Energetics and Kinetics of Interconversion of Two Myosin Subfragment-1·Adenosine 5′-Diphosphate Complexes As Viewed by Phosphorus-31 Nuclear Magnetic Resonance†

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ABSTRACT: The ^{31}P NMR spectrum of MgADP bound to myosin subfragment-1 (S-1) at 0 °C contains two resolved β-phosphate resonances corresponding to two interconvertible conformations of the S-1·ADP complex [Shriver, J. W., & Sykes, B. D. (1981) *Biochemistry 20*, 2004]. The two conformations, M_T*ADP and M_R*ADP, are in slow exchange on the NMR time scale, and the rates of interconversion are <20 s^{-1}. This is consistent with transient kinetic experiments reported in the literature and allows a determination of the rate constants of interconversion: $k+ \approx k- \approx 7\ s^{-1}$ at 0 °C. The relative population of the two conformations is highly temperature dependent, and only one form is significantly populated at 25 °C. Simulations of the ^{31}P NMR spectra are used to evaluate an equilibrium constant at various temperatures from 0 to 25 °C. The standard enthalpy and entropy differences for the R → T transition are determined from the variation of the relative free energies of the two states as a function of temperature: $\Delta H° = 15\ (\pm 2)$ kcal/mol and $\Delta S° = 55\ (\pm 5)$ cal/(deg mol) ($K = 1$ at 271 K). This suggests that a significant conformational change occurs in the R → T transition with MgADP bound in the active site. However, the entropy and enthalpy differences are nearly compensatory at physiological temperatures. At 25 °C the endothermic R → T transition is entropy driven, and $\Delta G° = 1.4$ kcal/mol.

Data from a wide variety of experiments have indicated that at least two distinctly different conformations of the myosin ATPase[1] are possible, depending on the nucleotide occupying the active site; e.g., the transition from the steady-state intermediate M**ADP·P[2] to M*ADP involves a significant conformational change as indicated by fluorescence spectroscopy (Werber et al., 1972; White & Taylor, 1976; Bechet et al., 1979), EPR (Seidel & Gergeley, 1971), UV difference spectroscopy (Morita, 1967), chemical modification (Onishi & Morales, 1976; Burke, 1980), and binding experiments (Wolcott & Boyer, 1974; Goody et al., 1977; Lowey & Luck, 1969). We have demonstrated by using ^{31}P NMR that both ADP and AMP·PNP bind to myosin S-1 to give two discrete, interconvertible conformations with a highly temperature-dependent equilibrium (Shriver & Sykes, 1980, 1981a). Preliminary ^{19}F NMR results with fluorine-labeled S-1 indicate that there are also two states, or conformations, of S-1 in the absence of nucleotide, again with a highly temperature-dependent equilibrium (J. W. Shriver and B. D. Sykes, unpublished experiments). These results emphasize that myosin and myosin–nucleotide complexes cannot be viewed as single, unique species but actually exist as an equilibrium mixture of at least two forms. Morita (1977) has recently shown that the myosin–AMP·PNP complex can be driven from an ATP-like complex to an ADP-like complex with decreasing temperature. We have argued from this and other data (Shriver & Sykes, 1981a) that there are two fundamental states of myosin and their relative population is determined by the nucleotide in the active site and the temperature.

Attempts to correlate biochemical states observed in solution with structural states (observed or proposed) in muscle have assigned chemical states with discrete angular configurations of myosin relative to actin, e.g., the M**ADP·P complex with actin is assigned at 90° configuration and the M*ADP–actin complex is assigned at 45° configuration (Eisenberg & Greene, 1980). Our results (Shriver & Sykes, 1981a) suggest that it is not the chemical states themselves which should be assigned to particular configurations of the actomyosin complex, but rather the two states of each myosin–nucleotide complex may correspond to the 45° and 90° states in the actomyosin complex.[3] This is supported by the correspondence between the ability of ADP and AMP·PNP to differently populate the two conformations and their differing ability to populate the two configurations in a muscle fiber reported by Marston et al. (1979). This makes the myosin scheme similar to models which have been proposed for energy-transducing ATPases of membranes, e.g., the E and *E forms of the Ca^{2+}–ATPase of sarcoplasmic reticulum which differ in their positioning of the Ca^{2+} binding site on the inner and outer membrane surface (deMeis & Vianna, 1979; Hill, 1977).

We present here a ^{31}P NMR study of the energetics of the interconversion of the two myosin S-1·ADP states, M_T*ADP and M_R*ADP. The temperature dependence of the relative populations of these two forms is used to determine the enthalpy and entropy differences between the M_T*ADP and

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[1] Abbreviations used: M, myosin; S-1, myosin subfragment-1; HMM, heavy meromyosin; ATPase, adenosine-5′-triphosphatase; ADP, adenosine 5′-diphosphate; ATP, adenosine 5′-triphosphate; P, orthophosphate; Pipes, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid); DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); S-1(A1), myosin subfragment-1 with the alkali-1 light chain; S-1(A2), myosin subfragment-1 with the alkali-2 light chain; EDTA, ethylenediaminetetraacetic acid; AP_5A, P^1,P^5-diadenosine 5′-pentaphosphate; AMP, adenosine 5′-monophosphate; $NaDodSO_4$, sodium dodecyl sulfate; DEAE, diethylaminoethyl.

[2] M**ADP·P designates a specific ternary complex of myosin with ADP and P which immediately follows M*ATP in the myosin ATPase reaction (Bagshaw & Trentham, 1974). Asterisks denote relative intrinsic fluorescence intensity. M*ADP designates a myosin–ADP complex which has a higher intrinsic fluorescence than free myosin. M*ADP is actually two complexes which we designate M_T*ADP and M_R*ADP (Shriver & Sykes, 1981a).

[3] Note that the numbers 90° and 45° are approximate values, at best, and are used here merely as a convenient means of referring to the two cross-bridge states before and after the power stroke.

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M_R*ADP states. In addition, information is gained on the rates of interconversion of the two states.

Materials and Methods

Myosin subfragment-1 was prepared as described previously by limited chymotryptic digestion of myosin filaments extracted from rabbit back and leg muscle (Shriver & Sykes, 1981a). The isozymes S-1(A1) and S-1(A2) were separated by DEAE-cellulose chromatography as described by Weeds & Taylor (1975). No DTNB light chain was present in these preparations as indicated by $NaDodSO_4$ gel electrophoresis (Weeds & Taylor, 1975). ADP was obtained from Sigma Chemical Co. (vanadium-free from yeast) and checked for purity by ^{31}P NMR; the only noticeable impurities being AMP and P. There was no evidence of AMP binding to S-1, and we have demonstrated that under the conditions used here P does not alter spectra of S-1·ADP complexes (Shriver et al., 1979; Shriver & Sykes, 1981a). D_2O was obtained from Bio-Rad Laboratories and stored over Chelex-100 to remove paramagnetic impurities. In addition, all spectra were run in the presence of 0.1 mM EDTA. AP_5A was obtained from Sigma Chemical Co.

ATPase activities were determined by using a Radiometer pH stat. Ca^{2+}–ATPase activity was measured in 0.6 M KCl, 10 mM $CaCl_2$, and 5 mM ATP at 25 °C and pH 7.9. K^+-EDTA–ATPase activity was measured in 0.6 M KCl, 5 mM EDTA, and 5 mM ATP at 25 °C and pH 7.9 Typically the Ca^{2+}– and K^+EDTA–ATPase activities were 1.1 and 13 s^{-1}, respectively.

^{31}P NMR spectra were collected on a Bruker HXS-270 instrument operating in the Fourier transform mode with quadrature detection. The spectrometer was interfaced to a Nicolet 1180 computer with a Diablo disc drive. Free-induction decay transients were stored digitally on high-density magnetic discs. Flat-bottomed 10-mm polished NMR tubes (Wilmad) were used throughout with a Teflon vortex suppression plug. The sample volume was typically 1 mL. Sweep widths of ±2500 Hz were used with an acquisition time of 0.46 s and a pulse delay of 1.6 s. The typical flip angle was 75° (17 μs pulse time). Generally 5000 transients were required to obtain a reasonable signal-to-noise ratio in the spectrum. The C6 methylene protons were routinely decoupled in all spectra. Chemical shifts were measured relative to an external standard of 85% H_3PO_4 in a capillary fixed in the center of the NMR tube.

The standard enthalpy difference between two myosin S-1 states observed by ^{31}P NMR was determined by using the van't Hoff expression

$$\left[\frac{\partial(R \ln K)}{\partial(1/T)}\right]_P = -\Delta H°$$

i.e., the slope of at least-squares fit of $-R \ln K$ vs. the reciprocal of the temperature in kelvin is $\Delta H°$. Equilibrium constants were derived from experimental spectra by using a Nicolet 1180 Basic computer program (written by L. Kay) which superimposed experimental and simulated spectra. Simulated spectra were calculated by assuming two-site exchange between two resonances which are doublets with an indirect spin–spin coupling constant of 17 Hz (the coupling constant observed in free MgADP). It was assumed that the temperature dependence of the chemical shifts was similar for the two sites. The standard entropy was then derived from

$$\Delta G° = \Delta H° - T\Delta S°$$

knowing that $\Delta G° = 0$ when $K = 1$. The temperature at which $K = 1$ was derived from a least-squares fit of the data.

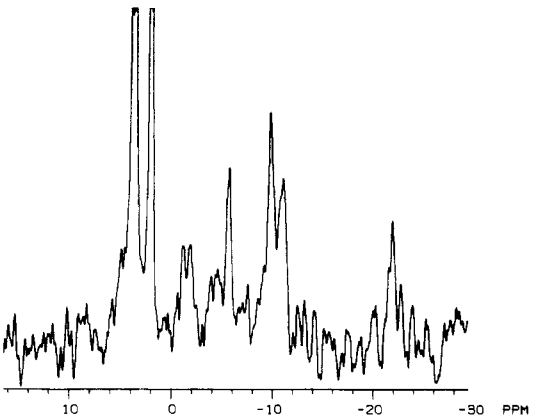

FIGURE 1: ^{31}P NMR spectrum of the myosin subfragment-1·ADP complex at 0 °C. A small amount of contaminating adenylate kinase coupled with the S-1 ATPase leads to ADP hydrolysis with production of AMP and P, seen at 3.5 and 2.0 ppm, respectively. An excess of ADP is present to allow sufficient time for spectral accumulation; free β-phosphate contributes at -5.84 ppm and free α-phosphate at -9.83 ppm. AP_5A has been added to inhibit adenylate kinase and contributes resonances at −11 and −22 ppm. The bound α-phosphate contributes at −9.8 ppm and can be seen as a difference between the free α- and β-phosphate resonances. The bound β-phosphate contributes two resonances at -1.15 and −1.9 ppm. Conditions: 0.5 mm S-1(A1,A2), 1 mM ADP, 5 mM $MgCl_2$; 0.1 mM EDTA, 0.1 M KCl, 50 mM Pipes, 100 μM AP_5A, and 50% D_2O, pH 7; 30 000 transients; 75° pulse angle; 0.41-s acquisition time; 1.6-s pulse delay; 25-Hz line broadening.

Results

Figure 1 shows the ^{31}P NMR spectrum of a solution of myosin subfragment-1 plus MgADP at 273 K. Even after the special precautions taken to purify S-1 used in these experiments, a small amount of myokinase still remained which when coupled with the S-1 ATPase resulted in hydrolysis of ADP with production of AMP and P. This activity was slowed, but not eliminated, by the addition of AP_5A, a myokinase inhibitor. P and AMP contribute resonances at 2.0 and 3.5 ppm, respectively. An excess of ADP over the S-1 ATPase sites was used to allow sufficient time for spectrum accumulation. Free ADP resonances can be seen at −5.84 (β-phosphate) and −9.83 ppm (α-phosphate). AP_5A resonances appear at −11.0 and −22 ppm. Bound ADP contributes an α-phosphate resonance at approximately −9.9 ppm which can be seen as a difference in the apparent intensities of the α- and β-phosphate resonances of free ADP. There are two β-phosphate resonances of bound ADP at −1.15 and −1.9 ppm, indicating two states for the S-1·ADP complex. It has been previously demonstrated that these are not due to A1 and A2 light chain heterogeneity or formation of the M·ADP·P ternary complex (Shriver & Sykes, 1981a). The temperature variation of the spectra is not consistent with heavy-chain heterogeneity. Analysis of the equilibrium between the two states is made by using the β-phosphate resonances.

Figure 2A shows the bound β-phosphate region of S-1·ADP spectra at various temperatures from 0 to 25 °C. As the temperature was increased from 0 °C the intensity of the low-field bound β-phosphate resonance decreased and the high-field resonance increased, which resulted in an increased signal-to-noise ratio and permitted shorter accumulation times. Because of the different accumulation times a quantitative comparison of the absolute intensities of the β-phosphate peaks at different temperatures cannot be made. We consider here the relative intensities of the two bound β-phosphate peaks in the region of 1.15–1.9 ppm. These data are consistent with

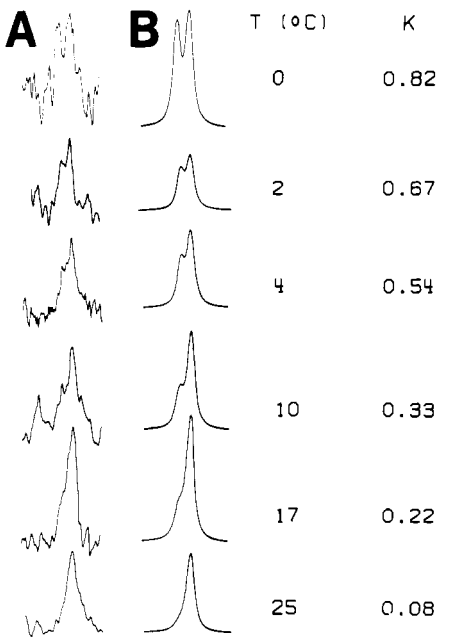

FIGURE 2: (A) Bound β-phosphate resonance region of the ^{31}P NMR spectra of myosin subfragment-1·ADP complex at various temperatures. Conditions were as described in Figure 1 except the temperature was varied from 0 to 25 °C as indicated and fewer transients were required at higher temperatures for spectra with adequate signal-to-noise ratios, e.g., at 25 °C only 3000 transients were accumulated. The spectra are shown with increasing vertical expansion factors with decreasing temperature so that the absolute intensity of the H peak is approximately the same throughout. (B) Simulations of the spectra shown in (A). Simulations were performed as described in the text. The relative populations of the two forms derived from the simulations were used to determine K(= [L]/[H]).

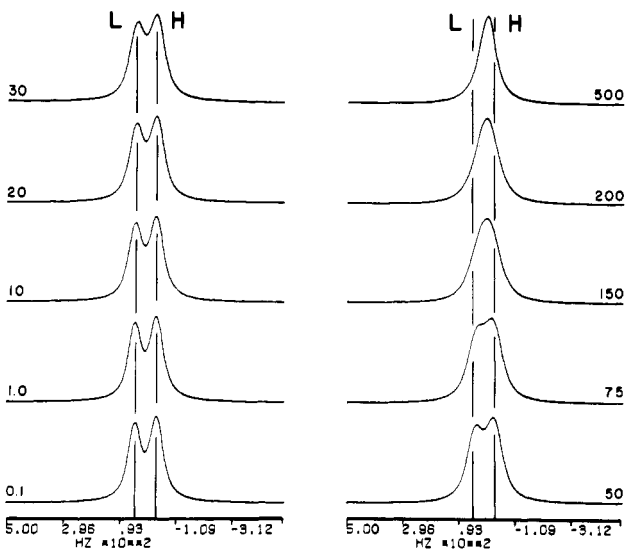

FIGURE 3: Simulations of β-phosphate resonances of the two ADP states, L and H, with various rates of interconversion. The L resonance is the left-most, low-field peak (see text). Both resonances are doublets with J = 17 Hz. The equilibrium constant, line separation, and natural line widths are assigned values which give the optimal fit to the 0 °C experimental spectrum: K = [L]/[H] = 0.82, linewidth of L = 47 Hz, line width of H = 53 Hz, and line separation = 80 Hz. The rate constant defining the conversion of H → L was varied as indicated. The spectra have been scaled so that the maximum peak height is constant.

the interconversion of two bound myosin ADP states with a highly temperature dependent equilibrium constant. The data do not rule out the possibility of other bound ADP states which might contribute to other regions of the spectrum.

With increasing temperature the myosin S-1 state resulting in the more shifted (low-field) resonance (−1.15 ppm) is driven into the state giving the least shifted (high-field) resonance (−1.9 ppm). For convenience of discussion we label the low-temperature (low-field) state the L state and the high-temperature (high-field) form the H state. From simulations of the experimental spectra, we can evaluate an equilibrium constant and obtain information on the magnitude of the rate constants defining the interconversion of the two forms. At 0 °C the two forms are in slow exchange

$$H \underset{k_-}{\overset{k_+}{\rightleftharpoons}} L$$

on the NMR time scale, i.e., k_+ and k_- are $<2\pi \times 80$ Hz, or 500 s^{-1}. The line width of the H form was shown to be 53 (±3) Hz from a simulation of the 25 °C spectrum.[4] This line width may be used to adequately fit either of the two resonances seen in the 0 °C spectrum, which indicates that slow exchange broadening does not make a significant contribution to the line widths at 0 °C. The equilibrium constant (K = [L]/[H]) at 0 °C was obtained from a fitting of the 0 °C spectrum by using the above line widths and a line separation of 80 Hz which is approximately equal to the observed separation. The ^{31}P NMR spectrum is a reliable representation of the relative populations if the two resonances were not saturated or, if there was saturation, the two forms have the same T_1 relaxation times. Determination of the T_1 times of the two forms was not practical due to the length of time needed to obtain a spectrum at 0 °C. However, negligible saturation is expected under the conditions used, and the two forms are expected to have essentially the same T_1 times. (Note that they have essentially the same T_2 relaxation times as indicated by the line widths.) In Figure 3 we show simulations of two exchanging resonances obtained by using the above values for the natural line widths, line separation, and equilibrium constant. The rate of conversion of the H form to the L form is varied from 0.1 to 500 s^{-1}, and the reverse rate constant is determined by the equilibrium constant. It can be seen that the apparent line widths and chemical shifts are influenced by the values of the rate constants for values greater than ~20 s^{-1}. Therefore the rate constants defining the interconversion of the two forms are less than 20 s^{-1} at 0 °C.

Simulations of the ^{31}P NMR spectra at temperatures above 0 °C (Figure 2B) were performed without modifying the chemical shift difference and natural line widths from the 0 °C simulation. A consistent but not unique set of simulations was obtained by making the temperature dependence of the rate constant defining the conversion of the L form to the H form result primarily from the temperature dependence of the equilibrium constant. It should be noted, however, that the data above 0 °C are not sufficient to define a unique set of rate constants but only the equilibrium constants. The spectra were simulated by assuming slow exchange between the two forms throughout the temperature range studied. The observed spectra are not consistent with simply increased rates of interconversion with increasing temperature and therefore exchange averaging of the two resonances, since the *relative* intensities are obviously changing over the observed temper-

[4] Note that both β-phosphate resonances are doublets due to spin–spin coupling with the α-phosphate. The doublets are not resolved due to the naturally broad lines and digital filtering of the free induction decay (which leads to an additional 30-Hz line broadening). The β-phosphate doublet of free MgADP is resolved in high-resolution spectra such as Figure 1A of Shriver & Sykes (1981a). Thus the observed line width of 53 Hz for the H form at 25 °C is a result of a natural line width of 23 (±3) Hz, a line broadening of 30 Hz, and a coupling constant of 17 Hz.

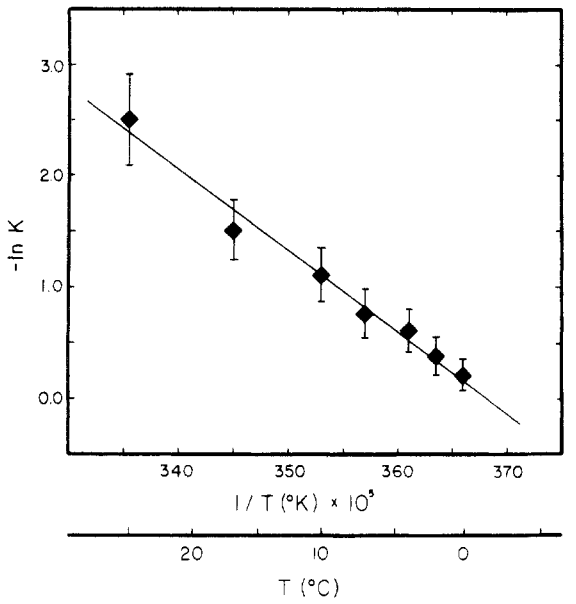

FIGURE 4: van't Hoff plot of the temperature dependence of the equilibrium constant K, which was derived from the fitting of ^{31}P NMR spectra of the β-phosphate of bound ADP. The estimated error is shown with errors bars.

ature range. Exchange averaging would result in a series of spectra similar to those shown in Figure 3, where the *relative* intensities of the two peaks remains constant.

Populations of the two forms at various temperatures were obtained from simulations of the ^{31}P NMR spectra (see Figure 2), and a plot of $-\ln K$ vs. the reciprocal of the temperature is given in Figure 4. A least-squares fit of the data results in $\Delta H° = -15$ (±2) kcal/mol (for the H → L transition) and $K_3 = 1$ at 271 K. Thus, $\Delta S° = -55$ (±5) cal/(deg mol). Spectra at pH 8 and 4 °C and 25 °C were essentially identical with those obtained at pH 7, implying that the thermodynamic parameters do not contain a significant contribution from buffer ionization. These values, particularly the large entropy difference, suggest a relatively large conformational change (both structurally and energetically) for the H → L transition (with MgADP in the active site). However, at 25 °C the two forms differ in free energy by only 1.4 kcal. Thus, although the H ⇌ L interconversion represents a significant conformational change, the entropy change is compensated by a similar enthalpy change and results in the two conformations having nearly equal free energies at temperatures near physiological temperatures.

Discussion

We have presented here evidence for two myosin subfragment-1·ADP states, the relative population of which is highly temperature dependent. Previous studies have presented spectroscopic data demonstrating a temperature-driven conversion of one myosin form to another with AMP·PNP in the active site. Bechet et al. (1979) have demonstrated that the fluorescence of HMM–AMP·PNP complexes relative to that of HMM increases with increasing temperature in a manner suggestive of a titration of one state to another. Similarly, Morita (1977) has shown a conversion of the UV difference spectrum of the S-1·AMP·PNP complex from an M·ADP-type spectrum at low temperatures to an M·ATP-type spectrum at high temperatures. Consistent with these data is the observation of two bound AMP·PNP states as seen by ^{31}P NMR with a temperature-dependent equilibrium constant (Shriver & Sykes, 1981a). ^{19}F NMR spectra of sulfhydryl-1-labeled S-1 in the absence of nucleotide also show two states with a temperature-dependent equilibrium constant.

Previous spectroscopic evidence for two states of the S-1·ADP complex is not as clear as that for AMP·PNP complexes. This may derive from the fact that both the fluorescence and UV difference spectroscopy data are measured relative to S-1 in the absence of nucleotides, which itself exhibits a thermotropic reaction. However, the ^{31}P NMR data presented here clearly demonstrate two states for the S-1·ADP complex. The data of Bechet et al. (1979) show a relative increase in fluorescence intensity with decreasing temperature. In addition, the data of K. Trybus and E. W. Taylor (unpublished experiments) appear to show an increase in the amplitude of the fluorescence transient of S-1 upon binding ADP with decreasing temperature [8.5% at 20 °C (extrapolated to infinite ADP dilition) and 10% at 3 °C]. Therefore we have tentatively assigned the low-temperature form (i.e., the L state above) to the higher fluorescence form of the two S-1·ADP complex states.

The existence of two states for myosin S-1 in both the presence and absence of nucleotides such as ADP and AMP·PNP implies that there are two fundamental interconvertible states of myosin S-1. Thus we have proposed that the conceptually simplest ADP binding scheme may be written as follows (Shriver & Sykes, 1981a,b)

$$\begin{array}{ccccc} M_T + ADP & \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} & M_T{\cdot}ADP & \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} & M_T{}^*ADP \\ k_{-0}\big\downarrow\big\uparrow k_0 & & k_{-6}\big\downarrow\big\uparrow k_6 & & k_{-3}\big\downarrow\big\uparrow k_3 \\ M_R + ADP & \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} & M_R{\cdot}ADP & \underset{k_{-5}}{\overset{k_5}{\rightleftharpoons}} & M_R{}^*ADP \end{array}$$

where M_T·ADP and M_R·ADP are loosely bound, transitory recognition complexes in fast NMR exchange with free S-1 and M_T*ADP and M_R*ADP are the two forms viewed by ^{31}P NMR. Using a spectrum-fitting program, we have shown here that M_T*ADP and M_R*ADP are in very slow exchange on the NMR time scale, i.e., the interconversion rates are <20 s^{-1}. M_R*ADP is assigned to be the high-fluorescence S-1·ADP complex, or the L state discussed under Results. This scheme is slightly different from that considered by K. Trybus and E. W. Taylor, and in the Appendix we show that the slow phase of the biphasic transient observed by Trybus and Taylor at 3 °C is given by $k_3 + k_{-3}$. Also, since the amplitude of the slow phase is positive, $A_2 > 0$; thus, $k_3' > \bar{k}_3$. Substitution shows that

$$k_{-0}/k_0 > k_{-3}/k_3$$

or

$$[M_R{}^*ADP]/[M_T{}^*ADP] > [M_R]/[M_T]$$

Thus, since $K_3 = 1$ at 0 °C, to a first approximation the binding process at 0 °C may be viewed as a three-step process with M_T predominating over M_R and reactions 1, 2, and 3 being the preferred pathway. The value of $\bar{k}_3$ ($=k_3 + k_{-3} = 15\ s^{-1}$) at 3 °C derived from the transient kinetics experiments is consistent with our NMR results. These two results allow us to calculate approximate values for k_3 and k_{-3} at 0 °C: $k_3 \approx k_{-3} \approx 7\ s^{-1}$. At temperatures above 10 °C the binding of ADP results in M_T*ADP primarily, and the binding enthalpies determined by various workers (Swenson & Ritchie, 1979; Kodama et al., 1977) are assigned to steps 1 and 2.

Morita (1977) has shown that heavy meromyosin–AMP·PNP complexes give an "M·ATP-type" UV difference spectrum (i.e., the same spectrum as obtained during the steady-state hydrolysis of ATP at 25 °C) at high temperatures and an "M·ADP-type" spectrum at low temperatures. Morita has interpreted this as AMP·PNP mimicking the M**ADP·P in-

termediate at high temperature and M*ADP at low temperature. However, the structure and charge of AMP·PNP are very similar to those of ATP, and while AMP·PNP may serve as an analogue of ADP·P in M**ADP·P, it is difficult to visualize how AMP·PNP could serve as an ADP analogue unless ATP could also. Both ^{31}P NMR and fluorescence spectroscopy indicate that there are two S-1·AMP·PNP states. ^{31}P NMR results presented here have provided important clear evidence that AMP·PNP is not unique in this property and ADP can also form two discrete M*ADP states. We have interpreted the AMP·PNP data therefore as indicating that there are two ATP bound states, both of which are significantly populated when the analogue AMP·PNP is used (Shriver & Sykes, 1981a). We have proposed that there are two fundamental conformations of myosin S-1, M_T and M_R, and at 25 °C ADP binds to M_T to give predominantly the M_T*ADP complex whereas AMP·PNP is able to crossover to the M_R*AMP·PNP state. An AMP·PNP-induced endothermic conformational change has been suggested by Swenson & Ritchie (1979) to explain the near zero binding enthalpy of AMP·PNP. At low temperature AMP-PNP cannot force the T ⇌ R reaction to the right, and only the M_T*AMP·PNP complex is significantly populated. The UV difference spectroscopy data of Morita (1977) indicate that M_T*AMP·PNP and M_T*ADP are structurally very similar and support the idea of two fundamental, marcomolecular states of myosin, with an equilibrium constant which is a function of the temperature and bound nucleotide.

The ^{31}P NMR evidence given here and in Shriver & Sykes (1981a) implies that the activated complex M**ADP·P is in the R form and M*ADP is in predominantly the T form at 25 °C. The presently accepted model of muscle contraction maintains that there are two discrete actomyosin states, the so called 90° and 45° configurations, and a transition from the 90° to the 45° state is the power stroke. It has been presumed by a number of workers that the power stroke occurs concomitantly with the release of phosphate (White & Taylor, 1976; Eisenberg & Greene, 1980) and therefore it would appear that the power stroke is an R → T transition. We have argued in Shriver & Sykes (1981a) that there is a direct correspondence between the two states observed in the presence and absence of actin and the R → T reaction represents an energy-transducing transition. It is interesting to compare the thermodynamic parameters for the myosin ATPase transition reported here with a possibly similar transition seen for the Ca^{2+}–ATPase of sarcoplasmic reticulum. Evidence has been presented for two functional forms of the sarcoplasmic reticulum Ca^{2+}–ATPase which differ in the positioning of the high-affinity Ca^{2+} binding site in the membrane. Inesi et al. (1976) have shown that for these two forms $\Delta H° = 13$ kcal/mol and $\Delta S° = 44$ cal/(deg mol).

Acknowledgments

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Appendix

We give here an expression defining the time dependence of the appearance of M_R*ADP, the more fluorescent of the two myosin–ADP complexes, upon mixing ADP with M_T and M_R. This expression is used to derive an approximate value for $k_3 + k_{-3}$ at 3 °C. We assume that the rates of interconversion of the two conformations are slow relative to ADP binding, i.e., ADP binds rapidly to a mixture of M_T and M_R to give M_T*ADP and M_R*ADP. The M_T*ADP and M_R*ADP mixture then relaxes to an equilibrium defined by K_3. k_{-2} and k_{-5} determine the off-rate of ADP and are small, approximately 1.4 s^{-1} at 20 °C and 0.07 s^{-1} at 5 °C (Bagshaw & Trentham, 1974). For a first approximation we may set $K_1 = K_4$ and $k_2 = k_5$; this allows for a simple kinetic expression, and the error introduced would appear predominantly in the fast phase as long as k_2 and k_5 are significantly greater than k_3 and k_{-3}. An expression describing the appearance of M_R*ADP may be derived by using the Laplace–Carson operator method (Roberts, 1977) and is given by

$$[M_R*ADP] = \frac{M_0K_3}{K_3 + 1}(1 + A_1e^{-\lambda t} - A_2e^{-\bar{k}_3 t})$$

$$\lambda = \frac{k_2K_1[ADP]}{K_1[ADP] + 1}$$

$$\bar{k}_3 = k_3 + k_{-3}$$

$$A_1 = \frac{k_3' - \lambda}{k_3'} \frac{\bar{k}_3}{\lambda - \bar{k}_3}$$

$$A_2 = \frac{k_3' - \bar{k}_3}{k_3'} \frac{\lambda}{\lambda - \bar{k}_3}$$

$$k_3' = k_3(1 + K_0^{-1})$$

$$K_i = \frac{k_i}{k_{-i}}$$

λ ($\approx k_2$) is the rate constant defining the fast phase and is 150–200 s^{-1}; $\bar{k}_3$ defines the slow phase and is 15 ($\pm$10) s^{-1}, from the results of Trybus & Taylor (1979).

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Different Polymeric Forms of Actin Detected by the Fluorescent Probe Terbium Ion†

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ABSTRACT: The interaction of actin with Tb^{3+} was studied by following the fluorescence emitted at 545 nm when the protein was excited at 285 nm in the presence of Tb^{3+}. It was shown that, at low ionic strength, each actin monomer binds, at saturation, six Tb^{3+} with an association constant of 0.8 μM^{-1}. In the presence of 0.1 M KCl the association constant decreases to 0.15 and 0.24 μM^{-1} at subcritical and overcritical actin concentrations, respectively; the number of the binding sites remains six. When polymeric actin is formed by the addition of 2 mM $MgCl_2$, the association constant drops to 0.008 μM^{-1} and the number of the binding sites to four. The lower number of the Tb^{3+} binding sites (four) in the actin polymerized by $MgCl_2$ as compared to the number of binding sites (six) of the actin polymerized by KCl is taken as evidence of a looser structure of this latter polymer. We have also shown that Tb^{3+} does not replace $^{45}Ca^{2+}$ at the single, "high-affinity" site of G-actin. Removal of this Ca^{2+}, in the presence of Tb^{3+}, destroys the typical G- and F-actin structures.

G-actin is polymerized by many agents: monovalent cations at high concentration, divalent cations (Kasai et al., 1962), polyamines (Oriol-Audit, 1978), and lanthanides (Barden & Dos Remedios, 1980). The nature of the polymer formed depends on the nature as well as on the concentration of the polymerizing agent. One millimolar Ca^{2+} and Mg^{2+} form double-stranded F-actin. When the concentration of the divalent cation is increased to 5 mM, paracrystals are formed. Ni^{2+} produces F-actin filaments which tend to break into short fragments. Zn^{2+} forms globular aggregates which coexist with the filaments (Strzelecka-Golaszewka et al., 1978). G-actin, in the presence of gadolinium ions, forms microcrystalline aggregates which, by addition of 0.1 M KCl, are converted into tubular structures (Dos Remedios & Dickens, 1978). In this work the study of the interaction between actin and Tb^{3+} reveals that the polymers formed by treating G-actin with either 0.1 M KCl or 2 mM $MgCl_2$ behave differently with respect to Tb^{3+} ions. These differences could be explained by a more compact structure of the polymer formed in the presence of Mg^{2+}. Studies are in progress to inquire whether the conformational differences of the two polymers are accompanied by functional differences.

Materials and Methods

G-actin from rabbit muscle was prepared according to Spudich & Watt (1971) and stored as a concentrated solution (5 mg/mL) at 0 °C in 0.2 mM ATP,[1] 0.2 mM $CaCl_2$, 0.5 mM mercaptoethanol, 0.5 mM NaN_3, and 2 mM Tris-HCl buffer, pH 8.2. Immediately before use the actin stock solution, diluted with an equal volume of 30 mM imidazole hydrochloride buffer, pH 7.0, was treated for 2 min at 2 °C with 1:15 (v/v) wet 100–200-mesh Dowex 1-X8 Cl form resin to remove free ATP. The solution was then separated from the resin by centrifugation and treated, by the same procedure, with 1:15 (v/v) wet 100–200-mesh Dowex 50-X2 K form resin to remove free or weakly bound Ca^{2+}, the resin being equilibrated in all the cases with 15 mM imidazole hydrochloride buffer, pH 7.0. Finally, the protein solution was adjusted to the desired concentration with 15 mM imidazole hydrochloride buffer, pH 7.0. By this procedure no appreciable loss in protein concentration or biological activity was observed. G-actin retained fully the capability to polymerize and to inhibit DNase I.

K-F-actin and Mg-F-actin were obtained by addition of 0.1 M KCl and 2 mM $MgCl_2$, respectively, to the G-actin treated with the ion-exchange resins. The formation of the polymer was followed until constant viscosity was reached. Alternatively, when low concentrations of actin were employed, the state of aggregation of the protein was ascertained by 90° light scattering measurements (incident light 580 nm, scattered light 580 nm). $TbCl_3$ was from Ventron-Gmbh, Karlsruhe, Germany; $^{45}CaCl_2$, carrier free, was from Amersham, England. Twice distilled water was used in all the operations, the first distillation being performed in the presence of $KMnO_4$.

Actin concentration was measured from the absorbance at 290 nm, the absorbance of 1 mg of pure actin/mL (light path 1 cm) being taken to be 0.62 (Gordon et al., 1976). Alternatively, the method of Lowry et al. (1951) was used. The molar concentration of G-actin was calculated on the basis of a molecular weight of 42 000 (Collins & Elzinga, 1975).

Calcium concentration was measured with a Perkin-Elmer atomic absorption spectrophotometer, Model 603, after deproteinization of the solution with 5% $HClO_4$. [$^{45}Ca^{2+}$]G-actin

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[1] Abbreviations used: ATP, adenosine 5′-triphosphate; Tris, tris(hydroxymethyl)aminomethane; DNase, deoxyribonuclease.

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