Analytical solution for steady-state populations in the self-assembly of microtubules from nucleating sites

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(Received 25 July 2001; published 31 December 2002)

In addition to the biological importance of microtubules, which form a portion of the cellular cytoskeleton and a network for intracellular transport, the kinetics of microtubule self-assembly have generated great interest because individual microtubules exist in growing and decaying phases, with randomly occurring interconversions between them. Although a great deal is known concerning the microscopic details of these growth and decay processes, no description is available for the steady-state microtubule concentrations that are observed experimentally when microtubules are grown from nucleating sites. We generalize Hill's two-state model to include the dependence of the rates on tubulin concentration for systems where microtubules are grown from nucleating sites. An analytic solution is provided here to the resulting nonlinear, doubly infinite set of kinetic equations for the steady-state concentrations of both the growing and decaying phase microtubules as a function of the degree n of self-assembly and of the tubulin concentration. We also discuss the conditions for the stability of the steady state.

DOI: 10.1103/PhysRevE.66.061916 PACS number(s): 87.10.+e, 82.35.Pq, 87.15.Rn

I. INTRODUCTION

Microtubules, along with actin, flagellin, fibrin, and tobacco mosaic virus, are examples of biological systems that self-assemble reversibly [1]. With the exception of bacteria, microtubules are found in all living cells where they form part of the cell structural support (the ''cytoskeleton'') and the ''rail'' network for intracellular transport of materials, and they participate in cell reproduction and motion. In the presence of GTP (guanosine triphosphate) and Mg^+ ions, and perhaps aided by microtubule-associated proteins, the protein tubulin self-assembles to form microtubules that are long hollow cylinders having an outer diameter of about 25 nm and a length that may span the cell. Microtubules may be grown either from nucleating sites or may be self-nucleated (at higher temperatures and/or tubulin concentrations). As shown by Fygenson, Braun, and Libchaber [2], microtubule formation from nucleating sites exhibits a steady-state limit for the microtubule length distribution at lower temperatures or tubulin concentrations. This steady-state behavior contrasts with the unbounded growth that occurs at higher temperatures/concentrations and for self-nucleated microtubules.

The dynamical growth pattern of microtubules has drawn considerable interest [3–5] since individual microtubules exhibit separate growth and decay phases with random transitions between these two phases and with a dynamical instability occurring when the growth becomes unbounded. Hill and co-workers [6] have introduced a minimal ''two-state'' model of the growth/decay kinetics of the microtubule mass distribution. The model involves a doubly infinite set of linear kinetic equations for the microtubule mass distributions of the growing and decaying microtubules, a model whose full analytical solution has recently been given by Rubin and co-workers [7]. The rate coefficients in Hill's model, consequently, are effective rate constants that depend on the concentrations of GTP, Mg^+, microtubule-associated proteins, etc., and that hide some of the interesting microscopic details of the microtubule growth and decay. As analyzed recently by Flyvbjerg and co-workers [8], the process of microtubule self-nucleation is quite complex. Restricting attention to situations in which only microtubule growth is possible, they model the nucleation kinetics with a set of coupled nonlinear equations that are rendered finite by considering the average microtubule size and lumping together all microtubules beyond a given size.

A full description of the growth kinetics (i.e., a description of the microtubule length distribution as a function of time) requires, in principle, the solution of a doubly infinite set of coupled nonlinear first-order differential equations. As noted above, the complexities posed by these equations have led most theoretical analyses to use either linearized models or nonlinear ones of finite dimensionality [9,10]. Here, we extend Hill's model to describe microtubule growth from nucleating sites. Both the latter extension and the inclusion of the explicit dependence of rate coefficients on the tubulin concentration render the doubly infinite set of coupled equations nonlinear. Despite this increased complexity, we resolve the major unsolved theoretical problem of determining the microtubule length distribution under steady-state conditions as a function of tubulin concentration for microtubule systems that are grown from nucleating sites. The analysis of the stability of the steady state can then readily be treated by considering the linear equations for small displacement from steady-state populations.

As recently demonstrated for the reversible self-assembly of the protein actin into *F*-actin filaments [11], experimental measurements for the extent of polymerization under steady-state conditions provide an excellent means to determine the thermodynamic parameters, such as reaction free energies, which depend on temperature, pH, salt concentration, etc. By analogy, similar studies of steady-state microtubule self-assembly should provide deeper understanding of the microtubule self-assembly mechanism, especially because the

steady-state behavior of actin and of microtubule self-assemblies can be interpreted using nonlinear algebraic equations that are far simpler to apply than the coupled nonlinear differential equations governing the kinetic behavior. Of particular importance in the analysis of steady-state data is a knowledge of the steady-state properties as a function of the initial concentration of tubulin monomers (before the self-assembly commences). The theory, predicting the variation of steady-state properties with initial tubulin concentration, may then be used for determining the rate constants for individual steps in the microtubule self-assembly process as a function of temperature, content of buffer solution, etc.

The focus of this paper lies in solving the equations governing the steady-state limit, so diffusion processes may be ignored because experiments [12] indicate that the attachment of tubulin dimers to the end of the microtubule is the rate determining step in the growth of microtubules. Thus, the doubly infinite set of coupled nonlinear algebraic equations suffices for describing the steady-state microtubule mass distribution. The steady-state equations are solved analytically.

We extend Hill's minimal model for the kinetics of individual microtubule formation. The treatment of nucleating sites and the explicit inclusion of the tubulin concentration implies that the twofold infinite set of coupled equations become nonlinear, providing the major mathematically complicated feature of modeling the microtubule dynamics. Extensions to produce more detailed models are briefly discussed.

II. MINIMAL MODEL FOR MICROTUBULE KINETICS

The minimal model describes the microtubule kinetics in terms of the concentrations $[T_n^+]$ and $[T_n^-]$ of n-mers (at time t) that are in growing and depolymerization stages, respectively. The individual ''monomers'' are $\alpha\beta$-tubulin dimers. The initial concentration of monomers is denoted as $[T]_0$, while its instantaneous concentration during the polymerization reactions (see below) is designated as $[T]$. The initial concentration of nucleating sites is $[N]_0$, while the concentration of free (i.e., no bound tubulin) nucleating sites is $[N]$. The growing state microtubule formed by the sequential attachment of n monomers to the nucleating site is designated as T_n^+, whose concentration at time t is denoted as $[T_n^+]$. The other essential reactions included in the model are the ''catastrophe'' process involving the transition from growing T_n^+ to decaying T_n^-, and the reverse ''rescue'' transition from T_n^- to T_n^+. We assume that GTP is present in excess, so the dependence of the rates on the GTP concentration need not be followed, nor is it necessary to distinguish between tubulin-GTP and tubulin-GDP concentrations (GDP is guanosine diphosphate).

This simple kinetic scheme is represented by the doubly infinite set of *unidirectional* reactions,

$$T+N\rightarrow T_1^+, \quad \text{binding of monomer to nucleating site with rate constant } k_n, \tag{1a}$$

$$T_n^+ + T\rightarrow T_{n+1}^+, \quad \text{polymerization or growth with rate constant } k_p, \tag{1b}$$

$$T_n^+\rightarrow T_n^-, \quad \text{catastrophe, with rate constant } k_c, \tag{1c}$$

$$T_n^-\rightarrow T_n^- + T, \quad \text{depolymerization with rate constant } k_d, \tag{1d}$$

$$T_n^-\rightarrow T_n^+, \quad \text{rescue with rate constant } k_r, \tag{1e}$$

with $n=1, 2, \ldots, \infty$, and $T_0^-\equiv 0$. The kinetics of reactions (1a)–(1e) are described by a twofold infinite set of coupled nonlinear differential equations,

$$\frac{d[T]}{dt} = -k_n[N][T] - k_p[T]\sum_{n=1}^{\infty}[T_n^+] + k_d\sum_{n=1}^{\infty}[T_n^-], \tag{2a}$$

$$\frac{d[T_1^+]}{dt} = k_n[N][T] - k_p[T][T_1^+] - k_c[T_1^+] + k_r[T_1^-], \tag{2b}$$

$$\frac{d[T_n^+]}{dt} = k_p[T][T_{n-1}^+] - k_p[T][T_n^+] - k_c[T_n^+] + k_r[T_n^-], \quad n>1, \tag{2c}$$

$$\frac{d[T_n^-]}{dt} = -k_d[T_n^-] + k_d[T_{n+1}^-] + k_c[T_n^+] - k_r[T_n^-], \quad n>0, \tag{2d}$$

where tubulin mass conservation imposes the constraint

$$[T]_0 = [T] + Q_+ + Q_-. \tag{3}$$

$[T]_0$ and $[T]$ are the initial and instantaneous concentrations of tubulin monomers, respectively, and the quantities $Q_+(1)$ and $Q_-(1)$ are weighted sums of the concentrations $[T_n^+]$ and $[T_n^-]$ of tubulin monomers in the growing and decaying states,

$$Q_+ = \sum_{n=1}^{\infty} n[T_n^+], \quad Q_- = \sum_{n=1}^{\infty} n[T_n^-]. \tag{3a}$$

The ''catastrophe'' rate k_c is observed to depend on the tubulin concentration [13], and this dependence $k_c([T])$ may be introduced into the final equation for determining $[T]$. No rate equation is necessary for the instantaneous concentration $[N]$ of available nucleating sites because the concentration of occupied nucleating sites, $[N]_0-[N]$, must equal the total concentration of microtubules chains, independent of size and whether they are growing or decaying,

$$[N]-[N]_0 = P_+ + P_-, \tag{4}$$

where the quantities P_+ and P_- are given by

$$P_+ = \sum_{n=1}^{\infty} [T_n^+], \quad P_- = \sum_{n=1}^{\infty} [T_n^-]. \tag{4a}$$

Nonlinearities appear in Eq. (3) from the explicit dependence on the monomer concentration $[T]$ and from the nucleation site ''balance equation'' in Eq. (4).

The average microtubule sizes for the growing microtubules, decaying microtubules, and for all microtubules are readily obtained from $P_\pm$ and $Q_\pm$, respectively, by

$$\langle n \rangle_+ = \frac{Q_+}{P_+}, \quad \langle n \rangle_- = \frac{Q_-}{P_-}, \quad \langle n \rangle = \frac{Q_+ + Q_-}{P_+ + P_-}. \tag{4b}$$

As noted above, the rate constants k_c and k_r for catastrophe and rescue steps, respectively, are understood to be functions of the concentrations [GDP] and [GTP], as well as of temperature and the concentrations of other components of the buffer solution (such as ions like Mg^+, the solution pH, and microtubule-associated proteins). Alternatively, the minimal kinetic model may be extended by including the explicit concentrations [GDP] and [GTP] in Eq. (1), but this extension presents no essential difficulty, again because of the stoichiometry constraints on the concentrations [GDP] and [GTP] (Ref. [12]). Experiments [2] show that the two ends of the microtubule grow at different rates, and this feature could likewise be incorporated with no difficulty into the model by introducing a slower rate (k_p^-) for growth at the ''minus'' end.

III. STEADY-STATE POPULATIONS

The steady-state limit is obtained from the kinetic equations (2) by setting the time derivatives on the left hand side to zero, i.e., by taking

$$\frac{d[T]}{dt} = \frac{d[T_n^+]}{dt} = \frac{d[T_n^-]}{dt} = 0, \quad n = 1,2, \ldots . \tag{5}$$

For notational simplicity, the steady-state values of the concentrations are henceforth also denoted as $[T]$, $[T_n^+]$, and $[T_n^-]$ in the following. The steady-state limits of equations (2) are rewritten in the more compact form,

$$k_n\{[N]_0 - P_+ - P_-\} = -k_p[T]P_+ + k_d P_-, \tag{6a}$$

$$[T_n^-] = a[T_{n-1}^-] + b[T_{n-1}^+], \quad n > 1, \tag{6b}$$

$$[T_n^+] = c[T_{n-1}^+] + d[T_n^-], \quad n > 1, \tag{6c}$$

$$[T_1^+] = f + d[T_1^-], \tag{6d}$$

where Eq. (4) has been used and where the reduced rate constants are defined as $a = (k_r + k_d)/k_d$, $b = -k_c/k_d$, $c = k_p[T]/(k_c + k_p[T])$, $d = k_r/(k_c + k_p[T])$, and $f = k_n[T]\{[N]_0 - P_+ - P_-\}/(k_c + k_p[T])$, some of which are still explicit functions of $[T]$.

Summing Eqs. (6b) and (6c) over n from $2 \to \infty$ and using the definitions in Eq. (4) provide the respective pair of equations,

$$(a-1)P_- + bP_+ = [T_1^-], \tag{7a}$$

$$-dP_- + (1-c)P_+ = [T_1^+] - d[T_1^-] = f, \tag{7b}$$

where application of Eq. (6d) provides the last equality in Eq. (7b). The set of equations (7a), (7b), and (6a) enable solving for $P_\pm$ and $[T_1^-]$,

$$P_+ = k_n[N]_0(k_r + k_d)/D, \tag{8a}$$

$$P_- = k_n[N]_0(k_c + k_p[T])/D, \tag{8b}$$

$$[T_1^-] = \beta = k_n[N]_0(k_c k_d - k_r k_p[T])/k_d D, \tag{8c}$$

$$D = (k_c + k_n)(k_d + k_n) + (k_n - k_r)(k_p[T] - k_n). \tag{8d}$$

Given the quantities in Eq. (8), it is possible to solve for the individual $[T_n^+]$ and $[T_n^-]$ by iteration, but the process is greatly facilitated by the use of generating function methods [14] as follows. Multiply Eqs. (6b) and (6c) by x^n and then summing from n from $2 \to \infty$ provides a pair of equations for the quantities $P_\pm(x) = \Sigma_2^\infty x^n[T_n^\pm]$, $(x \neq 1)$, which are the generating functions from which the solution to Eqs. (6) are found by differentiating, as

$$[T_n^-] = (ac)^{[(n-1)]/2}[\beta U_{n-1}(\lambda) + (ac)^{-1/2}(bf - c\beta)U_{n-2}(\lambda)], \tag{9a}$$

$$[T_n^+] = (ac)^{[(n-1)]/2}[(f + \beta d)U_{n-1}(\lambda) + (a/c)^{1/2} a f U_{n-2}(\lambda)], \tag{9b}$$

where $U_n(\lambda)$ are Chebyshev polynomials of the second kind,

$$U_n(\lambda) = \sin[(n+1)\arccos\lambda]/(1-\lambda^2)^{1/2}, \tag{10}$$

with the definitions $U_{-1}(\lambda) \equiv 0$ and $\lambda = (a + bd + c)/2(ac)^{1/2}$. The overall exponential factor in Eq. (9) is consistent with the exponential microtubule length distribution found in Ref. [2].

Since the steady-state concentrations $[T_n^+]$ and $[T_n^-]$ in Eqs. (9) are functions of $[T]$, it would appear that the mass conservation equation (3) could be used to determine the steady-state value for $[T]$ by using Eq. (3) in conjunction with equations for $Q_\pm$, formed by performing the indicated summations in Eq. (3a) on Eqs. (6b) and (6c), to eliminate $Q_\pm$ and leave a nonlinear algebraic equation for $[T]$. However, the pair of equations for $Q_\pm$ may be shown to be identical, leaving one equation short of that necessary for determining $[T]$. On the other hand, since the average degree of polymerization, $\langle n \rangle$, is measurable, we may use $\langle n \rangle$ to provide the remaining equation.

Substituting the mass conservation equation (3) and the solutions for $P_\pm$ from Eqs. (8a) and (8b) into the definition of $\langle n \rangle$ yields a readily solved quadratic equation for the steady state concentration $[T]$ as a function of $[T]_0$, $\langle n \rangle$, and the rate constants. If k_c is taken to depend on $[T]$, then the equation is no longer quadratic and therefore requires numerical solution. Insertion of this solution for $[T]$ into Eq. (9) yields the explicit steady-state mass distribution.

IV. STABILITY CONDITIONS

The steady state is stable, provided that small displacements from steady state die out with time. Experiments [2] indicate that this stability exists at a lower temperature/concentration range, while instability ensues at higher temperatures/concentrations. The stability limits are readily studied by expanding the nonlinear kinetic equations (2) about the steady-state solutions and by retaining only the linear contributions.

The steady state is stable, provided that small displacements from steady state die out with time or, perhaps, just oscillate (the ''dynamic instability''). Thus, the stability limits are obtained by expanding the nonlinear kinetic equations (2) about the steady-state solutions and retaining only the linear contributions. Define the deviations from steady state as, for example,

$$[\delta_n^+]=[T_n^+]_t-[T_n^+]_{ss}, \tag{11}$$

where now $[T_n^+]_t$ denotes the time-dependent concentration and $[T_n^+]_{ss}$ designates the time-independent steady-state limit. The linearized kinetic equations then become

$$\frac{d[\delta]}{dt}=-\Gamma-k_p[\delta]\sum_{n=1}^{\infty}[T_n^+]_{ss}-k_p[T]_{ss}\sum_{n=1}^{\infty}[\delta_n^+]+k_d\sum_{n=1}^{\infty}[\delta_n^-], \tag{12a}$$

$$\frac{d[\delta_1^+]}{dt}=\Gamma-k_p[\delta][T_1^+]_{ss}-k_p[T]_{ss}[\delta_1^+]-k_c[\delta_1^+]+k_r[\delta_1^-], \tag{12b}$$

$$\frac{d[\delta_n^+]}{dt}=k_p[\delta][T_{n-1}^+]_{ss}+k_p[T]_{ss}[\delta_{n-1}^+]-k_p[\delta][T_n^+]_{ss}-k_p[T]_{ss}[\delta_n^+]-k_c[\delta_n^+]+k_r[\delta_n^-],\quad n>1, \tag{12c}$$

$$\frac{d[\delta_n^-]}{dt}=-k_d[\delta_n^-]+k_d[\delta_{n+1}^-]+k_c[\delta_n^+]-k_r[\delta_n^-],\quad n>0, \tag{12d}$$

where

$$\Gamma\equiv k_n[\delta]\{[N]_0-P_{+,ss}-P_{,ss}\}-k_n[T]_{ss}\left\{\sum_{n=1}^{\infty}[\delta_n^+]-\sum_{n=1}^{\infty}[\delta_n^-]\right\}.$$

A growing exponential component to the solution of Eq. (12) indicates that the steady-state limit is unstable. While a linear set of first-order differential equations is readily solved, the doubly infinite character of the stability equations hampers practical computations. However, the infinite set of relaxation rates is really unnecessary. Instead, for example, a ''mode'' containing predominately the growing species, such as $\Delta_+=\Sigma_{n=1}^{\infty}[\delta_n^+]$, is expected to be one of the modes indicating the instability. It may be shown that the same final stability conditions ensue when the set of equations is expanded to include modes such as $\Omega_+=\Sigma_{n=1}^{\infty}n[\delta_n^+]$ involving the deviations from steady state of the total mass of microtubules.

Equations (12c) and (12d) are summed over n to reduce the stability conditions to a set of three equations. Let $\mathbf{V}^T$ denote the column vector,

$$\mathbf{V}^T(t)=(\Delta_+,\ \Delta_-,\ [\delta]), \tag{13}$$

and the equations may be represented in matrix form as

$$\frac{d\mathbf{V}(t)}{dt}=\mathbf{M}\mathbf{V}(t)+\mathbf{N}[\delta_1^-], \tag{14}$$

where the matrix $\mathbf{M}$ and vector $\mathbf{N}$ are given by

$$\mathbf{M}=\begin{pmatrix}-[k_n\{[N]_0-P_{+,ss}-P_{-,ss}\}+k_pP_+] & (k_n-k_p)[T]_{ss} & k_d+k_n[T]_{ss}\\ k_n\{[N]_0-P_{+,ss}-P_{-,ss}\} & -k_c-k_n[T]_{ss} & k_r-k_n[T]_{ss}\\ 0 & k_c & -k_r\end{pmatrix},\quad \mathbf{N}=\begin{pmatrix}0\\0\\-k_d\end{pmatrix}. \tag{15}$$

The solution to Eq. (14) may be written as

$$\mathbf{V}(t)=\exp(\mathbf{M}t)\mathbf{V}(0)+\int_0^t dt'\,\exp[\mathbf{M}(t-t')]\mathbf{N}[\delta_1^-]_{t'}, \tag{16}$$

where $\mathbf{V}(0)$ represents an arbitrary displacement from steady state. If an eigenvalue of $\mathbf{M}$ is positive, the term $\exp(\mathbf{M}t)\mathbf{V}(0)$ exhibits an instability of the steady state, so the behavior of the integral term is irrelevant to the stability consideration. The only other possibility for instability is if $[\delta_1^-]$ grows exponentially when all eigenvalues are negative, a situation that is physically unreasonable. Thus, the stability condition reduces to the determination of whether the matrix $\mathbf{M}$ has a positive eigenvalue. The characteristic equation $\det|\mathbf{M}-x\mathbf{1}|=0$ is cubic in x of the form, $x^3+a_2x^2+a_1x+a_0$, where (defining $[N]_{ss}=[N]_0-P_{+,ss}-P_{-,ss}$),

$$a_2 = k_n[N]_{ss} + k_p P_+ + k_c + k_r + k_n[T]_{ss}, \tag{17a}$$

$$a_1 = (k_n[N]_{ss} + k_p P_+)(k_c + k_r + k_n[T]_{ss}) + k_n[T]_{ss}(k_c + k_r), \tag{17b}$$

$$a_0 = k_n[N]_{ss}(k_r k_n[T]_{ss} - k_c k_d) + P_+ k_r k_n[T]_{ss}(k_c + k_r). \tag{17c}$$

The following situations cover all possibilities:

(a) If all roots are real and negative, the steady state is stable.

(b) If some roots are positive, there is unbounded growth, and the steady state is unstable. Because a_2 and a_1 are positive, at most one root is positive (instability), and this can arise only if a_0 is negative, which is highly unlikely.

(c) If two roots are complex conjugates, but the real parts are negative, the system oscillates about the steady state. If the real parts are positive, the system enters a domain of ''dynamic instability,'' oscillatory, unbounded growth.

While the conditions delineating these situations can be represented analytically, the large number of rate coefficients and the steady-state values involved render unwieldy the analytical analysis of these conditions. Rather, a detailed numerical analysis is required to map out the phase diagram as a function of the controllable system parameters, and this is beyond the scope of the present treatment.

Although steady-state microtubule growth is not observed experimentally to occur under conditions where the growth is self-nucleating, the dynamical equations should posses a steady-state solution whose resolution could provide insights into special conditions, such as those greatly increasing the catastrophe rate, where steady-state growth could be probed and thereby yield added understanding of self-nucleated growth. The treatment of self-nucleated steady-state growth follows identically as above [15] with the replacement $k_n[N][T] \rightarrow k_a[T]^m$, where m is the number of monomers required to form the nucleus. The final equation for $[T]$ is therefore no longer quadratic.

ACKNOWLEDGMENTS

This research was supported, in part, by Grant No. GM56678 from the National Institutes of General Medical Sciences of the NIH. I am grateful to Jack Douglas, Jacek Dudowicz, and Leif Matsson for helpful discussions, and to Thu Tran for help in catching the redundancy of the $Q_\pm$ equations.

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