Elongation of Actin Filaments is a Diffusion-limited Reaction at the Barbed End and Is Accelerated by Inert Macromolecules*  

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We used a fluorescence method to measure the rate constants for the elongation of pyrene-labeled actin filaments in a number of different solvents. The absolute values of the rate constants were established by electron microscopy. Using glycerol, sucrose, or ethylene glycol to vary the solution viscosity, the association rate constant \( k_a \) was \( 10^7 \) \( m^{-1} \) s\(^{-1} \) viscosity\(^{-1} \) (in centipoise). Consequently, plots of \( 1/k_a \) versus viscosity are linear and extrapolate to near the origin as expected for a diffusion-limited reaction where the rate constant approaches infinity at zero viscosity. By electron microscopy, we found that this inhibitory effect of glycerol is almost entirely at the fast growing, barbed end. For the pointed end, plots of \( 1/k_a \) versus viscosity extrapolate to a maximum rate of about \( 10^4 \) s\(^{-1} \) at zero viscosity, so that elongation at the pointed is not limited by diffusion. In contrast to these small molecules, polyethylene glycol, dextran, and ovalbumin all cause a concentration (and therefore viscosity)-dependent increase in \( k_a \). At any given viscosity, their effects are similar to each other. For example, at 3 centipoise, \( k_a = 2.2 \times 10^7 \) \( m^{-1} \) s\(^{-1} \). We presume that this is due to an excluded volume effect that causes an increase in the thermodynamic activity of the actin. If the proteins in the cytoplasmic matrix have a similar effect, the association reactions of actin in cells may be much faster than expected from experiments done in dilute buffers.

\[ k_a = 4s \cdot k_{free} \cdot b(D_m + D_p) \cdot N \cdot 10^{-9} \] (1)

where \( k \) is a unitless steric factor, \( k_{free} \) is a unitless electrostatic factor, \( b \) is the interaction radius in centimeters, \( D_m \) and \( D_p \) are the diffusion coefficients of the two reactants (monomers and filament ends, respectively), and \( N \) is Avogadro's number (see Berg and von Hippel (1985) for details). For the addition of an actin monomer to the end of an actin filament, \( D_m = 5 \times 10^{-7} \) \( cm^2 \) s\(^{-1} \) (Lanni et al., 1981; Tait and Frieden, 1982), \( D_p < 10^{-7} \) \( cm^2 \) s\(^{-1} \) (Tait and Frieden, 1982), and \( b \) is assumed to be \( 2 \times 10^{-7} \) \( cm \) based on the shape of the actin molecule (Smith et al., 1984). If \( k \) and \( k_{free} \) have values of unity, then the rate constant for elongation will equal that for collision and have a value of \( 7.5 \times 10^6 \) \( m^{-1} \) s\(^{-1} \). However, the correct orientation of the reacting molecules is essential so that \( k \) is more likely to have a value of \( 10^{-4} \) to \( 10^{-1} \) (Berg and von Hippel, 1985), so \( k_a \) will be in the range of \( 7.5 \times 10^5 \) to \( 7.5 \times 10^6 \) \( m^{-1} \) s\(^{-1} \). These predictions put the observed values for \( k_a \) well within the range for diffusion-limited reactions.

Since \( D \) is an inverse function of viscosity, one can test for a diffusion-limited process by evaluating the dependence of the reaction rate on viscosity (see Berg and von Hippel (1985) for a review of diffusion-limited processes). To do so for actin polymerization, we measured the elongation rate and rate constants in several different solutes with a range of viscosities and also over a range of temperatures. We also evaluated \( f_{free} \) by determining the rate constants over a wide range of salt concentrations.

We found that the rate constants for both subunit association and dissociation at the barbed end of actin filaments scale with inverse viscosity in glycerol, sucrose, and ethylene glycol, as expected for a diffusion-limited process. The dependence of the rate on the temperature is explained in part by the viscosity, but there is also a chemical activation process with an activation energy of about 3.5 kcal/mol. Above 50 mM KCl, salt inhibits elongation in a concentration-dependent fashion so electrostatic interactions are also an important factor in the process. Elongation at the pointed end also depends on the viscosity but is not diffusion-limited. Several macromolecules (ovalbumin, polyethylene glycol, and dextran) all increase the elongation rate in spite of the fact that they also increase the viscosity. This is presumably caused by an excluded volume effect that increases the thermodynamic activity of the actin (reviewed by Minton (1983)). Consequently, actin polymerization inside cells may occur much more rapidly than expected from previous work in dilute buffers.

**MATERIALS AND METHODS**

The solutes used in these experiments came from the following suppliers and were used without further purification: dextran T-40 (Pharmacia Fine Chemicals, Uppsala, Sweden); ethylene glycol, glycerol, polyethylene glycol 6000, and sucrose (J. T. Baker Chemical Co., Phillipsburg, NJ); ovalbumin (Sigma). Actin was pu-
rifed from rabbit skeletal muscle using gel filtration on Sephadex G-150 in 2 mM imidazole (pH 7.0), 0.5 mM dithiothreitol, 0.2 mM ATP, 0.1 mM CaCl₂, as the final step (MacLean-Fletcher and Pollard, 1980). *Acanthamoeba* actin was purified from sucrose extracts (Pollard, 1984). Part of the actin was labeled with pyrene iodoacetamide (Pollard, 1984) and used at 1–5% of the total actin in the fluorescence assay for polymerized actin (Koyama and Mihashi, 1981). Unless noted otherwise, the polymerization reactions were carried out in standard buffer (50 mM KCl, 1 mM MgCl₂, 1 mM EGTA, 0.5 mM dithiothreitol, 0.1 mM CaCl₂, 0.1–0.2 mM ATP, 10 mM imidazole (pH 7). The steady state extent of polymerization and initial rate of elongation from unlabeled actin filament nuclei were measured fluorometrically, usually at 20 °C (Pollard, 1983). Most of the solutes affected the fluorescence intensity of pyrene-labeled actin, so all data are corrected. All plots of elongation rate versus concentration of actin monomer were linear, except for those in high concentrations of ovalbumin. In that case, the elongation rate constants were calculated from the critical concentrations and rates at 1.2 μM actin. Absolute rates of elongation were measured by electron microscopy using bundles of actin filaments from *Limulus* sperm as nuclei (see Pollard and Cooper (1984)) except that the reaction was carried out directly on the electron microscopy grid and was stopped by inversion of the grid onto a large volume of 5 mM spermine (Sigma) in standard buffer to aggregate the filaments grown from the ends of the *Limulus* bundles. The details of this method will be presented in another paper. The viscosities of glycerol- and sucrose-containing solutions were taken from standard tables. The viscosities of the other solutions were measured in a Cannon-Manning semimicroviscometer (Cannon Instruments Co., State College, PA) at 20 °C. All linear plots were fit by least squares linear regression.

**RESULTS**

Sucrose, glycerol, and ethylene glycol all inhibit the elongation of rabbit muscle actin filaments but have little (sucrose) or no (glycerol and ethylene glycol) measurable effect on the critical concentration for polymerization evaluated by either steady state or initial rate methods (Table I). Similar results were obtained with glycerol solutions using *Acanthamoeba* actin. The rate constants for association and dissociation were determined from the dependence of the initial rate of elongation from actin filament nuclei on the actin monomer concentration. In all three solutes, *k₅* scales as an inverse function of viscosity (Fig. 1, open symbols). Plots of *k₅* versus viscosity are linear, and all extrapolate to near the origin (Fig. 2B). The origin corresponds to an infinite rate constant at zero viscosity. Since the critical concentration is constant, *k₅* also scales with viscosity in exactly the same way as *k₆*, otherwise the system would violate the principle of reversible thermodynamics. The absolute value of *k₅* in standard buffer at 20 °C was 10⁷ M⁻¹ s⁻¹ in parallel electron microscopy experiments, and all of these plots were normalized to this value.

Similar experiments by electron microscopy using glycerol to vary the viscosity showed that elongation at the barbed end is an inverse function of viscosity that plots of (*k₅*ₜₜ)⁻¹ versus viscosity extrapolate to near the origin (Fig. 2A (O) and Fig. 3). Elongation at the pointed end is only weakly dependent on the viscosity. Plots of (*k₅*ₜₜ)⁻¹ versus viscosity do not extrapolate to the origin (Fig. 2A (●)). Instead, the value of *k₅* at zero viscosity is about 10⁶ M⁻¹ s⁻¹.

In standard buffer, the elongation rate but not the critical concentration depends on the temperature (Fig. 4, A and B). This is true for both muscle and *Acanthamoeba* actin. From the dependence of *k₆* on the temperature, the activation energy for elongation is 8.5 kcal/mol (Fig. 4B, inset, and Table II). Using the temperature to vary the viscosity in standard buffer ±10% glycerol, plots of *k₅* versus viscosity are linear but do not extrapolate through the origin (Fig. 5). Above 20 °C, *k₅* is larger than expected from the temperature dependence of the viscosity alone; whereas below 20 °C, the opposite is true. If the values for *k₅*, as a function of temperature are corrected by a factor of viscosity⁻¹, the average activation energy is 3.5 kcal/mol.

Contrary to the effect of small solutes, all three macromolecular solutes that we tested increased the rate of elongation, in spite of the fact that they all increase the viscosity (Fig. 1, filled symbols). When plotted as a function of their viscosity, all three macromolecules had similar effects on elongation. Note that the difference between the small solutes and macromolecules can be considerable. For example, at a viscosity of 3 centipoise, elongation is almost 6 times faster in dextran, ovalbumin, or polyethylene glycol than in ethylene glycol, glycerol, or sucrose. Dextran and ovalbumin did not change the critical concentration for polymerization, whereas polyethylene glycol decreased the critical concentration to a small extent as described previously (Stromqvist et al., 1984). Our observations with polyethylene glycol differ from previously

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**Table I**

Effect of solutes on the critical concentration for the polymerization of actin

| Solute            | Viscosity | Critical concentration |
|-------------------|-----------|------------------------|
|                   |           | Steady state | Elongation rate |
| Standard buffer   | cP        | 0.15, 0.16     | 0.08, 0.10      | 0.12, 0.08     |
| Ethylene glycol (%)|          | 0.16          | 0.08            |
| 15                | 1.5       | 0.14          | 0.08            |
| 25                | 2.0       | 0.16          | 0.08            |
| 35                | 2.5       |              | 0.08            |
| 40                | 3.0       | 0.16          | 0.08            |
| Glycerol (%)      |           |              | 0.08            |
| 10                | 1.31      |              | 0.10            |
| 19*               | 1.65      |              | 0.10            |
| 27.5*             | 2.2       |              | 0.10            |
| 30                | 2.5       |              | 0.10            |
| 39                | 3.4       |              | 0.10            |
| Sucrose (%)       |           |              | 0.08            |
| 13.5              | 1.5       |              | 0.08            |
| 21                | 2.0       | 0.18          | 0.08            |
| 25.5              | 2.5       | 0.19          | 0.10            |
| 25                | 3.0       | 0.19          | 0.10            |
| Dextran (%)       |           |              | 0.10            |
| 5                 | 2.8       | 0.12          | 0.10            |
| 7                 | 4.25      |              | 0.10            |
| 10                | 7.5       |              | 0.10            |
| Ovalbumin (%)     |           |              | 0.10            |
| 5                 | 1.1       | 0.10          | 0.10            |
| 7                 | 1.25      | 0.10          | 0.10            |
| 10                | 1.5       |              | 0.10            |
| 15                | 2.0       |              | 0.10            |
| 20                | 3.3       |              | 0.10            |
| Polyethylene glycol (%) | |              | 0.10            |
| 1                 | 1.25      | 0.10          | 0.10            |
| 2                 | 1.60      | 0.10          | 0.10            |
| 3                 | 1.80      | 0.10          | 0.10            |
| 4                 | 2.7       | 0.08          | 0.10            |
| 6                 | 3.6       | 0.08          | 0.10            |
| 7                 | 4.25      | 0.08          | 0.10            |

The abbreviation used is: EGTA, ethylenebis(oxyethylene-nitrito)tetraacetic acid.

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The elongation rate constant ($k_+$) of actin filaments is diffusion-limited. Using low molecular weight solutes (open symbols) or macromolecular solutes (closed symbols), these rate constants were measured by the fluorescence method. $\circ$, rate constant in standard KCl/MgCl$_2$ buffer without added solutes. PEG 6K, polyethylene glycol 6000.

![Figure 1](image1.png)

**Figure 1.** Dependence of the elongation rate constant ($k_+$) ($\mu\text{M}^{-1}\text{s}^{-1}$) on the viscosity (centipoise, cP) using low molecular weight solutes (open symbols) or macromolecular solutes (closed symbols). These rate constants were measured by the fluorescence method. $\circ$, rate constant in standard KCl/MgCl$_2$ buffer without added solutes. PEG 6K, polyethylene glycol 6000.

![Figure 2](image2.png)

**Figure 2.** Plots of $1/k_+$ ($\mu\text{M}^{-1}\text{s}^{-1}$) versus viscosity. $A$, measurements at the two ends of the filament by electron microscopy using glycerol to vary the viscosity; $B$, measurements by the fluorescence method. $\circ$, standard KCl/MgCl$_2$ buffer without added solutes.

Published data describing only a small effect on the rate of elongation (Stromqvist et al., 1984).

To evaluate the electrostatic factor ($f_{\text{elec}}$), the rate constants were measured over a wide range of KCl concentrations (Fig. 6). The optimal concentration of KCl for elongation in 1 mM MgCl$_2$ is 50 mM, and $k_+$ declines at higher concentrations of KCl. Both $k_+$ and $k_-$ vary with salt concentration, but their ratio, the critical concentration, is relatively constant at 0.16–0.34 $\mu$M.

**DISCUSSION**

In the small solutes that we tested, the elongation rate at the barbed end ($R^b$) of an actin filament is

$$R^b = (k_+A_1 - k_-)\text{viscosity}^{-1}$$

with the viscosity in centipose. $A_1$ is the concentration of monomeric actin. This relationship, the extrapolation of $k_-$ versus viscosity to near the origin, and the large absolute value of $k_+$ all support the conclusion that the association...
TABLE II  
Activation energy for the elongation of actin filaments 

| Actin            | Temperature range °C | Activation energy kcal mol⁻¹ |
|------------------|----------------------|-----------------------------|
| Rabbit skeletal muscle       | 7.0-36.5             | 6.6                         |
|                   | 7.6-37.0             | 8.0                         |
|                   | 7.0-36.8             | 9.0                         |
|                   | 7.0-22.5             | 9.9                         |
| Acanthamoeba      | 7.5-25.0             | 10.1                        |
|                   | 6.2-28.5             | 7.1                         |
| Mean = 8.5        | S.D. = 1.5           |                             |

Fig. 5. Plots of 1/k⁺ versus viscosity with viscosity varied by temperature in standard buffer (●) or 10% glycerol in standard buffer (○). Measurement was by the fluorescence method. The dashed line is the average of the three plots in Fig. 2.

Elongation of Actin Filaments Is Diffusion-limited

reaction at the barbed end is highly favorable and under optimal conditions limited only by diffusion. Assuming that \( f_{inc} = 1 \), the steric factor \( k_s \) is rather large, about 0.02. Interpreted physically, 2% of the collisions of monomers with the end of a filament lead to binding, a high efficiency considering that the incoming subunit will rarely be oriented correctly to bind to the end of the filament.

At first glance, it seems remarkable that dissociation of subunits from the barbed end of the filament is also a diffusion-limited process. However, there are at least two reasonable explanations. First, the dissociation reaction must involve diffusion of the exiting subunit away from the vicinity of the filament end. To the extent that this movement is limited by the viscosity of the medium, the molecule may fail to move away fast enough to escape rather than rebinding. Second, the dissociation reaction may require that the terminal subunit be in a particular conformation to break its bonds with its neighbors. Since some intramolecular motions are driven by collisions of solvent with the surface, the rate of conformational fluctuations and therefore the frequency of some states will depend in part on the viscosity of the medium.

At the pointed end of actin filaments, the elongation reaction is probably not diffusion-limited since \( k_s^p \) is not an inverse function of viscosity and its absolute value is much smaller than at the barbed end. The mechanism can only be guessed at, but it is likely to be a multistep process with some steps independent of the viscosity. The extrapolated value of the rate constant at zero viscosity is about \( 10^8 \) M⁻¹ s⁻¹. This may be the chemical rate constant for a rate-limiting step. The existence of these additional steps may account for the slower rate compared with the barbed end where the collision rate seems to be the limiting step.

The activation energy of 8.5 kcal/mol is higher than expected for a diffusion-limited reaction (\(-5\) kcal/mol for reactions in water from 0 to 37 °C). When corrected for the effect of temperature on viscosity, actin filament elongation has a small activation energy of 3.5 kcal/mol that can be attributed to chemical activation of the reactants. Presumably, temperature affects the conformation and/or dynamics of either the monomers or filament ends or both. Although temperature affects the rates of the elongation reactions, it does not change the critical concentration in our standard polymerization buffer as it does under other conditions (see Korn (1982)). Hence, in MgCl₂, the apparent equilibrium constant is temperature-independent, \( \Delta H = 0 \), and the reaction is driven by \( \Delta S \) as shown previously by others (see Korn (1982)).

Electrostatic forces are a factor in the binding of subunits to the end of filaments since \( k_s \) declines toward zero at high concentrations of salt. Consequently, the \( f_{inc} \) term in Equation 1 is less than 1, and the unitless steric factor \( k \) in Equation 1 is actually larger than 0.02.

In terms of the biological functions of actin, the effect of macromolecules on elongation rates is probably our most important new observation. At a total protein concentration like that in the cytoplasm, elongation may be 10 times faster than expected for a solution of that viscosity. As in the case of some other biochemical reactions that are accelerated by inert macromolecules, this is probably an excluded volume effect (reviewed by Minton (1983)). The inert macromolecules increase the thermodynamic activity of the actin and thereby increase its effective concentration. This effect can be substantial, on the order of 10-fold. Consequently, inside cells, both association and dissociation may occur at vastly higher rates than expected from previous measurements of rate constants in dilute buffers. For example, Tilney and Inoue (1982)
calculated that diffusion alone could just barely account for
the rapid elongation of actin filaments in the acrosomal
process of Thyone sperm, but this required some generous
assumptions about the actin monomer concentration at the
base of the process. If the thermodynamic activity of actin is
10 times higher than its concentration, then even conservative
estimates regarding the monomer concentration are consist-
ent with the growth rate that they observed.

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