EVIDENCE FOR THE INVOLVEMENT OF LIGHT CHAINS IN THE BIOLOGICAL FUNCTIONING OF MYOSIN

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It is now well established that myosin, of molecular weight 470,000 (1) is comprised of an elongated helical core of two heavy chains, each of molecular weight 210,000, containing two to three light chains (2) of molecular weight 20,000 (1). By a variety of methods (3) the light chains may be dissociated from the heavy chains with a resultant loss in the ATPase and actin combining properties of the molecule. The light chains have also been shown to be present in subfragment-1, the biologically active fragment of myosin produced by papain digestion (4). Each mole of subfragment-1 (mol. wt. 105,000) has been shown to contain one light chain (5).

These earlier studies suggested that some functional role might exist for the light chains at or near the active site of the molecule and the evidence to be presented here shows that the presence of both light and heavy chains and their interaction are a necessary prerequisite for the hydrolysis of ATP by myosin and for its interaction with F-actin. For these studies the dis-

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sociation of light and heavy chains was carried out in 4 M LiCl, a method we had previously reported (3).

Material and Methods

Myosin and subfragment-1 were prepared as previously described (3). ATPase activities were determined in 0.5 M KCl-0.05 M Tris, pH 7.5-2.5 mM $CaCl_2$ at 25°C as described in previous articles (5).

Results and Discussion

Fig. 1 shows the loss in ATPase activity of myosin in 4 M LiCl as a function of time. Myosin was exposed to 4 M LiCl at 4°C for the prescribed time and the LiCl was removed within 2

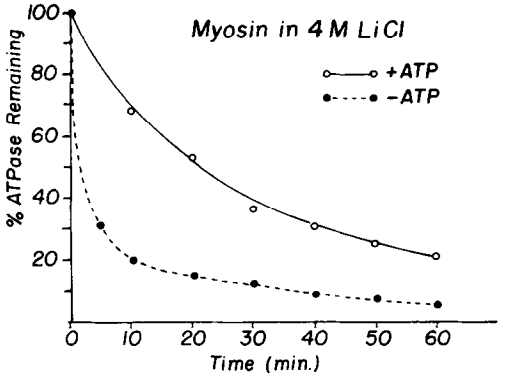

Fig. 1. Rate of loss of ATPase activity of myosin in 4 M LiCl.

minutes on a Sephadex G-10 column (0.9 x 8 cm). The ATPase activities of each sample were then determined. It can be seen that the presence of 2.5 mM ATP - 2.5 mM $MgCl_2$ retards the loss in ATPase and over 50% of the activity remains even after 20 minutes of exposure to LiCl at 4°C. A similar study was carried out with subfragment-1 which appears to be more resistant to loss

in ATPase in the presence of 4 M LiCl than is myosin. In the presence of ATP approximately 60% of the ATPase remains after 20 minutes of exposure (Fig. 2).

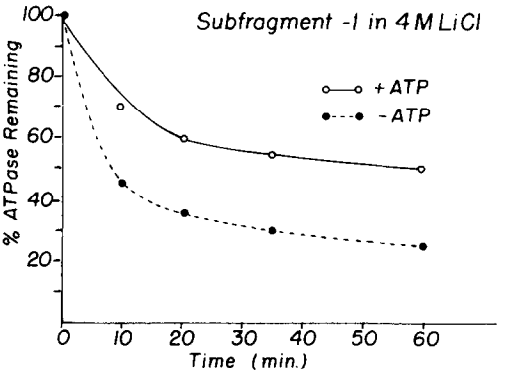

Fig. 2. Rate of loss of ATPase activity of subfragment-1 in 4M LiCl.

It was next necessary to demonstrate that under the conditions of these experiments complete dissociation of the light and heavy chains had actually occurred since if only 50% of the molecules were dissociated one would expect a 50% recovery of ATPase. Therefore, myosin in 4 M LiCl - 1.0 mM ATP - 1.0 mM $MgCl_2$ was subjected to gel filtration at 4°C on Sephadex G-200 equilibrated with the same solvent mixture. In Fig. 3 a typical separation of the light and heavy chains can be seen. The percentage of light chains, based on an extinction coefficient of 3.5 (6), was found to be 10.8, similar to that obtained in earlier sedimentation velocity and sedimentation equilibrium studies (1,2). These results suggested that the dissociation of the light and heavy chains was complete. The separated chains were then desalted on Sephadex G-10 and their ATPase activities determined. The entire procedure of separation

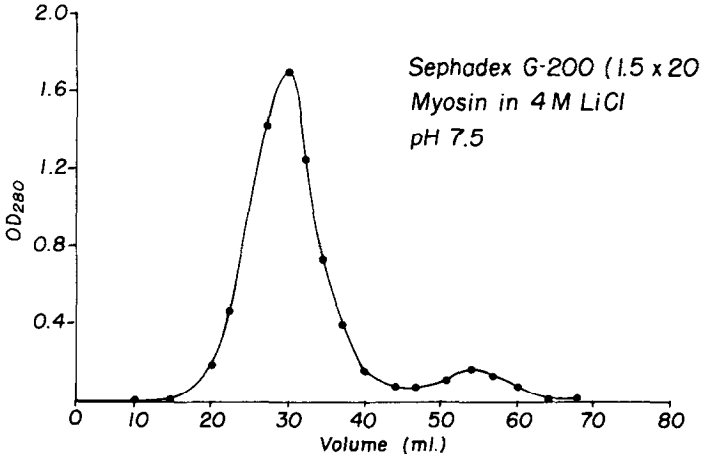

Fig 3. Gel filtration of myosin in 4 M LiCl. The eluting solvent also contained 2.5 mM ATP, 2.5 mM Mg^{++}, 25mM Tris, pH 7.5

and desalting could be accomplished within 20 minutes. Another sample of the separated chains in 4 M LiCl was remixed and dialyzed for 48-72 hours against 0.4 M KCl - 0.05 M Tris, pH 7.5 and the ATPase of this sample determined.

It can be seen in Table 1 that the unseparated chains of myosin and subfragment-1 retain approximately 50-60% of the control ATPase after 20 minutes of exposure to 4 M LiCl - 1.0 mM ATP - 1.0 mM $MgCl_2$. Twenty minutes was chosen as a comparison time since this was the minimum time necessary to separate the light and heavy chains from exposure to LiCl. The heavy and light chains themselves are devoid of all but traces of ATPase. Upon separation and remixing approximately 30% of the original myosin ATPase can be restored and 40% of the original subfragment-1 activity. The results represent the average of at least five experiments. The light chains which were not freshly prepared were not as effective in regenerating

Table 1.

Dissociation and Reconstitution of the Light and Heavy Chains of Myosin and Subfragment-1

	Myosin		Subfragment-1	
	ATPase umoles P_i/min/mg	%	ATPase umoles P_i/min/mg	%
Control	0.586	100	1.86	100
(H + L)*	0.304	52	1.12	60
H	0.029	5	0.15	8
L	0.000	0	0.04	2
(H + L)+	0.158	27	0.78	42

* Dissociated but not separated after 20 min in 4 M LiCl

\+ Separated on G-200 Sephadex and reconstituted

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the activity. In addition, light chains isolated from rabbit cardiac myosin restored only 10% of the original myosin ATPase suggesting some species specificity. The ability of myosin to combine with F-actin was restored to the same extent as the ATPase suggesting that both biological functions of myosin in the contractile process are mediated through an interaction of the light and heavy chains. The inability to obtain higher recoveries of activity is no doubt associated with the fact that upon removal of the light chains a rapid irreversible association of the heavy chains occurs presumably preventing their reassociation with the light chains at the proper site.

Fredericksen and Holtzer (7) have recently reported that up to 70% of the myosin ATPase could be restored after 100 minutes exposure to pH 11.0, conditions under which it is known that light chains are dissociated from myosin (2). Both before and after this report appeared our attempts to obtain similar results have been unsuccessful. Complete, irreversible loss in ATPase occurred within 15-20 minutes at pH 11.0. In addition, their recovery of activity was not obtained by an actual separation and reassociation of the light and heavy chains but only by raising and lowering the pH of the same solution.

The studies presented here clearly demonstrate that the light chains can be considered an integral part of the subunit structure of myosin. Their presence as part of the native molecule constitutes a necessary prerequisite for the biological functioning of myosin.

Acknowledgements

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