THE CONFORMATION OF MYOSIN DURING THE STEADY STATE OF ATP HYDROLYSIS: STUDIES WITH MYOSIN SPIN LABELED AT THE S_1 THIOL GROUPS[1]

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Summary

MgATP produces a large change in the ESR spectrum of myosin that has been spin labeled at the S_1 thiol groups. This change, indicative of increased mobility of the label, persists during the steady state of the hydrolysis of ATP, and when ATP has been depleted the spectrum changes to one identical with that observed on adding MgADP. It appears that the binding or hydrolysis of ATP by myosin in the presence of Mg induces a conformational change in myosin that persists during the steady state. If, as proposed by Taylor and coworkers (1,2) the predominant species present during the steady state is the myosin-ADP complex, then the conformation of myosin in this complex depends on whether the complex was formed with added ADP or in the course of the hydrolysis of ATP.

Introduction

Our earlier ESR studies on myosin spin labeled at the S_1 thiol group showed that either ATP or ADP increases the mobility of the label (3). This effect, which has been confirmed by Stone (4), was attributed to changes in myosin conformation upon combination with the nucleotide. Morita (5) observed that ATP and ADP produce different changes in the ultraviolet absorption spectrum of heavy meromyosin (HMM). ATP and ADP differ also in their effect on the fluorescence of ANS (8-anilino-1-naphthalene sulfonate) bound to myosin (6). The presumed changes in protein conformation accompanying these changes which, as suggested by several authors may be involved in the molecular mechanism of muscle contraction, appear to be localized, since these

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nucleotides have little or no effect on the optical rotatory dispersion of myosin or HMM (7,8). The present report deals with a change in the ESR spectrum of S_1 spin labeled myosin brought about by interaction with MgATP under conditions of steady state hydrolysis of ATP.

Results and Discussion

If the ESR spectrum of myosin labeled at the S_1 thiol group with an iodoacetamide spin label [N-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl) iodoacetamide] is recorded within two or three minutes after the addition

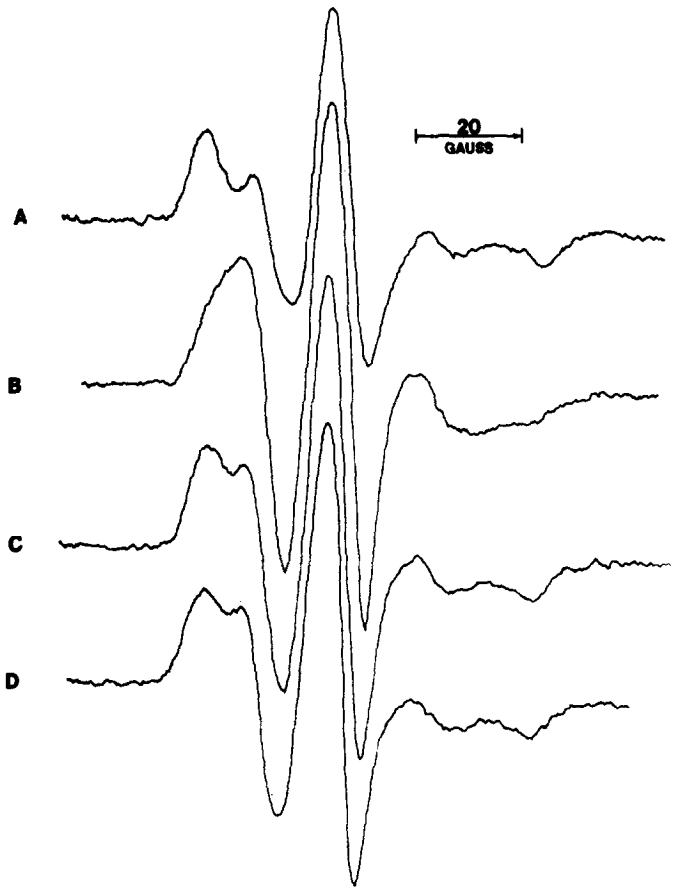

Fig. 1. Effect of MgATP and MgADP on ESR spectra of spin labeled myosin. Selective spin labeling at the S_1 thiol groups with N-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl) iodoacetamide was carried out as previously described (1). Spectra of solutions containing S_1 labeled myosin, 14 mg/ml, 0.4M KCl and 0.04M Tris, pH 7.5, were recorded at room temperature. A, no further addition; B, 5 mM $MgCl_2$ and 5 mM ATP, recorded 2 min. after addition of ATP; C, as B, recorded 10 min. after addition of ATP; D, 5 mM $MgCl_2$ and 5 mM ADP.

of MgATP, a much larger spectral change is found (Fig. 1A,B) than that previously observed (3,4). Spectrum B (Fig. 1) indicates the presence of labels whose mobility is intermediate between that of free labels and that of labels bound to S_1 groups in the absence of nucleotides. As the hydrolysis of ATP proceeds, the spectrum eventually changes to that shown in Fig. 1C, which is essentially the same as that observed on addition of MgADP (Fig. 1D). If one follows the time course of the spectral change by setting the magnetic field at the maximum of peak 2, a steady signal is observed that drops rapidly to the final level after about five minutes (Fig. 2). The time at which this change occurs is doubled by halving the myosin concentration or by doubling the concentration of ATP; thus the change seems to occur when the concentration of ATP decreases to a critical level. In the light of these findings it appears that the spectra observed earlier on adding nucleotides to spin labeled myosin were recorded at a time when essentially all the added ATP had been hydrolyzed. Spectra of the type shown in Fig. 1B were not observed in the absence of Mg^{++}, even when recorded within two minutes after adding ATP; under these conditions the spectra were similar to those shown in Fig. 1C and D.

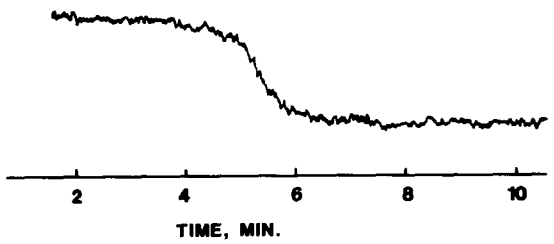

Fig. 2. Time course of change in ESR spectrum of spin labeled myosin following addition of MgATP. To a solution of myosin containing 0.4M KCl, 0.04M Tris, pH 7.5, 5 mM $MgCl_2$, and 14 mg of protein/ml, 5 mM ATP was added at 0 time.

Taylor and coworkers (1,2) found that the kinetics of the hydrolysis of ATP by myosin (M) in the presence of Mg^{++} or Ca^{++} could be described by a scheme in which rapid hydrolysis of ATP is followed by a slower rate

limiting desorption of products:

$$M + ATP \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} M\text{-}ATP \xrightarrow{k_2} M\text{-}ADP\text{-}P \xrightarrow{k_3} M + ADP + P_i \ ,$$

the rate constants k_2 and k_3 being 50 sec^{-1} and 0.02 sec^{-1}, respectively. In this scheme the predominant species during steady state hydrolysis is the myosin-ADP complex. If this interpretation is correct, the changes observed by Morita (5) and Cheung (6) on adding ATP to HMM or myosin should be attributed to the ADP complex rather than to the ATP complex of myosin, since both sets of experiments were carried out under conditions when the predominant form of myosin would be the ADP complex.

On the assumption that also with S_1 modified myosin the rate limiting step in the hydrolysis of ATP is the dissociation of the products, our results can be interpreted in the following way. The ESR spectrum observed during the steady state would be attributable to the myosin-ADP complex resulting from the hydrolysis of ATP. Since this spectrum differs from that obtained on adding MgADP to myosin, the conformation of myosin in the ADP complex resulting from the hydrolysis of ATP must be different from that in the complex formed by adding MgADP to myosin.

If this interpretation is correct, two possibilities have to be considered with regard to the origin of the myosin-ADP complex produced by hydrolysis of ATP. The first would ascribe the change in conformation to the formation of an ATP complex; the new conformation would be stabilized by the ADP produced as a result of the hydrolysis of ATP. The other possibility would be that the spectral change results from a conformational change directly associated with the hydrolysis of ATP, which would persist until the products are desorbed.

Although at present no direct data are available on the detailed kinetics of the hydrolysis of ATP catalyzed by S_1 modified myosin, the assumption that the dissociation of the products is rate limiting appears reasonable. For the ATPase activity of S_1 modified myosin is further accelerated by actin, which, in the case of unmodified myosin (cf. 1,2),

acts by increasing the rate of dissociation of the products. On the other hand, the kinetics of the hydrolysis of ATP by S_1 modified myosin are similar in many respects to those of the K^+(EDTA) activated ATPase of unmodified myosin (9). In the case of the K^+-ATPase the pre-steady state rate of hydrolysis is slower than the steady state rate (1) suggesting that the dissociation of products may not be rate limiting in this case. If this also applies to the S_1 modified myosin, then the ESR spectrum observed during the steady state of the ATP hydrolysis catalyzed by modified myosin may be due to an ATP-myosin complex. Detailed studies of the kinetics of ATP hydrolysis catalyzed by modified myosin and ESR studies under conditons permitting more rapid observation of spectral changes might resolve these questions.

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