The Flexibility of Actin Filaments as Revealed by Fluorescence Resonance Energy Transfer

THE INFLUENCE OF Divalent Cations*

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The temperature profile of the fluorescence resonance energy transfer efficiency normalized by the fluorescence quantum yield of the donor in the presence of the acceptor, $f^*$, was measured in a way allowing the independent investigation of (i) the strength of interaction between the adjacent protomers (intermonomer flexibility) and (ii) the flexibility of the protein matrix within actin protomers (intramonomer flexibility). In both cases the relative increase as a function of temperature in $f^*$ is larger in calcium-F-actin than in magnesium-F-actin in the range of 5–40 °C, which indicates that both the intramonomer and the intermonomer flexibility of the actin filaments are larger in calcium-F-actin than those in magnesium-F-actin. The intermonomer flexibility was proved to be larger than the intramonomer one in both the calcium-F-actin and the magnesium-F-actin. The distance between Gln41 and Cys374 residues was found to be cation-independent and did not change during polymerization at 21 °C. The steady-state fluorescence anisotropy data of fluorophores attached to the Gln41 or Cys374 residues suggest that the microenvironments around these regions are more rigid in the magnesium-loaded actin filament than in the calcium-loaded form.

The tension generation in the striated muscle is performed through a series of chemical reactions by cyclic interaction of myosin with ATP and actin, and at least six intermediates are proposed for actomyosin ATPase in solution (1–3). On a cellular level in supramolecular complexes where stabilizing forces may modulate the hydrolysis process, some contribution from actin flexibility and dynamics to the contraction process cannot be excluded. This statement is supported by earlier and recent suggestions about the role of actin during the force development in muscle (4).

Flexural rigidity experiments suggested that the actin filament was extensible (5). These findings were supported by electron microscopic measurements on the sarcomere in rigor fibers (6). The extensibility of the thin filaments was also suggested by the changes of the spacings of the x-ray diffraction pattern during contraction (4, 7). Actin filaments were shown to be elastic and extensible by measuring the stiffness of the actin-tropomyosin complex with in vitro nanomanipulation (8).

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model have led to suggestions of a more complex model of the muscle contraction. This model involves large scale conformational changes of myosin head in the light chain-binding domain that rotates relative to the actin-binding portion of the catalytic domain (28–30). The closure of the cleft on the actin-binding domains, which follows the release of the P, results in a specific interaction between the two proteins, and this interaction might be modulated by the actual dynamic and conformational states of both proteins.

Although there are strong indications that actin is an active part of the contracting system, we are still far from understanding the details of the biological function of this abundant protein. The lack of complete understanding of the function of the actin in the contracting system can emphasize the importance of further investigations dealing with this matter.

The principal aim of this study was to characterize the effect of divalent cations on the internal flexibility and the conformational states of actin filaments using the method of fluorescence resonance energy transfer. According to the fluorescence resonance energy transfer data presented in this paper, the calcium-F-actin is proved to be more flexible than the magnesium-F-actin in either the intermonomer or the intramonomer protein flexibility. The intermonomer flexibility is larger than the intramonomer one, regardless of the nature of the bound cation. In accordance with these flexibility data the steady-state anisotropy experiments indicate that the microenvironments of the Gln41 and Cys574 residues are more rigid in the Mg2+-saturated filaments than in calcium-F-actin.

MATERIALS AND METHODS

Reagents—KCl, MgCl2, CaCl2, Tris, N-(iodoacetyl)-N’-(5-sulfo-1-naphthyl)ethylendiamine (IAEDANS),1 quinine (hemisulfate salt), dimethylformamide, guinea pig liver transglutaminase (TGase), and EGTA were obtained from Sigma. Iodoacetamide 5-fluorescein (IAF) and fluorescein cadaverine (FC) were purchased from Molecular Probes (Eugene, OR); adenosine 5’-triphosphate (ATP) and ␦-mercaptoethanol were obtained from Merek (Darmstadt, Germany); the Bradford protein assay reagent was purchased from Bio-Rad (Munich, Germany), and NaN3 was from Fluka (Buchs, Switzerland).

Protein Preparation—Acetone-dried powder of rabbit skeletal muscle was obtained as described by Feuer et al. (31). Rabbit skeletal muscle actin was prepared according to the method of Spudich and Watt (32) and stored in 2 mM Tris/[HCl buffer (pH 8.0) containing 0.2 mM ATP, 0.1 mM CaCl2, 0.1 mM ␦-mercaptoethanol, and 0.02% NaN3 (buffer A).

Labeling of the Cys574 residue (Fig. 1A) with IAEDANS was performed as described earlier (33), and FC (2 mg/ml) was incubated with 10-fold molar excess of IAEDANS for 1 h at room temperature. Labeling of the same residue in separate samples with IAF was carried out in the following way: monomeric actin (2 mg/ml) was mixed with the 10-fold molar excess of IAF over the protein and incubated for 3–4 h at room temperature. Then the actin was polymerized for 12–16 h at 4 °C. After the labeling procedures, the samples were centrifuged at 100,000 × g for 2 h at 4 °C. The pellets were dissolved in buffer A and dialyzed overnight against buffer A (in the case of IAF-labeled actin the dialyzing buffer contained 1% (w/v) dimethylformamide as well). The Gln57 residue (Fig. 1A) was modified with FC by the use of the procedure of Takashi (34), and G-actin was incubated with 10-fold molar excess of the dye in the presence of 1 mg/ml TGase. The labeling was carried out for 16 h at 4 °C. Unbound FC was removed similarly as described in the case of IAEDANS and IAF labeling procedures.

The G-actin concentration was determined with a Shimadzu UV-2100 spectrophotometer by using the absorption coefficient of 6100 M−1 cm−1 at 290 nm (35). The concentration of actin was between 15 and 100 μg/ml, unless stated otherwise.

Fluorescence Experiments—The concentration of actin was between 36 and 100 μg/ml, unless stated otherwise. The fluorescence emission spectra of the donor were recorded at temperatures ranging between 5 and 40 °C with a Perkin-Elmer LS50B luminescence spectrometer in the presence and the absence of the appropriate acceptor (FC in the experiments dealing with intramonomer flexibility and IAF in the study of intermonomer flexibility). The excitation wavelength for the IAEDANS was 380 nm. The slits were set to 3 nm in both the excitation and emission paths. The spectra were corrected for the inner filter effect as described earlier (42). To calculate fluorescence resonance energy transfer efficiency (see Equation 2), the under-curve areas of these emission spectra were calculated between 380 and 460 nm. In this wavelength range, the contribution of the acceptor (either the FC or the IAF) to the measured fluorescence is negligible.

The steady-state fluorescence anisotropy of the donor and acceptor molecules was calculated from the polarized emission components (FHV, FVV, FHV, and FVV, where the subscripts indicate the orientation of the excitation and emission polarizers) as follows,

\[
r = \frac{F_{FV}}{F_{FV}}/F_{FV} + 2 F_{FV}
\]

where \( G = F_{FV}/F_{FV} \). In the case of actin-bound IAEDANS, the excitation wavelength was 360 nm, and the emission wavelength was 460 nm, while for the fluorescein derivatives the excitation monochromator was set to 493 nm, and the emission was measured at 520 nm. The slits were set to 3 nm. In these experiments the concentration of the actin was decreased to 5 μg/ml after the polymerization by diluting the sample with the appropriate buffer. In this way the depolarizing effect of light scattering was reduced to a negligible level.

The corrected fluorescence emission spectra of IAEDANS-F-actin was recorded in the absence of the acceptor at an excitation wavelength of 360 nm to obtain the fluorescence quantum yield of the donor molecule. The quantum yield of quinine sulfate (0.53 in 0.1 N H2SO4) was used as a reference (43).

To test the reversibility of the temperature-induced changes in fluorescence, the samples were re-measured by cooling back the solution to the initial low temperature (7 °C) or repeating the measurements after overnight dialysis. The errors of the measured data presented in this paper are mean ± S.E. calculated from the results of three to five independent experiments.

Donor-Acceptor Distance—The transfer efficiency of the fluorescence resonance energy transfer occurring between a single donor and single

1 The abbreviations used are: IAEDANS, N-(iodoacetyl)-N’-(5-sulfo-1-naphthyl)ethylendiamine; FC, fluorescein cadaverine; IAF, 5-idoacetamidofluorescein; G-actin, monomeric actin; F-actin, actin filament.
acceptor can be calculated from the fluorescence intensities as follows,
\[ E = 1 - \frac{(F_{DA}/F_{DA0})(F_D/F_D0))}{\beta} \] (Eq. 2)
where \( F_{DA} \) and \( F_D \) are the fluorescence intensities of the donor molecule in the presence and the absence of the acceptor, respectively; \( \beta \) symbolizes the acceptor/monomer molar ratio. \( c_D \) and \( c_{DA} \) are the concentrations of the donor molecule in the samples indicated by the subscripts. By knowing the fluorescence energy transfer efficiency \( E \), it is possible to determine the distance \( R \) between the donor and acceptor molecules from the following equation:
\[ E = R_c^6(R_c^6 + R^6) \] (Eq. 3)
where \( R_c \) is the Förster's critical distance defined as the donor-acceptor distance where the fluorescence resonance energy transfer efficiency is 50%. The use of Equation 3 requires the calculation of \( R_c \) as follows,
\[ R_c^6 = (8.79 \times 10^{-11})n^{-4}\beta\phi_fJ \] (Eq. 4)
where \( n \) is the refractive index of the medium, \( \chi^2 \) characterizes the relative orientation of the donor and acceptor molecules, \( \phi_f \) is the fluorescence quantum yield of the IAEDANS in the absence of acceptor, and \( J \) is the overlap integral given in \( \text{m}^{-1} \text{cm}^{-1} \text{nm}^2 \). The overlap integral \( J \) is defined as follows,
\[ J = \int F_D(\lambda)\epsilon_D(\lambda)\lambda^4d\lambda \int F_A(\lambda)\lambda^4d\lambda \] (Eq. 5)
where \( F_D(\lambda) \) is the corrected fluorescence emission spectra of the donor, and \( \epsilon_D(\lambda) \) is the molar extinction coefficient of the acceptor.

**Normalized Transfer Efficiency**—The temperature profile of the normalized energy transfer parameter \( f \), defined as the ratio of the transfer efficiency and the fluorescence quantum yield of the donor in the presence of acceptor \( (E) \), is proportional to the mean value of the energy transfer rate constant, \( <k> \), which has been shown to be an appropriate parameter for monitoring intramolecular fluctuations and/or conformational changes of a macromolecule (44).
\[ f = \frac{(<k>\Phi_D)}{(k_f/k_i)} = C(R_c^{-6}\chi^2) \] (Eq. 6)
where \( \Phi_D \) is the fluorescence quantum yield of the donor in the presence of acceptor, and \( k_i \) is the rate constant of the fluorescence emission. According to earlier publications the value of \( k_i \) is fairly constant under a wide variety of experimental conditions (see e.g. Ref. 45), therefore here its value is taken as constant. The subscript "i" indicates the value of the given parameter for the \( i^{th} \) population, taking a momentary picture, and \( C \) is a constant involving the refractive index \( (n) \) and the overlap integral \( (J) \), which were assumed to be constant (44). The sensitivity of this parameter to temperature is able to provide information regarding the flexibility of the protein matrix between the two fluorophores. It should be noted that \( f \) is sensitive to changes in the donor-acceptor distance originating from any kind of intramolecular motions. Thus, the temperature profile of this parameter provides information about the average flexibility of the protein matrix located between the two labels.

The method was developed for systems where the energy transfer occurred between a single donor and a single acceptor (44). However, in the present experiments dealing with intermonomer energy transfer, one should take into account that the donor can transfer energy to acceptors located on more than one neighboring actin protomers, i.e. the transfer is directed to a multiple acceptor system. Considering the helical structure of the actin filament, it seems reasonable that the acceptor population can be divided into two characteristically different groups: 1) acceptors on the closest protomer in the single-started genetic helix and 2) acceptors affecting the fluorescence of the donor from the double-started long-pitch helix. It is also assumed that acceptors on more distant protomers are not efficient in the reduction of the donor fluorescence. Accordingly, the donor-acceptor system can be described with two different equilibrium donor-acceptor distance distributions. It could be easily shown by using simple mathematical transformations that the measured normalized energy transfer parameter of the system having a single donor interacting with two different groups of acceptor molecules is the sum of the normalized energy transfer efficiencies characterized by the individual donor-acceptor systems (see also Ref. 46).
\[ f = f_1 + f_2 \] (Eq. 7)

Considering that the value of the fluorescence quantum yield is proportional to the fluorescence intensity measured at a given wavelength, it is usually more convenient to determine the value of the \( f \), which is defined as follows (44),
\[ f = \frac{<E>}{F_{DA}} = C'(<E>)/(\Phi_D) \] (Eq. 8)
where \( F_{DA} \) is the fluorescence intensity of the donor in the presence of acceptor, and \( C' \) is a constant that is proportional to the \( C \) used in Equation 6.

**RESULTS AND DISCUSSION**

The actin monomer has one high-affinity and three or more lower-affinity (i.e., intermediate- and low-affinity) cation-binding sites (see Ref. 47 for review). It is very likely that in vivo the high-affinity site is occupied by Mg\(^{2+}\), and the Mg\(^{2+}\) and K\(^+\) ions compete for the lower-affinity binding sites (47). The ion composition of the buffer that was used in this study to prepare magnesium-F-actin can be considered as a reasonable model for the free ion concentrations of Mg\(^{2+}\) and K\(^+\) in the cytosol (47). This preparation resulted in a magnesium-F-actin that contains Mg\(^{2+}\) at the high-affinity binding site and probably either Mg\(^{2+}\) or K\(^+\) at the lower-affinity sites. According to earlier publications the type of the cation at the lower-affinity binding sites might have an important biological effect (48). The calcium-actin filaments were polymerized in the presence of millimolar concentration (2 mM) of CaCl\(_2\). Following this procedure the Ca\(^{2+}\) in calcium-F-actin, similar to the Mg\(^{2+}\) in magnesium-F-actin samples, occupies the high-affinity binding site and probably competes with the K\(^+\) for the lower-affinity binding sites.

In the present work we explored the differences between flexibilities of filaments polymerized from calcium-actin and magnesium-actin by investigating separately the intermonomer and the intramonomer flexibilities. To examine intermonomer flexibilities the donor IAEDANS and the acceptor IAF are attached to different actin protomers within the filament. The relatively low donor ratio in these samples (compared with that of actin without the donor) assures that there is no acceptor in the actin filament, which is in resonance transfer with two donor molecules (see Fig. 1B). Accordingly, in these experiments one is dealing with a single donor-multiple acceptor system (see “Materials and Methods”). In a different experimental setup, the double labeling of the actin monomer makes it possible to study intramonomer flexibility within the actin filament. In this case it was necessary to dilute the samples with unlabeled actin to exclude the possibility of interaction between donor and acceptor molecules located on neighboring protomers. Considering the atomic model of the actin filament (49), it is very likely that the 10-fold dilution of the double labeled actin monomers with unlabeled monomers accurately separates the labeled monomers within the double helix of actin filaments (Fig. 1C). The experiments designed to monitor the reversibility of the temperature-induced changes in the fluorescence parameters gave evidence that the changes were reversible.

The distance between the donor (IAEDANS at Cys\(^{374}\)) and acceptor (FC at Glu\(^{34}\)) molecules is \( 4.46 \pm 0.07 \) nm and \( 4.49 \pm 0.06 \) nm in the Ca\(^{2+}\) - and Mg\(^{2+}\)-loaded forms of the monomer, respectively, indicating that the exchange of the bound cation does not influence the relative position of the Glu\(^{34}\) and Cys\(^{374}\) residues in the actin monomer. The data are in good accordance with the results of Moraczewska et al. (50), who found that the replacement of Ca\(^{2+}\) with Mg\(^{2+}\) produced no essential change in the distance between Glu\(^{41}\) and Cys\(^{374}\). These results are also in agreement with our recent observation that the distance between Lys\(^{51}\) and Cys\(^{374}\) of the actin monomer is cation-independent (51). The distance between Glu\(^{41}\) (Co) and Cys\(^{374}\) (Sp) residues is 4.1 nm according to the x-ray diffraction experiments (52). The value of this parameter resolved in our experiments is somewhat longer. The relatively small difference between the x-ray and the fluorescence data might be due to the size of the applied fluores-
cent probes.

The donor-acceptor distances (between residues Cys^{374} and Gln^{41}) in the filament at room temperature are 4.45 ± 0.08 nm and 4.59 ± 0.09 nm in calcium-F-actin and magnesium-F-actin, respectively (Table I.), which indicates that the polymerization does not affect significantly the donor-acceptor distance. This is in agreement with Miki’s conclusion (53) that the small domain does not affect significantly the donor-acceptor distance. This is respectively (Table I.), which indicates that the polymerization intermonomer fluorescence energy transfer experiments (amino acids, which were labeled to carry out the intramonomer or intermonomer energy transfer measurements (B), and the doubly labeled monomers are also marked (DA) in the case of intramonomer measurements (C). The ratio of the labeled and unlabeled monomer populations in the pictures is approximately the same as it is after the preparation procedure described under “Materials and Methods.”

**Table 1**

| Temperature | $\Phi_0$ | $R_s$ | $R$ |
|-------------|----------|-------|-----|
| °C          | Ca$^{2+}$ | Mg$^{2+}$ | Ca$^{2+}$ | Mg$^{2+}$ | Ca$^{2+}$ | Mg$^{2+}$ | Ca$^{2+}$ | Mg$^{2+}$ |
| 7           | 0.55     | 0.53   | 5.06 (±0.05) | 5.01 (±0.06) | 66.8 (±1.6) | 62.1 (±2.0) | 4.50 (±0.8) | 4.58 (±0.8) |
| 11          | 0.54     | 0.53   | 5.04 (±0.04) | 5.00 (±0.06) | 66.3 (±2.0) | 62.7 (±1.8) | 4.50 (±0.9) | 4.58 (±0.9) |
| 16          | 0.51     | 0.52   | 4.99 (±0.06) | 4.99 (±0.09) | 66.1 (±2.2) | 62.1 (±1.5) | 4.47 (±0.8) | 4.59 (±0.8) |
| 21          | 0.49     | 0.51   | 4.96 (±0.08) | 4.97 (±0.09) | 65.7 (±2.1) | 61.6 (±1.5) | 4.45 (±0.8) | 4.59 (±0.9) |
| 26          | 0.47     | 0.50   | 4.92 (±0.08) | 4.96 (±0.07) | 65.6 (±2.1) | 60.8 (±1.4) | 4.42 (±0.8) | 4.61 (±1.0) |
| 32          | 0.44     | 0.49   | 4.87 (±0.06) | 4.93 (±0.09) | 65.2 (±1.7) | 60.9 (±1.2) | 4.38 (±0.9) | 4.61 (±0.9) |
| 39          | 0.41     | 0.47   | 4.82 (±0.09) | 4.91 (±0.09) | 64.5 (±1.4) | 58.7 (±0.5) | 4.36 (±0.8) | 4.63 (±0.9) |

The S.E. of the mean are given in parentheses, except for the quantum yield where the error appears in the third digit.

**Figure 1.** A, the schematic representation of the atomic model of monomeric actin reconstructed according to the results of Kabach et al. (60). The subdomains are labeled with numbers 1–4, and the positions of the amino acids, which were labeled to carry out the intramonomer or intermonomer fluorescence energy transfer experiments (i.e. Gln^{41} and Cys^{374}), are marked with dark surfaces. B and C show the simplified picture of the actin filament. The approximated position of the labeled monomers is shown within the actin filament reconstruction of the results of Kabsch (60). The circles are representing monomers within the polymer. Capital letters within the circles stand for the monomer (i.e. Gln^{41} and Cys^{374}), which are representing monomers within the polymer. Capital letters within the circles stand for the monomer (A) molecules in the intermonomer energy transfer measurements (B), and the doubly labeled monomers are also marked (DA) in the case of intramonomer measurements (C). The ratio of the labeled and unlabeled monomer populations in the pictures is approximately the same as it is after the preparation procedure described under “Materials and Methods.”

**Table 1**

The temperature dependence of the fluorescence quantum yield of the LAEDANS ($\Phi_0$), the Förster’s critical distance of the LAEDANS-FC pair ($R_s$), the transfer efficiency measured in the intramonomer transfer experiments ($E$), and the calculated donor-acceptor distances ($R$) in calcium-F-actin and magnesium-F-actin.

The S.E. of the mean are given in parentheses, except for the quantum yield where the error appears in the third digit.

The cation dependence of the flexibility of the actin protomer within the filament can be characterized by measuring the temperature profile of the normalized transfer efficiency (Equations 6 and 8). In experiments dealing with intraprotem interactions the temperature dependence of the relative $f'$ is proved to be substantially larger in calcium-F-actin than in magnesium-F-actin between 5 and 40 °C (Fig. 2A). The total change of 5% in the Mg$^{2+}$-saturated form faces the 30% increase in the Ca$^{2+}$-saturated form. The data set suggests that the protomer structure is more flexible in the Ca$^{2+}$-loaded form of the actin filament than that in the magnesium-loaded form. The change in the relative $f'$ is very similar in calcium-F-actin to what was observed in the case of actin monomer by using a similar donor-acceptor pair (51). Accordingly, the flexibility of the small domain does not seem to be sensitive to polymerization in calcium-actin. Contrary to this, the relative change of $f'$ is smaller in magnesium-F-actin than that in magnesium-G-actin (51), indicating that in the Mg$^{2+}$-loaded form this protein segment is more rigid in the filament than it is in the monomer.

According to the results of intermonomer transfer experiments, the change of the relative $f'$ is larger in the calcium-F-actin than in the magnesium-F-actin (Fig. 2B), which suggests that the strength of the intermonomer interaction is stronger in the Mg$^{2+}$-saturated filament. By comparing the data obtained in the experiments addressing intramonomer and intermonomer fluorescence energy transfer, one can conclude that the intramonomer flexibility is smaller than the intermonomer flexibility for both the calcium-F-actin and magnesium-F-actin (Fig. 2, A and B). Considering that in intramonomer energy transfer the contributions of the two kinds of acceptor populations (see also “Materials and Methods”) to the measured fluorescence energy transfer efficiency are probably similar (49), in these experiments it is not possible to separate the flexural...
properties of the genetic helix and the two-started long-pitch helix. The increase in the amplitude of the relative fluctuation of the donor and acceptor molecules should result in an increase of the mean value of the energy transfer rate constant, \( k_{ti} \), and therefore the measurable donor-acceptor distance, even if the equilibrium distance between the two labels remains unchanged (44). In the light of our present data regarding the cation-dependent flexural properties of the filament, it seems possible that the slight temperature dependence of the donor-acceptor distance measured in the calcium-F-actin is partly the result of a temperature-induced increase in the amplitude of the relative fluctuation of the donor and acceptor molecules.

The interpretation of the results described above requires further spectral considerations. Both the temperature- and cation-induced changes in the shape of the emission spectra of the donor and the absorption spectra of the acceptor are negligible (data are not shown). Accordingly, the value of the overlap integral (Equation 5.) depends on neither the temperature nor the nature of the bound cation. Therefore it cannot contribute to the observed changes of \( f' \) in the filaments. However, the value of the \( f' \), and hence the relative \( f' \), might depend on the orientation factor (\( \kappa^2 \)). Although this is the only parameter in the fluorescence energy transfer experiments which cannot be measured properly, the measurements of the steady-state anisotropy of both the donor and the acceptor molecules might provide information regarding the behavior of \( \kappa^2 \). The anisotropy of IAEDANS and FC is cation-dependent in the actin filament (Fig. 3, A and B). The measured anisotropy values are larger in the Mg\(^{2+}\)-saturated form than in the Ca\(^{2+}\)-saturated one for both IAEDANS and FC, which can be taken as an indication of conformational differences between the calcium-F-actin and magnesium-F-actin. Interestingly, similar cation-induced change was not observed in the case of IAF (Fig. 3C). Taking into account that both IAEDANS and IAF are connected to the same amino acid (Cys374), the different cation sensitivity possibly originates from the application of different fluorophores. According to the results of Orlova and Egelman

**Fig. 2.** A, the cation dependence of the temperature profile of the relative \( f' \) in F-actin resolved in the experiments dealing with intramonomer flexibility. The donor was IAEDANS, and FC served as an acceptor. The actin concentration was 30–40 \( \mu \)M, while the labeled actin was present at 1–3 \( \mu \)M. B, the temperature profile of the relative \( f' \) in calcium-F-actin and magnesium-F-actin resolved in the experiments dealing with intermonomer flexibility. The value of this parameter was calculated from the results of experiments with the IAEDANS-IAF donor-acceptor pair. The actin concentration was 30–40 \( \mu \)M.

**Fig. 3.** The temperature dependence of the steady-state fluorescence anisotropy of IAEDANS (A), FC (B), and IAF (C) in calcium-F-actin (filled circles) and magnesium-F-actin (open circles). The concentration of actin was 5 \( \mu \)M in these experiments (see "Materials and Methods").
(54), there is a high-density bridge between the two strands of filament when the high-affinity cation-binding sites are occupied by Ca\(^{2+}\). This density bridge was not observed in magnesium-F-actin. They proposed that the presence of this bridge could be the result of the shift in the position of the C terminus. Therefore, the cation dependence of the fluorescence anisotropy in the case of IAEFANS and FC might reflect the cation-induced intramolecular rearrangement of the C-terminal segment in the actin filament. Although the exact nature of this rearrangement is not known, it seems to be possible that the formation of the density bridge in calcium-F-actin involves the modification of some of the connections between the C-terminal segment and the small domain of either the same or the neighboring protomers. The subdomain 1 (involving the Cys\(^{374}\) residue) is in close contact with the subdomain 2 (which contains the Gln\(^{41}\) residue) of the subsequent protomer within the long-pitch helix (49). Accordingly, the formation of the high-density bridge in calcium-F-actin can result in a conformation where the microenvironments of the Cys\(^{374}\) and the Gln\(^{41}\) residues are more flexible than in magnesium-F-actin. Furthermore, the results of the steady-state anisotropy measurements support the conclusion that the intramonomer and intermonomer flexibilities are larger in calcium-F-actin than in magnesium-F-actin. The temperature sensitivity of the fluorescence anisotropy of all fluorophores is similar (Fig. 3, A–C), which suggests that the temperature-induced change in the value of the orientation factor is also similar in these cases. Accordingly, the change in the \(x^2\) is probably not the source of the apparent cation-dependent variation in the value of the relative \(f\) in either the intermonomer or the intramonomer energy transfer experiments. All these data allow the conclusion that both the intramonomer and intermonomer flexibilities are larger in calcium-F-actin than in magnesium-F-actin. Furthermore, the results of the steady-state anisotropy measurements support the conclusion that the microenvironments of the Gln\(^{41}\) and Cys\(^{374}\) residues are more rigid in the magnesium-F-actin than in the calcium-F-actin.

The bending and torsional flexibility of calcium-F-actin was found to be smaller (21) or similar (28–31) to that of magnesium-F-actin. However, we have shown here and in our previous work (26) that the flexibility characteristic for intramolecular motions on a nanosecond time scale is larger in calcium-F-actin than in the Mg\(^{2+}\)-saturated form of the filament. We have suggested (26) that the apparent conflict could be resolved considering that the methods applied in our experiments and those used in the cited articles (21, 28–31) provide information about intramolecular motions on a substantially different time scales.

The structure of the filament-F-actin can be taken as a model of the thin filament in the relaxed state. The changes in the actin-associated layer lines in x-ray diffraction pattern during muscle activation (56, 57) and the differences of the layer lines observed between magnesium-F-actin and calcium-F-actin (54) are similar. Relying on these data Egelman and Orlova (58) proposed that the structure of the calcium-F-actin was corresponding to the thin filaments in the activated state. It is very likely that due to the slow exchange of the tightly bound divalent cation in actin the replacement of Mg\(^{2+}\) by Ca\(^{2+}\) does not occur under physiological conditions (47). Accordingly, Egelman and Orlova (58) concluded that the activated state of the thin filament was probably induced by the binding of myosin. One might assume that the similarity of the structure of calcium-F-actin and the structure of the F-actin in the activated thin filaments can extend to intramolecular dynamic events occurring on a nanosecond timescale. Thus, considering that actin-myosin interaction can possibly utilize the strain energy stored in actin filaments (59), the divalent cation-dependent changes in the intramolecular flexibility described in this study might be important in the efficient energy transduction of the muscle contraction.