TWO FORMS OF THE P LIGHT CHAIN OF MYOSIN IN RABBIT AND BOVINE HEARTS

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Received 13 April 1982

1. Introduction

Heavy and light chains of myosin exist in a number of forms of different primary structure, various combinations of which are responsible for the large number of isoenzymic forms of the molecule present in muscle [1–11]. The precise combination of these forms of the components of the myosin molecule is characteristic of the muscle type and its stage of development. Changes in the isoenzymic composition of myosin can be observed, for example, after exposure to hormones such as thyroxine [12,13], after changes in innervation [14,15] and after chronic overloading in the heart [16].

Several forms of the alkali light chains are often present in a given skeletal muscle cell type [17,18]. We have demonstrated that the P light chain exists in two forms, P1 and P2, in ventricular muscle from several species [19]. This observation is supported by the recent report of two P light chains of different amino acid sequence in chicken ventricular myosin [20].

We now present evidence that the two forms of P light chain of myosin in bovine ventricle also have a different sequence and that the relative amounts of the two P light chains change during development in the rabbit heart.

2. Materials and methods

2.1. *Analysis of rabbit hearts*

Hearts from animals killed by asphyxia in CO_2 were used immediately or stored at −20°C. They were prepared for electrophoresis in two dimensions on polyacrylamide gels as in [19].

2.2. *Preparation of myosin and myosin light chains*

Hearts from cattle killed at a local slaughterhouse were immediately chilled in ice and myosin prepared the same day from ventricular muscle as in [19]. The freshly prepared myosin was dissolved in 9 M urea/37.5 mM Tris–HCl (pH 7.0)/7.5 mM 2-mercaptoethanol/0.25 M KCl and the whole light chain fraction isolated by collecting the fraction precipitated between 63–80% saturation with ethanol [21]. This was dissolved in water and dialyzed against 2 changes of 20 mM $(NH_4)HCO_3$/15 mM 2-mercaptoethanol for 2 h at 4°C. After centrifugation for 15 min at 20 000 × *g* the supernatant containing the light chain fraction was freeze-dried and stored at −20°C. The light chain preparation was completed within 36 h of the death of the animal.

Light chains P1 and P2 were isolated from bovine whole light chain fractions by isoelectric focusing on polyacrylamide gels with the capacity to separate 100–200 μg protein. After staining with Coomassie Blue G250 the protein bands corresponding to each P light chain were cut out, washed with 70% (v/v) ethanol and stored at −20°C.

2.3. *Peptide mapping of enzyme digests*

Slices obtained from the isoelectric focusing gels containing the individual P light chains from bovine ventricular myosin were partially digested and analysed by a method similar to that in [22], with the modification that digestion and subsequent electrophoresis was carried out in urea rather than in sodium dodecyl sulphate (SDS). Gel compositions were: main gel – 15% (w/v) acrylogel (BDH Chemicals Ltd., Poole, Dorset)/8 M urea/560 mM Tris–HCl (pH 8.6); stacking gel – 10% (w/v) acrylogel/4 M urea/12.5 mM Tris–HCl (pH 6.8)/40 mM glycine/1 mM EDTA. The gel slices were equilibrated in 2 M urea/10% (v/v) glycerol/120 mM Tris–HCl (pH 6.8)/1 mM EDTA and positioned in the wells of the stacking gel. To each slice was added 25–50 μl of a solution of the

Published by Elsevier Biomedical Press
00145793/82/0000–0000/$02.75

enzyme (200 μg/ml) dissolved in sample buffer containing 20% (v/v) glycerol. The whole was then overlayered with 0.7% agarose/25 mM Tris/190 mM glycine (pH 8.3) containing bromophenol blue. With 25 mM Tris–190 mM glycine (pH 8.3) as running buffer, the proteins were electrophoresed into the stacking gel and the current was switched off for 30 min to allow proteolytic digestion of the proteins to occur. The current (30 mA) was then reapplied for 20 h. After staining the gels, slices containing the complete digest were made and subjected to second dimensional gel electrophoresis in SDS as in [23,24].

3. Results and discussion

The two-dimensional electrophoretic pattern of proteins from ventricles of late foetal and neonatal rabbits was very similar to that obtained with the adult animal. Two differences in the light chain pattern could, however, be observed. An extra protein, similar in isoelectric point to alkali chain A1, but of smaller M_r, was seen in all foetal and neonatal hearts until ~3 weeks after birth. This protein could represent an embryonic form of light chain A1, similar to that in [25,26] where embryonic forms of light chain A1 were reported in both rat and human hearts.

Immediately before and after birth there was virtually no detectable P2 form of the P light chain or its phosphorylated derivative, P4 (fig.1). With increasing age the relative proportion of the light chain P2 + P4 increased so that 50–60 days after birth the ratio of the 2 forms of the P light chain reached that reported in [19] for adult rabbits.

In these studies no attempt was made to assess the precise level of phosphorylation of the P light chain since the hearts were homogenized in 9 M urea; a procedure that has been shown not to completely inhibit myosin light chain phosphatase activity [19,21]. Under these conditions of analysis the total P light chain fraction was observed to be <10% phosphorylated in hearts up to 20 days after birth. Subsequently the level of phosphorylation rose rapidly to 30%.

The light chains of myosin are known to undergo modification on handling that can lead to changes in electrophoretic mobility [28,29]. Indeed such a modification was considered to be responsible for the dual forms of the P light chain originally described in [30]. Nevertheless, the very consistent values obtained for the ratio of the two forms, P1 and P2 obtained on

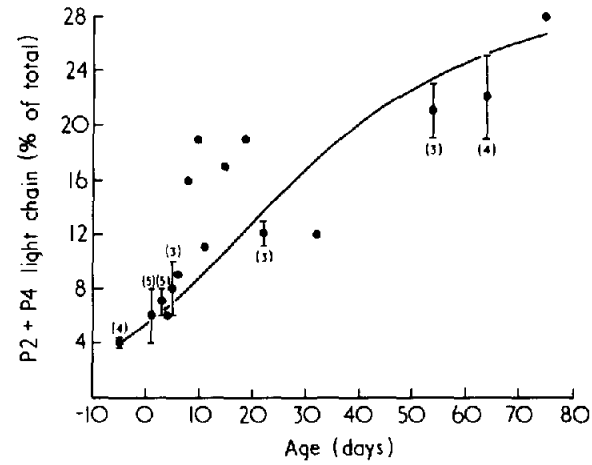

Fig.1. Changes in relative proportions of the 2 forms of the P light chain of myosin in rabbit ventricle during development. See text for experimental details. Values represent the proportion of the total P light chain in the form of P2 + P4 (phosphorylated form of P2). Bars represent SEM and numbers of hearts analysed are indicated in parenthesis where >2.

analysis of hearts in a variety of conditions and the change in ratio observed during development led to the suggestion [19] that light chains P1 and P2 are indeed different gene products.

Primary sequence differences between the 2 peptides were not readily apparent from amino acid analysis of the 2 forms of the bovine ventricular P light chain isolated by isoelectric focusing (table 1). In this respect the analyses are very similar to those in [20] for the 2 forms of P light chains of myosin from the chicken ventricle. The differences in the alanine and histidine contents of the 2 forms present in the bovine ventricle are possibly significant.

Differences were observed, however, in the peptide composition of the 2 forms of the P light chain after partial digestion with trypsin or thermolysin. Electrophoresis in urea gels after partial tryptic digestion (see section 2) revealed differences in the band patterns of the digests of the light chains P1 and P2 (fig.2a(i,ii)). The differences in the peptides of the 2 light chains were more clearly revealed on subsequent SDS electrophoresis of peptides initially separated by electrophoresis in urea gels. Although the general pattern of the peptide maps were similar, at least two peptides of low M_r were unique to light chain P1 (fig.2b,c).

With thermolysin a very acidic peptide was present in digests of light chain P1 after 30 min, but not in digests of light chain P2. After 60 min digestion of light chain P2, small amounts of an acidic peptide

Table 1
Comparison of the amino acid analysis of bovine ventricular light chains P1 and P2 with chicken ventricular light chains L2A and L2B

Amino acid	Bovine PLC-1	Chicken[a] L2B	Bovine PLC-2	Chicken[a] L2A
Asx	23.5	21	24.3	21
Thr	8.8	9	8.7	7
Ser	6.3	4	7.3	5
Glx	27.8	25	28.9	25
Pro	7.0	7	7.3	7
Gly	15.0	12	16.4	11
Ala	13.0	10	14.7	13
Val	7.8	7	7.8	7
Met	3.5	7	3.6	7
Ile	9.0	10	8.9	11
Leu	10.3	10	11.3	10
Tyr	1.4	3	1.5	2
Phe	10.3	14	9.8	14
His	2.0	2	2.7	2
Lys	16.5	17	16.6	16
Arg	5.5	4	6.9	5
Cys	n.d.	1	n.d.	0

[a] From sequence data in [20]

Analysis performed in duplicate on 2 preparations as in [31]; n.d., not determined

appeared, but by this time more acidic peptides had appeared in the digest of light chain P1. These studies suggest that differences exist in the structures of the 2 light chains which result in light chain P1 being more susceptible to digestion with thermolysin in the presence of 4 M urea.

Digestion with chymotrypsin, subtilisin, or *Staphylococcus aureus* V8 protein was not satisfactory for detecting differences in the primary structures of the 2 forms of the P light chain.

This study supports the hypothesis that the P light chain of bovine and rabbit heart exist in 2 forms that are different gene products. Taken with the results in [20] it suggests that polymorphism of the P light chain is a general property of myosin from the vertebrate heart, although the adult rat and adult mouse hearts are exceptions to this rule [19]. Both forms of P light chain can be phosphorylated in situ, but their roles are as yet uncertain. Recently, we have shown that slow skeletal muscle in the rabbit also contains 2 forms of the P light chain [32]. This observation and the fact that rapidly beating hearts contain less of the P2 form of the P light chain suggests that P light chain polymorphism may be characteristic of the slower types of striated muscle.

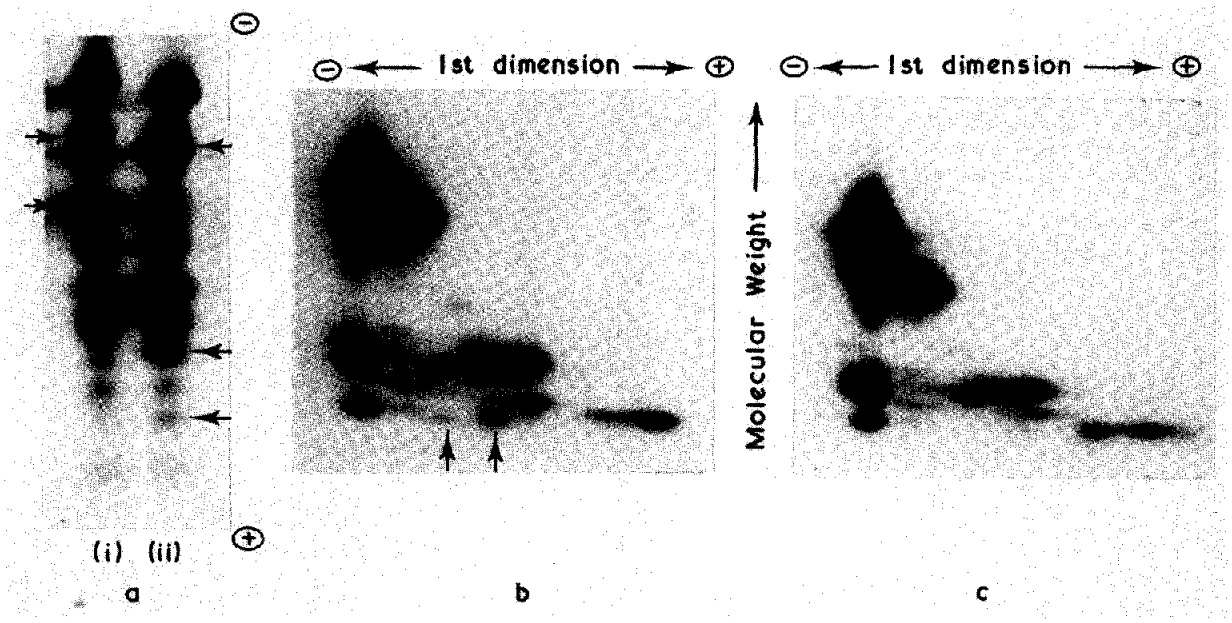

Fig.2. Electrophoresis of partial tryptic digests of cardiac P light chains. See text for experimental details. (a) Electrophoresis in 8 M urea pH 8.6; (i) light chain P1; (ii) light chain P2. Differences in peptide patterns indicated by arrows. Electrophoresis in 8 M urea (pH 8.6) followed by electrophoresis in 10–20% polyacrylamide–SDS gels: (b) light chain P1; (c) light chain P2. Peptides clearly unique to each digest are indicated by arrows.

Acknowledgements

We are grateful to Ms Sue Brewer for performing the amino acid analyses. This work was supported by a Programme Grant from the Medical Research Council.

References

[1] Trayer, I. P., Harris, C. I. and Perry, S. V. (1968) Nature 217, 452–453.
[2] Masaki, T. and Yoshizaki, C. (1974) J. Biochem. 76, 123–131.
[3] Whalen, R. G., Butler-Browne, G. S. and Gros, F. (1978) J. Mol. Biol. 126, 415–431.
[4] Flink, I. L., Rader, J. H. and Morkin, E. (1979) J. Biol. Chem. 254, 3105–3110.
[5] Hoh, J. F. Y. (1979) FEBS Lett. 98, 267–270.
[6] Hoh, J. F. Y. and Yeoh, G. P. S. (1979) Nature 280, 321–323.
[7] Whalen, R. G., Schwartz, K., Bouveret, P., Sell, S. M. and Gros, F. (1979) Proc. Natl. Acad. Sci. USA 76, 5197–5201.
[8] Obinata, T., Masaki, T. and Takano, H. (1980) J. Biochem. 87, 81–88.
[9] Dalla Libera, L. and Sartore, S. (1981) Biochim. Biophys. Acta 669, 84–92.
[10] Stockdale, F. E., Raman, N. and Baden, H. (1981) Proc. Natl. Acad. Sci. USA 78, 937–935.
[11] Ueda, S. and Yazaki, Y. (1981) J. Biochem. 90, 555–558.
[12] Hoh, J. F. Y., McGrath, P. A. and Hale, P. T. (1978) J. Mol. Cell. Cardiology 10, 1053–1076.
[13] Johnson, M. A., Mastalgia, F. L., Montgomery, A., Pope, B. and Weeds, A. G. (1980) in: Plasticity of Muscle (Pette, D. ed) pp. 607–615, Academic Press, New York.
[14] Buller, A. J., Mommaerts, W. F. H. M. and Seraydarian, K. (1969) J. Physiol. (London) 205, 581.
[15] Weeds, A. G., Trentham, D. R., Kean, C. J. C. and Buller, A. J. (1974) Nature 247, 135–139.
[16] Lompre, A. M., Schwartz, K., D'Albis, A., Lacombe, G., Van Thiem, N. and Swynghedauw, B. (1979) Nature 282, 105–107.
[17] Weeds, A. G., Hall, R. and Spurway, N. C. S. (1975) FEBS Lett. 49, 320–324.
[18] Pette, D. and Schnez, U. (1977) FEBS Lett. 83, 128–130.
[19] Westwood, S. A. and Perry, S. V. (1981) Biochem. J. 197, 185–195.
[20] Matsuda, G., Maita, T., Kato, Y., Chen, J. and Umegane, T. (1981) FEBS Lett. 136, 232–236.
[21] Grand, R. J. A. and Perry, S. V. (1982) in preparation.
[22] Cleveland, D. W., Fischer, S. G., Kirschner, M. W. and Laemmli, U. K. (1977) J. Biol. Chem. 193, 265–275.
[23] Anderson, N. G. and Anderson, N. L. (1978) Anal. Biochem. 85, 331–340.
[24] Anderson, N. L. and Anderson N. G. (1978b) Anal. Biochem. 85, 341–354.
[24] Whalen, R. G. and Sell, S. M. (1980) Nature 286, 731–733.
[26] Price, K. M., Littler, W. A. and Cummins, P. (1980) Biochem. J. 87, 81–88.
[27] Stull, J. T., Manning, D. R., High, C. W. and Blumenthal, D. K. (1980) Fed. Proc. FASEB 39, 1552–1557.
[28] Perrie, W. T. and Perry, S. V. (1970) Biochem. J. 119, 31–38.
[29] Manning, D. R., DiSalvo, J. and Stull, J. T. (1980) Mol. Cell. Endocrinol. 19, 1–19.
[30] Frearson, N. and Perry, S. V. (1975) Biochem. J. 151, 99–107.
[31] Wilkinson, J. M., Perry, S. V., Cole, H. A. and Trayer, I. P. (1972) Biochem. J. 127, 215–228.
[32] Westwood, S. A., Hudticken, O. and Perry, S. V. (1982) unpublished.