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Biophysical Chemistry 94 (2001) 41–46

Biophysical Chemistry

www.elsevier.com/locate/bpc

The interdomain motions in myosin subfragment 1

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Received 12 July 2001; accepted 7 August 2001

Abstract

The interdomain motions in myosin subfragment 1 (S1) were studied by steady-state and time-resolved fluorescence of tryptophan residues and *N*-(iodoacetyl)-*N′*-(5-sulfo-1-naphtyl)ethylenediamine (AEDANS) attached to Cys178 of alkali light chain 1 (A1) exchanged into S1. The efficiency of fluorescence resonance energy transfer (FRET) from tryptophan residues of motor domain to AEDANS at A1 decreased dramatically after addition of ATP to S1A1-AEDANS. The efficiency of FRET calculated from the crystal structure of chicken S1 corresponded to the experimental one measured in the presence of ATP. The results showed that AEDANS at Cys178 of A1 became more mobile and distant from the motor domain of S1 upon ATP binding. These findings led to the suggestion that a release of the products of ATP hydrolysis and power stroke might be associated with movement of light chain-binding domain towards the N-terminal domain of S1.

Keywords: Myosin; Fluorescence; Tryptophan; *N*-(iodoacetyl)-*N′*-(5-sulfo-1-naphtyl)ethylenediamine (AEDANS); Energy transfer; ATP hydrolysis

1. Introduction

Muscle contraction results from cyclic interactions between myosin and actin driven by ATP hydrolysis. The fundamental problem of muscle contraction is how the hydrolysis of ATP is coupled to force generation. There is no consensus on how energy of ATP hydrolysis is stored in myosin and what is a maximal translocation distance per hydrolyzed ATP molecule [1]. It is well known that ATP can dissociate myosin from actin filament, while little is known about what conformational changes occur in myosin upon ATP hydrolysis. In the popular swinging lever-arm hypothesis, ATP may induce a rotation of the C-terminal domain of myosin subfragment 1 containing light chains, and the release of ATP hydrolysis products may cause a reversal rotation,

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0301-4622/01/$ - see front matter
PII: S0301-4622(01)00210-1

which might be associated with force generation and translocation of actin filaments [2,3]. The tryptophan fluorescence and other data indicated the presence of several intermediate states of myosin during ATP hydrolysis [4]. The structural studies of different myosin molecules with various nucleotide analogs revealed at least three distinct conformations of myosin subfragment 1 [5]; however, the relation between these structures and intermediate states of myosin during ATP hydrolysis is still uncertain.

In this work, we studied ATP-induced conformational changes in S1-containing alkali light chain 1 by steady-state and time-resolved fluorescence of tryptophan residues located in the motor domain of S1 and AEDANS attached to Cys178 of A1, which contains no tryptophan residues. We observed that during steady-state hydrolysis of ATP by S1A1-AEDANS, the efficiency of fluorescence resonance energy transfer from tryptophan residues to AEDANS of A1 decreased, while the mobility of AEDANS increased. The results indicated that the S1 lever arm moved in the opposite direction to that predicted by the swinging model [2].

2. Materials and methods

2.1. Reagents

N-(iodoacetyl)-*N′*-(5-sulfo-1-naphtyl)ethylenediamine (AEDANS) was purchased from Molecular Probes Inc. ATP and ADP were from Sigma.

2.2. Preparation of proteins

Myosin was prepared from rabbit skeletal muscle according to the method of Tonomura and co-workers [6]. S1 was obtained by chymotryptic digestion of myosin and separated into two isoforms by DEAE-cellulose chromatography [7]. S1 carrying the alkali light chain 1 (A1) was used in the experiments. Skeletal myosin light chains and acetylcysteine–AEDANS were a generous gift from Dr Takashi (Baylor Research Institute, Dallas, TX). A1 light chain was labeled with AEDANS at a single cysteine residue (Cys178) and exchanged into S1 according to Marsh and Lowey [8]. The protein concentrations were measured spectrometrically using absorbance (1%) at 280 nm: 6.0 cm^{-1} for myosin, 7.5 cm^{-1} for S1 and 2.2 cm^{-1} for A1. AEDANS concentration was determined from absorbance at 337 nm (6.3 mM^{-1} cm^{-1}). The concentration of proteins labeled with AEDANS was calculated from absorbance values after correction for the absorbance of AEDANS at 280 nm. Unless otherwise indicated, all experiments were carried out in a solution containing 50 mM KCl, 1 mM $MgCl_2$, 0.2 mM dithiothreitol and 10 mM Tris–HCl, pH 7.5.

2.3. Fluorescence measurements

All fluorescence measurements were carried out on a multi-frequency cross-correlation phase-modulation spectrofluorometer ISS-K2 (ISS Inc, Champaign, IL). Steady-state fluorescence of tryptophan residues and AEDANS were excited at 295 and 360 nm, respectively. The spectral width of the excitation and emission slits was 4 nm. The polarizers in the excitation and emission paths were set at 54.7 and 0° from the vertical orientation, respectively. Steady-state anisotropy of AEDANS fluorescence was excited at 390 and measured at 490 nm. The fluorescence lifetime of AEDANS was measured at 360-nm excitation and the emission was selected by a bandpass filter with transmission at 480 ± 10 nm. Acetylcysteine–AEDANS was used as a reference sample with a single lifetime of 9.9 ns. Rotational correlation times were obtained at similar settings as for lifetime measurements, except that polarizers were inserted in the excitation and emission pathways and the excitation wavelength was set at 390 nm.

2.4. Fluorescence data analysis

The anisotropy decay [$r(t)$] can be described by exponential functions:

$$r(t) = r_0 \cdot \sum_i g_i \cdot \exp(-t/\theta_i) = \Sigma r_{0i} \cdot \exp(-t/\theta_i) \quad (1)$$

where $r_0 = \sum_i r_{0i}$ is the fundamental anisotropy in the absence of rotational diffusion, θ_i is the fractional rotational correlation time, and g_j is the associated fractional anisotropy amplitude. The data were fitted using two models, where r_0 was a fixed or a variable parameter. The fixed value, $r_0 = 0.32$, was taken from [9].

The efficiency of fluorescence energy transfer (E) can be calculated from donor emission in the absence (I^{D}) and presence of acceptor (I^{DA}):

$$E = \frac{I^{\mathrm{D}} - I^{\mathrm{DA}}}{I^{\mathrm{D}}} \quad (2)$$

On the other hand, E depends on the donor–acceptor distance (R):

$$E = \frac{1}{1 + (R/R_0)^6} \quad (3)$$

where R_0 is the Förster distance for the given donor–acceptor pair at which the efficiency of energy transfer is 50%.

3. Results and discussion

3.1. Mobility of AEDANS at Cys178 of A1

We measured the steady-state anisotropy, rotational correlation time and lifetime of A1-AEDANS alone and S1A1-AEDANS in the presence and absence of ATP or ADP (see Table 1). Nucleotides have no effect on fluorescence of A1-AEDANS alone. The fluorescence decay data of both A1-AEDANS and S1A1-AEDANS could be well fitted with a single fluorescence lifetime of ca. 13.2 ns. The fluorescence intensity and lifetime of the excited state of AEDANS in S1A1-AEDANS were not affected by nucleotides, in agreement with earlier work [9]. In contrast to the lifetime and intensity of fluorescence, the steady-state anisotropy and rotational correlation time decreased when nucleotides bound to S1A1-AEDANS. The rotational correlation times were calculated using two fitting models, where r_0 was a fixed or a variable parameter. Both models gave very similar results (the values obtained with a fixed r_0 are given in Table 1). The fact that anisotropy and rotational correlation time decreased while lifetime values were not changed indicated the increasing of mobility of AEDANS in S1A1-AEDANS upon ATP binding.

3.2. Fluorescence resonance energy transfer from tryptophan residues to AEDANS

A possible movement of alkali light chain 1 relative to the motor domain upon ATP binding and hydrolysis was studied by fluorescence resonance-energy transfer from tryptophan residues located in the motor domain to AEDANS attached to Cys178 of A1 containing no tryptophans. In contrast to labeling of the motor domain, the labeling and exchange of A1 into S1 did not modify its functions [8]. We avoided the use of fluorescent analogs of ATP in this work, due to their possible non-specific binding to S1 [9]. Tryptophan fluorophore and AEDANS form a good donor–acceptor pair with a Förster radius (R_0) of 22 Å for randomly oriented dipoles [10]. The tryptophan fluorescence spectra of S1A1 and

Table 1
The steady-state anisotropy (r), lifetime (τ), fractional anisotropy amplitudes (r_{01}, r_{02}) and rotational correlation times (θ_1, θ_2) of AEDANS-Cys178 of A1 alone, or incorporated into S1 in the presence or absence of nucleotides

	r	τ	r_{01}	r_{02}	θ_1	θ_2
A1-AEDANS	0.071	13.1	0.170	0.150	0.29	6.46
S1A1-AEDANS	0.163	13.3	0.166	0.154	1.89	59.9
(S1A1-AEDANS) + ATP	0.125	13.1	0.154	0.166	0.94	33.1
(S1A1-AEDANS) + ADP	0.142	13.3	0.150	0.170	1.01	35.0

Fundamental anisotropy (r_0) was a fixed parameter (0.32).

S1A1-AEDANS in the presence and absence of ATP were recorded and normalized to the area under the spectrum (from 320 to 380 nm) of each protein denatured in 6 M urea. Under denaturing conditions, S1 and A1-AEDANS are completely dissociated, and thus there is no energy transfer, and all tryptophan residues have the same quantum yield. S1A1-AEDANS spectra were also corrected for incomplete labeling with AEDANS (60% labeling). The normalized and corrected spectra are shown in Fig. 1 (the intensity of S1A1 was taken as 1.0). It is clear that the quenching of tryptophan fluorescence in S1A1-AEDANS is much stronger in the absence than in the presence of ATP. If there were one tryptophan residue, the distances would be 28.6 and 37.4 Å in the absence and presence of ATP, respectively. However, chymotryptic myosin S1 has five tryptophan residues: Trp113, Trp131, Trp440, Trp510 and Trp595, all of which might be potential donors.

The apparent efficiency of energy transfer (E_{ap}) represents the relative change in the total fluorescence intensity (I_{tot}):

$$E_{ap} = \frac{\Delta I_{tot}}{I^D_{tot}} = \frac{\sum_{i=1}^{5} I_i^D - \sum_{i=1}^{5} I_i^{DA}}{\sum_{i=1}^{5} I_i^D} \quad (4)$$

where D and DA denote the fluorescence intensity in the absence and presence of acceptor, respectively, and I_i is the intensity of an individual tryptophan residue.

The efficiency of FRET from individual tryptophan residues is defined as:

$$E_i = \frac{I_i^{\mathrm{D}} - I_i^{\mathrm{DA}}}{I_i^{\mathrm{D}}} \quad (5)$$

As can be noted, E_{ap} is not equal to the sum of E_i. Using Eq. (3), we calculated the probability of energy transfer based on the distance (R) between the centers of the indole rings of each tryptophan residue and the sulfur atom of Cys178 of A1,

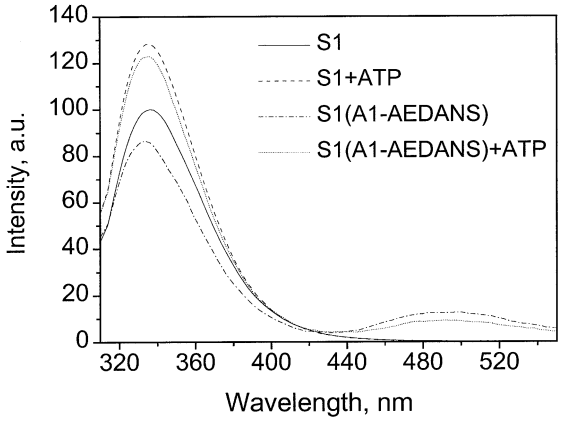

Fig. 1. Steady-state fluorescence spectra of S1 and S1A1-AEDANS in the absence and presence of ATP. The excitation wavelength was 295 nm.

calculated from the crystal structure of chicken skeletal S1 (see Table 2). It follows that energy transfer from Trp440, Trp510 and Trp595 to AEDANS is almost negligible. We assumed that the main contributors to energy transfer are Trp113 and Trp131. The ATP-induced increase in fluorescence of S1 can be mostly attributed to Trp440 and Trp510 [11]. Therefore, all changes in the quantum yields of Trp113 and 131 would be caused by energy transfer to AEDANS. In this case, Eqs. (4) and (5) can be combined and simplified to:

$$E_{\mathrm{ap}} = \frac{E_{\mathrm{Trp113}} I^{\mathrm{D}}_{\mathrm{Trp113}} + E_{\mathrm{Trp131}} I^{\mathrm{D}}_{\mathrm{Trp131}}}{I^{\mathrm{D}}_{\mathrm{tot}}} \quad (6)$$

In order to estimate which state (−ATP, or +ATP) the crystal structure of chicken S1 corresponds to, we compared the experimental values of apparent energy transfer, which are 17 and 4% in the absence and presence of ATP, respectively, with E_{ap} calculated using Eq. (6). E_{Trp113} and E_{Trp131} were taken as 9.52 and 5.06% (see Table 2), respectively. I_{tot} was taken as 1.0 in the absence of ATP and 1.2 in the presence of ATP, since ATP induced a 20% increase in the total fluorescence intensity. Previously, it was demonstrated that the fractional contribution of both Trp113 and 131 is 0.41 of the total fluorescence of S1 in

Table 2
The efficiency of energy transfer (E) calculated using Eq. (3) based on the distance (R) between the centers of the indole rings of each tryptophan residue and the sulfur atom of Cys178 of A1, calculated from the crystal structure of chicken skeletal S1

	Trp113	Trp131	Trp440	Trp510	Trp595
R (Å)	32.0	35.9	65.8	40.1	65.9
E (%)	9.52	5.06	0.14	2.64	0.14

the absence of ATP [12]. Quantum yields of Trp113 and 131 are not sensitive to nucleotide binding [12], and therefore the fractional contribution of these tryptophan residues in the presence of ATP (where $I_{tot} = 1.2$) is 0.34. Since we did not know the contribution of each tryptophan (Trp113 and 131) to the fluorescence spectrum, we considered three cases: (i) equal contributions from Trp113 and 131; (ii) the contribution of Trp113 is zero; or (iii) the contribution of Trp131 is zero. The values calculated for the efficiency of energy transfer for the three cases were 3.0, 2.1 and 3.9% (−ATP), or 2.0, 1.4 and 2.6% (+ATP), respectively. Thus, based on the distances between Cys178 and Trp113 and Trp131 calculated from the crystal structure, we obtained an average value of the efficiency of energy transfer of $2.6 \pm 1.3\%$, which is very similar to that obtained from experimental data in the presence of ATP (4.0%). It seems that the crystal structure of chicken S1 might correspond to a structure with bound ATP. Indeed, Rayment and co-authors [13] suggested that due to the presence of SO_4 molecules in the active site of crystallized chicken S1, its structure probably corresponded to S1 with a bound nucleotide.

It is evident that in the absence of ATP, when the efficiency of energy transfer is 17%, the distances between AEDANS and Trp113 and 131 should be shorter than in the presence of ATP, when the efficiency of energy transfer is 4%. Assuming that the chicken crystal structure corresponds to the ATP-bound state of S1, we can try to estimate a distance change after the release of ATP hydrolysis products. The distances from Trp113 and Trp131 to AEDANS in the absence of ATP might be presented as:

$$R_{\mathrm{Trp113}} = 32.0 - \Delta R_1 \tag{7a}$$

$$R_{\mathrm{Trp131}} = 35.9 - \Delta R_2 \tag{7b}$$

where the values 32.0 and 35.9 Å were taken from Table 2, and ΔR_1 and ΔR_2 are the changes in distances from Trp113 and Trp131 to AEDANS, respectively. In the general case, we have a set of two equations with four variables (ΔR_1, ΔR_2, $I^{\mathrm{D}}_{\mathrm{trp113}}$, $I^{\mathrm{D}}_{\mathrm{trp131}}$):

$$\begin{cases} E_{\mathrm{ab}} = \dfrac{I^{\mathrm{D}}_{\mathrm{trp113}}}{1 + \left(\dfrac{32 - \Delta R_1}{R_0}\right)^6} + \dfrac{I^{\mathrm{D}}_{\mathrm{trp131}}}{1 + \left(\dfrac{35.9 - \Delta R_2}{R_0}\right)^6} \\ I^{\mathrm{D}}_{\mathrm{trp113}} + I^{\mathrm{D}}_{\mathrm{trp131}} = 0.41 \end{cases} \tag{8}$$

Assuming that $\Delta R_1 \approx \Delta R_2 = \Delta R$ and considering three sets of parameters: $I^{\mathrm{D}}_{\mathrm{trp113}} = I^{\mathrm{D}}_{\mathrm{trp131}} = 0.205$; $I^{\mathrm{D}}_{\mathrm{trp113}} = 0.41$, $I^{\mathrm{D}}_{\mathrm{trp131}} = 0$; and $I^{\mathrm{D}}_{\mathrm{trp113}} = 0$, $I^{\mathrm{D}}_{\mathrm{trp131}} = 0.41$, we can obtain three solutions for ΔR of 10.5, 8.6 and 12.5 Å, respectively. Thus, our estimation showed that AEDANS on Cys178 of A1 became closer by 10.5 ± 2.0 Å to the motor domain of S1 upon release of ATP hydrolysis products.

We have demonstrated for the first time that ATP decreased the anisotropy of fluorescence of AEDANS at Cys178 of A1 and the efficiency of FRET from tryptophan residues of the motor domain of S1 to AEDANS-A1. The fluorescence energy transfer mostly occurred from Trp113 and Trp131, which are the nearest tryptophan residues to Cys178 of A1. The FRET data indicated that ATP induced an increase in the distance from these tryptophan residues to AEDANS at Cys178 for approximately 10 Å. A reverse movement of A1, together with the C-terminal part of S1 upon release of ATP hydrolysis products, might contribute to force generation.

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