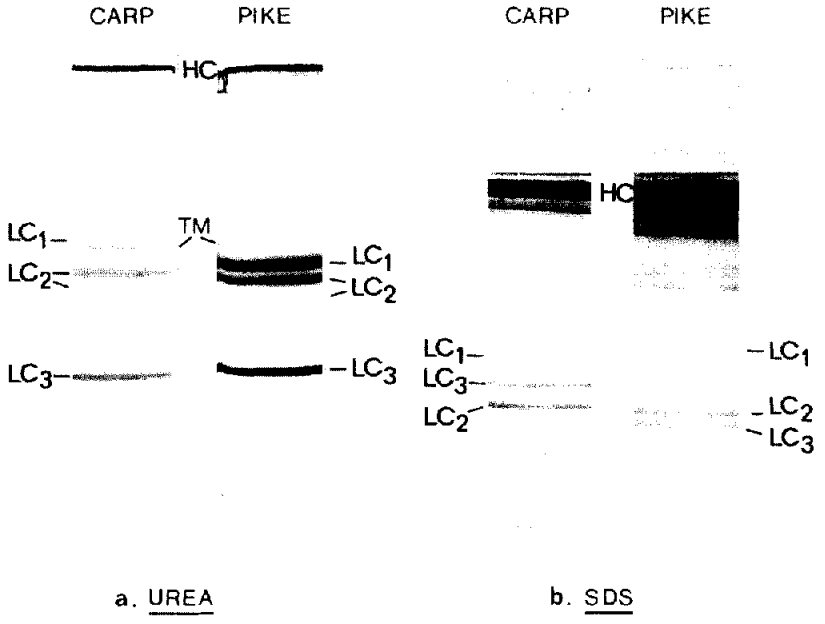

Fig.1. Polyacrylamide gel electrophoresis of myosin extracted from carp and pike white muscle. (a) 10% polyacrylamide gels, 8 M urea, 0.02 M Tris–0.12 M Glyc. (pH 8.6). (b) Discontinuous polyacrylamide gels in the presence of 0.1% SDS. Main gel 15%, polyacrylamide 0.4244 M Tris–0.0308 M HCl (pH 9.18). HC: heavy chains; LC: light chain; TM: tropomyosin.

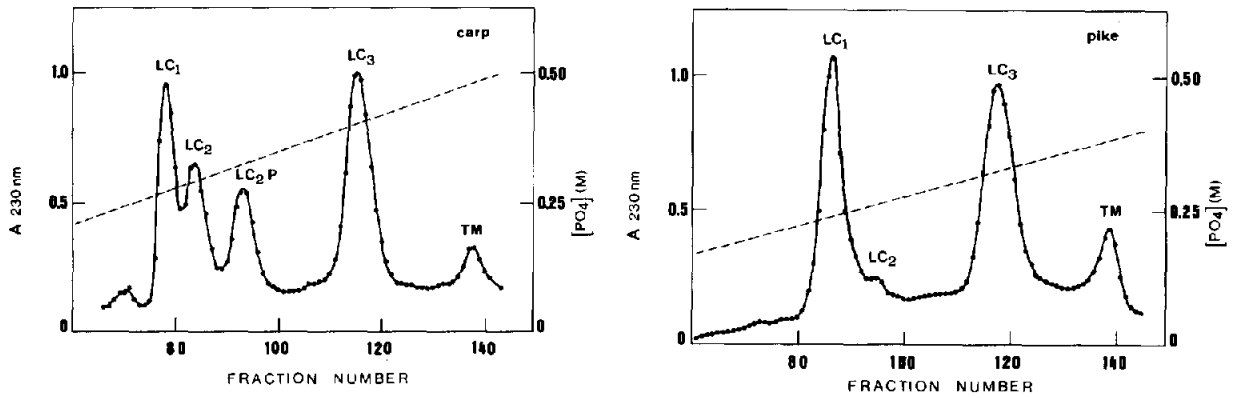

Fig.2. Elution profiles of the fractionation of light chains of carp and pike white muscle myosin on DEAE-cellulose Whatman DE-52. The proteins (~ 50 mg of light chains) were eluted with a linear gradient to 0.5 M potassium phosphate pH 5.9. 8 ml fractions were collected at a flow rate of 40 ml/h.

preparations revealed that all the slow moving component of LC_2 was converted into the fast moving one, which is thus the phosphorylated form of LC_2.

The fast component, LC_3, from carp and pike was purified by an ion exchange chromatography on cellulose DE-52. Light chains are eluted with a good yield in a single run (fig.2).

The mol. wts. of the two LC_3 are obviously different. Both LC_3s display similar ultraviolet spectra. Peaks at 253, 259, 265 and 268 nm at neutral pH reflect a high content in phenylalanine. Maximum at 276 nm which is shifted to 295 nm at alkaline pH corresponds to tyrosine. Moreover these spectra suggest a lack of tryptophan. Extinction coefficients $E^{1\%}_{1\,cm}$ at 280 nm

Table 1
Amino acid composition of myosin light chains

	Residues per molecule of protein			
Amino acid	Carp LC_3	Pike LC_3	Rabbit Alk_1[a]	Rabbit Alk_2[a]
Lys	14.5	10.3	21.0	11.6
His	2.3	1.0	2.0	1.9
Arg	6.0	4.9	4.2	4.3
Cys	0.3	0.3	1.0	0.9
Asx	28.7	23.2	19.8	18.3
Thr	7.9	5.8	8.0	7.1
Ser	7.6	5.0	8.8	8.2
Glx	25.6	19.6	29.2	24.4
Pro	5.4	4.8	12.1	3.7
Gly	15.5	14.0	12.0	12.0
Ala	13.2	10.1	22.8	12.5
Val	15.1	10.8	10.6	10.2
Met	8.2	5.6	6.0	5.6
Ile	9.1	6.9	9.0	7.0
Leu	13.4	16.2	13.8	12.8
Tyr	3.3	2.4	3.0	3.0
Phe	9.4	7.5	8.1	8.5
Mol. wt.	20 500	16 300	21 000	17 000

[a]The values for rabbit myosin light chains are taken from [1].

at neutral pH amount to about 3.7 for carp and 3.1 for pike. The amino acid compositions of the carp and pike LC_3 given in table 1 are very similar. They confirm the relatively high phenylalanine to tyrosine ratio shown by the ultraviolet spectra. Comparison with amino acid compositions of Alk_1 and Alk_2 rabbit light chains homologates the fast moving light chain of fish to the rabbit Alk_2. Indeed this fish light chain does not have the characteristic high lysine, proline and alanine content of the additional peptide of the rabbit Alk_1 [15].

By their acidic nature, their relatively high content in phenylalanine and the apparent lack of tryptophan the fish light chains may be compared to parvalbumins, which occur in large amounts in fish white muscles. Further similarities in amino acid sequences have been found between rabbit alkali light chains, rabbit troponin C and carp parvalbumins [16,17]. Our characterization of fish light chains as well as that of carp troponin C by Drabikowsky [18] allow now to extent the comparison to the primary structure of these proteins originating from a single species, the carp.

In conclusion myosins from fish white muscle have a light chain pattern like that of higher vertebrates. As in mammals, the 18 000 daltons light chain can be removed by DTNB and phosphorylated. It is interesting to note that this DTNB light chain is found with the same mol. wt. in myosin from all striated muscles [9]. The two other fish light chains, similar to rabbit 'alkali' light chains, are subject to larger specific variations in mol. wt. and charge than their mammalian counterparts and are thus a good criterium for the characterization of fish species [19].

Acknowledgements

We are greatly indebted to Professor G. Hamoir for encouragement and support and to Dr Ch. Gerday for assistance with amino acid analysis.

We wish to thank Professor S.V. Perry of the Department of Biochemistry of the University of Birmingham (UK) for his hospitality and facilities given to one of us (B.F.) for the experiments on phosphorylation.

References

[1] Weeds, A. G. and Lowey, S. (1971) J. Mol. Biol. 61, 701–725.
[2] Perrie, W. T., Smillie, L. B. and Perry, S. V. (1973) Biochem. J. 135, 151–164.
[3] Hamoir, G., McKenzie, H. A. and Smith, M. B. (1960) Biochim. Biophys. Acta 40, 141–149.
[4] Connell, J. J. (1961) Biochem. J. 80, 503–509.
[5] Syrovy, I., Gaspar-Godfroid, A. and Hamoir, G. (1970) Arch. Internat. Physiol. Bioch. 75, 919–934.
[6] Huriaux, F. and Focant, B. (1974) Arch. Internat. Physiol. Bioch. 82, 991–992.
[7] Focant, B., Huriaux, F. and Hogge, J.-M. (1974) Arch. Internat. Physiol. Bioch. 82, 985–987.
[8] Gazith, J., Himmelfarb, S. and Harrington, W.F. (1970) J. Biol. Chem. 245, 15–22.
[9] Perrie, W. T. and Perry, S. V. (1970) Biochem. J. 119, 31–38.
[10] Neville, D. M. Jr. (1971) J. Biol. Chem. 246, 6328–6334.
[11] Gornall, A. G., Bardawill, C. S. and David, M. M. (1949) J. Biol. Chem. 177, 751–766.
[12] Benson, J. V. and Patterson, J. A. (1965) Analyt. Chem. 37, 1108–1110.
[13] Hirs, C. H. W. (1956) J. Biol. Chem. 219, 611–621.
[14] Pires, E., Perry, S. V. and Thomas, M. A. W. (1974) FEBS Lett. 41, 292–296.
[15] Frank, G. and Weeds, A. G. (1974) Eur. J. Biochem. 44, 317–334.
[16] Weeds, A. G. and McLachlan, A. D. (1974) Nature 252, 646–649.
[17] Collins, J. H. (1974) Biochem. Biophys. Res. Commun. 58, 301–308.
[18] Drabikowski, W., Barylko, B. and Dabrowska, R. (1975) in: Calcium binding proteins (Drabikowsky, W., Strzelecka-Golaszewska, H. and Carafoli, E., eds.), pp. 69–107, Elsevier, Amsterdam.
[19] Focant, B., Huriaux, F. and Johnston, I. A. (1975) unpublished.