Cardiac Contractile Proteins

Adenosine Triphosphatase Activity and Physiological Function

JAMES SCHEUER AND ASHOK K. BHAN

IN RECENT years there has been an active search for a link between the enzymatic activity of the contractile proteins and increased or decreased myocardial function. Barany's observation of a general parallelism between the actomyosin or myosin adenosine triphosphatase (ATPase) activity and the speed of shortening among different skeletal muscles has provided logic for such a linkage (Barany, 1967; Barany et al., 1967), and the observation by Alpert and Gordon (1962) that myofibrillar ATPase activity was reduced in samples of myocardium of patients who died with cardiac failure indicated that such relationships may be important in the pathogenesis of human cardiac disease. This brief review will focus on factors that may influence the ATPase activity of cardiac contractile proteins in different physiological and pathophysiological states. It will discuss how changes in overall ATPase activity may be influenced rather than describe detailed kinetic mechanisms. A comprehensive review of other aspects of cardiac contractile proteins has been published recently (Leger et al., 1975).

Control of myocardial function might be exerted at numerous sites in the cell, and it appears unlikely that any one of these is the sole controlling factor under all physiological conditions. It is more likely that when chronic adaptations occur of the type that may alter ATPase activity of the contractile proteins, changes in other factors that affect contractile performance also may be present. In addition to myosin, the loci of control that might be important in either acute or chronic alterations in contractile function include the ionic movements and electrical currents controlled by the sarcolemma, the energy-dependent uptake and perhaps the release of calcium by the sarcoplasmic reticulum, the sensitivity of the troponin-tropomyosin system to alterations in calcium concentration in the cell, and perhaps a role for mitochondria in the control of intracellular calcium concentration.

Nevertheless, the final reaction must involve the interaction of myosin and actin, the resultant contraction, and the simultaneous splitting of ATP. Since this is the final reaction in the pathway, it is tempting to postulate that the activity of the myosin ATPase will be an important control factor in the whole complex chain of events known as excitation-contraction coupling.

Different ATPase Measurements

A major problem in trying to relate ATPase activities to contractile function in different physiological and pathophysiological states has been our lack of knowledge about which ATPase to measure. The various measurements and their advantages and disadvantages are outlined in Table 1. Early workers used the ATPase activities of myofibrils as activated by magnesium or calcium. Myocardial myofibrillar preparations are relatively crude fractions which contain mitochondria, sarcoplasmic reticulum, and sarcolemma, all of which have their own ATPase activities. Although in earlier studies the contractile protein ATPase activity may have predominated, small alterations in myofibrillar ATPase activity might have been due to changes in factors unrelated to the myosin enzymatic site. More recently, methods have been developed to eliminate the sarcolemmal, sarcoplasmic reticulum, and mitochondrial ATPases by treatment of the myofibrils with the non-ionic detergent Triton X-100 (Solaro et al., 1971), so that a meaningful measurement of the myofibrillar ATPase can be obtained. However, in comparative studies, a large change in the content of proteins other than myosin...
### Table 1 Contractile Protein Preparations Used for ATPase Measurements

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Conditions of ATPase assay</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myofibrils</td>
<td>Low ionic strength, + Ca(^{2+}) and Mg(^{2+})</td>
<td>Reflects the ATPase of the integrated contractile element. Presumably closest to the true physiological ATPase</td>
<td>Mitochondrial, sarcoplasmic reticulum, and sarcolemmal contamination. Washing of myofibrils with Triton will eliminate the membrane ATPase</td>
</tr>
<tr>
<td>Actomyosin</td>
<td>Low ionic strength + Ca(^{2+}) and Mg(^{2+})</td>
<td>Relatively cleaner system than the myofibrils. Reflects the actin-activated ATPase of myosin</td>
<td>Relatively long extraction procedures may alter or modify some proteins. Contaminating ATPase can be removed by washing the myofibrils with Triton prior to extraction of actomyosin</td>
</tr>
<tr>
<td></td>
<td>High ionic strength + Ca(^{2+})</td>
<td>Reflects the ATPase of myosin alone</td>
<td></td>
</tr>
<tr>
<td>Myosin</td>
<td>Ca(^{2+}), ATPase, high ionic strength, K(^{-})-EDTA ATPase, high ionic strength</td>
<td>Advantage of having a pure protein to work with</td>
<td>Use of Ca(^{2+})-ATPase alone or K(^{-})-EDTA ATPase as correlates of contractile behavior is not adequate under certain conditions. These may remain unaltered whereas the actin-activated ATPase will show significant changes</td>
</tr>
<tr>
<td></td>
<td>Actin-activated ATPase in low ionic strength with Ca(^{2+}) and Mg(^{2+})</td>
<td>Actin-activated ATPase provides a useful index of actin-myosin interaction. Proportions of various proteins in the reconstituted system can be carefully controlled, thus providing considerable flexibility in the study of protein-protein interaction</td>
<td></td>
</tr>
<tr>
<td>HMM and SF</td>
<td>Ca(^{2+}) and K(^{-})-EDTA ATPase measured under same conditions as those for myosin and provide similar information</td>
<td>The proteins are soluble at low ionic strength. Particularly useful for the measurement of actin-activated ATPase</td>
<td>Proteolysis of myosin for the preparation of HMM and SF often leads to degradation of 20,000-dalton subunits in cardiac myosin. This artifact may create significant alteration in the actin-myosin interaction</td>
</tr>
<tr>
<td></td>
<td>Actin-activated ATPase, low ionic strength</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SF = subfragment.

still might lead to inaccurate estimates of the myofibrillar ATPase activity.

At the other end of the purity scale one can study the ATPase activity of pure myosin or of its proteolytic subfragments. The globular head of myosin contains sites both for the enzymatic splitting of adenosine triphosphate (ATP) and for binding to actin. Therefore, proteolytic fragments of myosin such as heavy meromyosin (HMM), subfragment 1 (SF1) (Lowey and Cohen, 1962; Lowey et al., 1969), or other small subfragments (Bhan and Malhotra, 1976) can be prepared with elimination of much of the enzymatically inactive portion of the molecule but retention of the active enzymatic site. The study of the ATPase activity of pure myosin, HMM, or SF, might provide a more definitive result with respect to adaptations in the contractile proteins. Studies using myosin ATPase activity have been useful, but it is important to document that the myosin is pure and that its sulfhydryl groups are in the reduced state (Kielley and Bradley, 1956; Reisler et al., 1974). Purity of the myosin can be demonstrated by careful electrophoretic analysis and by analysis for nucleoproteins. Oxidation of the free cysteine residues can profoundly affect the apparent ATPase activity.

A variety of cationic conditions have been used for measuring the myosin ATPase activity. However, different results may be obtained depending on whether the reaction is carried out in the presence of calcium or potassium. Magnesium is the important cation for physiological activity of actin-regulated myosin ATPase, yet magnesium inhibits pure myosin ATPase activity and therefore has not been used in measuring myosin ATPase activity. Thus the ionic conditions that most nearly reflect
the physiologically important ATPase cannot be employed when studying pure myosin in the absence of actin.

The splitting of ATP by myosin in the intact cell is modulated by several other factors. The effect of actin on the myosin ATPase and the roles of various activators and inhibitors of actin and myosin interaction such as the troponin-tropomyosin system all are important considerations when evaluating contractile protein enzyme activity. Therefore it is unlikely that myosin ATPase activity alone can provide information that is definitive regarding the contractile process.

Actomyosin ATPase activity should provide a more integrated measure of the physiologically important reaction. However, preparations of actomyosin also may be contaminated by other ATPases. These can be eliminated only partially by including selective inhibiting agents such as azide for mitochondria and ouabain for sarcolemma. Also, since the intact actomyosin system will contain the troponin-tropomyosin complex, and this may be modified by the isolation process, the sensitivity of the actomyosin ATPase activity could be altered during isolation in ways that may make the final measurements difficult to interpret. In this regard the degree of phosphorylation of troponin I, and possibly of myosin, might change during isolation, and other modifications may occur that could alter the apparent ATPase activity.

Probably the closest approach to the physiologically important contractile protein ATPase activity is achieved by isolating the components of the contractile proteins and then reconstituting the system. Myosin can be purified and its activation then studied in the presence of purified actin, troponin, and tropomyosin. Studies along these lines have only just begun to be reported for heart, but it is likely that they will lead to greater insights into physiological mechanisms.

Finally, it is possible that most investigators are measuring the ATPase over too long a duration. It has been observed in skeletal muscles that there is an initial burst of ATPase activity in the presence of magnesium and calcium (Lynn and Taylor, 1970). This activity might relate more to physiological control than to the steady state activities most investigators measure. Initial burst activities probably also are important in cardiac myosin (Tada, 1967). However, most studies are conducted under steady state conditions.

**Conditions of ATPase Assay**

A variety of conditions are reported for studies on myofibrillar, actomyosin, and myosin ATPase, and it is difficult to extrapolate from one to the other. Barany (1967) showed a general parallelism between actin-activated magnesium ATPase, myosin ATPase activity in the presence of potassium and EDTA (K⁺-EDTA ATPase), and calcium-activated ATPase (Ca²⁺-ATPase). Ammonium also has been used to activate the ATPase (Wikman-Coffelt et al., 1975b). ATP splitting by myosin in the presence of divalent cations involves more complex reactions than that with monovalent cations (Lynn and Taylor, 1970). In the intact myofibril, it is most likely that myosin ATPase is activated in the presence of actin and magnesium, and it is this ATPase activity that most probably is physiologically important. However, only a few studies with heart myosin have used this measurement (Banerjee and Morkin, 1977; Alpert and Mulieri, 1977; Litten et al., 1978).

Distinct differences may be observed depending upon the myosin ATPase activity measured. For instance, it has been observed that when modification of some of the sulphydryl groups of myosin occurs, the Ca²⁺-ATPase activity may rise and the K⁺-EDTA ATPase may fall (Kielley and Bradley, 1956; Reisler et al., 1974).

We recently have observed that removal of light chain 2 (LC₂) from cardiac myosin results in no change in Ca²⁺-ATPase activity, an increase in actin-activated ATPase activity, and a fall in K⁺-EDTA ATPase activity (Malhotra et al., 1979). Since these ATPase activities can vary in different directions, the most intact system should be employed to try to explain physiological changes.

Actomyosin is a relatively intact system, but as mentioned above, ATPase activity may vary if modifications take place in the regulatory proteins during purification. Also, the solutions of high ionic strength that frequently are employed prevent the interaction of the actin with the myosin, and thus the ATPase activity measured is not the true actomyosin ATPase.

The relation of the cations to each other also should be considered. Fenner et al. (1973) demonstrated that, although both calcium and potassium could activate myosin ATPase alone, they were mutually inhibitory, and Solaro and Shiner (1976) reported that magnesium shifts the ATPase response to calcium differently in skeletal and cardiac myofibrillar preparations. Thus it is not sufficient to state that an ATPase activity of a contractile protein preparation does or does not shift in the same direction as a physiological alteration, but the type of activating system must be clarified and compared from study to study.

**Correlations of ATPase Activity with Physiological Functions**

Despite the problems mentioned above, it is of great interest that studies of contractile protein ATPase activity have correlated so well with physiological and pathophysiological states, as shown in Table 2. The table illustrates correlations of increased cardiac contractile performance and myofibrillar, actomyosin, and myosin ATPase activities in different species as a function of age, when con-
TABLE 2 Correlations between Cardiac Contractile Protein ATPase Activity and Various Physiological States

<table>
<thead>
<tr>
<th>Author</th>
<th>Date</th>
<th>Species</th>
<th>Preparation studied</th>
<th>Correlation with physiological state</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yazaki and Raben</td>
<td>1974</td>
<td>Mouse</td>
<td>Myosin</td>
<td>Decreasing Ca²⁺-ATPase activity (mouse &gt; rat &gt; dog &gt; rabbit)</td>
</tr>
<tr>
<td>Delcayre and Swynghedauw</td>
<td>1975</td>
<td>Rat</td>
<td>Myosin</td>
<td>Decreasing Ca²⁺-ATPase activity correlated with decreasing Vmax of myocardial contraction (rat &gt; guinea pig &gt; dog &gt; rabbit) described by Henderson et al. (1970)</td>
</tr>
<tr>
<td>Alpert et al.</td>
<td>1967</td>
<td>Rat</td>
<td>Actomyosin</td>
<td>Decreasing ATPase activity 100–1000 days of age correlated with decreased velocity of contraction</td>
</tr>
<tr>
<td>Heller and Whitehorn</td>
<td>1972</td>
<td>Rat</td>
<td>Actomyosin</td>
<td>Decreasing ATPase activity 53–151 days correlated with decreased velocity and extent of shortening</td>
</tr>
</tbody>
</table>

**Species differences**

A. Physical training

<table>
<thead>
<tr>
<th>Author</th>
<th>Date</th>
<th>Species</th>
<th>Preparation studied</th>
<th>Correlation with physiological state</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wilkerson and Evonuk</td>
<td>1971</td>
<td>Rat</td>
<td>Actomyosin</td>
<td>Increased ATPase activity</td>
</tr>
<tr>
<td>Bhan and Scheuer</td>
<td>1972</td>
<td>Rat</td>
<td>Actomyosin</td>
<td>Increased ATPase activity correlated with duration of training (swimming) with speed of superprecipitation. Increased contractile performance demonstrated</td>
</tr>
<tr>
<td>Bhan and Scheuer</td>
<td>1975</td>
<td>Rat</td>
<td>Myosin HMM</td>
<td>Increased ATPase activity correlated with duration of training (swimming). Increased contractile performance demonstrated</td>
</tr>
<tr>
<td>Goodkind et al.</td>
<td>1974</td>
<td>Guinea pig</td>
<td>Myofibrils and myosin</td>
<td>Increased ATPase activity in hyperthyroid guinea pigs, correlated with increased dT/dt of papillary muscles</td>
</tr>
<tr>
<td>Yazaki and Raben</td>
<td>1975</td>
<td>Rat</td>
<td>Myosin</td>
<td>Increased ATPase activity in hyperthyroid rabbits and decreased ATPase activity in hypothyroid rats</td>
</tr>
<tr>
<td>Banerjee et al.</td>
<td>1976</td>
<td>Rabbit</td>
<td>Myosin</td>
<td>Increased ATPase activity in hyperthyroidism</td>
</tr>
<tr>
<td>Conway et al.</td>
<td>1976</td>
<td>Dog</td>
<td>Myosin</td>
<td>Increased ATPase activity in hyperthyroidism</td>
</tr>
<tr>
<td>Banerjee and Morkin</td>
<td>1977</td>
<td>Rabbit</td>
<td>Actin activation of myosin</td>
<td>Increased ATPase activity in hyperthyroidism</td>
</tr>
<tr>
<td>Banerjee et al.</td>
<td>1977</td>
<td>Rabbit</td>
<td>Actin activation of HMM</td>
<td>Increased ATPase activity in hyperthyroidism</td>
</tr>
</tbody>
</table>

B. Other hormonal changes

<table>
<thead>
<tr>
<th>Author</th>
<th>Date</th>
<th>Species</th>
<th>Preparation studied</th>
<th>Correlation with physiological state</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rovetto et al.</td>
<td>1970</td>
<td>Cat</td>
<td>Myofibrils Actomyosin Myosin</td>
<td>Decreased ATPase activity in adrenalectomy reversed by dexamethasone treatment</td>
</tr>
<tr>
<td>Hjalmarson et al.</td>
<td>1970</td>
<td>Rat</td>
<td>Actomyosin Myosin</td>
<td>Decreased ATPase activity in hypophysectomy; normal ATPase activity with hypophysectomy plus thyroxin; decreased ATPase activity with hypophysectomy plus growth hormone; all correlated with maximum dP/dt</td>
</tr>
<tr>
<td>Rovetto et al.</td>
<td>1972</td>
<td>Rat</td>
<td>Myosin</td>
<td>Increased ATPase activity with growth hormone and thyroxine; decreased ATPase activity with hypophysectomy; decreased ATPase activity with hypophysectomy and growth hormone; normal ATPase activity with hypophysectomy, growth hormone, and thyroxine; decreased ATPase activity with propylthiouracil; decreased ATPase activity with adrenalectomy</td>
</tr>
<tr>
<td>Dowell</td>
<td>1976</td>
<td>Rat</td>
<td>Myosin</td>
<td>Decreased ATPase activity with chronic sympathectomy and with adrenalectomy and amyautectomy; correlated with reductions in maximum dP/dt</td>
</tr>
</tbody>
</table>
### Table 2 (Continued)

<table>
<thead>
<tr>
<th>Author</th>
<th>Date</th>
<th>Species</th>
<th>Preparation studied</th>
<th>Correlation with physiological state</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. Hemodynamic overload: hypertrophy and cardiac failure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alpert and Gordon</td>
<td>1962</td>
<td>Human</td>
<td>Myofibrils</td>
<td>Decreased ATPase activity in patients with cardiac failure due to hypertensive cardiovascular disease</td>
</tr>
<tr>
<td>Gordon and Brown</td>
<td>1966</td>
<td>Human</td>
<td>Myofibrils</td>
<td>Decreased ATPase activity in patients with cardiac failure due to hypertensive cardiovascular disease</td>
</tr>
<tr>
<td>Chandler et al.</td>
<td>1967</td>
<td>Cat</td>
<td>Myofibrils</td>
<td>Decreased ATPase activity in cats with pulmonary artery banding; ATPase activity decreased in hypertrophy and failure in proportion to decreased mechanical performance</td>
</tr>
<tr>
<td>Luchi et al.</td>
<td>1969</td>
<td>Dog</td>
<td>Myosin</td>
<td>Decreased ATPase activity in dogs with spontaneous heart failure</td>
</tr>
<tr>
<td>Draper et al.</td>
<td>1971</td>
<td>Guinea pig</td>
<td>Myofibrils and actomyosin</td>
<td>Decreased ATPase activity in guinea pigs with ascending aortic banding</td>
</tr>
<tr>
<td>Swynghedauw et al.</td>
<td>1971</td>
<td>Rabbit</td>
<td>Myofibrils</td>
<td>No change in myofibrillar ATPase in recent aortic regurgitation; no change in actomyosin ATPase in recent and decreased in chronic aortic regurgitation</td>
</tr>
<tr>
<td>Berson and Swynghedauw</td>
<td>1973</td>
<td>Rat</td>
<td>Myofibrils</td>
<td>Decreased ATPase activity in rats with aortic stenosis and regurgitation</td>
</tr>
<tr>
<td>Swynghedauw et al.</td>
<td>1973</td>
<td>Rabbit</td>
<td>Myofibrils</td>
<td>Decreased ATPase activity in rabbits with chronic aortic regurgitation</td>
</tr>
<tr>
<td>Oganesyan et al.</td>
<td>1973</td>
<td>Dog</td>
<td>Rabbit</td>
<td>Early decreased ATPase inactivity after overload of the left ventricle, returning to normal with time</td>
</tr>
<tr>
<td>Katagiri and Morkin</td>
<td>1974</td>
<td>Calf</td>
<td>Myosin</td>
<td>Decreased ATPase activity after coarctation of the aorta</td>
</tr>
<tr>
<td>Shiverick et al.</td>
<td>1976</td>
<td>Rabbit</td>
<td>Myosin</td>
<td>Decreased ATPase activity in rabbits with pulmonary artery banding paralleled by decreased shortening velocity</td>
</tr>
<tr>
<td>Alpert and Mulieri</td>
<td>1977</td>
<td>Rabbit</td>
<td>Myosin</td>
<td>Depressed actin-activated ATPase activity after pulmonary artery banding</td>
</tr>
<tr>
<td>Litten et al.</td>
<td>1978</td>
<td>Rabbit</td>
<td>SF,</td>
<td>Depressed actin-activated ATPase activity after pulmonary artery banding</td>
</tr>
</tbody>
</table>

Tractable performance is increased, as after physical training in the rat, and in hyperthyroidism, or when contractile performance is decreased, as in several hormonal deficiencies, decreased sympathetic states, and in profound cardiac hypertrophy and heart failure. However, these correlations do not constitute proof that the ATPase activity controls muscle function.

It should be noted that most animal studies of hyperthyroidism employ massive doses of the exogenous hormone, and the results might not be representative of natural hyperthyroidism.

Table 2 is not all-inclusive but presents studies that in our opinion lend support to the probability of a relationship between contractile protein ATPase activity and physiological function. Studies of human cardiac myosin have not been included. However, reports by Klotz et al. (1975) and Malhotra et al. (1977) indicate that the myosin ATPase activity is similar to that found in dogs and pigs. Of interest is the finding that at least a 20-hour delay after death does not affect the basic properties of the Ca²⁺-ATPase. Therefore it may be possible to study the effect of various disease states in humans on contractile proteins.

Reports of decreased ATPase activity in cardiac hypertrophy or failure are of particular interest. In several older studies, depressed ATPase activities were not observed (Benson, 1955; Davis et al., 1960). However, as shown in Table 2, changes usually have been found when more modern biochemical techniques were employed. In addition, a number of studies appear to show that the severity of overload correlates with the change in ATPase activity. Mild or early overload may cause a rise in ATPase activity, whereas severe or late overload causes a depression (Meerson, 1969; Wikman-Coffelt et al., 1978). However, since contractile performance was not defined in these experimental models, and therefore precise physiological correlations were not available, those studies have not been included in Table 2. It appears most likely that a mild or physiological overload such as physical training in the rat or mild pulmonic stenosis is associated with...
enhanced ATPase activity and, at least in physical training, enhanced contractile function, whereas in abrupt or severe hemodynamic overload, ATPase activity and contractile function are depressed.

Alpert et al. (1974) recently have reviewed the relationship between the depressed ATPase activity observed in myocardial hypertrophy and failure and the diminished contractility frequently observed. Henry et al. (1972) studied simultaneous ATP hydrolysis and isometric tension development in muscles from rabbit hearts with right ventricular overload. Although depressed mechanical function of papillary muscles from these hearts was observed, the tension response of glycerinated fibers to varying calcium concentrations was not different from that of fibers from control hearts, and the change in tension development was paralleled by a similar change in ATP splitting in both control and hypertrophied fibers. Myofibrillar ATPase activity was depressed in the presence of high magnesium in the preparations from hypertrophied hearts, but not when calcium responses were measured in the absence of magnesium. This study might suggest that the ATPase activity is dissociated from contractile performance in hypertrophied myocardium. However, the isometric contractile function of the glycerinated fibers was not depressed. This does not agree with previous findings of depressed function of fibers from failing hearts (Benson et al., 1958; Bing and Kako, 1961) and suggests that the hypertrophy preparation may not have been representative enough to answer the question of whether depressed ATPase activity is responsible for myocardial failure. The paper does indicate that studies of papillary muscles may not always be representative of what is happening at the sarcomere level, a fact confirmed by Krueger and Pollack (1975), and raises the point that a test tube measurement of ATPase activity may not be representative of ATP splitting in the intact contracting fibril.

Basic Considerations in the ATPase Reaction

The confusing state regarding ATPase activity of various preparations is not surprising in view of the known complexity of the ATPase reaction and the gaps that persist in our knowledge of the contractile process of striated muscle. Despite recent questions that have been raised about the crossbridge theory of muscle contraction (Noble and Pollack, 1977), for the purposes of this review we will assume that the theory is correct. The current concept is that, in the resting state, tropomyosin prevents the binding of the myosin crossbridge to the actin receptor site. This state is maintained by the inhibitory component of troponin (Tn-I). When activation of the cell occurs and leads to a rapid increase in calcium in the region of the myofibril, the calcium binds to its troponin receptor (Tn-C), changing the conformation of the troponin, removing the inhibition of troponin I and tropomyosin, and permitting the exposure of the myosin-binding site on actin.

A scheme for intermediate reactions found during ATP splitting by actomyosin and myosin is outlined in Figure 1A (Lymn and Taylor, 1971; Taylor and Lynn, 1972), along with the postulated relation of these reactions to actin and myosin mechanical interactions (Fig. 1B) (Taylor and Lynn, 1972; Huxley, 1969). In the first two reactions, ATP is bound to the actomyosin (reaction 1a), and then actin is rapidly dissociated from the complex (reaction 1b). The mechanical correlate is the release of the myosin crossbridge from its actin-binding site. This is followed by the hydrolysis of the ATP (reaction 2) to form a myosin-ADP-Pi complex and is accompanied by reorientation of the crossbridge opposite another actin-binding site. Next there is reassociation of actin with the myosin-ADP-Pi complex (reaction 3), which is accompanied by the rebinding of the crossbridge to the actin-binding site. Finally, the release of the ADP and Pi (reaction 4) is accompanied by the reorientation of the crossbridge to cause movement (sliding) of the actin filament and shortening of the sarcomere or tension development. The rate-limiting reaction in the actomyosin-ATPase scheme appears to be the final step, in which ADP and Pi are released (reaction 4), although evidence for rate limitation earlier in the scheme also has been reported (Eccleston et al., 1976; Chock et al., 1976). Several reactions in the actomyosin ATPase system are not represented in the myosin ATPase system, and therefore it would not be surprising if studies of actomyosin ATPase activity yielded results that were quite different than studies of myosin ATPase activity. In fact, since the rate-limiting reaction in the actomyosin ATPase scheme is not the same as in the myosin ATPase scheme, the two measurements might yield markedly different results. Although there has been

![Figure 1](http://circres.ahajournals.org/lookup/suppl/doi:10.1161/01.RES.45.1.6/-/DC1/fig_1){:style="width:500px;"}

**Figure 1** Proposed chemical steps involved in the actomyosin and myosin ATPase reactions (A) and proposed interactions between actin and myosin filaments (B). The numbers marking the steps in panel A correspond to those in panel B. Details are described in the text. These schemata are patterned after those proposed by Lynn and Taylor (1971), Taylor and Lynn (1972), and Huxley (1969).
a general qualitative correlation between the various ATPase activities with physiological measurements, as demonstrated in Table 2, it is quite conceivable that the measurement of myosin ATPase activity may give variable results as compared to ATPase activity measured in the presence of actin.

The direct extrapolation of ATPase activity to physiological function is difficult. Theoretically, measurements of actin-activated myosin ATPase activity should reflect the turnover of crossbridge interaction with actin, and therefore the speed of contraction of the unloaded muscle (V_{max}). However, since the mechanical function of cardiac muscle cannot be measured at true zero load, and since crossbridge function in loaded muscle has not been elucidated, a direct explanation of physiological function of the muscle based upon its enzymatic activity is not possible at this time.

**Myosin Structure and Function: The Role of Light Chains**

In view of the reports on correlations between contractile function and myosin ATPase activity, a review of the structure of myosin is in order. Myosin is a hexameric molecule with a molecular weight in the range of 480,000. It is made up of a long helical tail and a globular head, which contains the ATPase-active site and the actin-binding site. The head region also contains the light chains, which are likely to modulate the ATPase activity. White skeletal muscle appears to have three types of light chains; cardiac myosin has two classes of light chains (Lowey and Risby, 1971; Weeds and Pope, 1971; Sarkar et al., 1971). The light chains of cardiac myosin have molecular weights in the range of 27,000 (LC1) and 19,000–20,000 (LC2), as measured by sodium dodecyl sulfate gel electrophoretic techniques, although lower values are obtained using peptide analysis or ultracentrifugation (Leger et al., 1975).

The role of the light chains in controlling ATPase activity of cardiac myosin is unclear; however, they appear to be immunospecific (Wikman-Coffelt et al., 1973) and probably play an important functional role. Studies of Stracher (1969), Kim and Mommaerts (1971), and Drienen and Gershman (1970) suggested that the presence of light chains and the type of light chains present govern the level of myosin ATPase activity. When light chains were isolated from fast skeletal muscle myosin and added to heavy chain from slow muscles, the resultant myosin ATPase activity became similar to that of fast muscle myosin (Kim and Mommaerts, 1971). When cross-innervation studies were performed so that fast skeletal muscle was innervated with a nerve to a slow muscle and the slow muscle was innervated with nerves to fast muscle, the contractile activities shifted to those appropriate to the respective nerve and the ATPase activities of the myosins changed similarly (Buller et al., 1969; Barany and Close, 1971; Hoh, 1975). Evidence was cited that a change in light chains accompanied these alterations (Weeds et al., 1974; Sreter and Gergely, 1974).

More recent hybridization experiments by Wagner and Weeds (1977) on a myosin subfragment, using a skeletal SF1 cardiac LC1 hybrid or a cardiac SF1-skeletal light chain hybrid, show that the actin-activated ATPase level rather than the Ca2+-ATPase is determined by the type of light chain present. However, it must be emphasized that the level (or expression) of the ATPase might be a function of the type of heavy chain in addition to the light chain, rather than of the light chain alone.

The 18,000- to 20,000-dalton light chain is a common feature of myosin from various sources. This light chain has been shown to be necessary for calcium binding and Ca2+ sensitivity in scallop muscle (Szent-Gyorgyi and Szentkiralyi, 1973; Kendrick-Jones, 1976), and in nonmuscle myosin phosphorylation of this subunit sensitizes the actin-activated ATPase to calcium (Adelstein and Conti, 1975). Smooth muscle actin-activated myosin ATPase also is potentiated by phosphorylation of light chains (Gorecka et al., 1976; Small and Sobieszek, 1977). The 18,000-dalton subunit from different sources shows a considerable degree of amino acid sequence homology (Jakes et al., 1976; Leger and Elzinga, 1977). Thus there is evidence that the light chains may be important in modulating myosin or actomyosin ATPase activity, although direct evidence for this in cardiac myosin is only beginning to emerge. In this regard, preliminary studies by (Malhotra et al., 1979) indicate that removal of LC2 from cardiac myosin does not alter the calcium ATPase activity but results in enhanced actin-activated ATPase activity.

McPherson et al. (1974) observed that LC1 forms multiple bands on two-dimensional urea gel electrophoresis. They reported that LC1 has four differently charged components. LC2 gives multiple bands on charge electrophoresis, and this appears to be due to the presence of phosphorylated and nonphosphorylated forms of this subunit. Light chains of different mobilities may be seen in myosin of different species (Delcayre and Swynghedauw, 1975; Malhotra et al., 1977). Light chains have been demonstrated to have slower protein turnover rates in cardiac muscle than do heavy chains (Yazaki and Raben, 1978; Wikman-Coffelt et al., 1973). Thus light chains and heavy chains appear to be under separate genetic control.

It has been postulated that changes in the light chains could account for the differences in ATPase activities observed in various physiological and pathophysiological states. One group has reported that the ATPase activity of right ventricular myosin is lower than that of left ventricular myosin (Wikman-Coffelt et al., 1970a, 1975c), and that this is associated with a larger proportion of light chain material found in myosin from right rather than from left ventricle. These investigators also ob-
observed that when mild pulmonic stenosis was produced there was an increase in the myosin ATPase activity of the right ventricle in about 3 weeks that was associated with a decrease in one of the components of LC (Wikman-Coffelt et al., 1975). They postulated that the decrease in the LC$_{1}$ isomer was responsible for the greater ATPase activity found in the hypertrophied right ventricle (Wikman-Coffelt et al., 1975). However, these investigators reported in detail only K$^{+}$-EDTA ATPase activity, and, as mentioned previously, it is not certain how this measurement correlates with physiological function. An extra light chain also was reported by Henry and Sobel (1973) in myosin from hypertrophied hearts. However, most observers have not found any alterations in the ratios of light chains to heavy chains in cardiac hypertrophy (Swynghedauw et al., 1973; Katagiri and Morkin, 1974; Shiverick et al., 1976) or other conditions that change cardiac contractile performance (Bhan and Scheuer, 1975). Reports of extra light chains must be viewed with caution, since myosin may undergo proteolytic degradation during its purification (Siezen and Dreizen, 1978, Bhan et al., 1978).

In addition to different numbers of light chains or in the ratios of light chains to each other or to heavy chains, alterations in phosphorylation of light chains might be important. It has been observed that the 19,000-dalton light chain of skeletal muscle exists in phosphorylated and nonphosphorylated forms (Perrie et al., 1973) and also that cardiac LC$_{2}$ becomes phosphorylated (Frearson and Perry, 1975). Phosphorylation of smooth muscle myosin LC$_{2}$ by a specific protein kinase has been reported to cause an increase in actin-activated myosin ATPase activities that parallels the phosphorylation (Gorecka et al., 1976; Small and Sobieszek, 1977). Barany and Barany (1977) showed that phosphorylation of myosin light chain in frog skeletal muscle was associated with increased contractile force. On the other hand, Frearson et al. (1976) found an inverse relation between light chain phosphorylation and contractile force when hearts were perfused with epinephrine. It should be stressed that at the present time a physiological role for phosphorylation of the light chain has not been established, and it is not known whether the degree of phosphorylation differs in different physiological or pathophysiological states.

**Sulfhydryl Modulation**

Myosin contains sulfhydryl groups as part of the cysteine residues, and, as mentioned previously, care must be taken during the purification procedure to prevent modification of these groups, which would spuriously affect the measured ATPase activity. There are two classes of sulfhydryl groups: those that react quickly with sulfhydryl-directed agents (S1 groups), and those that react more slowly (S2 groups) (Kiely and Bradley, 1956; Reisler et al., 1974). Reaction of the S1 groups increases the Ca$^{2+}$-ATPase activity and decreases the K$^{+}$-EDTA activity. Once the S1 groups are bound, the binding of S2 groups inhibits the Ca$^{2+}$-ATPase activity.

It is possible that these sulfhydryl groups naturally modulate the ATPase activity of myosin, serving to change myosin reactivity in various physiological or pathophysiological states. This possibility is supported by the observations that binding of SH sites causes differential changes in ATPase activity in various physiological states. For instance, the Ca$^{2+}$-ATPase activity of rabbit cardiac myosin is increased markedly by reacting it with the sulfhydryl-binding agent N-ethyl maleimide (NEM), but rat cardiac myosin, which already has very high activity, is not activated by this agent (Yazaki and Raben, 1974). However, myosin from hyperthyroid rabbits, in which cardiac contractility and myosin ATPase activity are both increased does not have the same degree of activation as myosin from normal rabbits (Yazaki and Raben, 1975; Banerjee et al., 1976). Cardiac myosin from hyperthyroid rabbits reacted to NEM much as did myosin from euthyroid rats (Yazaki and Raben, 1975), and myosin from hypothyroid rats reacted to NEM much as did myosin from euthyroid rabbits. Myosin from rats can be activated by the sulfhydryl-binding agent iodoacetamide (Bhan et al., 1975), but myosin from hearts of physically trained rats, which already has a higher natural ATPase activity, cannot be activated to the same degree by the iodoacetamide. Furthermore, in the myosin from the hearts of the conditioned rats, the effects of sulfhydryl-modifying agents can be blocked by incubation of the myosin with Mg$^{2+}$-ATP, suggesting that the modified sulfhydryl group is in the area that can be affected by nucleotide binding. Cardiac myosin from hypertrophied myocardium due to systolic overload of the right ventricle has depressed Ca$^{2+}$-ATPase activity. However, the percent activation of the ATPase when the S1 groups are bound is greater than in myosin from control animals, but the peak activities reached are the same as controls (Shiverick et al., 1976). These studies with sulfhydryl-binding agents using cardiac myosin from hyperthyroid hearts, myosin from hearts of physically trained animals, and myosin from hypertrophied hearts all support the concept that the altered ATPase activities in these conditions may be related to a different conformation of sulfhydryl residues that changes the reactivity at or near the active site of the myosin. It should be stressed that these measurements of sulfhydryl modification mainly report changes in Ca$^{2+}$-ATPase activity.

Recently, Banerjee and Morkin (Banerjee and Morkin, 1977; Banerjee et al., 1977) have studied actin activation of myosin and HMM from hyperthyroid animals. They observed that the increased actin-activated ATPase activity of myosin from hyperthyroid animals can be abolished by prior treatment of the myosin with NEM, suggesting that sulfhydryl modifications may be related to the
physiological alterations in contraction and ATP splitting. The change in the reactivity of the sulfhydryl groups probably is indicative of an altered conformation of these groups that might arise out of modifications elsewhere in the molecule.

Thus, sulfhydryl changes could play a role in the physiological adaptation of myosin ATPase that may partially account for altered myocardial function observed in the various physiological and pathophysiological states. However, as stressed by Banerjee and Morkin (1977), it is unlikely that sulfhydryl modification per se is entirely responsible for such differences.

Differences in the Primary Structure of Myosin

It has been postulated that production of a new type of myosin or changes in the ratios of myosin isoenzyme forms might account for alterations in ATPase activities (Swynghedauw et al., 1976). Whether changes in the primary structure might relate to alterations in the physiological function has not been established.

Since the amino acid turnover of light chains and heavy chains may differ in different physiological states (Sims et al., 1978), changes in the amino acid composition of myosin might be anticipated. However, Katagiri and Morkin (1974) reported no change in amino acid composition of light chains from hyper trophyed hearts, but recently Raszkowski et al. (1977) found that cardiac myosin from dogs with congestive heart failure had a decreased level of cystine/2 residues. Earlier, Thyrum et al. (1970) reported a change in amino acid content of myosin from hyperthyroid rabbits, but Yazaki and Raben (1975) were unable to confirm this. Recently, Flink et al. (Flink and Morkin, 1977; Flink et al., 1979) have provided more conclusive evidence of a new species of myosin in hearts from hyperthyroid rabbits.

Methylation of Myosin

The role of posttranslational modifications of myosin needs closer attention if the mechanism of altered ATPase activities of cardiac myosin is to be understood. Recent studies have shown the lack of 3-methyl histidine and N-methyl lysine in the heavy chains of cardiac myosin. This finding apparently reflects the deficiency of specific methylating enzymes in cardiac muscle (Huszar, 1975). Whether this has any physiological significance is unknown.

Regulatory Proteins

Despite the central roles these compounds play in controlling the interaction of actin and myosin, only a few biochemical studies have been performed on these components of cardiac contractile proteins. As in skeletal muscle, cardiac troponin contains three subunits: an inhibitory unit (Tn-I), a calcium-binding unit (Tn-C), and a tropomyosin-binding unit (Tn-T) (Brekke and Greaser, 1976). Tn-C is sensitive to calcium within the physiological range, and probably serves as the calcium receptor site for activation of actin and myosin interaction in the intact myofibril. Tn-I can be phosphorylated readily by a cyclic AMP-dependent kinase (Perry and Cole, 1974; Cole and Perry, 1975; Reddy, 1976). Tn-I is rapidly phosphorylated when hearts are perfused with epinephrine (England, 1975; Solaro et al., 1976). However, as with phosphorylation of light chains, the physiological role of Tn-I phosphorylation remains a mystery.

Tropomyosin inhibits the Ca^{2+}-ATPase activity of pure actomyosin and the Mg^{2+}-ATPase activity of actomyosin in the presence of tropomyosin (Schaub and Perry, 1971). Whether tropomyosin changes as the heart adapts to different physiological states and how such alterations might mediate myocardial function is completely unknown at this time.

Other possible regulatory compounds are found in extracts of contractile proteins. These include α actinin and C protein. How these might relate to physiological function is unknown.

Summary

This review has pointed out the good correlation frequently observed between ATPase activity of various contractile protein preparations and contractile function of various muscles including the myocardium. Some of the variables in the measurement of the various ATPases and the relationship of these measurements to physiological ATPase in the intact myofibril have been mentioned. The possible roles of changes in the light chains and of sulfhydryl groups in the control of ATPase activity have been outlined. The possibility that phosphorylating reactions might exert control over physiological activity remains to be clarified.

It is evident that, despite the large amount of research that has been done, our understanding of how the biochemistry of contractile proteins relates to physiological function is in its infancy, and only with a more complete elucidation of the underlying biochemistry of the components of contractile proteins and how they interact will the biochemical basis of physiological and pathophysiological adaptations become evident.

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