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The kinetics of a two-state transition of myosin subfragment 1

A temperature-jump relaxation study

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Temperature-jump measurements were carried out on myosin subfragment 1 (S1) labeled at Cys-707 with 5-(iodoacetamido)fluorescein (S1-AF). The relaxation was monitored by following the increase in the fluorescence intensity of the attached probe after a jump of 5.8°C. A single relaxation process was observed over a range of final temperatures, and the relaxation time decreased from 16.69 ms at 15°C to 3.91 ms at 27°C. The relaxation results are interpreted in terms of a two-state transition: $(S1\text{-}AF)_L \underset{k_-}{\overset{k_+}{\rightleftharpoons}} (S1\text{-}AF)_H$, and the observed single relaxation time (τ) equals $1/(k_+ + k_-)$. The individual first-order rate constants, k_+ and k_-, were calculated from τ and the equilibrium constant previously determined. The activation energy was 21.9 kcal/mol for the forward reaction and 9.3 kcal/mol for the reverse reaction, corresponding to an enthalpy value of 12.6 kcal/mol for the two-state transition. The results provide, for the first time, direct kinetic evidence of a two-state transition of S1 in the absence of bound nucleotide, and support a two-state model of unliganded myosin subfragment 1.

Temperature jump; Myosin subfragment 1

1. INTRODUCTION

Multiple conformational states of the actomyosin complex and the myosin–nucleotide complex have been demonstrated in a variety of equilibrium [1–3] and kinetic [4–9] studies. These states are components of the actomyosin ATPase pathway and are involved in force generation in muscle. The existence of two temperature-sensitive equilibrium states of the complex formed between myosin subunit 1 (S1) and nucleotide was reported in studies with ^{31}P NMR [3,10] and with εADP [11,12]. Two approaches have been used with extrinsic probes covalently linked to Cys-707 (SH_1) of S1 to demonstrate a two-state equilibrium of S1 in the absence of bound nucleotide. These are ^{19}F NMR [13] and the fluorescence of iodoacetamidofluorescein (IAF) [11]. The use of IAF has additionally allowed detection of two equilibrium states of S1 liganded to actin and actin plus ADP, and determination of the energetics of these two-state transitions [14]. Elucidation of the mechanism of actomyosin ATPase is dependent upon an understanding of the equilibrium and kinetic properties of the various states of these complexes. The enthalpy values for some of the transitions are positive and large, and suggest that the kinetics of these transitions can be studied by temperature-jump relaxation. This method is particularly useful for a simple unimolecular transition. In this communication we report on a preliminary study of the kinetics of the two-state transition of S1 labeled with IAF at Cys-707. The results show a well-defined single relaxation, providing the first direct kinetic evidence of two states of unliganded myosin subfragment 1.

Abbreviations: S1, myosin subfragment 1; IAF, 5-(iodoacetamido)-fluorescein; S1-AF, S1 labeled with IAF; TES, *N*-[tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid; εADP, 1,N^6-ethenoadenosine 5′-diphosphate.

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2. EXPERIMENTAL

2.1. *Materials*

The preparation of S1 and its labeling with IAF at Cys-707 were previously described [14]. The degree of SH_1 labeling was in the range of 0.90–0.95, as determined by the absorbance of the probe. IAF was purchased from Molecular Probes (Junction City, OR), and all other chemicals and reagents were of reagent grade.

2.2. *Temperature-jump measurements*

Temperature-jump relaxation measurements were carried out on a Joule heating Hi-Tech TJ/SF-54 spectrometer. A temperature rise of 5.8°C was achieved in 10 μs by discharging 11 kV through the sample in a volume of 0.1 ml. We found that the original 75 W xenon lamp was inadequate as the excitation light source for temperature-jump measurement because of the generally small change in fluorescence intensity resulting from small perturbations. For the measurements reported here, the small lamp was replaced by a 150 W Osram XOB

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xenon lamp, which was mounted on a separate optical track and focused on the excitation monochromator. A quartz light guide was used to transmit the excitation light to the 3 × 3 mm observation chamber. Samples were excited at 490 nm and the emission of the fluorophore was isolated at a right angle with a OG-530 (Schott) cut-off filter, and detected by a Hamamatsu R-1463 photomultiplier tube. The transients were digitized at a typical rate of 1 MHz and stored on a 80386 microprocessor-based PC. The signal-to-noise ratio was generally poor, and multiple tracings (8–12) were used for signal averaging. The traces were fitted to a sum of exponential terms:

$$F(t) = A + \sum_{i=1} B_i \exp(-k_i \cdot t)$$

where the ith rate constant, k_i is related to the ith relaxation time τ_i by $\tau_i = 1/k_i$. In a typical experiment a sample of S1-AF was rapidly injected into the temperature-jump cell via the stopped-flow mode, and the injected sample was allowed to equilibrate before the spark gap inside the cell was triggered. The goodness of a fit was judged by (i) the residual plot, (ii) the reduced chi squares ratio, and (iii) the Durbin–Watson parameter.

3. RESULTS AND DISCUSSION

In a previous study we showed that the enthalpy change for the two-state transition of S1-AF was non-zero. This was the basis for the temperature-jump measurements. Upon increasing the temperature, the equilibrium of the transition $(S1\text{-}AF)_L \rightleftharpoons (S1\text{-}AF)_H$ shifted in favor of the high-temperature state with an increase in fluorescence [14]. After jumping the temperature, an increase in fluorescence was also observed during the short period when the system returned to the new equilibrium at the higher temperature. The intensity change was of the order of 2–3% at the lower temperatures and 4–5% at the higher temperatures. A typical temperature-jump trace obtained at a final temperature of 27.0°C is shown in Fig. 1. The trace could not be fitted to either a two- or three-exponential function, but could be fitted with a single exponential function with acceptable statistics. The trace is characterized by a rate constant of 255.7 s^{-1} corresponding to a relaxation time of 3.91 ms. The relaxation time was independent of the concentration of S1-AF. A single relaxation was also observed at lower temperatures, and the relaxation times obtained over the temperature range of 15–27°C are given in Table I. These data were collected with a total of three preparations of S1-AF.

A simple unimolecular reaction such as:

$$(S1\text{-}AF)_L \underset{k_-}{\overset{k_+}{\rightleftharpoons}} (S1\text{-}AF)_H$$

has only one relaxation time. The relaxation time is related to the two first-order rate constants by $\tau = 1/(k_+ + k_-)$ and is independent of concentration. The observed relaxation data are compatible with a unimolecular transition between two states of S1-AF, as shown above. The equilibrium constants for this transition were previously reported [14] over the temperature range of 7–27°C. Those equilibrium constants can be used to calculate k_+ and k_- from the observed relaxation

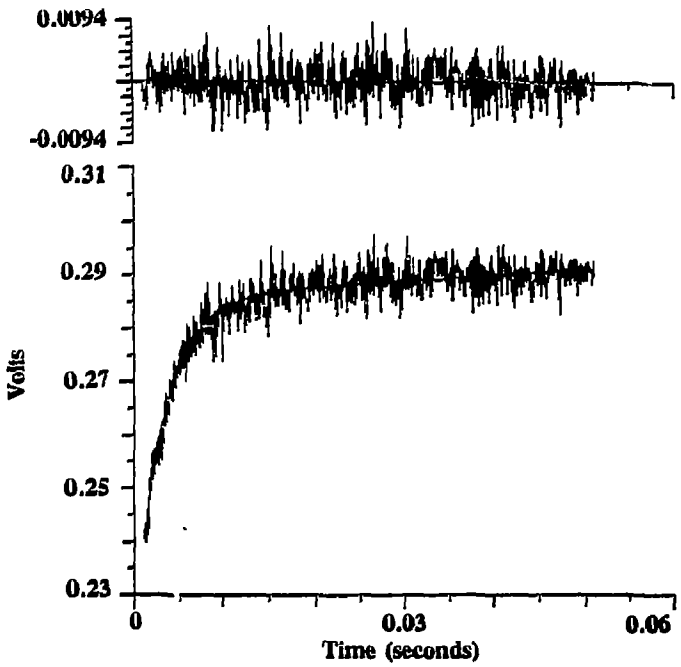

Fig. 1. A representative temperature-jump tracing obtained with 6 μM S1-AF in 60 mM KCl, 2 mM $MgCl_2$, 30 mM TES, pH 7.5. The initial temperature was 21.2°C, and the final temperature after the jump was 27°C. The progress of relaxation toward the new equilibrium was monitored by the increase in fluorescence immediately following temperature jump. The solid curve is the best fit of the trace to a single exponential function, yielding a relaxation time of 3.7 ms. The upper tracing across the plot is the deviation between the calculated and experimental results. χ^2_R = 1.08, D–W parameter = 1.99.

times. The calculated rate constants are also given in Table I.

Fig. 2 shows the temperature dependence of the two rate constants. The dependence is of the Arrhenius type. The activation energy is 21.9 kcal/mole for the forward

Table I

Kinetic parameters from temperature-jump measurements of myosin subfragment 1 labeled with IAF

Temp. (°C)	Relaxation time τ (ms)	$1/\tau$ (s^{-1})	Forward rate k_+ (s^{-1})	Reverse rate k_- (s^{-1})
15	16.69	59.9	44.1 ± 5.6	15.8 ± 2.0
17	11.92	83.9	63.9 ± 4.5	20.0 ± 1.4
19	9.27	107.9	84.6 ± 4.9	23.3 ± 1.4
21	8.17	122.4	97.8 ± 5.8	24.6 ± 1.5
23	6.62	151.8	126.1 ± 7.3	25.0 ± 1.5
25	5.08	196.7	162.2 ± 8.0	29.5 ± 1.4
27	3.91	255.7	220.1 ± 11.2	32.6 ± 1.7

The temperature is the final temperature at which the system returned to equilibrium. Measurements were carried out with S1-AF (6 μM) in 60 mM KCl, 2 mM $MgCl_2$, 30 mM TES, pH 7.5. Control experiments indicated that the pH of the system was essentially constant over the narrow temperature range within which the temperature-jump experiments were carried out.

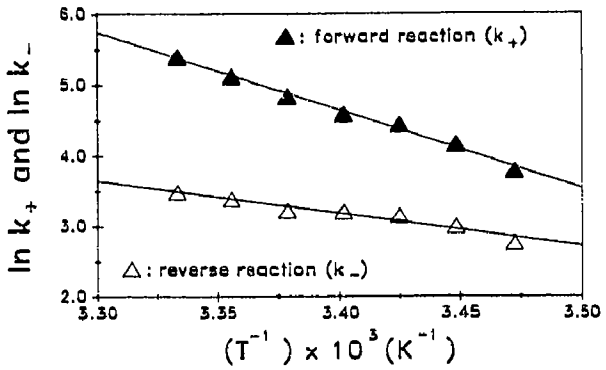

Fig. 2. Temperature dependence of the rate constants for the transition $(S1\text{-}AF)_L$ $(S1\text{-}AF)_H$. ▲, forward reaction (k_+); △, reverse reaction (k_-). The slopes of the lines are the activation energy values (ΔE) for the two reactions: $\Delta E_+ = 21.9$ kcal/mol, $\Delta E_- = 93$ kcal/mol.

reaction (ΔE_+) and 9.3 kcal/mole for the reverse reaction (ΔE_-). The enthalpy change for the transition is given by $\Delta H = \Delta E_+ - \Delta E_-$ and is 12.6 kcal/mol. This kinetically determined value is in agreement with that obtained from equilibrium measurements, suggesting that the relaxation is adequately described by a unimolecular reaction.

The existence of two equilibrium states of the S1–ADP complex on the basis of fluorescence data was previously demonstrated by the biexponential decay of εADP bound to S1. The two lifetimes were little affected by changes in temperature, but the amplitudes of the two lifetimes changed in a systematic manner [12]. These results are indicative of two conformations of S1 in the ground state, not a temperature effect on the excited-state properties of the fluorophore, and enabled determination of the equilibrium constant for interconversion between the two conformations. Unliganded S1-AF and the complex formed between S1-AF and ADP each displayed two fluorescence lifetimes whose properties were similar to those observed with S1–εADP [11,14]. The enthalpy and entropy changes for interconversion between two states in all three systems are very similar, and suggest a similar mechanism for the three transitions.

The transition of S1-AF between two states which are at equilibrium at a given temperature is unimolecular in both directions. The rate constants of the reaction cannot be determined in a conventional experiment. Relaxation measurement is a convenient approach to evaluate the kinetics and to establish that the signal change due to a small perturbation arises from a unimolecular reaction. While it is well known that S1 modified at Cys-707 has enhanced Ca^{2+}-ATPase activity, and the activity of S1-AF is also enhanced by a factor of 3 [11], S1-AF binds actin with an affinity that is only 12% smaller than native S1 [15]. This finding suggests that S1-AF may be considered very similar to unmodified S1. Thus, the present relaxation results are the first kinetic evidence of a two-state transition of unliganded S1. While multiple states of actomyosin have been proposed as integral components of the actomyosin ATPase mechanism [16] it is not clear what role a two-state equilibrium of S1 may play in the mechanism. In a recent report of the interaction of actin with S1-AF we [17] showed that a two-state equilibrium of unbound S1 must be included in a minimum kinetic scheme, and each S1 state can interact with actin with comparable bimolecular binding rates.

Temperature-jump measurements have not been previously reported on other contractile proteins in solution. A recent laser temperature-jump study by Davis and Harrington [18] on skinned muscle fibers showed multiphase relaxations in force generation with relaxation times in the range of 2–12.5 ms in one phase and about 100 ms in a slow phase. These investigators attributed the relaxation of the first phase following temperature-jump to thermoelastic expansion of the component proteins. While their temperature-jump results cannot delineate relaxations from specific proteins it is tempting to speculate that the relaxation observed with a fragment of myosin in the present work may have some relationship to those detected in the more complex and organized system.

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