Studies on Myosin from Red and White Skeletal Muscles of the Rabbit

II. INACTIVATION OF MYOSIN FROM RED MUSCLES UNDER MILD ALKALINE CONDITIONS

(Received for publication, June 6, 1967)

J. C. SEIDEL†

From the Department of Muscle Research, Retina Foundation, Institute of Biological and Medical Sciences, Boston, Massachusetts 02114

SUMMARY

The rapid loss of ATPase activity of myosin from the red, or slow, skeletal muscles of the rabbit under mild alkaline conditions has been studied in detail. The loss of ATPase activity is irreversible and consistent with a mechanism involving two first order reactions with rate constants of 0.3 and 0.007 min⁻¹.

No gross change in molecular structure accompanies inactivation, as judged by the lack of major change in optical rotatory dispersion or sedimentation. Slight increases in the rate of tryptic digestion and reactivity of sulfhydryl groups which may or may not be involved in the loss of enzymic activity are observed after inactivation. It appears that the molecular changes involved in loss of enzymic activity are relatively small and may be localized at or near the catalytic site. Myosin from rabbit cardiac muscle loses ATPase activity at pH 9.0 at the same rate as myosin from red skeletal muscles.

METHODS

Most experiments were done with myosin prepared according to Szent-Györgyi's dilution and precipitation procedure (see Reference 6). Extraction with KI (7) was used when myosin was prepared from individual muscles. Red muscles used for the preparation of myosin were the soleus, semitendinosus, crureus, and intertransversarius; white muscles were the adductor magnus and the vastus lateralis. Actin was prepared from mixed skeletal muscles (8) (extraction at 0-4°C), purified by centrifugation and polymerization (9), and further purified by partial polymerization with MgCl₂ (10).

ATPase activities were measured in the presence of either 1 mM EDTA and 0.5 M KCl or 10 mM CaCl₂ and 0.025 M KCl; in either case 0.2 mg of myosin per ml, 5 mM ATP, and the appropriate buffer were used. Buffers were 0.05 M Tris at pH 7.5, and 0.05 M Tris plus 0.05 M glycine at pH 9.0. The incubation was stopped by adding an equal volume of 10% trichloroacetic acid, and the phosphate was determined by the method of Fiske and SubbaRow (11). Optical rotatory dispersion was measured in a Jasco spectropolarimeter. The reaction of -SH groups with mercuribenzoate was measured by the method of Boyer (12) and the reaction with 5,5'-dithiobis(2-nitrobenzoate) by the method of Ellman (13). The reaction with 5,5'-dithiobis(2-nitrobenzoate) was carried out in a solution containing 1 mM EDTA and 0.1 M potassium phosphate, pH 7.0.

Mercuribenzoate was obtained from Sigma; 5,5'-dithiobis(2-nitrobenzoic acid) from Aldrich; ATP, ADP, and AMP from P-L Biochemicals, Milwaukee, Wisconsin; and trypsin from Nutritional Biochemicals.

RESULTS

Kinetics of Inactivation at pH 9.0—About 80% of the Ca++-activated ATPase and 90% of the EDTA-K⁺-activated ATPase

Myosins isolated from red and white skeletal muscles of the rabbit exhibit several marked differences in their enzymic properties (2, 5), probably resulting from differences in the molecular structure of the two proteins (2, 5). One of the most striking differences between the two enzymes is the lability of the adenosine triphosphatase of myosin-R1 under mild alkaline conditions (1, 2).
of myosin-II were lost in 10 min on exposure of the enzyme to a pH of 9.0 (Fig. 1). In contrast, less than 10% of the activity of myosin-W was lost during this time.

The data in Fig. 1 can be represented as the sum of two exponential terms, as

\[ A = c_1e^{-rt_1} + c_2e^{-rt_2} \]  

where \( A \) is the ratio of activity at time \( t \) to that at time 0, \( c_1 + c_2 = 1 \), and \( c_1, c_2, r_1, \) and \( r_2 \) are positive numbers. The dashed and solid straight lines in Fig. 1 are described by Equations 2 and 3, respectively

\[ \log A_1 = -r_1t/2.3 + \log c_1 \]  

\[ \log A_2 = -r_2t/2.3 + \log c_2 \]  

and \( A = A_1 + A_2 \).

The data in Fig. 1 are consistent both with a mechanism involving two parallel reactions

\[ B \xrightarrow{k_1} C \text{ and } D \xrightarrow{k_2} E \]

and with one involving two consecutive reactions

\[ F \xrightarrow{k_1'} G \xrightarrow{k_2'} H \]

In the case of parallel reactions it is stipulated that the enzyme exists in two active forms, \( B \) and \( D \), which are inactivated with rate constants \( k_1 \) and \( k_2 \), respectively; if only one active form existed and it underwent inactivation by two pathways, the process of inactivation would follow simple first order kinetics with a single rate constant, \( k \), where \( k = k_1 + k_2 \).

The two exponential terms in Equation 1 express the activities of the two forms, and \( k_1 = r_1, k_2 = r_2 \) are obtainable from Equations 2 and 3; \( c_1 \) and \( c_2 \) represent the activities of the forms at \( t = 0 \).

In the case of two consecutive reactions the concentrations of the forms \( F \) and \( G \) at time \( t \) are given (14) by

\[ c_F = c_0e^{-k_1't} \]  

\[ c_G = c_0 \left( \frac{k_1'}{k_2'} - \frac{k_1'}{k_2} \right) (e^{-k_2't} - e^{-k_1't}) \]

where \( c_0 \) is the total protein concentration. If one assumes that the specific activity of form \( F \) is \( s_F \), that of form \( G \) is \( s_G \), and that form \( H \) is inactive, the activities per unit volume attributable to \( F \) and \( G \) are given by

\[ a_F = s_Fc_F \]  

\[ a_G = s_Gc_G \]

respectively. The fraction of initial activity at time \( t \) is

\[ A = \frac{a_F + a_G}{s_Fc_0} \]

By substituting from Equations 4, 5, 6, and 7 into Equation 8 and rearranging, one obtains

\[ A = \left( 1 - \frac{s_Fk_1'}{s_F(k_1' - k_2')} \right) e^{-k_1't} + \frac{s_Fk_1'}{s_F(k_1' - k_2')} e^{-k_2't} \]

By comparing Equation 9 with Equation 1 it appears again that the \( k_1' = r_1 \) and \( k_2' = r_2 \). Thus the rate constants can be determined from the slopes of Equation 2 and 3, but one cannot distinguish between the two types of mechanisms on the basis of the kinetic data. It should be noted that if the mechanism of inactivation involves two consecutive reactions, the intercepts, \( \log c_1 \) and \( \log c_2 \), in Equations 2 and 3 have no simple meaning. Since both \( c_1 \) and \( c_2 \) are positive, by comparing the coefficients of the exponentials in Equations 1 and 9, one sees that the following relations must hold:

\[ \frac{s_Fk_1'}{s_F(k_1' - k_2')} (1 \text{ and } k_1')k_2' \]

The rate constants, \( k_1 = k_1' \) and \( k_2 = k_2' \), determined from Fig. 1 are 0.3 min\(^{-1}\) and 0.007 min\(^{-1}\), respectively.

The relationship between the initial rate of inactivation and protein concentration indicates a first order reaction (Fig. 2).

The kinetic behavior illustrated in Fig. 1 is not the result of an inhibition of the inactivation by the reaction products, since the rate of inactivation of native myosin-II was unchanged by the addition of inactivated myosin-II (Fig. 3).

The inactivation produced by incubating myosin-II at pH 9
FIG. 2. Dependence of initial rate of inactivation on myosin concentration. Myosin-R was added to a medium containing 0.5 M KCl, 0.1 M Tris, and 0.1 M glycine at pH 9.0 and 25°. With concentrations of myosin-R of 1 mg per ml or greater, aliquots were taken at appropriate times and added to the assay system, containing 0.5 M KCl, 0.1 M Tris, 1 mM EDTA, and 5 mM ATP, pH 7.5. At lower myosin concentrations the rate of loss of activity was determined by a prior incubation of myosin (incubation method) in the ATPase assay system (pH 9.0) for various times, at the end of which ATP was added to initiate the ATPase assay. The reaction was stopped after 5 min with trichloracetic acid. The rate constant of inactivation was determined from plots of the log of the ATPase activity against incubation time. The different symbols represent results of different experiments.

FIG. 3. Effect of inactivated myosin-R on the inactivation of native myosin-R. Myosin-R (5 mg per ml) was inactivated at 25° for 30 min by raising the pH to 9.0 with 0.4 M glycine (pH 9.5) and bringing the pH back to 7.0 with 0.4 M imidazole buffer (pH 6.5). In the case of native myosin-R the same volumes of glycine and imidazole were first mixed, then added to the myosin, while the pH was maintained at 7.0. Equal volumes of control and inactivated myosin-R were mixed, and the rates of loss of ATPase activity of the native, inactivated, and mixed myosins were determined by the incubation method described in the legend to Fig. 2. ○, native myosin-R; □, inactivated myosin-R; △, mixed native and inactivated myosin-R, expressed per mg of native myosin-R.

FIG. 4. Irreversibility of inactivation of myosin-R. Myosin-R (4 mg per ml) was adjusted to pH 9.0 with 0.4 M glycine (pH 9.5) and incubated at 25° for 3 or 10 min. The pH was returned to 7.0 with 0.4 M imidazole (pH 6.5), and the myosin was placed in ice. Aliquots were taken immediately and at the indicated times and added to a medium containing 0.6 M KCl, 0.05 M Tris, 5 mM EDTA, and 5 mM ATP at pH 7.5 and 25° for the determination of ATPase activity. Arrows indicate the return of pH to 7.0.

and 25° for 3 or 10 min was not reversed by reducing the pH to 7 (Fig. 4). Even after overnight storage at pH 7.0 and 4°, no reactivation was observed.

The temperature dependence of the rate constant for alkaline inactivation was determined from data obtained in four experiments and is shown in Fig. 5. The apparent activation energy of the process determined from these data was 26 kcal per mole.

In contrast to the difference between myosin-W and myosin-R in the rates of inactivation at pH 9, the rates of inactivation of myosin-R and myosin-W at pH 7.0 were essentially the same (Fig. 6).

Myosin Prepared from Individual Red Skeletal Muscles and Cardiac Muscle—Myosin was prepared from each of the four red muscles individually and from rabbit cardiac muscle by the KI extraction method of Szent-Györgyi (7), and the ATPase activities and rates of alkaline inactivation were compared. The results, shown in Table I, indicate that the myosin from each of the four red skeletal muscles is essentially the same with respect to ATPase activity and the rate of alkaline inactivation. Myosin from rabbit cardiac muscle had an ATPase activity slightly lower than that of myosin-R, but was inactivated at pH 9 at the same rate as myosin-R.

Protection against Inactivation—To determine whether the inactivation of myosin-R could be attributed to a contaminant in the myosin-R preparation which might also inactivate myosin-W, myosin-R and myosin-W were mixed and the inactivation at pH 9 was measured. It was found that the presence of myosin-R did not lead to inactivation of myosin-W. The data suggest that myosin-W slightly protects myosin-R against inactivation. However, this effect was small and the loss of ATPase activity...
Temperature dependence of the rate of inactivation at pH 9.0. The rate of inactivation at each temperature was determined as described in the legend to Fig. 1. ATPase activity was measured at 25° and pH 7.5. Rate constants were determined from plots of the log of ATPase activity with respect to time.

![Temperature dependence of the rate of inactivation at pH 9.0.](image)

**Fig. 5.** Temperature dependence of the rate of inactivation at pH 9.0. The rate of inactivation at each temperature was determined as described in the legend to Fig. 1. ATPase activity was measured at 25° and pH 7.5. Rate constants were determined from plots of the log of ATPase activity with respect to time.

**Fig. 6.** Inactivation of myosin-R and myosin-W at pH 7.0. Myosin (4 mg per ml) in 0.5 M KCl and 0.05 M imidazole, pH 7.0, was heated at 40° for the times indicated, and 0.1 ml was added to the assay system containing 0.05 M Tris, 10 mm CaCl₂, and 5 mm ATP (total volume, 2 ml) at pH 7.5 and 25°. Initial activities were 1.1 and 0.56 μmoles of P_i per mg of myosin per min for myosin-W and myosin-R, respectively. ○, myosin-W; □, myosin-R.

of these mixtures at pH 9 was nearly equal to the sum of the activity lost by each myosin measured separately (Fig. 7). This was true for mixtures varying in composition from 4:1 to 1:1 (milligrams of myosin-R per mg of myosin-W).

Actin reduced, but did not completely prevent, the inactivation of myosin-R at pH 9 (Fig. 8). There was a stimulation of ATPase activity of native myosin-R by actin in the presence of 10 mm CaCl₂, probably owing to the presence of traces of Mg²⁺ added with the actin preparation; however, this stimulation was lost after 2 min at pH 9. Maximum protection was observed with a myosin to actin ratio of 3:1 (Table II), but even with a 2:1 myosin to actin ratio some inactivation was observed. In contrast, bovine serum albumin at a concentration of 1 mg per ml had no effect on the rate of alkaline inactivation.

ATP, ADP, and pyrophosphate protected against inactivation at pH 9, while AMP had no effect (Table III). The results were essentially the same in the presence or absence of Mg²⁺, which was added to reduce the hydrolysis of ATP during the inactivation.

**Table I**

<table>
<thead>
<tr>
<th>Muscle</th>
<th>ATPase activity before alkaline treatment</th>
<th>Rate constant for inactivation at pH 9</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μmoles P_i/mg myosin/min</td>
<td>min⁻¹</td>
</tr>
<tr>
<td>Semitendinosus</td>
<td>0.80</td>
<td>0.30</td>
</tr>
<tr>
<td>Soleus</td>
<td>0.86</td>
<td>0.22</td>
</tr>
<tr>
<td>Intertransversarius</td>
<td>1.20</td>
<td>0.24</td>
</tr>
<tr>
<td>Crureus</td>
<td>0.84</td>
<td>0.24</td>
</tr>
<tr>
<td>Mixed red skeletal myosin</td>
<td>0.78</td>
<td>0.24</td>
</tr>
<tr>
<td>Cardiac</td>
<td>0.61</td>
<td>0.27</td>
</tr>
</tbody>
</table>

The ATPase assay medium contained 0.05 M Tris, 0.05 M glycerine, pH 9.0, 0.5 M KCl, 1 mm EDTA, 5 mm ATP, and 0.2 mg of myosin per ml. The rate of inactivation was determined by the incubation method (see the legend to Fig. 2). Myosin from individual muscles was prepared by the KI extraction method (7), owing to the small amount of tissue available, particularly with cardiac muscle. Mixed red skeletal myosin was prepared by mixing equal parts by weight of each of the four myosins prepared from red skeletal muscles.
ATPase Activity of Myosin-R Stable at pH 9—The residual activity of myosin-R after 20 min at pH 9 and 25° usually amounted to 10 to 20% of the initial activity, except that when the ATPase activity before inactivation was low, it amounted to 30 or 40% of the initial activity. The Ca++-activated specific activity after alkaline inactivation was always between 0.05 and 0.1 μmole of P_i per mg of protein per min. This residual activity was due to myosin and not to a contaminating particulate ATPase, as seen from the effect of various moderators on the residual activity (Table IV). CaCl_2 or EDTA plus KCl stimulated the ATPase activity, while MgCl_2 inhibited Ca++-activated ATPase. Activation of this residual activity by magnesium was of an order of magnitude less than the produced by KCl plus EDTA or by CaCl_2. Heating of inactivated myosin-R at 100° for 15 min caused complete loss of the residual activity. No attempt was made to purify the alkaline stable ATPase from alkaline treated myosin-R.

It seemed possible that myosin-R preparations might be contaminated with some myosin-W, which would be slowly inactivated and which might account for the pH 0 stable ATPase activity of myosin-R. If this were so, one would expect that inactivation would alter the apparent enthalpy of activation (AH*) for Ca++-activated hydrolysis of ATP, since AH* for this process is lower for myosin-W than for myosin-R (2). However, the temperature dependence of Ca++-activated ATPase activity was essentially unchanged by incubation of myosin-R at pH 9.0 for 30 min, although the activity was reduced by about 70%. The values of ΔH* were 8.2 and 7.1 kcal per mole, before and after alkaline inactivation, respectively. After similar treatment of myosin-W the value of ΔH* remained unchanged at 4 kcal per mole.

Properties of Inactivated Myosin-R The sedimentation pattern of myosin-R which had been inactivated for 10 min at pH 9 and then neutralized to pH 7 showed a large peak sedimenting at nearly the same rate as the native myosin-R and a small, faster moving component (Fig. 9). This faster component was present in the native myosin-R, but appeared to be increased in amount.

**TABLE II**

<table>
<thead>
<tr>
<th>Myosin to actin ratio</th>
<th>Loss of ATPase activity in 10 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg myosin/mg actin</td>
<td>%</td>
</tr>
<tr>
<td>∞</td>
<td>62</td>
</tr>
<tr>
<td>8:1</td>
<td>48</td>
</tr>
<tr>
<td>5:1</td>
<td>40</td>
</tr>
<tr>
<td>4:1</td>
<td>44</td>
</tr>
<tr>
<td>3:1</td>
<td>24</td>
</tr>
<tr>
<td>2:1</td>
<td>20</td>
</tr>
</tbody>
</table>

**TABLE III**

Effect of adenine nucleotides and pyrophosphate on alkaline inactivation of myosin-R

Myosin-R (4 mg per ml) was inactivated by incubation in 0.5 mM KCl, 0.04 mM Tris, and 0.04 mM glucose, at pH 9.0 and 25°. Additions of nucleotides or pyrophosphate were made at 1 mM final concentration in the presence of 1 mM MgCl_2, and at 5 mM final concentration in the absence of MgCl_2. After a 5-min incubation at pH 9, 0.1 ml of myosin was added to the assay system containing 0.5 mM KCl, 1 mM EDTA, 5 mM ATP, and 0.04 mM Tris, at pH 7.5 and 25°.

<table>
<thead>
<tr>
<th>Addition</th>
<th>Loss of ATPase activity in 5 min % initial activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>64</td>
</tr>
<tr>
<td>ATP</td>
<td>22</td>
</tr>
<tr>
<td>ADP</td>
<td>22</td>
</tr>
<tr>
<td>AMP</td>
<td>58</td>
</tr>
<tr>
<td>PP</td>
<td>0</td>
</tr>
</tbody>
</table>

**TABLE IV**

ATPase activity of myosin-R after inactivation at pH 9.0

Myosin-R (3.15 mg per ml) was inactivated in 0.04 mM Tris, 0.04 mM glucose, and 0.45 mM KCl, at pH 9.0 and 25°, for 45 min and 0.2-ml aliquots were taken for ATPase assay. The ATPase activity was measured in 0.05 mM KCl, 5 mM ATP, and 0.1 mM Tris, pH 7.5, incubated at 25° for 15 min.

<table>
<thead>
<tr>
<th>Additions</th>
<th>ATPase activity</th>
<th>Relative activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.002 μmole P_i/mg myosin/min</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>0.081 μmole P_i/mg myosin/min</td>
<td>0.11</td>
<td>0.11</td>
</tr>
<tr>
<td>0.023 μmole P_i/mg myosin/min</td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>0.200 μmole P_i/mg myosin/min</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>0.018 μmole P_i/mg myosin/min</td>
<td>0.09</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Fig. 8. Effect of actin on the inactivation of myosin-R at pH 9.0. The inactivation was measured by the incubation method (see the legend to Fig. 2) in an assay medium containing 0.05 mM Tris, 0.05 mM glucose, 0.025 mM KCl, and 10 mM CaCl_2, at pH 9.0. ATP was added at 5 mM concentration, myosin-R at 0.18 mg per ml, and actin at 0.1 mg per ml. O, myosin alone; △, actin present during inactivation; □, actin absent during inactivation but added with ATP to begin the ATPase assay.
after inactivation. No component sedimenting more slowly than myosin appeared.

The reaction of the -SH groups of native and inactivated myosin-R was followed spectrophotometrically by the use of mercuribenzoate and 5,5'-dithiobis(2-nitrobenzoate). Six to eight -SH groups per 10^6 g of myosin-R reacted with mercuribenzoate, and no difference was observed between the native or alkaline inactivated enzyme. While most of the -SH groups of myosin-R reacted almost instantaneously with mercuribenzoate, the reaction with 5,5'-dithiobis(2-nitrobenzoate) was much slower and the rate could be followed spectrophotometrically. There was a small but consistent increase in the rate of reaction of -SH groups after alkaline inactivation of myosin-R (Fig. 10), while the total number of -SH groups reacting with 5,5'-dithiobis(2-nitrobenzoate), approximately 8/10^6 g of myosin-R, was not changed by alkaline inactivation. Sulphydryl-protecting agents, 2-mercaptoethanol (0.04 M) or 1,4-dithiothreitol (0.01 M), had no effect on the rate of the alkaline inactivation of myosin-R.

The digestion of myosin-R with trypsin as followed in the pH-stat revealed a small increase in the rate of digestion with inactivated myosin-R (Fig. 11). The optical rotation of native myosin-R and of myosin-R inactivated at pH 9 was the same. The optical rotatory dispersion curves from 220 to 300 nm for native and inactivated myosin-R were superimposable, and the specific rotation at 233 nm was -8000° per g for both native and inactivated myosin-R.

![Figure 10](http://www.jbc.org/)

**Figure 10.** Reaction of 5,5'-dithiobis(2-nitrobenzoate) with native and alkaline inactivated myosin-R. Myosin-R (9 mg per ml) was inactivated as described in the legend to Fig. 3 at 25° for 10 min. The reaction was carried out in a solution containing 0.5 M KCl, 10^{-3} M EDTA, 10^{-3} M 5,5'-dithiobis(2-nitrobenzoate), 0.1 M potassium phosphate, and myosin (0.74 mg per ml) at pH 7.0 and room temperature (about 20°). The reaction was followed by the change in absorbance at 412 nm, measured in a Zeiss PMQ II spectrophotometer. The blanks used were identical with the samples, except for the absence of myosin. The molar extinction was estimated by allowing an excess of 5,5'-dithiobis(2-nitrobenzoate) to react with reduced glutathione, and a value of $E_m = 1.35 \times 10^4$ per cm was obtained, comparing favorably with the value of 1.36 $\times 10^4$ per cm reported by Ellman (13). O, native myosin-R; □, myosin-R inactivated at pH 9.0 for 10 min.

![Figure 11](http://www.jbc.org/)

**Figure 11.** Tryptic digestion of native and alkaline inactivated myosin-R. Myosin-R (11 mg per ml) was inactivated as described in the legend to Fig. 3. Tryptic digestion was carried out in a solution containing 0.5 M KCl, 1.6 mg of myosin per ml, and 0.067 mg of trypsin per ml at pH 7.0 and 25° in a total volume of 3 ml. The reaction was followed in a Radiometer pH-stat, with 0.002 M NaOH in 0.5 M KCl as titrant. The instrument was calibrated before use by titrating a standard solution of potassium acid phthalate. O, native myosin-R; □, myosin-R inactivated at pH 9.0 for 10 min.

**DISCUSSION**

The fraction of remaining ATPase activity in Fig. 1 is the sum of two exponential terms having positive coefficients $c_1$ and $c_2$ expressed by Equation 1. Therefore, the kinetics of inactivation of myosin-R is consistent with a mechanism involving the presence of two forms of the enzyme being inactivated at different rates or the same form of the enzyme undergoing inactivation in
two steps, the first step resulting in a partially active form. The possibility that the slowing of the rate of inactivation indicates an approach to an equilibrium is unlikely in view of the irreversibility of the inactivation upon returning to pH 7.0. Another possibility that can be ruled out is that the inactivated enzyme protects the active enzyme against inactivation, since the addition of inactivated myosin-R does not alter the rate of inactivation of native myosin-R. The presence of actin (a likely impurity in myosin-R which would protect against inactivation and which, if present, might account for the observed kinetics) is ruled out by the lack of magnesium-activated ATPase activity, the lack of superprecipitation of myosin-R on addition of magnesium and ATP, and the lack of a precipitation reaction with antiserum prepared against actin. The possibility that the residual ATPase is due to contaminating mitochondrial or microsomal ATPase also is ruled out by the lack of Mg++ activation.

The possibility that myosin-R contains a contaminating substance producing inactivation at alkaline pH or that myosin-W contains a protecting contaminant is unlikely in view of the experiments in which the inactivation of mixtures of myosin-W and myosin-R was measured. Previous results (2) also show no evidence for the presence in red muscle of a substance producing inactivation of myosin either at alkaline or at neutral pH. Thus the presence of such a substance must be considered highly unlikely and the differences in alkaline lability of myosin-W and myosin-R probably represent differences in structure or stability of the myosin molecule itself. The most likely explanation of the kinetic results is either that myosin-R preparations contain an alkaline stable and an alkaline labile form of myosin or that the inactivation proceeds in a series of consecutive reactions.

The lack of change of $\Delta \Pi^*$ of Ca++-activated ATPase of myosin-R on alkaline inactivation argues against the possibility that the slowly inactivated fraction of myosin-R might be due to the presence of contaminating myosin-W.

The lack of change in the number of $-SH$ groups of myosin-R reacting with mercuribenzoate or 5,5'-dithiobis(2-nitrobenzoate) after inactivation and the absence of any effect of sulphydryl-protecting agents on the rate of inactivation argue against the view that loss or modification of $-SH$ groups might be involved in the inactivation.

Limited sedimentation studies were carried out with a view toward determining whether the inactivation of myosin-R might involve the formation of slower sedimenting fragments of the type observed on prolonged treatment of myosin at pH values above 10 (15-17). The sedimentation pattern shows no evidence of a slowly sedimenting component. Although the area under the peak of the more rapidly sedimenting component, perhaps representing aggregation of myosin (18), is increased on inactivation, this increase could not account for the loss of 80% of the ATPase activity. One cannot rule out the possibility that aggregated myosin-R might be more stable at alkaline pH than the myosin-R monomer and that the alkaline stable ATPase activity might be due to the presence of aggregated myosin-R. Further work is needed to determine whether molecular changes in monomeric myosin-R may be reflected in the sedimentation behavior.

Taken together, the small changes in sedimentation pattern, the rate of reaction of $-SH$ groups, the rate of tryptic digestion, and the lack of a detectable change in optical rotatory dispersion curves suggest that, at most, only limited changes in secondary or tertiary structure occur on alkaline inactivation of myosin-R. The slight changes in tryptic digestibility and in the rate of reaction with 5,5'-dithiobis(2-nitrobenzoate) might be taken to indicate a small degree of unfolding of the structure of myosin-R during alkaline inactivation. Whether or not these changes are related to loss of enzymic activity, however, must remain an open question.

The similarity between myosin-R and cardiac myosin has been discussed earlier (5), and the present results show that these two types of myosin are also similar with respect to their instability under mild alkaline conditions.

REFERENCES

CORRECTIONS

In the paper by J. C. Siedel (Vol. 242, No. 23, Issue of December 10, 1967, page 5623), on page 5628, left-hand column, the second line from the bottom, "mean residue rotation" should be substituted for "specific rotation" and "degree per g" deleted, so that the last sentence under "Results" reads as follows:

"The optical rotatory dispersion curves from 220 to 300 nm for native and inactivated myosin-R were superimposable, and the mean residue rotation at 233 nm was −8000 for both native and inactivated myosin-R."

In the paper by Richard L. Momparler and Glenn A. Fischer (Vol. 243, No. 16, Issue of August 25, 1968, page 4298), the $K_i$ value for cytosine arabinoside should read "$3.6 \times 10^{-4} M$" instead of "$3.6 \times 10^{-5} M$" and the $K_i$ value for deoxycytidine should read "$1.3 \times 10^{-4} M$" instead of "$1.3 \times 10^{-5} M$" throughout the article.
Studies on Myosin from Red and White Skeletal Muscles of the Rabbit: II.
INACTIVATION OF MYOSIN FROM RED MUSCLES UNDER MILD
ALKALINE CONDITIONS
J. C. Seidel


Access the most updated version of this article at http://www.jbc.org/content/242/23/5623

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/242/23/5623.full.html#ref-list-1