Kinetics of Association of Myosin Subfragment-1 to Unlabeled and Pyrenyl-labeled Actin*

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The kinetics of reaction of myosin subfragment-1 (S1) with F-actin have been monitored by the changes in light scattering and in pyrenyl-actin fluorescence at 20 °C, pH 7.5, and physiological ionic strength. The association rate constant of S1 to F-actin decreases about 10-fold as the molar ratio of bound S1 increases from 0 to 1. This decrease in kₚ is most likely due to the steric hindrance of available binding sites by initially bound S1. The apparent rate constant for association of S1 to bare filaments is 9 μM⁻¹ s⁻¹, a value 1 order of magnitude higher than the one previously estimated from experiments in which S1 was in excess over F-actin. The anticooperative binding kinetics of S1 to F-actin are consistent with the negative cooperativity displayed in the equilibrium binding curves of S1 to pyrenyl-F-actin.

Fluorescence titration curves of partially labeled pyrenyl-F-actin by S1 are sigmoidal, consistent with a 4-fold higher affinity of S1 for unlabeled than for labeled actin. This conclusion is strengthened by kinetic data of S1 binding to partially labeled F-actin, which exhibit a biphase behavior due to the slower dissociation of S1 from unlabeled than from labeled actin.

The interaction of the myosin head (myosin subfragment-1, S1) with the actin filament (F-actin) is central to the ATP-driven vectorial movement of myosin along the filament and the resulting production of force by muscle. The mechanism of interaction of S1 with F-actin, the nature of the different complexes formed with or without nucleotide bound to S1 and their relative stabilities have been analyzed by a variety of kinetic and equilibrium methods. In the absence of ATP or ADP, S1 forms a high affinity “rigor” complex (K = 10⁷ M⁻¹) with F-actin, with a maximal binding stoichiometry of one S1 per F-actin subunit (1–4). Formation of the F-actin-S1 rigor complex can be monitored by the associated increase in light scattering or by the 80% quenching of fluorescence of the pyrenyl probe covalently bound to Cys-374 of actin. The kinetic analysis of increase in light scattering (5, 7) or of decrease in pyrenyl fluorescence (6–11) provided a description of the mechanism of actin-S1 complex formation in terms of 2 consecutive reactions, a rapid bimolecular reaction followed by an isomerization process as follows, where S and A represent myosin subfragment 1 and the actin subunit in the filament.

\[
\begin{align*}
A + S & \rightarrow AS \rightarrow AS^* \\
& k_1 \quad k_2 \\
& k_1 \quad k_2
\end{align*}
\]

SCHEME I

The kinetic data, however, were sometimes more complex than expected within Scheme I, and simplifying assumptions were often made in the interpretation of the data, or conditions were chosen under which the experimental complexity appeared lower, allowing a satisfactory description of the data by Scheme I. For instance, biphase fluorescence changes (8) or deviations from simple exponentials (11) have been reported under conditions where a pseudo-first order process was expected, which could not be explained by S1 microheterogeneity; agreement has not been reached on the values found for the rate constants involved in Scheme I, under similar ionic conditions (7, 8, 10); non-linearity has been observed in binding measurements of heavy meromyosin to F-actin (12). Other experiments showed that functional parameters, e.g. the Kₘ of ATP in the actomyosin ATPase (13) and the protection afforded by actin against proteolytic degradation of S1 (14, 15) were dependent on the saturation of the filament by S1. It was proposed recently (11) that the existence of two rigor complexes in which S1 would interact either with one or with two adjacent F-actin subunits in the filament would account for many of the aforementioned deviations from Scheme I. This model uses the facts that the myosin head can form a ternary complex with two G-actin molecules in a low ionic strength buffer where actin alone remains monomeric (16–18) and that the reconstructions of the actin-myosin interface (19) using the crystallized structures of G-actin (20) and of S1 (21) and the atomic model of the filament (22) indicate that S1 can make rigor contacts with two actin subunits interacting with each other via longitudinal bonds along the long pitch helix.

In the present work, we have used light scattering and pyrenyl fluorescence to monitor the kinetics of interaction of S1 with F-actin in rigor. Experiments have been performed at different actin/S1 molar ratios, and both light scattering and fluorescence kinetic data have been analyzed in a comprehensive fashion. We find that the rate constant for association of S1 to F-actin depends on the extent of saturation of the filament by S1. Our results also show evidence for a ~5-fold lower affinity of S1 for pyrenyl-labeled actin than for unlabeled actin.

MATERIALS AND METHODS

Proteins—Actin was purified from rabbit back muscle acetone powder (23) and isolated as CaATP G-actin by Sephadex G-200 chromatography (24) in G buffer (5 mM Tris-Cl, pH 7.8, 0.1 mM CaCl₂, 0.2 mM DTT, 0.2 mM ATP, 0.01% NaN₃). Actin was pyrenyl-labeled in the F-actin form as described (6). The extent of labeling was 0.9 to 1.0. CaATP-G-actin 1:1 complex was prepared free of ATP by Dowex-1 treatment (16, 25). Myosin was purified from rabbit back muscle, and dithymotypic and dithymotypic S₁ was resolved into S₁(A₁S₁) isomers by SP-Trisacryl chromatography in 10 mM MOPS buffer, pH 7.0, 1 mM DTT as described (18). Most experiments were carried out with the S₁(A₁S₁) isomer.

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‡The abbreviations used are: S₁, subfragment-1; DTT, dithiothreitol; MOPS, 4-morpholinepropanesulfonic acid.
concentrations of G-actin and S1 isomers were determined spectrophotometrically using extinction coefficients of 0.617 mg\(^{-1}\) cm\(^{-1}\) at 290 nm for actin (26), and 0.75 mg\(^{-1}\) cm\(^{-1}\) at 277 nm for S1(A2) (27).

Proteins were kept on ice at a concentration of 50–80 \(\mu\)M and used within 2 weeks following purification.

Static Fluorescence Measurements—Equilibrium binding of S1 to F-actin in the rigor state was monitored by the quenching of fluorescence of pyrenyl-F-actin upon binding S1. CaATP-G-actin (1:1 complex (20 \(\mu\)M) containing known proportions of pyrenyl-G-actin was polymerized by addition of 0.1 M KCl and 2 mM MgCl\(_2\), followed by the addition of 1.5 mM equivalent phallolidin. Samples were prepared by diluting the stock F-actin-phallolidin 10-fold in F\(_0\) buffer (5 mM Tris-Cl, pH 7.8, 0.1 mM CaCl\(_2\), 0.2 mM DTT, 2 mM MgCl\(_2\), 0.1 M KCl) containing different amounts of S1(A2) in the range (0–3 \(\mu\)M). The fluorescence of pyrenyl-F-actin was monitored at 20 °C in a Spex fluorolog spectrofluorimeter with excitation and emission wavelengths set at 366 and 387 nm, respectively, after incubation of samples at room temperature for a few minutes.

Rapid Kinetics—The kinetics of interaction of F-actin with S1(A2) in the absence of ATP was monitored in the stopped-flow apparatus (DX.17 MV, Applied Photophysics) at 20 °C. Light scattering was monitored at 90° to the incident light at a wavelength of 400 nm, and slits of 1 mm. Pyrenyl fluorescence was monitored using an excitation wavelength of 366 nm (slit 0.5 mm) and a quanta 380 Schott filter on the emission beam. One of the drive syringes contained F-actin stabilized by phallolidin in F\(_0\) buffer, prepared as described above. The other drive syringe contained S1(A2) also in F\(_0\) buffer. In light scattering measurements, either unlabeled or fully (90%) pyrenyl-labeled F-actin was used. In fluorescence measurements, fully labeled pyrenyl-F-actin was mainly used, except in the experiment described in Fig. 5.

In all kinetic measurements, four to six consecutive shots were performed and the traces were averaged before being analyzed. Kinetic data were routinely analyzed within first order processes, when appropriate, using the software attached to the instrument. When the data deviated from the exponential behavior, a model was proposed based on the qualitative examination of the trend shown by the data upon changing concentrations of either actin or S1, and the kinetic curves were analyzed within the equation describing the new model. In the absence of analytical expression of the time dependence of the intensity of scattered light or of fluorescence, simulation of the kinetic curves was carried out using the HOPKINSIM simulation program, and appropriate values of the rate constants were adjusted by hand to obtain a satisfactory superimposition of a large number of experimental curves obtained at series of concentrations of actin and S1 onto the corresponding theoretical kinetic curves.

RESULTS

Light Scattering Measurements of the Kinetics of Interaction of F-actin with S1(A2): The Process Shows Negative Cooperativity—The time course of increase in light scattering linked to the formation of the F-actin-S1 rigor complex was measured either at constant F-actin and increasing S1, or at constant S1 and increasing F-actin. The increase in intensity of scattered light was a simple first order process only when F-actin was in excess over S1, conditions leading to partially decorated actin filaments, with final molar ratios of bound S1 per F-actin of 0.30. In contrast, when S1 was equal to or in excess over F-actin, conditions under which the molar ratio x of bound S1 increased from 0 to 1 during the binding process, the reaction was not first order. In addition, the extent of increase in the intensity of scattered light per unit of F-actin-S1 complex formed at the end of the reactions, was ~10% higher when fully decorated filaments were formed (S1 in excess over F-actin) than when partially decorated filaments were formed (F-actin in excess over S1). Typical traces obtained for the formation of 2 \(\mu\)M F-actin-S1 from either 2 \(\mu\)M S1 and 6 \(\mu\)M F-actin, or from 2 \(\mu\)M F-actin and 6 \(\mu\)M S1, are shown in Fig. 1 and are clearly not superimposable. The overall reaction was slower when S1 was in excess over F-actin, and the fit to a monoexponential was poor (variance = 2.10\(^{-3}\)) as compared to the good fit (variance = 6.10\(^{-4}\)) obtained when F-actin was in excess over S1 (Fig. 1, insets). The pseudo first order rate constant obtained when F-actin was in excess over S1 varied linearly with F-actin in the range 0–15 \(\mu\)M, as shown in Fig. 2. A value of 9 \(\mu\)M\(^{-1}\) s\(^{-1}\) for the apparent bimolecular association rate constant of S1 to F-actin was derived from the data. The value of the dissociation rate constant (intercept) was too low (\(<1\) s\(^{-1}\)) to be determined with accuracy.

The large difference between the two time courses shown in Fig. 1 arises from the two following points.

First, the increase in intensity of light scattered at 90°, \(R_{90°}\), is not a linear function of x, as described by the following equation (28, 29).

\[
R_{90°} = K P_x (M_A + x M_B)^2 F_0 x
\]

(Eq. 1)

K is a constant proportional to the square of the refractive index increment, \(P_x\) is the shape factor of a filament containing on average x molecules of S1 bound/F-actin subunit, \(M_A\) and \(M_B\) are the molecular masses of actin (42 kDa) and S1 (130 kDa), respectively, and \(F_0\) is the total concentration of F-actin subunits. Because the intensity of scattered light increases cooperatively with x, as is apparent in titration curves (5), the final increase in light scattering when F-actin is in excess over S1 (curve a in Fig. 1) is lower than when S1 is in excess over F-actin (curve b in Fig. 1). When F-actin is in excess over S1, x remains small (0 \(< x \leq 0.2\)) during 80% of the whole reaction, and \(R_{90°}\) can be assumed to be proportional to x, but this assumption is no longer valid when S1 is in excess over F-actin.

Second, comparison of curves a and b indicates that the rate of association of S1 to F-actin declines as the density of bound S1 increases. Hence, the rate constant derived from experiments in which F-actin is in excess over S1 (Fig. 2) refers to the association rate constant \(k^1_s\) of S1 to undecorated or poorly decorated filaments (x < 0.2). To analyze the kinetic curves obtained when F-actin was reacted with an excess of S1, we took into account the non-linearity of \(R_{90°}\) with x, and we tentatively assumed a simple linear decrease of \(k^1_s\) from \(k_0^1\) to \(k_1^1\) as x increases from 0 to 1, as follows.

\[
k_s^1 = (k_1^1 - k_0^1)x + k_0^1
\]

(Eq. 2)
Association of Myosin Subfragment-1 to F-actin

Assuming the binding of S₁ to F-actin to be essentially irreversible, and S₂ to be in excess over F-actin,

\[
dx \over dt - k^3 [S_0][1 - x] = [S_0][k^2 - k^{1}][x^2 + (k^{1} - 2 k^{2})x + k^{0}] \quad \text{(Eq. 3)}
\]

where [S₀] represents the total concentration of S₁, which leads to Equation 4.

\[
x = (k^{0} / k^{2} - 1) \exp [-[S_0][k^{2}][t][1 - \exp [S_0][k^{1}][t] - 1] \quad \text{(Eq. 4)}
\]

The time dependence of x described by Equation 4 was combined with Equation 1 to derive the time dependence of the intensity of scattered light upon binding of S₁ to F-actin under conditions where x varies from 0 to 1 during the reaction.

Fig. 3 shows the good fit of the simulated time courses to experimental traces at three sets of F-actin and S₁ concentrations. The value of k^{2} was imposed equal to 9 \mu M^{-1} s^{-1} as derived from Fig. 2; the fit was good for all curves using the same value of 0.5 ± 0.5 \mu M^{-1} s^{-1} for k^{1}. Values of k^{0} higher than 1 \mu M^{-1} s^{-1} did not fit the data. Hence, the results are consistent with the view that, as the actin filament becomes increasingly saturated by S₁, steric hindrance slows down further binding of S₁ to available sites.

Kinetics of the Change in Pyrenyl-F-actin Fluorescence upon Binding S₁.—The same experiments as the ones described in the previous section were carried out using the change in pyrenyl-actin fluorescence to monitor the binding of S₁ to F-actin. A preliminary experiment showed that the kinetics of change in light scattering were identical, using 90–100% labeled pyrenyl-actin, to the one observed with unlabeled actin. The changes in fluorescence recorded upon adding 5 \mu M S₁(A₂) to 1 \mu M pyrenyl-F-actin, or 5 \mu M pyrenyl-F-actin to 1 \mu M S₁(A₂) are shown in Fig. 4. Again the two time courses corresponding to the formation of 1 \mu M pyrenyl-F-actin-S₁ were not superimposable. In agreement with the light scattering results described above, the reaction was slower when S₁ was in excess over F-actin, and the time courses could be well analyzed within a first order process only when F-actin was in excess over S₁. The pseudo first order rate constant then varied linearly with pyrenyl-F-actin concentration and the data points superimposed with those derived from light scattering kinetics (Fig. 2), indicating that the same rate-limiting process was monitored by either light scattering or pyrenyl fluorescence. In order to appreciate whether the length of the filaments affected the kinetics of interaction with S₁, gelsolin was added to F-actin in a proportion varying between 1:500 to 1:50. The kinetics were unaffected by the length of the filaments in this range.
Typical time courses of the decrease in pyrenyl fluorescence upon mixing 2 μM pyrenyl-F-actin with 6 μM S1(A1) or 1 μM pyrenyl-F-actin with 3, 4, or 5 μM S1(A2) (top to bottom). Noisy curves are experimental traces (average of a minimum of four shots). Smooth curves are calculated time courses using Equations 4 and 5.

![Figure 5](image)

**Figure 5.** Analysis of the change in pyrenyl fluorescence upon reaction of fully labeled pyrenyl-F-actin with an excess of S1.

Experimentally, fluorescence titration curves are shown in Fig. 6 at 2 and 0.4 μM pyrenyl-F-actin. Although the difference between the theoretical best fits provided by a regular hyperbolic and an anticooperative binding scheme is small, clearly the data are accurate enough to demonstrate that the anticooperative binding scheme provides a better fit to the experimental curve. The theoretical hyperbolic best fit (K = 0.02 μM) indeed crosses the experimental curve twice. The anticooperative binding curve was calculated using the following classical equation (30), where Kc and Kr represent the equilibrium dissociation constants for binding of S1 to an unlabeled or a filament containing a proportion, x, of F-actin-S1 subunits.

\[ K_x = K_c e^{-w(x)} \]  

(Eq. 6)

The nature of the function \( \psi(x) \) being unknown, the simple linear function \( \psi(x) = ax \) was tried. The best fit (shown in Fig. 6) was obtained for Kc = 0.01 μM and \( a = -2.3 \pm 0.2 \). Note that this value of \( a \) also corresponds to a 10-fold decrease in affinity of S1 for F-actin as the saturation of the filament by myosin heads increases from 0 to 1.

Other experiments done at higher ionic strength (0.3 M KCl) yielded binding curves that were consistent with a lower overall affinity, but showed no increased anticooperativity.

All the above experiments have been unable to detect an appreciable difference between unlabeled and labeled actin in reacting with S1. However, the affinity of S1 for both actins is so high that a 10-fold difference in the apparent rate constants of dissociation of S1 from F-actin or pyrenyl-F-actin would not have been detected.

Reaction of S1 with Mixtures of Unlabeled and Pyrenyl-labeled F-actin—Samples of 2 μM F-actin containing different proportions (C, 90% [ ], 40% [m], 53%) of pyrenyl-F-actin were titrated by S1(A1). Open and closed circles represent two sets of independent experiments. The fluorescence of pyrenyl-F-actin was measured at equilibrium as described under "Materials and Methods." Experimental conditions are Fpes buffer, 20 ºC. Symbols represent the data. Solid lines are calculated curves within the competition scheme proposed in the text and using \( K_c/K_o = 4 \) and \( K_o = 0.05 \mu M \).
data shows that the observed quenching of fluorescence of a
given pyrenyl-labeled F-actin subunit upon binding S1 is inde-
pendent of the nature (labeled or unlabeled) of the neighboring
subunits in the filament. The data shown in Fig. 7 were there-
fore analyzed within the following simple scheme, where F and
F* represent unlabeled and pyrenyl-labeled F-actin, respecti-
vively, and FS and *FS their complexes with S1.

\[
F + S \leftrightarrow FS, \quad K_f = \frac{[F][S]}{[FS]}
\]

\[
*F + S \leftrightarrow *FS, \quad K_{f} = \frac{[*F][S]}{[*FS]}
\]

**Scheme II**

The relative change in pyrenyl fluorescence, \( Y \), reflected the
binding of S1 to pyrenyl-F-actin, as described by the following
equation, where \( \phi(0) \), \( \phi(S_0) \) and \( \phi(\infty) \) represent the
fluorescence intensity observed in the absence of S1, or at a given
total concentration \( S_0 \) or at a saturating concentration of S1,

\[
Y = \frac{\phi(0) - \phi(S_0)}{\phi(0) - \phi(\infty)} \quad \text{[Eq. 7]}
\]

The combined equations for mass conservation lead to:

\[
[S] = [S_0] - [FS] - [F] + [F0] \quad \text{[Eq. 8]}
\]

where \([S]\) and \([S_0]\) represent the free and total concentrations
of S1, \([F_0]\) and \([*F_0]\) the total concentrations of unlabeled and
labeled F-actin, respectively. The value of \([S]\) is the solution of
a cubic equation. A simple fitting procedure was used by gen-
erating a series of incremented values of \([S]\), and calculating
the corresponding values of \([S_0]\) according to Equation 8. The
theoretical curves representing Y versus \([S_0]\) were adjusted to
match the ensemble of experimental curves obtained at differ-
ent percentages of pyrenyl-actin by changing the values of \(K_f\)
and \(K_{f'}\), knowing that both values have to be lower than 0.1 \(\mu\)M;
hence, only the ratio \(K_f/K_{f'}\) can actually be derived from these
measurements. The value of \(K_f/K_{f'}\) had to be in the range of 4 - 5
to provide a satisfactory fit to all the curves taken together. The
\(\chi^2\) value increased when values of 3.5 or 5.5 were used for
\(K_f/K_{f'}\).

In conclusion, equilibrium binding measurements of S1 to
mixtures of unlabeled and pyrenyl-labeled F-actin demonstrate
that the affinity of S1 for labeled actin is 4-fold lower than for
unlabeled actin. In conclusion, derivatizing Cys-374 alters the
interaction of S1 with F-actin. Kinetic evidence for a lower
affinity of S1 for pyrenyl-actin was provided by observing the
time course of pyrenyl fluorescence upon mixing S1 with par-
tially labeled F-actin; Fig. 8A shows that when S1 was substoi-
chiometric with respect to total F-actin, a biaphasic change was
observed, a rapid quenching of fluorescence being followed by a
slower recovery to a higher final fluorescence intensity. This
biaphasic change indicates that the rates of S1 association to
labeled or unlabeled F-actin both are very fast, but the rate of
dissociation of the F-actin-S1 complex is lower for unlabeled
actin. The extent of fluorescence recovery was lower when the
cular ratio of S1 to F-actin increased; no fluorescence recovery
was observed when the concentration of S1 was sufficient to
bind to both labeled and unlabeled actin. As shown in Fig. 8B,
the amplitude of the rapid transient decrease in fluorescence
varied linearly with S1 until the 1:1 molar ratio to total F-actin
was reached, then remained constant, giving a high affinity
titration curve within which pyrenyl-F-actin would react with
S1 with the same affinity as unlabeled F-actin. The final fluo-
rescence in turn confirmed the sigmoidal titration curve dis-
played in equilibrium measurements (Fig. 7). The slow recov-
ergy of fluorescence was a first order process of rate constant \(k
= 0.16 \text{ s}^{-1}\), which is the rate-limiting process in the dissocia-
tion of S1 from pyrenyl-F-actin.

**Temperature Dependence of the Kinetics of Binding of S1 to
F-actin**—All kinetic data obtained thus far at 20 °C under
physiological conditions (Fig. 2) show no evidence for a higher
limit in the pseudo first order rate constant for the reaction of
S1 with F-actin. A limit of 200 s^{-1} has been measured by others
at 20 °C and low ionic strength (10). At physiological ionic
strength, no rate-limiting step could be measured (8), in agree-
ment with the present data. However, the existence of a con-
formational change of F-actin-S1 following the formation of a
rapid equilibrium collision complex was inferred from two ob-
servations. First, the value found for the apparent second order
rate constant for association of S1 to F-actin at physiological
ionic strength was typically \(10^6 \text{ M}^{-1} \text{ s}^{-1}\) at 20 °C, which
is lower than expected for a diffusion-controlled reaction (5, 8,
30). Second, the binding reaction had a very strong tempera-
ture dependence, indicative of the involvement of a conforma-
tional change in the measured process (31). It should be noted,
however, that the low value found in previous works (5, 8, 31)
for the apparent second order rate constant under conditions
similar to ours (physiological pH and ionic strength, 20 °C) was
derived from the analysis of turbidity or pyrene fluorescence
changes, in excess of S1 over F-actin, within a simpler first order
Association of Myosin Subfragment-1 to F-actin

The equilibrium and kinetics of binding of myosin subfragment-1 to F-actin have been investigated with the aim to understand whether the binding was a reaction described by a single equilibrium dissociation constant, as concluded from earlier works (1-5), or whether the equilibrium binding might be non-Michaelian, and associated to more complex kinetics, as suggested by more recent works, which pointed to the nonlinear dependence of physical parameters monitoring F-actin-S1 interaction on the extent of S1 bound to F-actin (11, 32-35). We find that the rate of S1 binding to F-actin depends on the extent of bound S1, steric hindrance of the first bound S1 molecules preventing further binding of S1 to the partially decorated filament. Both the kinetics of changes in light scattering or pyrenyl-actin fluorescence (Figs. 1 and 3-5) and the equilibrium binding measurements (Fig. 6) can be quantitatively accounted for, assuming that the apparent bi-molecular association rate constant decreases linearly with the molar ratio of bound S1. A good fit to the data was obtained assuming a 10-fold decrease in the association rate constant \( k_1 \) of S1 to F-actin as the saturation of the filament by myosin heads increases from 0 to 1. The corresponding 10-fold decrease in affinity of S1 for F-actin, over the whole titration curve, was confirmed in the analysis of the equilibrium binding data (Fig. 6).

Evidence for negative cooperativity in binding kinetics was provided by the observation that the F-actin-S1 complex is formed faster when F-actin is in excess over S1 than in the opposite situation, and that the process is not first order when S1 is in excess over F-actin. Such non-exponential reactions have been noticed in the past (8, 11). One of the main points of the present paper is to propose a quantitative analysis of this phenomenon. The kinetic data were analyzed assuming a linear dependence of \( k \), on the stoichiometry \( x \) of S1 bound per F-actin subunit. This linear dependence was chosen, in the absence of any experimental evidence for a defined binding scheme, because it provided a simple analytical solution (Equation 4) that allowed simulation and fitting of the non-exponential binding kinetics. A more physically reasonable analysis of the data, taking into account the polarity of the filament, would imply to define different rate constants for association of S1 to F-actin subunits having either no neighboring bound S1, or a neighboring bound S1, ethed on the barbed or on the pointed end side of the subunit to which S1 is binding. Consideration of the helical nature of the filament, in which each actin subunit in fact four neighbors, would introduce further splitting of the different association rate constants. A complete analysis would also require a statistical analysis of the distribution of S1 bound at each value of \( x \), and Monte Carlo methods would be necessary to model the binding scheme adequately. However, we are not sure that fitting such a complex model to the present data would provide an unambiguous set of association rate constants.

Our results and conclusions are in partial agreement and in partial disagreement with the recent work of Andreev et al. (11). While the equilibrium and kinetic data of S1 association to F-actin are in agreement with the anti-cooperativity in S1 binding demonstrated by the sedimentation data of Andreev et al. (11), our kinetic observations and our interpretation differ. Andreev et al. reported that the time course of quenching in pyrene fluorescence upon binding S1 was biphasic when F-actin was in excess over S1, which was interpreted as a two-step binding of S1 to one F-actin subunit, followed by an isomerization consistent with binding of S1 to a second actin subunit in the filament. Our observations are somewhat different, since a
simple exponential binding process was recorded when F-actin was in excess over S1 (Figs. 1 and 4), and the reaction deviated from a monoequalnal when S1 was in excess over F-actin. The data presented by Andreev et al. with S1 in excess over F-actin (Fig. 2A in Ref. 11) actually show a very poor fit to a monoequalnal (in agreement with our data), the experimental trace intersecting the exponential best fit several times. Although our data do not support the two-step binding model proposed by Andreev et al., dearly the basic observations of nonlinear kinetics and anticooperative equilibrium binding are confirmed in the present work, with a different interpretation.

In most kinetic studies of S1 binding carried out thus far, the concentration of S1 was varied, in excess over F-actin. The data therefore referred to a high molar ratio of bound S1, and were assumed to be well described by monoequalnals, which now appears to be incorrect (see curves b, residuals, in Figs. 1 and 4). Within our interpretation, the rate constant derived from such kinetic data, analyzed within a single first order process, reflects the average rate of binding of S1 to a partially decorated filament, and is therefore lower than the rate constant for association of S1 to a bare filament, which we find equal to 9 μM⁻¹ s⁻¹ at 20 °C under physiologival conditions. This value is within the range expected for a diffusion-controlled reaction; however, the high activation energy of the reaction strongly suggests that a structural change of the F-actin S1 complex is involved, in agreement with previous works, and also with the model derived from the kinetics of interaction of G-actin with S1 (18).

The present work gives the first quantitative estimate of the difference in affinity of S1 for pyrenyl-labeled and unlabeled actin. This difference was previously thought to be insignificant, because S1 reacts at apparently identical rates with either unlabeled or fully labeled F-actin. However, the reaction of S1 with mixtures of labeled and unlabeled actins clearly shows evidence, both in the equilibrium binding curves and in the kinetics, for a 4-5-fold difference in affinity, most likely accounted for by a difference in the rate constant k⁻² in Scheme I.

The conclusion that labeling of Cys-374 on actin interferes with S1 binding is in agreement with predictions made in the reconstruction of the actin-myosin head interface from the crystallographic images of actin and S1 (19) and using the atomic model of the actin filament (22). It also agrees with the fact that in F-actin, Cys-374 can be N,N'-paraphenylenediamineimide cross-linked to Lys-191 of the adjacent subunit along the short pitch helix (36), and this cross-link is inhibited by S1 binding (37), indicating S1 binding to F-actin perturbs the region of the C terminus of F-actin. It should be noted that the difference in affinity of labeled and unlabeled actin for S1 is not displayed by G-actin (16, 18). Accordingly S1 can be cross-linked to Cys-374 in the G-actin-S1 complexes, not in the F-actin-S1 complex (38).

Finally our results suggest that the cross-bridges might operate differently when the thin filaments are poorly or fully saturated by myosin heads, a conclusion in agreement with biochemical data (13) and structural evidence (39) for the interaction between adjacent heads bound to the filament at high S1/actin ratios.

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