Conformational Distributions and Proximity Relationships in the Rigor Complex of Actin and Myosin Subfragment-1*

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Cyclic conformational changes in the myosin head are considered essential for muscle contraction. We hereby show that the extension of the fluorescence resonance energy transfer method described originally by Taylor et al. (Taylor, D. L., Reider, J., Spudich, J. A., and Stryer, L. (1981) J. Cell Biol. 89, 362–367) allows determination of the position of a labeled point outside the actin filament in supramolecular complexes and also characterization of the conformational heterogeneity of an actin-binding protein while considering donor-acceptor distance distributions. Using this method we analyzed proximity relationships between two labeled points of S1 and the actin filament in the acto-S1 rigor complex. The donor (N-[(iodoacetyl)amino]ethyl]-5-naphthylamine-1-sulfonate) was attached to either the catalytic domain (Cys-707) or the essential light chain (Cys-177) of S1, whereas the acceptor (5-(iodoacetamido)fluorescein) was attached to the actin filament (Cys-374). In contrast to the narrow positional distribution (assumed as being Gaussian) of Cys-707 (5 ± 3 Å), the positional distribution of Cys-177 was found to be broad (102 ± 4 Å). Such a broad positional distribution of the label on the essential light chain of S1 may be important in accommodating the helically arranged acto-myosin binding relative to the filament axis.

The cyclic interaction between actin and myosin is thought to provide the molecular basis for muscle contraction (2). There have been numerous attempts to characterize the molecular events underlying the contractile acto-myosin interaction (3). The recent availability of high resolution atomic structures of the actin monomer (4), the actin filament (5–7), myosin S1 (8), and the acto-S1 complex (9) provided a framework for the description of conformational changes associated with the contractile cycle.

The atomic models permit the study of proximity relationships between various sites in the acto-myosin complex. An alternative method for studying proximal relationships within a macromolecule is FRET1 spectroscopy. Several interprotein distances within the acto-myosin complex have been already determined with this latter method (10–14). The distance between Cys-374 of actin and Cys-707 of S1 under rigor conditions was found to be 60 (11), 50 (12, 15), and 51 Å (14). By the use of scallop myosin hybrid molecules this distance was determined to be 45 Å (16). The distance between Cys-374 of actin and Cys-177 in the ELC of S1 in rigor was measured to be 50 (11) and 60 Å (10). FRET spectroscopy was applied to determine the radial coordinate, i.e. the distance from the imaginary axis of a labeled side chain within the actin filament, where special symmetry conditions are fulfilled (1). The radial coordinate of a number of points in the actin filament and proximal relationships in the S1- and heavy meromyosin-decorated actin filament was determined by this approach (1, 17–23).

Although FRET spectroscopy and x-ray crystallography are significantly different methods, there is usually a good correlation between the distances calculated from the atomic model and the ones obtained by FRET (24–26). Accordingly, most of the FRET data obtained in the case of actin and acto-myosin are in a reasonably good agreement with the distances obtained by using the atomic models (5–7, 9). However, an exception is the distance between Cys-177 on ELC of S1 and Cys-374 on the actin filament, because FRET revealed 50–60 Å (10, 11), whereas the atomic model resulted in ~89 Å (9). Although this discrepancy may originate from biologically irrelevant factors (e.g. the non-zero size of the FRET probes (24)), the problem deserves further investigations because of the central role of the light chain-binding domain of S1 in the force generating process (9, 27, 28).

In the present work we analyze the proximity relationships in the rigor acto-S1 complex, with a particular focus on the conformational distributions of the light chain-binding domain associated with a donor-acceptor distance distribution. The FRET method, developed originally by Taylor et al. (1) for radial coordinate determinations in actin filament, was extended to obtain the positional distribution of points in S1 relative to the axis of the actin filament. The applicability and limitations of the extended method are described for different scenarios, e.g. for various spatial conditions and spectral parameters. The proximity relationships between the axis of the actin filament and either Cys-707, or Cys-177 of S1 were studied in rigor complex at 20 °C. The distance distribution describing the donor position was approximated as Gaussian with a mean value of Rg and full width at half-maximum of σ. In the case of donor on Cys-707 (in the catalytic domain) Rg was 52 Å, in good agreement with earlier data (11, 12, 14–16). This distance distribution was substantially narrow, with σ of 5 Å. In case of donor bound to Cys-177 (in the ELC), Rg was 73 Å, which is greater than the ones obtained by using FRET technique (50–60 Å) (10, 11) and correlates more with the 89 Å based on the atomic model of the acto-myosin complex (9). The simulated transfer efficiencies fit the experimental data only in the case when a wide positional distribution of the label on the light chain-binding domain was assumed (σ = 102 Å). These

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results are compatible with earlier observations according to which there might be a direct interaction between the ELC of S1 and the C-terminal region of actin (29, 30). In addition to this our data can support the hypothesis stating that during the power stroke, the light chain-binding domain tilts against the catalytic domain, that is connected to the actin filament in a fixed orientation (9, 27, 28).

EXPERIMENTAL PROCEDURES

Materials—KCl, MgCl₂, CaCl₂, TES, Tris, EGTA, 1-fluro-2,4-dinitrobenzene, PIPES, MOPS, dithioerythritol, apyrase, and IAEDANS were obtained from Sigma. ATP, ADP, and β-mercaptoethanol were obtained from Merck, and NaN₃ was from FLUKA (Switzerland, Buch). IAF was purchased from Molecular Probes (OR, Eugene). The Bradford protein assay reagent was purchased from Bio-Rad.

Protein Preparation and Labeling—Actin (31, 32) and myosin (33) were prepared from rabbit skeletal muscle. S1 was prepared by chymotryptic digestion according to Weeds and Taylor (34). Labeling of Cys-707 of S1 was carried out as described earlier (14). Labeling of Cys-374 of S1 (27) was performed as published recently (35, 36). This light chain labeling did not involve the commonly employed light chain exchange procedure, which has been reported to distort its structure (37). Following labeling, S1 was dialyzed overnight against rigor buffer (25 mM TES, pH 7.0, 80 mM KCl, 2 mM EGTA, 4 mM β-mercaptoethanol, and 5 mM MgCl₂) and were clarified by a 1-h centrifugation at 100,000 × g prior to fluorescence experiments. Actin was labeled on the Cys-374 residue with IAF as described previously (38), but the incubation time was extended to 28–30 h. Labeled actin monomer solution was clarified by a 2-h centrifugation at 100,000 × g. The clarified actin was polymerized by the addition of 2 mM MgCl₂ and 100 mM KCl to 2 h at room temperature and then dialyzed overnight against rigor buffer to remove free nucleotides (14).

The absorption measurements were carried out with a Shimadzu UV-2100 spectrophotometer. The following absorption coefficients were used to estimate the labeling stoichiometry: S1, ε₁₀₀₀ nm = 7.5 (39); actin monomer, ε₃₅₅ nm = 0.63 (40); IAEDANS, ε₃₅₅ nm = 6100 M⁻¹ cm⁻¹ (41); and IAF, ε₄₉₄ nm = 77000 M⁻¹ cm⁻¹ (11). Relative molecular masses of 42,300 Da and 115,000 Da were used for uncomplexed actin monomer and S1, respectively. The optical density of the labeled protein solution was corrected for the contributions of the fluorescence dyes. In these samples the protein concentration was also determined occasionally by the Bradford micro-assay (42). The following labeling ratios were determined: S1 labeled with IAEDANS on the Cys-707, 0.8–0.9; S1 labeled with IAEDANS on Cys-177, 0.5–0.7; and actin labeled with IAF on Cys-374, 0.75–0.8.

ATPase Assays—Labeled S1 was routinely characterized by measuring the K⁺/EDTA-ATPase and Ca⁺²-ATPase activity (43). The assays were performed at room temperature in 50 mM Tris-HCl, pH 8.0, 0.6 mM MgCl₂, 25 mM ATP, and either 10 mM EDTA or 9 mM CaCl₂. The ATPase activities were measured simultaneously for unlabeled S1 and served as reference.

Fluorescence Spectroscopic Experiments—Steady-state fluorescence experiments were carried out with a Perkin-Elmer LS50B luminescence spectrometer. Prior to the fluorescence measurements, protein solutions were dialyzed overnight against rigor buffer. The concentrations of actin and S1 in the samples were 10 and 2 μM, respectively. Under these conditions the concentration of uncomplexed S1 is negligible (12, 14). The solution of S1 and labeled and unlabeled actin filaments were mixed appropriately to obtain the desired protein concentrations and acceptor molar ratios (ratio of acceptor to actin monomer concentration). The samples were then incubated at room temperature for 4–5 h. Consideration of the tread-milling rate of the actin filament (44) suggests that this time is long enough to reach an equilibrium state. To test this assumption, the samples were remeasured following overnight incubation, resulting in no deviation from the data obtained after 4–5 h incubation time. To estimate the undesired effect of contaminating nucleotides (ATP or ADP) in control experiments, the FRET measurements were carried out in the presence of apyrase (1 units/ml, 3-h incubation time), an enzyme that can hydrolyze either ATP or ADP.

Theoretical Considerations—FRET efficiency was determined from the decrease of the donor fluorescence intensity as follows.

\[ E = 1 - \frac{(F_D)_{obs}}{(F_D)} \]  

(1)

where \( (F_D)_{obs} \) and \( (F_D) \) are the donor fluorescence intensities in the presence and the absence of acceptor, respectively. Fluorescence emission of IAEDANS was recorded at excitation and emission wavelengths of 400 and 470 nm, respectively. Excitation and emission slits were set to 5 nm. Under these conditions the contribution of acceptor fluorescence to the measured fluorescence emission was excluded. Fluorescence intensities were corrected for the inner filter effect (45). Because the highly reactive cysteine (Cys-707) of S1 becomes labeled up to about 10% (35) even when Cys-177 of the ELC is targeted, the observed transfer efficiencies were corrected for the heterogeneous donor labeling as follows.

\[ E_{corr} = \frac{(E_{observed} - \gamma E_{Cys-707})/(1 - \gamma)}{E_{Cys-707}} \]  

(2)

where \( E_{observed} \) is the measured transfer efficiency, \( E_{Cys-707} \) is the transfer efficiency between the donor on the Cys-707 and acceptors on the actin filament, and \( \gamma \) is the ratio of Cys-707-bound donor concentration to S1 concentration (35). The actual value of \( \gamma \) was obtained from the results of ATPase activity experiments.

The distance between donor and acceptor molecules was calculated by using the transfer efficiency and Förster’s critical distance (\( R_0 \)). \( R_0 \) is defined as the donor-acceptor distance at which the FRET efficiency is 50%. The values of \( R_0 \), for the IAEDANS-IAF pair between the Cys-374 on actin and Cys-707 on S1 were found previously to be 52 (11) and 48–49 Å (12). For the same pair labeling Cys-707 of ELC and Cys-374 of actin \( R_0 \) was found to be 47 Å (10). Accordingly, we used a consensus value of 50 Å. From the transfer efficiency (\( E \)) and Förster’s critical distance (\( R_0 \)), the distance between the donor and acceptor molecules (\( R \)) for a single donor-single acceptor system can be calculated from the following.

\[ R = \left( \frac{R_0^6}{E} \right)^{1/6} \]  

(3)

The calculation becomes more complicated if a single donor may transfer energy to more than one acceptor. In this case Equation 3 transforms to (46, 47) the following.

\[ E = \sum \frac{[R_i/R_0]^6}{[1 + \sum [R_i/R_0]^6]} \]  

(4)

where \( R_i \) denotes the individual donor-acceptor distances. For the analysis of experimental data we assumed that the donor molecule in S1 might transfer energy to acceptors located on the five nearest actin monomers, the cumulative transfer efficiency (\( E_{corr} \)) can be calculated using Equations 4 and 6 as follows.

\[ E_{corr} = \sum \frac{[R_i/R_0]^6}{[1 + \sum [R_i/R_0]^6]} \]  

(5)

where \( i \) takes the integer value from 1 to 5 and refers to individual actin monomers, and \( r \) is the radial coordinate of Cys-374. If the acceptor molar ratio (\( r \)) is known, the probability (\( p_i \)) of the individual arrangements could be obtained from the binomial distribution as follows.

\[ p_i = \begin{pmatrix} 5 \cr r \end{pmatrix} (1 - p)^{5 - r} \]  

(6)

Knowing the positions of the potential acceptor labeling sites on the five actin monomers, the cumulative transfer efficiency (\( E_{corr} \)) at a given acceptor molar ratio (\( r \)) can be calculated using Equations 4 and 6 as follows.

\[ E_{corr} = \sum p_i E_i \]  

(7)
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probability of the \( k \)th arrangement (Equation 6). By applying these equations, one can simulate the transfer efficiency over a range of virtual donor positions as a function of acceptor molar ratio. These simulations were carried out in MATLAB 5.2 (MathWorks Inc., MA). Comparison of the simulated curves with the experimental data allows the determination of the physically verifiable position of the donor. The set of coordinates that best describes the donor position was determined by minimizing the value of the error parameter (\( d^2 \)) defined as follows.

\[
d^2 = \sum (\alpha_i - \beta_i)^2/n
\]

where \( \alpha_i \) and \( \beta_j \) are the measured and simulated transfer efficiencies, and \( n \) is the number of experimental data. To minimize the value of \( d^2 \), the surface of errors (\( d^2 \)) was generated over a wide range of Descartean coordinates, and the global minimum of this surface was determined. The proper set of coordinates was determined with initial parameters in the vicinity of the global minimum using a fitting algorithm provided by the software (MATLAB 5.2). This two-step analysis allowed the best parameter set to be found even if local minimums were present on the \( d^2 \) surface. It should be noted that the errors presented here for the distance parameters were given by the software used for analyzing the data and calculated by neglecting the experimental errors attributed to the transfer efficiency values (see Table 1). Therefore, these values provide an underestimation for the real errors.

The mathematical treatment described above was unable to account for the distance distribution related to the conformational heterogeneity of the protein matrix. To accommodate conformational distribution as well, the donor-acceptor distance distributions must also be taken into account. We have assumed in the analysis that the transitions between the conformational states are slow on a nanosecond time scale. To describe the distance distribution characteristic for a donor-acceptor system, it is necessary to approximate its actual shape by a mathematical function. The complexity of the conformational and dynamic events occurring within a protein did not allow us to use the exact form for the distribution, and therefore, the application of a simple model was required. Among the simple models, which might serve as good approximations of the donor-acceptor distance distribution, the Gaussian model seemed to be appropriate in earlier studies (48). Accordingly, the distribution of the donor-acceptor distances (\( d(R) \)) was approximated here with a Gaussian function, with a mean of \( R \) and the full width at half-maximum of \( \sigma \).

\[
d(R) = \frac{1}{\sigma \sqrt{2\pi}} \exp \left[ -\frac{(R - \bar{R})^2}{2\sigma^2} \right]
\]

Although this model very likely allows the proper determination of the mean donor-acceptor distance (\( \bar{R} \)), the width of the distribution (\( \sigma \)) may only be interpreted qualitatively.

Because the sensitivity of the method along the \( x \) or \( z \) axis proved to be rather small (see "Results and Discussion"), only the distribution of the \( z \) coordinate was involved in the simulations. In these simulations the summation of the individual transfer efficiencies (see Equation 7) was carried out as follows.

\[
E_i = \sum \rho_i d(R_i)E \alpha_i
\]

where \( d(R_i) \) is the probability that the donor-acceptor distance is \( R_i \) (at the \( i \)th position of the distance distribution). In the simulations the position of the donor was allowed to probe all the locations around the equilibrium position between \( (R_i - 3\sigma) \) and \( (R_i + 3\sigma) \).

RESULTS AND DISCUSSION

Characterization of Labeled S1—Labeling of Cys-707 of S1 with IAEDANS inhibited the K\(^+\)/EDTA ATPase activity by 80–90% and increased the Ca\(^{2+}\)-ATPase activity by a factor of 2–3. These results support previously published observations (14). When S1 was labeled with IAEDANS on Cys-177 of the ELC, the K\(^+\)/EDTA ATPase activity decreased only by 3–12%, which is in agreement with the data obtained by Smyczynski and Kasprzak (35). The comparison of SDS-polyacrylamide gel electrophoresis to K\(^+\)/EDTA ATPase results revealed that this small reduction of the K\(^+\)/EDTA ATPase activity is due to undesired labeling of the Cys-707 residue (35). Under these conditions the Ca\(^{2+}\)-ATPase activity decreased to 30% approximately. Because this observation cannot be explained by either Cys-697 or Cys-707 modification, the decrease of the Ca\(^{2+}\)-ATPase activity was probably due to light chain modification. The labeling ratio in our study was less (0.5–0.7) than the one published recently (0.88–1.08) (35), where the isolated A1 isoform (S1A1) of the S1 was modified with IAEDANS (35). It was shown by SDS-polyacrylamide gel electrophoresis on proteins extracted from muscle fibers that the affinity of IAEDANS to the S1A2 isomer is smaller than to S1A1 (49); therefore, the S1A1 isoform labels were probably dominating in our IAEDANS-labeled samples.

To estimate the undesired effect of the presence of contaminating nucleotides or dissociated ELCs, control experiments were carried out to characterize the rigor complex of actin (10 \( \mu \)M) and ELC-labeled S1 (2 \( \mu \)M) by centrifugation at 100,000 \( \times \) g for 4 h. The corrected fluorescence intensity of the IAEDANS was decreased to less than 5% in the samples after centrifugation, indicating that the concentration of uncomplexed S1 or dissociated light chains was negligible. This conclusion is further supported by the fact that the presence of apyrase in the samples did not influence either the results of these sedimentation experiments or the FRET efficiency values measured as a function of acceptor molar ratio.

The Applicability and Limitations of the Extended Method—

Taylor et al. (1) have provided a mathematical treatment to determine the radial coordinate of a labeled side chain in the actin filament from the dependence of FRET efficiency on the acceptor molar ratio. In these experiments the donor and acceptor molecules were located on helically symmetric positions within the actin filament. We extended the method to supramolecular systems where the donor is located outside the filament and the acceptors are on identical residues of different protomers along the actin filament. The comparison of simulated and experimental FRET data allowed the determination of the coordinates of the donor relative to the filament axis. To estimate the sensitivity of the FRET efficiency (\( E \)) to the donor coordinates, we probed possible donor positions over a realistic range by changing the values of the three coordinates (\( x, y, \) and \( z \)) in equidistant steps. The size of these steps was typically 1 Å. The change in the transfer efficiency (\( \Delta E \)) was calculated between neighboring steps, and a surface of \( \Delta E \) along the coordinates was generated. The maximum on this surface indicated the position of the donor molecule where the FRET efficiency was most sensitive to changes in donor position. According to the simulations, the transfer efficiency (\( E \)) was the most sensitive along any of the three coordinates (i.e., \( x, y, \) and \( z \)) if the individual donor-acceptor distances were close to Förster’s critical distance (\( R_0 \)), as expected. The sensitivity of \( E \) to the donor position increased by the decrease of \( R_0 \). The change in transfer efficiency was the greatest along the \( x \) axis, whereas it was much less affected by changing the \( y \) and \( z \) coordinates. The effects of the \( y \) and \( z \) axes were approximately equal. Furthermore, the analysis was unable to differentiate between opposite signs (i.e., + or −) of the \( y \) and \( z \) coordinates, probably because of the helical symmetry of the actin filament.

Experimental Results—In the present experiments IAF was located on the actin filament (Cys-374), and IAEDANS was attached to either the Cys-707 or the Cys-177 residue of the S1 (Fig. 1). The FRET efficiencies were determined (Equations 1 and 2) for the IAEDANS \( \gamma \) pair as a function of acceptor molar ratio (Table I). For a comparison, the transfer efficiency was also calculated by using the atomic coordinates of the labeled side chains in the acto-myosin complex (9) (Fig. 2). In the latter calculation we assumed that the donor and acceptor positions were fixed in the acto-myosin complex, i.e., the effects of conformational heterogeneity were not considered. The correlation
between the measured transfer efficiencies and the ones calculated from the atomic model was closer in the case of donor attached to Cys-707 than in the case of donor on the ELC of S1 (Fig. 2). This observation supports earlier results (10–12, 14–16).

When the donor was attached to Cys-707 of S1, the measured transfer efficiency increased proportionally to the acceptor molar ratio, which is evidenced by a good linear fit (Fig. 2). This observation supports earlier results (10–12, 14–16). (Fig. 2). The linear fits, which would be expected for a single donor-single acceptor system (continuous lines), and the transfer efficiencies calculated using the atomic model of the acto-S1 complex (dashed lines) (9). In this latter calculation the following distances were used: \( R_5 = 63.9 \, \text{Å}, R_2 = 99.8 \, \text{Å}, R_3 = 51.7 \, \text{Å}, R_4 = 103.7 \, \text{Å}, \) and \( R_6 = 88.8 \, \text{Å} \) for the Cys-707 and \( R_1 = 92.17 \, \text{Å}, R_5 = 134.7 \, \text{Å}, R_6 = 88.8 \, \text{Å}, R_4 = 138.89 \, \text{Å}, \) and \( R_5 = 119.9 \, \text{Å} \) for the Cys-177, where \( R_i \) is the distance between the donor and the acceptor on the \( i \)th monomer.

**Analysis of the Experimental Results**—The analysis of the measured transfer efficiency data was carried out by assuming either homogenous S1 population, where all the S1s are in identical conformation, or by considering a conformational distribution of the labeled protein resulting in a donor-acceptor distance distribution. In the former case it was assumed that the donor position could be described with single set of \( x, y, \) and \( z \) coordinates for the entire S1 population. This approximation resulted in a good fit for the donor on Cys-707 (Fig. 3a). The value of \( x, y, \) i.e. the distance of Cys-707 from the filament axis was 77 ± 3 Å. The values of \( y \) and \( z \) were 5 ± 5 and 3 ± 4 Å, respectively. We were unable to determine whether the fit results were positive or negative because of the symmetric arrangement of the acceptors along the \( y \) and \( z \) axes of the actin filament (see above). However, the atomic model of the actomyosin complex (9) and the definition of the coordinate system used in this study (Fig. 1, b and c) suggest that both \( y \) and \( z \) are positive. The \( x, y, \) and \( z \) coordinates correspond to 52 ± 3 Å distance between the donor and the acceptor on the nearest actin monomer by considering the 25 Å consensus radial coordinate of Cys-374 in the actin filament. This result is in agreement with earlier publications (11, 12, 14–16). In these reports the authors assumed in the calculations that the energy trans-

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**TABLE I**

| Acceptor molar ratio | \( \varepsilon_{\text{Cys-707}} \) | \( \varepsilon_{\text{Cys-177}} \) |
|----------------------|-----------------|-----------------|
| 0.12                 | 6.84 (0.17)     | 9.74 (4.97)     |
| 0.25                 | 12.03 (1.46)    | 17.21 (4.62)    |
| 0.37                 | 18.56 (0.17)    | 22.89 (2.24)    |
| 0.50                 | 23.52 (1.72)    | 27.68 (3.72)    |
| 0.62                 | 29.23 (1.88)    | 30.69 (1.35)    |
| 0.75                 | 36.07 (0.78)    | 33.33 (2.55)    |

The experiments were carried out under rigor conditions at 20 °C. Standard deviations are given in parentheses.
fer occurred between a single donor on the S1 and a single acceptor on the actin filament. The good accordance between our results and these referenced data supports further the conclusion that the contributions of acceptors on more distant actin monomers are negligible. In the case of transfer between the donor on Cys-177 and acceptors on actin, the assumption of homogenous S1 population failed to give a good fit (Fig. 3b). Consideration of the fitting algorithm (see “Experimental Procedures”) suggests that the lack of good fitting parameters is not due to the inability of the mathematical algorithm.

Deviation from the linear fit could be in principle explained by nonrandom actin polymerization resulting in an inhomogeneous acceptor distribution (21). To estimate the effect of nonrandom filament assembly, we polymerized the actin in the presence of phalloidin. Such preparation was shown to result in a random monomer assembly (21). Because the results obtained in the presence and absence of phalloidin were indistinguishable from each other, the effect of nonrandom actin assembly was excluded. The consideration of these results and the inability of the simulations assuming homogenous S1 population to approximate the experimental results in the case of ELC suggested that the conformation of the light chain-binding domain of S1 was heterogeneous.

To test the effect of heterogeneous S1 population, the distance calculations were carried out with the assumption that the S1 population could adopt a wide range of conformations characterized by a donor-acceptor distance distribution (Fig. 3). The interconversion rate among these conformations was assumed to be slow on a nanosecond time scale. In the case of donor on the catalytic domain (Cys-707), the assumption of homogeneous S1 population resulted in good fit of the simulated data to the experimental ones. Thus, it might be expected that the positional distribution of the S1 population is narrow even if such a distribution is assumed. The distribution of distances between Cys-707 of S1 and the z axis was centered at 77 ± 2 Å. Considering the radial coordinate of Cys-374 of actin the distance between Cys-707 of S1 and Cys-374 on the closest actin monomer was calculated to be 52 ± 2 Å, which is in good agreement with calculations that assumed a homogeneous S1 population. The width of this distance distribution was 5 ± 3 Å. The relatively small width indicates that the positional distribution between Cys-707 in S1s catalytic domain and the actin filament is narrow.

In the case of energy transfer between the donor on Cys-177 of the ELC and the acceptors on actin, the simulation gave a remarkably good fit if heterogeneity of the S1 population was assumed (Fig. 3b). The distance distribution between Cys-177 of the ELC and the z axis of the actin filament was centered at 98 ± 3 Å with a width of 102 ± 4 Å. Accordingly, the mean distance between Cys-177 of ELC and Cys-374 on actin was 73 ± 3 Å. The wide positional distribution of the ELC, and therefore probably the light chain-binding domain of S1, reflects either the large flexibility of the protein matrix or the presence of a large number of distinct conformations of a relatively rigid protein matrix. Further experiments are required to distinguish between the alternative explanations.

Relationship to Other Studies—When a conformational distribution accompanied with a distance distribution of the light chain-binding domain of S1 was considered in the analysis of the FRET data, the mean distance was 73 Å between the donor on Cys-177 and acceptors on actin. The distance resolved in the present analysis (73 Å) is longer than those (50–60 Å) published previously by other laboratories (10, 11) and correlates better with the distance (∼89 Å) calculated from the atomic model of the acto-myosin complex (9). The nonzero size of the probes applied in the FRET experiments (5–15 Å) may explain (24) the perishing difference between determinations based on FRET data and the atomic model.

In the present work the extension of the method of Taylor et al. (1) revealed that in the acto-S1 rigor complex, the positional distribution of Cys-707 in the catalytic domain was narrow relative to the actin filament axis, whereas the ELC could adopt a wide donor-acceptor distance distribution. According to the “rotating lever arm” model of muscle contraction, the light chain-binding domain of the myosin head tilts against the catalytic domain (9, 27, 28). Tilting of the lever arm is coupled to the ATPase cycle (8, 9). In the model, the catalytic domain is connected to the actin filament in a fixed orientation during the power stroke (9, 27, 28). The use of x-ray and mechanical data from muscle fibers showed that the myosin head performed bending and tilting motions during muscle contraction (50). These experiments did not resolve which part of the S1 was responsible for the emergence of elastic strain.

FRET experiments could detect the swinging motion of the light chain-binding domain and provided evidence for the close coupling between the isomerization of myosin head and the phosphate release step (51). The ability of the catalytic and light chain-binding domains to rotate relative to each other was also shown by using conventional EPR spectroscopy (52). In accordance with this observation, saturation transfer-EPR measurements suggested that the catalytic and light chain-binding domains were connected by a flexible hinge in the myosin head (53). In good correlation with these observations, our results indicate that the dynamic properties of the protein matrix of S1 allow the independent rotation of the light chain-binding domain relative to the catalytic domain, which is fixed in a unique conformation to the actin filament under rigor conditions. Interestingly, in saturation transfer-EPR measurements, the difference between the mobility of the catalytic and light chain-binding domains existed only in the filament form of myosin (53) and disappeared in either the monomeric form or when the myosin was bound to actin. In the light of this result it seems to be important to determine by other experimental methods whether the large flexibility of the protein matrix was responsible for the wide distance distribution of the light chain-binding domain in our experiments or a relatively rigid protein experienced a number of distinct conformations separated by relatively high free energy barriers.

Recently, EPR spectroscopy revealed that there were two distinct myosin isoforms with 36° axial separation between the two orientations of the light chain-binding domain in muscle fibers (54). The two orientations did not change upon muscle activation; only the fractions of myosin heads shift between these orientations (54). Under experimental conditions similar to the ones used in our study, i.e. in rigor, the light chain-binding domain populates only one of these orientations, namely the strong binding state (54). The mean axial angle of this domain was 38° with a substantially large width of 49° (54). The broad conformational distribution of the light chain-binding domain, resolved in our analysis, agrees well with the observed wide axial angle distribution of this domain (54).

Conclusions—The method originally described by Taylor et al. (1) was extended here to provide coordinates of a labeled point on an actin-binding protein in a coordinate system confined to the actin filament axis (Fig. 1). Furthermore, we were able to describe the conformational heterogeneity of a labeled segment of the S1 by assuming a distribution of the donor-acceptor distances. Therefore, such combination of FRET experiments and computer simulations seems to be a powerful tool in the investigation of dynamic characteristic of supramolecular complexes having an axial symmetry.

The results of our FRET experiments indicate that the ori-
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entation of the catalytic domain is fixed relative to the actin filament under rigor conditions, and the light chain-binding domain of S1 experiences a wide positional distribution relative to either the actin filament or the catalytic domain. These observations are in accordance with a number of earlier reports (8, 9, 27, 28, 50–54) and seem to be compatible with the recently established model of muscle contraction, which suggests that the power-stroke-associated conformational changes within the S1 are of interdomain nature.

The consideration of conformational distribution in our analysis resulted in a mean distance of 73 Å between Cys-177 on the ELC and Cys-374 of actin, which provides good correlation with the data (89 Å) calculated by using the atomic model of the acto-myosin complex (9). The wide conformational distribution of the ELC is compatible with earlier observations according to which the ELC of S1 might get, at least transiently, in contact with either the actin filament or the catalytic domain. These domain of S1 experiences a wide positional distribution relative to the C-terminal region of actin (29, 30). The different helical symmetry of the actin and myosin filaments suggests that the presence of this wide conformational distribution may be important for the formation of rigor cross-bridges between the two filament systems.

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