Actin from Heart Muscle:
Sulfhydryl Groups

By Arnold M. Katz, M.D., and Joyce B. Maxwell

A comparison of a number of chemical features of actin prepared from cardiac and skeletal muscle has been undertaken in an attempt to evaluate the role that this contractile protein may play in determining specific physiological responses by the two types of muscle. Analyses of physicochemical properties and amino acid compositions failed to reveal significant differences between these proteins, although minor dissimilarities of amino acid sequence, which could be of functional significance, were not excluded. In extending this comparison, the reactions of the sulfhydryl groups (SH) of cardiac and skeletal actins have been investigated because these groups play an important role in polymerization and provide a sensitive indication of changes in the protein occurring as a result of binding of nucleotides and bivalent cations. Comparison of the number and reactivities of the SH groups of cardiac and skeletal actin preparations failed to reveal differences. Furthermore, the inhibition of SH reactivity by Ca++ and ATP, and the reversal of the Ca++-induced inhibition by Mg++, previously noted in the case of skeletal actin preparations, were also seen in these studies with cardiac actin.

Methods

Actin was prepared from the hearts and quadriceps femoris muscles of individual mongrel dogs and from batches of approximately forty fresh-frozen rabbit hearts, using the methods previously described. Determinations of sulfhydryl content were carried out by measuring the spectral shift of PMB* at 255 nm and the loss of absorbancy of NEM at 300 nm. The protocol followed in the sulfhydryl titrations and in the determinations of the time course of the reaction of SH groups with PMB has been described elsewhere.

All results are calculated on the basis of a molecular weight for actin of 60,000; this is slightly less than the value determined for dog cardiac actin (62,300) but has been used in order to facilitate comparison of results obtained with rabbit skeletal actin.

Results

The results of sulfhydryl analyses of rabbit cardiac actin are presented in table 1.

### TABLE 1

<table>
<thead>
<tr>
<th>Determinations</th>
<th>SH groups measured</th>
<th>No. SH groups/60,000 g protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. PMB in 0.01 M Tris nitrate</td>
<td>Total</td>
<td>5.9</td>
</tr>
<tr>
<td>2. PMB in 0.01 M Tris nitrate plus 5 M urea</td>
<td>Total</td>
<td>5.9</td>
</tr>
<tr>
<td>3. NEM in 0.1 M phosphate plus 0.2 M SDS</td>
<td>Total</td>
<td>5.8</td>
</tr>
<tr>
<td>4. Same as 3</td>
<td>Total, less</td>
<td>5.6</td>
</tr>
<tr>
<td>5. NEM in 0.1 M phosphate</td>
<td>&quot;Fast-reacting&quot;</td>
<td>1.9</td>
</tr>
<tr>
<td>6. PMB after prior reaction with NEM</td>
<td>Total, less</td>
<td>4.2</td>
</tr>
<tr>
<td>7. Sum of 5 and 6</td>
<td>Total</td>
<td>6.1</td>
</tr>
</tbody>
</table>

* Abbreviations: PMB, p-mercuribenzoate; NEM, N-ethylmaleimide; ATP, adenosinetriphosphate; Tris, tris(hydroxymethyl)aminomethane; SDS, sodium lauryl sulfate; EDTA, ethylenediaminetetraacetic acid.
Sulfhydryl Contents of Actin (per 60,000 g Protein) from Dog Heart and Skeletal Muscle

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Method</th>
<th>SH groups determined</th>
<th>Mean</th>
<th>No. of determinations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>PMB in urea</td>
<td>Total</td>
<td>6.2±0.3*</td>
<td>5</td>
</tr>
<tr>
<td>Heart</td>
<td>PMB</td>
<td>Total</td>
<td>5.7±0.6</td>
<td>27</td>
</tr>
<tr>
<td>Skeletal</td>
<td>PMB</td>
<td>Total</td>
<td>6.2±0.7</td>
<td>12</td>
</tr>
<tr>
<td>Heart</td>
<td>NEM in SDS</td>
<td>Total</td>
<td>5.5±0.7</td>
<td>9</td>
</tr>
<tr>
<td>Heart</td>
<td>NEM</td>
<td>&quot;Fast-reacting&quot;</td>
<td>1.9±0.2</td>
<td>8</td>
</tr>
</tbody>
</table>

* Standard error of the mean.

The total number of SH groups was approximately 6 per mole, of which two reacted with NEM when the actin was in the native state. These two groups were not essential for polymerization since cardiac actin previously reacted with NEM still polymerized upon the addition of salt.

Analyses of the sulfhydryl contents of actin preparations from 20 dog hearts and 12 samples of dog skeletal muscle are summarized in Table 2. The best integral value for total SH content, both for cardiac and skeletal actins, was 6 moles per 60,000 grams of protein and the number of SH groups of the native protein reacting with NEM was approximately two.

Determinations of the SH content of dog actins showed variations which appeared to represent differences between individual preparations. Thus, repeated determinations, by both PMB and NEM methods, on a single actin preparation varied less than did determinations carried out on different actin preparations. In an attempt to define the cause of these discrepancies in observed sulfhydryl content, the possible role of several factors was examined. A series of hearts and samples of skeletal muscle, extracted by the Bárány method 1 showed variability of SH content similar to that noted when the actin powders were made by the Straub method employed in the majority of these experiments. No effect on sulfhydryl content could be attributed to freezing of the hearts before preparation of the actin powders and the rate of reaction of sulfhydryl groups with PMB was not influenced by prior freezing (fig. 1).

![Rates of reaction of dog heart actin with PMB](http://circres.ahajournals.org/)

**Figure 1**
Rates of reaction of dog heart actin with PMB. Actin prepared from fresh heart muscle (○) is compared with actin from heart muscle frozen for 72 hours (●). Reactions were carried out simultaneously at room temperature in 0.012 mM ATP, 1.1 mM Tris nitrate at pH 7.6, using 0.4 mg/ml actin and approximately twofold excess of PMB to actin SH groups.

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allowing the heart to remain unfrozen on ice for 48 hours did not affect the reaction of the SH groups in actin. In the case of two dogs, the beating heart was transected and the apical portion immediately chilled and extracted, while the basal part of the heart was allowed to remain in situ for 20 minutes. No loss of titratable SH groups could be attributed to deterioration of the cardiac muscle allowed to fibrillate without coronary flow (table 3). Variations in apparent sulfhydryl content could not be attributed to folding of the peptide chain, since the number of titratable SH groups did not increase in the presence of denaturing agents (table 2), and disulfide formation was unlikely because amino acid analyses of these preparations failed to demonstrate significant amounts of cystine where the number of cysteine residues was somewhat less than 6 per mole. It appears most likely that the discrepancies may have arisen from the variation of some aspect of the preparative technique or to contamination by traces of heavy metal. Similar problems have been encountered in studies of rabbit skeletal actin preparations (see below).

Measurements of the time course of the reaction with PMB of the SH groups of dog cardiac actin revealed that approximately one-third of the reaction was completed within the first ten minutes (fig. 1). This initial phase was abolished by prior reaction of the native actin with NEM (fig. 2) and probably repre-
represented the titration of two fast-reacting SH groups.

The rate of the reaction with PMB of the SH groups of cardiac and skeletal actins was the same in highly purified preparations (fig. 3). In the presence of 0.1 mM CaCl₂, the reactivity of the SH groups of cardiac actin was inhibited; 0.1 mM MgCl₂ partially reversed the inhibitory action of CaCl₂ (fig. 4). Adenosinetriphosphate also reduced the SH reactivity of cardiac actin; this inhibitory action was potentiated by low concentrations of CaCl₂ (fig. 5).

Discussion

In previous studies of rabbit skeletal actin estimated values of sulfhydryl content have ranged from four to seven SH groups per mole.4-8, 13-16 and the total number of half-cystine residues has been found to be six to seven.13, 14, 16 While there is no satisfactory explanation for the apparent differences in SH content, several recent studies, including those carried out in this laboratory, favor a value of six to seven.7, 8, 15, 16 It is evident that not too much emphasis can be placed upon an accurate integral value in view of the variations in determination of sulphydryl groups as well as uncertainty regarding the precise molecular weight of both cardiac and skeletal actins.1

The present data, which suggest a value of six SH groups per mole of cardiac actin (tables 1 and 2), agree well with determinations carried out under similar conditions in the case of skeletal actins from rabbits8 and dogs (table 2). In all actins studied, approximately two SH groups reacted with NEM. As in the case of rabbit skeletal muscle actin, these were the SH groups of dog heart actin which reacted rapidly with PMB (fig. 2), and blocking of these two SH groups did not prevent subsequent polymerization on addition of salt.

The rates of reaction with PMB of the SH groups of cardiac and skeletal actin preparations were identical (fig. 3) and the effects of Ca++ and Mg++ on the reactivities of the
ACTIN FROM HEART MUSCLE

Influence of Ca++ and Mg++ on the SH reactivity of rabbit cardiac actin. Effects of 0.1 mM CaCl₂ (•) and 0.1 mM MgCl₂ (○) are compared with a control reaction (□). Conditions were as described in legend to figure 1 except for ATP concentration which was 0.013 mM.

SH groups of cardiac actin were similar to those previously noted in the case of skeletal actin (fig. 4). The inhibitory action of ATP on SH reactivity of cardiac actin, and the potentiation of this inhibition by Ca++ (fig. 5) were also similar to the effects of these substances on skeletal actin. Thus, these reactions of the SH groups in actin as well as those aspects of molecular structure previously examined demonstrate no differences between cardiac and skeletal actins.

Summary

The number and reactivity of the sulfhydryl groups of cardiac and skeletal actin preparations have been examined, using material from dogs and rabbits. The number of sulfhydryl groups of all actins was approximately six per mole, two of which could be characterized as fast-reacting and were not essential for polymerization. The time course of the reaction of the sulfhydryl groups of heart and skeletal actins with p-mercuribenzoate were identical, and the effects of Ca++, Mg++, and adenosinetriphosphate upon this reaction were similar for actins from the two tissues. In accord with previous structural comparisons, no difference between cardiac and skeletal actin has been detected.

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