CROSS-HYBRIDIZATION OF LIGHT CHAINS OF CARDIAC MYOSIN ISOZYMES: ATRIAL AND VENTRICULAR MYOSINS

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1. Introduction

It was shown earlier that an increase in temperature in the absence of divalent cations was conducive to light chain dissociation [1], and that the affinity of myosin for divalent cations decreases at lower pH values, i.e., pH 6.5 [2], thus facilitating the release of light chains [2,3]. High salt concentration promotes light chain dissociation, especially light chain LC_1, either in the form of NH_4Cl [4] or LiCl [5]. Following dissociation of the myosin oligomer, reassociation occured with a decrease in temperature (4°C), additional excess light chains, and the presence of divalent cations [5]. In the studies of Wagner and Weeds [4] and Okamoto and Yagi [5], the myosin oligomer was both dissociated and reassociated in the presence of excess light chains, either native or foreign. Thus, for maximum dissociation of the myosin oligomer and reassociation of myosin light chains with isozymal heavy chains without denaturing myosin, i.e., losing actin + Mg^{2+}-stimulated ATPase activity, the procedures of Higuchi et al. [1] were coupled with those of Wagner and Weeds [4]; this provided a system to study the role of light chains in regulation of ATPase activity. For these analyses the three myosin isozymes, sheep atrial and ventricular myosins, and rabbit skeletal muscle myosin light chains were used because of their obvious difference in ATPase activity [6,7], and their variance in light chain electrophoretic mobility [6,7]. These differences allowed the monitoring of cross-hybridization by assessing ATPase activity and mobility of light chains on SDS–polyacrylamide gel electrophoresis of the hybrid myosin.

2. Materials and methods

Sheep atrial and ventricular myosins, as well as rabbit skeletal muscle myosin, were purified as described in earlier reports [6,8]. Myosin light chains for cross-hybridization studies were prepared with 8 M urea as described earlier [8], except that at the end of the 2-h incubation with 8 M urea, myosin heavy chains were precipitated with 45% $(NH_4)_2SO_4$ and the light chains remaining in the supernatant after centrifugation were precipitated by dialysis overnight in saturated $(NH_4)_2SO_4$. The precipitated light chains were then centrifuged and dialyzed in a large volume of 0.05 M TES (pH 7.0). Myosin and its light chains were identified by electrophoresis on 5–20% polyacrylamide gradient slab gels [8]. Protein in the individual bands were quantitated by eluting the dye with 25% pyridine as described earlier [1].

Calcium-activated ATPase activity at high ionic strength was measured in a medium containing 5 mM ATP, 0.5 M KCl, 10 mM $CaCl_2$, 0.02 M Tris–HCl (pH 7.6 at 30°C). Calcium-activated ATPase activity at low ionic strength was assayed in 0.2 M Tris–maleate (pH 6.5), 5 mM ATP, 0.01 M KCl, 10 mM $CaCl_2$ at 30°C. Actin + Mg^{2+}-activated myosin ATPase activity was measured in a medium containing 0.05 M TES (pH 7.0), 0.05 M KCl, 4 mM $MgCl_2$, 1 mM ATP, 0.1 mg/ml myosin, and 0.1 mg/ml actin at 30°C. All other conditions for assaying ATPase activity were as in [7].

For cross-hybridization studies the following conditions were used: 2 ml of light chains (2 mg/ml) were stirred at 23°C with 0.5 ml buffer (Stock Buffer:

0.5 M TES (pH 6.8), 1.5 M KCl and 10 mM DTT); to this was added 0.5 ml H_2O and 1 ml ATP (0.4 g ATP was dissolved in 10 ml 0.5 M EDTA, pH 6.8). After stirring for 1 min, 1 ml myosin (8.0 mg/ml) was added, stirred for another minute, and then 1.5 g NH_4Cl was added to all samples except the control. Control samples were analyzed with and without NH_4Cl; there was no denaturation due to the high salt concentration as long as ATP was present and incubation was not excessively long. Light chains were added to the control in order to treat all samples similarly. After addition of NH_4Cl, myosin was stirred for 3–8 min, and then immediately transferred to a dialysis bag and dialyzed in a large volume of water at 4°C for 2 h; myosin was then further dialyzed in 0.05 M TES (pH 7.0), and 1 mM $CaCl_2$ overnight. Myosin was then precipitated the next day by centrifugation and dissolved in 0.75 ml 0.5 M KCl, and 0.05 M TES (pH 7.9). Myosin was dissociated during the brief 23°C treatment and then reassociated in the overnight dialysis.

3. Results

Experimental conditions for dissociation and reassociation of the myosin oligomer as described in [1–6] were used for the cross-hybridization studies described here. An increase in temperature altered the conformation of myosin thus facilitating light chain release; the presence of substrate protected the active site [1]. The presence of chelating agents promoted light chain LC_2 release and a large concentration of NH_4Cl facilitated light chain LC_1 dissociation [1,4]. Instead of precipitating light chain-deficient myosin at the end of the incubation period with $(NH_4)_2SO_4$ followed by addition of foreign light chains, an excess of foreign light chains was initially added during both the dissociation and reassociation incubation periods as described in [4,5].

Gel electrophoretic patterns of cross-hybridized rabbit skeletal muscle myosin light chains with ventricular myosin heavy chains are shown in fig.1; the interchange was carried out at 23°C and 4°C, and at increasing incubation times as shown in figure legends. As shown in table 1, there was no significant difference in the ATPase activities of the variously treated myosins, including the control; all expressed values similar to that of native ventricular myosin. Maximum reassociation with foreign light chains occured with an 8 min incubation at 23°C (fig.1A, slot 4).

Similarly, ventricular (fig.2), and skeletal muscle myosin light chains (fig.3), could be cross-hybridized with atrial myosin heavy chains. (The doublet for sheep atrial light chain LC_2 consistently occurs on the gradient gels used here; such a doublet was not obtained with dog atrial myosin light chain LC_2.) Again the largest degree of reassociation occurred at

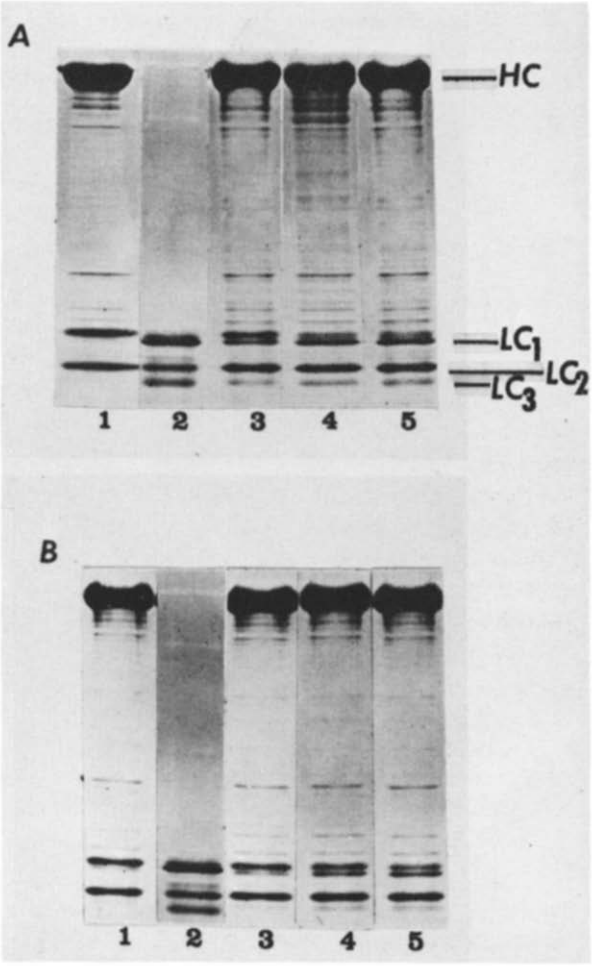

Fig.1. Cross-hybridization of ventricular myosin heavy chains with rabbit skeletal muscle myosin light chains at 23°C (A) and 4°C (B). In both (A) and (B) control myosin is shown in (1) and rabbit skeletal muscle myosin light chains in (2). In (A) incubation at 23°C is for 3 min (3); 5 min (4); and 8 min (5). In (B) incubation is at 4°C for 10 min (3); 20 min (4); and 40 min (5). The most complete hybridization is 23°C for 8 min shown in (A) (5); of the light chain protein present in (A) (5) 3% is in ventricular light chain LC_1; 39% in skeletal light chain LC_1; 50% present in skeletal + ventricular light chains LC_2; and 8% present in skeletal light chain LC_3. The position of the heavy chain (HC), and the three light chains, LC_1, LC_2, and LC_3, are indicated.

Table 1
Cross-hybridization with rabbit skeletal muscle myosin light chains

Conditions	Ventricular myosin ATPase (μmol P_i . mg myosin^{-1} . min^{-1})		
	Ca^{2+}-activated (low salt)	Ca^{2+}-activated (high salt)	Actin-activated
(4°C)			
10 min incubation	1.03	0.39	0.13
20 min incubation	1.04	0.45	0.14
40 min incubation	1.02	0.45	0.14
Control	1.02	0.44	0.16
(23°C)			
3 min incubation	0.94	0.46	0.14
5 min incubation	0.86	0.45	0.13
8 min incubation	0.94	0.45	0.14
Control	1.02	0.45	0.12

The electrophoresis gels for this study is shown in fig.1

23°C and an incubation time of 8 min (fig.2, slot 4) and (fig.3b, slot 2). Native ATPase activity was retained by atrial myosin, irrespective of the cross-hybridized light chains as shown in table 2, even though rabbit skeletal muscle myosin expresses a higher ATPase activity as compared to atrial myosin, and ventricular myosin a lower ATPase activity relative to that of the atria. These data indicate that

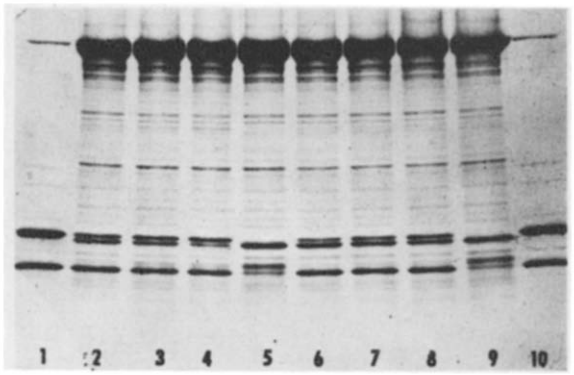

Fig.2. Cross-hybridization of atrial myosin (5,9) with ventricular myosin light chains (1,10). Incubation is at 23°C (2–4) and 4°C (6–8). Incubation at 23°C was for 3 min (2); 5 min (3) and 8 min (4). Incubation at 4°C was for 10 min (6); 20 min (7) and 40 min (8). The most complete hybridization is 23°C for 8 min (4). Of the light chains present in (4) 41% was present in ventricular myosin light chain LC_1; 7% in atrial myosin light chain LC_1, and 48% in ventricular light chain LC_2.

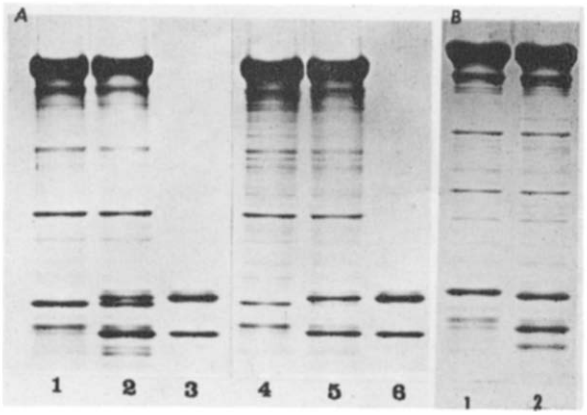

Fig.3. Cross-hybridization of atrial myosin (A) (1,4) with ventricular myosin light chains (3,6); atrial myosin following light chain interchange is shown in 2 and 5. Additional atrial myosin light chains were added to the cross-hybridization incubation of 1 and 2. Protein concentration was reduced to 0.8 mg/ml during the interchange incubation in 4 and 5, and no additional native atrial light chains added in order to decrease degree of native light chain reassociation. Incubation is 23°C for 8 min. In (1) 100 light chain LC_1 is present and in (4) 50% of light chain LC_1 is present. In (B) atrial myosin (1) was incubated with rabbit skeletal muscle myosin light chains; cross-hybridized atrial myosin is shown in (2). Protein was 1.6 mg/ml during the interchange incubation; no additional atrial light chains were added to (1) or (2) during the incubation. Skeletal muscle myosin light chains were added to the interchange incubation of (2). The ATPase activity for such interchange is shown in table 2.

Table 2
Cross-hybridization with rabbit skeletal muscle myosin and ventricular myosin light chains

	Atrial myosin ATPase (μmol P_i . mg myosin^{-1} . min^{-1})		
Rabbit skeletal muscle myosin LC	Ca^{2+}-activated (low salt)	Ca^{2+}-activated (high salt)	Actin-activated
(4°C)			
10 min incubation	1.70	0.86	0.26
20 min incubation	1.76	0.91	0.24
40 min incubation	1.53	0.86	0.23
Control	2.10	0.91	0.30
(23°C)			
3 min incubation	1.93	0.82	0.26
5 min incubation	1.75	0.77	0.24
8 min incubation	1.70	0.86	0.24
Control	2.05	0.79	0.26
Ventricular myosin light chains			
(4°C)			
10 min incubation	1.93	0.91	0.27
20 min incubation	1.78	0.86	0.23
40 min incubation	1.40	0.81	0.18
	1.94	0.79	0.28
(23°C)			
3 min incubation	1.84	0.92	0.28
5 min incubation	1.75	0.98	0.29
8 min incubation	1.72	0.95	0.27
Control	1.99	0.92	0.28

Analyses shown are averages of 2 experiments and 2 determinations for each experiment. Gel electrophoresis pictures of one of these experiments is shown in fig.2

Table 3
Cross-hybridization with ventricular myosin light chains

	Atrial myosin ATPase (μmol P_i . mg myosin^{-1} . min^{-1})		
(23°C)	Ca^{2+}-activated (low salt)	Ca^{2+}-activated (high salt)	Actin-activated
Control myosin Additional atrial light chains (100% LC_1 present)	2.1	0.87	0.25
8 min incubation with additional ventricular and atrial myosin light chains	1.8	0.86	0.22
Control myosin No additional light chains (50% LC_1 present)	2.2	0.87	0.25
8 min incubation with additional ventricular myosin light chains	2.3	0.89	0.24

The electrophoresis gels for this study is shown in fig.3A

both ventricular and atrial myosin continue to express their respective native ATPase activities regardless of the type of light chains present.

It was necessary to have a large concentration of foreign light chains present in the incubation media in order to observe maximum cross-hybridization. Myosin reassociated stoichiometrically when additional light chains were present. Where no additional light chains were added, and reassociation was carried out at a low protein concentration, the atrial oligomer was light chain-deficient (fig.3), however, even under these conditions myosin retained a normal ATPase activity (table 3), as observed in earlier studies [1]. Previously, it was shown that 20% of light chain LC_1 of cardiac myosin and 75% of light chain LC_2 could be dissociated from myosin heavy chains with no corresponding decrease in ATPase activity [1]. In these analyses where dissociation was carried out at a high salt concentration, 50% of light chain LC_1 could be removed with no corresponding loss in ATPase activity.

4. Discussion

Hybridization experiments were performed using light and heavy chains from different types of myosins to explore the role and influence of the different light chains on the ATPase activity of myosin. The two chemically homologous light chains, LC_1 and LC_3 [9], of rabbit skeletal muscle myosin replace stoichiometrically cardiac myosin light chain, LC_1, in both atrial and ventricular myosins. The data offer unequivocal evidence that cross-hybridization of cardiac myosin with rabbit skeletal muscle myosin light chains readily occurs. These hybridization experiments between light and heavy chains from different muscle sources show that the light chains do not modify the steady state ATPase activity of Ca^{2+}-stimulated ATPase activity at either high or low ionic strength, nor do they modify actin-activated ATPase activity of atrial and ventricular myosins as shown in tables 1 and 2. These data on cardiac myosin confirm the recent studies of P. D. Wagner on skeletal muscle myosin where he showed that light chains from different myosin sources did not alter the myosin ATPase activity of rabbit skeletal muscle myosin. (Private communication concerning a recently submitted manuscript.) When actin-activated ATPase activity of light chain-deficient myosin was assayed at 30°C for 5 min as described here, there was no decrease in ATPase activity as compared to when similar assays were carried out at 35°C for 10 min [6,7]. Light chain-deficient myosin was shown earlier to be heat-labile [1].

Although there are large differences in the Ca^{2+}-stimulated and actin-activated ATPase activities of atrial and ventricular myosins [7,10,11], nevertheless, the light and heavy chains of the two myosins can be nearly completely exchanged with no subsequent alteration in the steady state ATPase activity of native myosin of the heavy chains. Because K^+/EDTA-stimulated ATPase activity is similar between the two cardiac myosins [7,11], this type of cation-stimulated ATPase activity was not analyzed.

Myosin presumably goes through a conformational change in the absence of divalent cations when the temperature is increased to 23°C, similar to the temperature-dependent change which occurs in the spectrin oligomer, which in turn promotes dissociation in the latter [12]. A transition in the pH activity curve of rabbit skeletal muscle heavy meromyosin and cardiac myosins with increases in temperature have been reported [1,13,14]. High concentrations of salt, known to promote conformational changes in the Ca^{2+} binding light chain [15], were necessary to release light chain LC_1 with an increase in temperature as described here. As discussed by Okamoto and Yagi [5], ionic strength appears to be critical for light chain exchange. As shown here, both a high salt concentration and an increase in temperature were necessary to obtain complete cross-hybridization of foreign light chains with native cardiac myosin heavy chains. The light chains do not appear to modulate the ATPase activity of either myosin or actin-activated myosin at steady state activity. Also, as described here, 50% of light chain LC_1 can be dissociated from the cardiac oligomer with no alteration in such light chain-deficient myosin. Likewise, light chains can be exchanged from myosins demonstrating large differences in ATPase activity with no subsequent change in the steady state ATPase activity of the native myosin of the heavy chains.

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References

[1] Higuchi, M., Fabian, F., Wandzilak, T., Mason, D. T. and Wikman-Coffelt, J. (1978) Eur. J. Biochem. 92, 317–323.
[2] Wikman-Coffelt, J. (1980) Biochem. J. 185, 265–268.
[3] Srivastava, S., Muhlrad, A. and Wikman-Coffelt, J. (1980) Biochem. J. (in press).
[4] Wagner, P. D. and Weeds, A. G. (1977) J. Mol. Biol. 109, 455–473.
[5] Okamoto, Y. and Yagi, K. (1977) J. Biochem. 82, 17–23.
[6] Wikman-Coffelt, J., Srivastava, S. and Mason, D. T. (1979) Biochimie 61, 1309–1314.
[7] Wikman-Coffelt, J. and Srivastava, S. (1979) FEBS Lett. 106, 207–212.
[8] Long, L., Fabian, F., Mason, D. T. and Wikman-Coffelt, J. (1977) Biochem. Biophys. Res. Commun. 76, 626–635.
[9] Sarkar, S., Sreter, F. A. and Gergely, J. (1971) Proc. Natl. Acad. Sci. USA 68, 946–950.
[10] Yazaki, Y., Udea, S., Nagai, R. and Shimada, K. (1979) Circ. Res. 45, 522–527.
[11] Flink, I. L., Rader, J. F., Banerjee, S. K. and Morkin, E. (1978) FEBS Lett. 94, 125–130.
[12] Ralston, G., Dunbar, J. and White, M. (1977) Biochim. Biophys. Acta 491, 345–348.
[13] Ishigami, F. and Morita, F. (1977) J. Biochem. 81, 297–303.
[14] Mrakovcic, A., Oda, S. and Reisler, E. (1979) Biochemistry 26, 5960–5965.
[15] Higuchi, M., Stewart, D., Mason, D. T., Ikeda, R. and Wikman-Coffelt, J. (1978) J. Comp. Biochem. Physiol. 60B, 495–499.