Thermodynamic regulation of actin polymerization

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(Received 21 March 2001; accepted 1 May 2001)

A Flory–Huggins-type lattice model of actin polymerization under equilibrium conditions is employed to analyze new spectroscopic measurements for the extent of actin polymerization Φ as a function of temperature T, salt concentration [KCl], and the initial concentration of actin monomers $[G_0]$. The theory subsumes existing mechanisms for actin monomer initiation, dimerization, and chain propagation. The extent of polymerization Φ increases with T to an unanticipated maximum, and the calculations explain this unusual effect as arising from a competition between monomer activation, which diminishes upon heating, and propagating chain growth, which increases upon heating. The actin polymerization is described as a rounded phase transition, and the associated polymerization temperature T_p depends strongly, but nearly linearly on $[G_0]$ and [KCl] over the concentration regimes investigated. Our findings support the suggestion that physicochemical changes can complement regulatory proteins in controlling actin polymerization in living systems. © *2001 American Institute of Physics.*
[DOI: 10.1063/1.1380693]

The polymerization of monomeric *G*-actin into *F*-actin filaments is a paradigm for the reversible polymerizations of many other biological systems at equilibrium, such as tubulin, flagellin, fibrin, and tobacco mosaic virus.[1] Moreover, actin polymerization exhibits unique features not present in other ''living'' polymerization systems, e.g., the reversible polymerization of sulfur[2] which also polymerizes upon heating. For example, the measurements described below indicate that the fraction of monomers polymerized (Φ) exhibits a maximum as a function of temperature T, and this unusual feature has prompted the theoretical portion of the present investigation. An additional difference is the presence of a reversible dimerization that produces a low temperature tail in Φ vs T. The competition between this dimerization and the chain propagation is of interest in biological contexts since it serves to regulate the chain length far from the polymerization transition.

Virtually all prior attempts[3] at elucidating the mechanism for actin polymerization have studied the polymerization kinetics under physiological conditions, requiring the tedious fitting of experimental data to the solutions of coupled nonlinear differential equations that depend on at least six unknown rate constants (associated with the three reversible reaction steps). We show that by using temperature and other thermodynamic variables rather than time as the measurement variables, we more effectively determine the mechanism and the equilibrium energetic parameters governing the polymerization process. There are still six energetic parameters to be determined, but strong constraints tie the entropy and enthalpy parameters for each of the three essential processes (activation, dimerization, propagation), leading effectively to three adjustable parameters. These basic energetic parameters are shown to exhibit strong variations with added salt concentration [KCl] and with initial *G*-actin concentration $[G_0]$, thereby supporting prior suggestions[4–6] for a physiochemical component to the control of actin polymerization in nonmuscle cells. The thermodynamic approach also emphasizes the nature of the underlying phase transformation which is neglected in kinetic studies.

The first stage of actin polymerization is believed to in-

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volve initiation and dimerization of initiated actin monomers,[7,8] followed by the growth of actin filaments (*F*-actin).[9,10] The activated monomer and dimer react to form trimers (the nucleus), and the trimers associate with monomers to yield polymers.[1] We consider solutions where activation and propagation occur under equilibrium conditions determined from the minimal reaction scheme,

$$A_1 \rightleftharpoons A_1^* \quad \text{(monomer activation)}, \tag{1}$$

$$A_1^* + A_1^* \rightleftharpoons A_2 \quad \text{(dimerization)}, \tag{2}$$

$$A_2 + A_1^* \rightleftharpoons A_3 \quad \text{(trimer formation)}, \tag{3}$$

$$A_i + A_1 \rightleftharpoons A_{i+1}, \quad i = 3,4,\ldots,\infty \quad \text{(propagation)}, \tag{4}$$

where A_1 designates a *G*-actin monomer, an asterisk denotes an activated species, and the subscript i indicates the degree of polymerization. A similar hierarchy of reactions for actin polymerization is employed by Cooper *et al.*[3] in kinetic studies of actin polymerization, and the evidence for this reaction scheme is discussed elsewhere.[7–12]

For simplicity, the free energies associated with the propagation reactions (3) and (4) are taken as identical, so the distribution of actin species at a given temperature T is governed by three equilibrium constants or, equivalently, by three free energies: the free energy of initiation $\Delta G^0_{\rm init} = \Delta H^0_{\rm init} - T\Delta S^0_{\rm init}$, the free energy of dimerization $\Delta G^0_{\rm dim} = \Delta H^0_{\rm dim} - T\Delta S^0_{\rm dim}$, and the free energy of propagation $\Delta G^0_{\rm prop} = \Delta H^0_{\rm prop} - T\Delta S^0_{\rm prop}$. The equilibrium system is described by an incompressible Flory–Huggins (FH) type lattice model[13–15] in which each A_i species occupies i lattice sites. For mixtures of particles with disparate sizes, FH theory implies that the natural composition variable is the volume fraction ϕ, with ϕ_i designating the volume fraction of A_i.

The theory classifies equilibrium polymerization as equivalent to a *phase transition* subject to an applied field (initiation) which ''rounds'' the clustering transition to a greater or lesser degree.[15] Transition ''rounding'' influences the sharpness of the polymerization transition and the temperature range over which it occurs.[15] The extent of polymerization Φ is a key quantity in both theoretical and experimental studies of this transition.

While the FH model of Refs. 13–15 enables computation of several thermodynamic properties, we provide a simplified treatment that suffices for determining the equilibrium extent of polymerization. The equilibrium volume fractions $\{\phi_i\}$ satisfy the conditions

$$\phi_1^*/\phi_1 = K_{\rm init}(T) \equiv \exp[-\Delta G^0_{\rm init}/RT], \tag{5}$$

$$\phi_2/(\phi_1^*)^2 = 2K_{\rm dim}(T) \equiv 2\exp[-\Delta G^0_{\rm dim}/RT], \tag{6}$$

$$\phi_3/(\phi_2\phi_1^*) = (3/2)K_{\rm prop}(T) \equiv (3/2)\exp[-\Delta G^0_{\rm prop}/RT], \tag{7}$$

$$\phi_{i+1}/(\phi_i\phi_1) = [(i+1)/i]K_{\rm prop}(T) \equiv [(i+1)/i]\exp[-\Delta G^0_{\rm prop}/RT], \quad i>3, \tag{8}$$

where RT designates molar thermal energy, $K_{\rm init}(T)$, $K_{\rm dim}(T)$, $K_{\rm prop}(T)$ are the equilibrium constants for the activation, dimerization, and propagation reactions, respectively, and the free energies $\Delta G^0_{\rm init}$, $\Delta G^0_{\rm dim}$, $\Delta G^0_{\rm prop}$ are molar quantities. The factors of 2, 3/2, and $(i+1)/i$ in Eqs. (6)–(8) reflect the different volumes of the actin *r*-mers, as included in similar theoretical treatments of protein polymerization[1] and the living polymerization of α-methylstyrene.[13–15]

The extent of polymerization Φ is the fraction of monomers converted into polymers,

$$\Phi = (\phi_1^0 - \phi_1 - \phi_1^*)/\phi_1^0, \tag{9}$$

where $\phi_1^0 \equiv [G_0]$ is the initial *G*-actin volume fraction before polymerization, and ϕ_1 and ϕ_1^* are the equilibrium volume fractions of nonactivated and activated actin monomers, respectively. Conservation of actin mass requires,

$$\phi_1^0 = \phi_1 + \phi_1^* + \phi_2 + \sum_{i=3}^{\infty} \phi_i. \tag{10}$$

Substituting Eqs. (5)–(8) and performing the summation in Eq. (10) yields

$$\phi_1^0 = \phi_1 + K_{\rm init}\phi_1 + 2K_{\rm dim}\phi_1^2 + \frac{K_{\rm init}K_{\rm dim}K_{\rm prop}(3-2K_{\rm prop}\phi_1)\phi_1^3}{(1-K_{\rm prop}\phi_1)^2}. \tag{11}$$

Equation (11) is solved numerically for the equilibrium *G*-actin monomer volume fraction ϕ_1 in terms of the equilibrium constants $K_{\rm init}$, $K_{\rm dim}$, and $K_{\rm prop}$ or, alternatively, in terms of the dimensionless free energies $\Delta G^0_{\rm init}/RT$, $\Delta G^0_{\rm dim}/RT$, and $\Delta G^0_{\rm prop}/RT$. Invoking the relation $\phi_1^* = K_{\rm init}\phi_1$ enables the computation of the extent of polymerization $\Phi(T)$ as a function of temperature for a given set of enthalpies and entropies for initiation, dimerization, and propagation.

Rabbit muscle actin is prepared by the method of Pardee and Spudich,[16] with a final purification by size exclusion chromatography (Sephacryl S-200, Pharmacia).[17,18] The method of Kouyama and Mihashi[19] is used to add the fluorescent label *N*-(1-pyrenyl)iodoacetamide (Molecular Probes, Eugene, OR) to *G*-actin. The labeled *G*-actin is purified on a column. The labeled and unlabeled *G*-actins are Sephacryl mixed to produce a mixture of 3% labeled and 97% unlabeled.

The fluorescence intensity is measured by an Aminco Bowman Series 2 Luminescence Spectrometer with the excitation wavelength set at 365 nm, resulting in emission wavelengths at 387 and 407 nm.[20] At each temperature T, the fluorescence signal at 407 nm, $I(T)$, is followed with time until it reaches steady state (25 min). For each *G*-actin concentration, the fluorescence intensity at 407 nm is also measured as a function of T for a sample containing no KCl [we denote it as $I_G(T)$]. Measurements of $I_G(T)$ show little T dependence. After taking measurements at the highest T, each sample is completely polymerized by bringing the concentration of $MgCl_2$ to 15 mM; this provides the fluorescence intensity I_F at 407 nm for the fully polymerized sample. I_F does not change appreciably with T and does not depend on the molecular weight distribution since the label is a local effect in the molecule. The extent of polymerization $\Phi(T)$ is obtained from $I(T) = \Phi(T)I_F + [1-\Phi(T)]I_G(T)$.

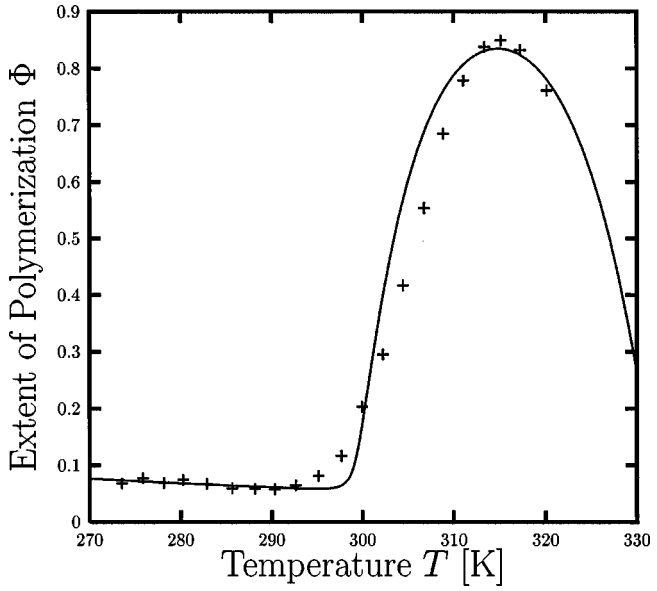

FIG. 1. Extent of polymerization Φ vs temperature. Plus symbols are experimental data (2 mg/ml of actin and 9 mM of KCl), and the solid line is the theoretical fit to the data.

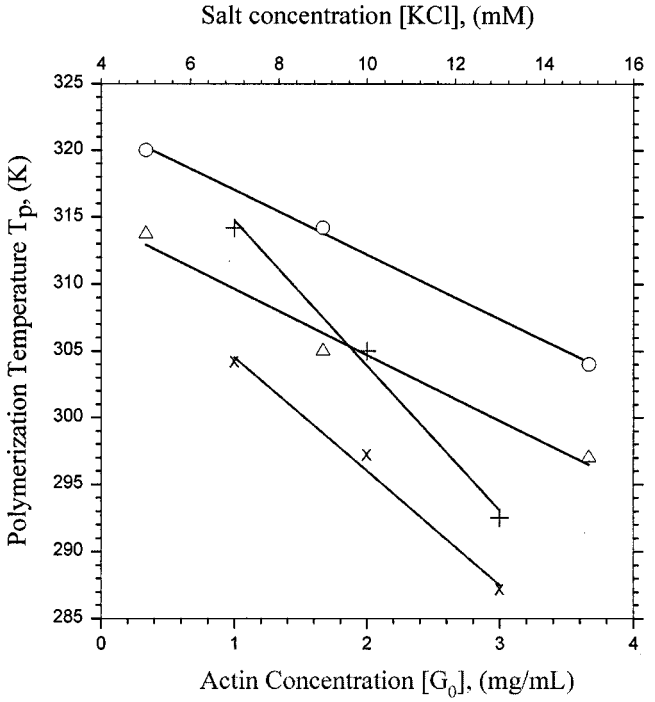

FIG. 2. The experimental polymerization temperature T_p as a function of the initial *G*-actin concentration $[G_0]$ and the salt concentration. + and × correspond to 9 mM KCl and 15 mM KCl, respectively. △ and ○ refer to $[G_0]=1$ mg/ml and $[G_0]=2$ mg/ml, respectively. Lines are least-squares fits to the data. The maximum uncertainty in our graphical extrapolation estimate of T_p equals ±3 °C.

The precision for measurements of $\Phi(T)$ for a given sample is about 1%, but the reproducibility among protein samples is about 10%.[20]

The experimental data for the extent of polymerization $\Phi(T)$ are fitted by Eq. (9) using the six parameters of the theory (i.e., $\Delta H^0_{\rm init}$, $\Delta S^0_{\rm init}$, $\Delta H^0_{\rm dim}$, $\Delta S^0_{\rm dim}$, $\Delta H^0_{\rm prop}$, and $\Delta S^0_{\rm prop}$). An example of these fits is depicted in Fig. 1 for the sample containing 2 mg/ml of *G*-actin and 9 mM of KCl. The salt concentrations are chosen to have the polymerization occur in a convenient experimental range. The experimental polymerization temperature T_p (see below) provides a *strong constraint* between $\Delta H^0_{\rm prop}$ and $\Delta S^0_{\rm prop}$; the maximum in $\Phi(T)$ occurs when $\Delta G^0_{\rm init}\approx 0$; and the low temperature portion of $\Phi(T)$ constrains the relative magnitudes of $\Delta H^0_{\rm dim}$ and $\Delta S^0_{\rm dim}$. The fits are quite sensitive to changes as small as 1% in the individual parameters. The six enthalpies and entropies are summarized in Table I for all samples studied. At fixed salt concentration, $\Delta H^0_{\rm prop}$ and $\Delta S^0_{\rm prop}$ are roughly independent of $[G_0]$, whereas the enthalpies and entropies of both initiation and dimerization are sensitive to $[G_0]$. The precise mechanistic origin of this dependence remains to be analyzed.

TABLE I. Free energy parameters for actin polymerization.

	9 mM KCl Actin concentration $[G_0]$			15 mM KCl Actin concentration $[G_0]$		
Free energy parameters	1 mg/ml	2 mg/ml	3 mg/ml	1 mg/ml	2 mg/ml	3 mg/ml
$\Delta H^0_{\rm init}$ [kJ/mol]	179	250	351	350	450	500
$\Delta S^0_{\rm init}$ [J/(mol K)]	561	794	1160	1118	1445	1695
$\Delta H^0_{\rm dim}$ [kJ/mol]	−369	−508	−710	−711	−908	−1008
$\Delta S^0_{\rm dim}$ [J/(mol K)]	−1067	−1529	−2265	−2185	−2831	−3331
$\Delta H^0_{\rm prop}$ [kJ/mol]	122	124	138	180	180	180
$\Delta S^0_{\rm prop}$ [J/(mol K)]	518	532	591	724	725	744

Given an uncertainty of ±10% in the experimental determination of $\Phi(T)$, the main features of the temperature variation of $\Phi(T)$ are well reproduced by the theory, with both a low temperature tail and the unusual[13,15] high temperature maximum present. The nonzero $\Phi(T)$ for polymerization at low T arises in our model from dimerization, which increases upon cooling. The maximum in Fig. 1 occurs due to the competition between the decreasing concentration of nonactivated actin monomers and the increasing volume fraction of activated monomer species upon polymerization [see Eq. (9)].

The polymerization transition temperature T_p is identified as the temperature at which $\Phi(T)$ exhibits an inflection point.[13] [$\Phi(T)$ in Fig. 1 may possibly exhibit another inflection point at high T, signaling re-entrant depolymerization, but our data are insufficient to confirm this possibility.] Figure 2 presents the experimentally determined T_p as a function of actin concentration $[G_0]$ for fixed salt concentration. (Equivalently, this plot provides the temperature dependence of the ''critical concentration''[13] for actin polymerization.) T_p decreases roughly linearly (solid lines) with $[G_0]$. A decrease of T_p (''floor temperature''[21]) with the concentration of associating species is also typical for systems that cluster reversibly upon heating.[14] Figure 2 also describes the variation of T_p with salt concentration for fixed $[G_0]$. Added salt (for constant $[G_0]$) enlarges the magnitudes of the enthalpies and entropies for all three polymerization processes. The actin monomers are polyelectrolytes, and added salt should modify the composition and extent of the counterion clouds about both the monomers and polymers.[22] This effect natu-

rally changes the polymer rigidity and the energetics for propagation and, thus, alters T_p. Similar shifts of phase transformation temperatures (phase separation, crystallization, gelation) with salt concentration are observed in protein solutions.[23,24]

In conclusion, spectroscopic measurements for the extent of polymerization Φ of actin in solution as a function of temperature and salt concentration [KCl] are compared to theoretical calculations based on a lattice model of equilibrium polymerization.[13–15] The actin polymerization scheme incorporates the initiation of *G*-actin monomers and the formation of actin dimers from activated monomers, followed by chain propagation, with a trimer as the nucleus.[25–27] The inclusion of an activation step for *G*-actin monomers is a crucial ingredient in the polymerization mechanism as is the requirement that ΔG for activation changes sign as temperature is increased (i.e., the activation step has its own ''floor temperature''[21]). The latter produces the unusual maximum in the extent of polymerization Φ as a function of temperature. Similarly, distinct free energies for dimerization and propagation are necessary to describe the low temperature tail in Φ. This model, in conjunction with simple FH theory, leads to the novel view of actin polymerization as a rounded phase transition.[15] Moreover, the analysis solidifies essential features of the polymerization mechanism.

We find that the polymerization transition temperature T_p decreases strongly and linearly with $[G_0]$ and [KCl] over the concentration range investigated. The strong dependence of T_p on salt and initial *G*-actin concentrations may have implications for the regulation of actin polymerization in living systems. Since the hypothesis of physiochemical regulation of actin polymerization[4–6] requires the transition temperature to be sensitive to the physiochemical control variables, our demonstration of this sensitivity suggests that purely physiochemical changes can complement the specific action of regulatory proteins in controlling actin polymerization for a wide range of biological processes. The equilibrium theory and the equilibrium energetic parameters determined should aid the molecular modeling of actin polymerization in cellular processes occurring under nonequilibrium conditions.

This work is supported, in part, by the Chemistry Division of the National Science Foundation and by the National Institute of Arthritis and Musculoskeletal and Skin Diseases (AR45191) and the National Institutes of General Medical Sciences (GM56678) of the National Institutes of Health. We thank N. Blough for the use of his spectrometer, M. Alessi for assistance with actin purification, and S. De for help with the calculations.

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