THEORETICAL DESCRIPTION OF RELEASE, UPTAKE, AND PULSE CHASE OF LABELED SUBUNITS OF ACTIN OR A MICROTUBULE THAT UNDERGOES HEAD-TO-TAIL POLYMERIZATION

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ABSTRACT The notion of the number of once-visited sites by a one-dimensional random walker is used to describe the amount of release, uptake, and pulse chase of labeled subunits of actin, or a microtubule that undergoes head-to-tail polymerization or treadmilling. Exact formulae for any number of steps are given as well as all the possible asymptotic forms of these quantities. Numerical calculations are carried out using hypothetical and experimentally measured values of rate constants.

INTRODUCTION

Head-to-tail polymerization of actin or microtubules is attracting considerable attention from researchers owing to its possible biological importance as well as its own fascinating features. Theoretical aspects of the phenomenon have been treated by Wegner (1) and Hill (2, 3) from a chemical kinetic and thermodynamic point of view. Recently, several experimental studies on the release and uptake of labeled subunits, radioactive or fluorescent, of those protein polymers have been carried out (4–6). The main point of these experiments is to confirm qualitatively that actin or microtubules really undergo head-to-tail or treadmilling polymerization. In the present paper we describe head-to-tail polymerization as a random walk problem, and present exact formulae for the amount of release and uptake of labeled subunits. This is done by applying results previously found (7) for the quantity of once-visited sites by a random walker. Our formulae cover not only stages after very many steps but also transient stages with a finite number of steps by the walker. By varying the relative values of the rate constants for polymerization and depolymerization, and the monomer concentration in the solution, we see various different asymptotic expressions emerge for the quantities of interest. We list these exhaustively. We also treat the number of remaining labeled subunits observed in pulse-label experiments. Numerical calculations are done using hypothetical values of rate constants and experimentally measured values by Pollard and Mooseker (8), discussed below.

Random walk models have been applied to the problem of release or uptake of labeled subunits of microtubules by Zeeberg et al. (4) and Hill and Kirschner (9). The former derived an asymptotic expression for the average amount of label incorporation using a continuous time random walk. This approach was also used by Hill and Kirschner (9) who used the well-known treatment of random walks with absorbing barriers given by Chandrasekhar (10) to derive an asymptotic formula for the mean number of labeled monomers lost from the polymer. Here we make explicit use of the quantity called once-visited sites by a random walker, which turns out to provide quite a simple way to calculate the amount of release or uptake of labeled subunits. We recover the same formulae for the quantities of interest as the above authors in asymptotic cases. An advantage of our formulation is that, owing to the fact that we used a discrete-time random walk, our formulae for transient states contain only summations. These are more suitable for numerical calculation than the integrations that appear in the works of Zeeberg et al. and Hill and Kirschner. Throughout the paper the following assumptions are made: (*a*) polymers of actin or microtubules are very long. (*b*) No breakage of the polymer at an interior part can take place. (*c*) No association of two or more polymers to form a longer polymer can take place.

RANDOM WALK DESCRIPTION OF HEAD-TO-TAIL POLYMERIZATION

Following Wegner (1) and Hill (2), basic rate equations at an assembling end of a protein polymer (we call it the

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A-end) and a disassembling end (D-end) can be written as

$$\frac{dN_A}{dt} = K_A c - k_A \tag{1}$$

$$\frac{dN_D}{dt} = K_D c - k_D \tag{2}$$

where N_A and N_D represent the numbers of subunits taken in at the A-end or D-end, K_A and K_D are the rate constants for addition of a subunit, k_A and k_D are the rate constants for deletion of a subunit at the respective ends, and c stands for the free concentration of the subunits in the solution. We will see later that differentiation of the A-end from the D-end is only for convenience. Roles of these two ends can be inverted under different conditions. We measure the length of a polymer in units of an increment in the length when attachment of one subunit to one of the two ends takes place. We assume that assembling or disassembling of a unit at the A- or D-end takes place in a helical manner if the protein polymer under consideration consists of more than one strand. This means that we regard, e.g., a microtubule that consists of 13 strands, as a tube that is made up of one helically wound strand. This may or may not be a good approximation for the microtubule case. However, it is reasonable for F-actin, which consists of only two strands, and it is exact for an idealized one-strand polymer. If we regard each end of actin or a microtubule as a random walker, then addition of one subunit corresponds to a one-step-long movement of the walker in, say, the plus direction and deletion of a unit corresponds to a step in the minus direction. If the protein polymer is long enough, two random walkers at the A- and D-end can be treated independently. The transition probability p_A of the walker stepping in the plus direction is

$$p_A = \frac{K_A c}{K_A c + k_A} \tag{3}$$

whereas the probability q_A of a step in the minus direction, corresponding to release of a subunit, is

$$q_A = \frac{k_A}{K_A c + k_A}. \tag{4}$$

The transition probability p_D of stepping in the plus direction for a walker at the D-end, corresponding to deletion of a subunit at the D-end, is

$$p_D = \frac{k_D}{K_D c + k_D}. \tag{5}$$

The probability q_D for the minus direction is

$$q_D = \frac{K_D c}{K_D c + k_D}. \tag{6}$$

The steady-state condition or constant mean length condition for the polymer is given by

$$K_A c - k_A = k_D - K_D c \tag{7}$$

or

$$s_A(p_A - q_A) = s_D(p_D - q_D) \tag{8}$$

where

$$s_A = K_A c + k_A \text{ and } s_D = K_D c + k_D, \tag{9}$$

which can be interpreted as the number of steps in a unit time taken by the walkers at the A- and D-end, respectively.

INITIALLY LABELED POLYMER CASE

Release of Labeled Subunits

First we consider a case in which polymers have been prepared for complete labeling when the measurement starts. There must be situations in which not all the constituents of the polymers can be labeled. However, we consider only an ideal case in which all components of the polymers are labeled, because otherwise exact calculation of the number of released labeled monomers is difficult. However, the more general problem can be handled approximately by introducing one more parameter representing the ratio of the number of initially labeled units in a polymer to the number of unlabeled ones.

We take the one-dimensional coordinate along a labeled polymer as shown in Fig. 1. Because the polymer is assumed to be very long, each end can be taken as an origin, O_A and O_D, respectively, where each random walker starts with the transition probability shown in the figure. Although polymerization or depolymerization transitions at each end of the polymer occur at random intervals, we describe the system by a conventional discrete-time random walk.

The number of released labeled subunits after time t is the sum of the number $L^-_{n_A}$ of once-visited sites in the minus direction by the random walker at the A-end and the number $L^+_{n_D}$ of once-visited site in the plus direction by the walker at the D-end, where n_A (n_D) is the number of steps taken by the walker at the A-end (D-end) during the time

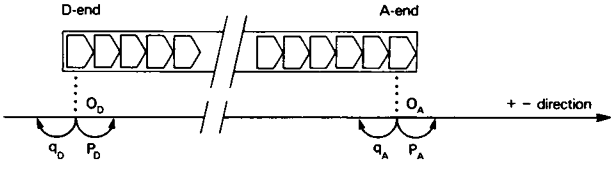

FIGURE 1 An initially labeled polymer along the x-axis. Because the polymer is assumed to be very long, each end can be taken to have an independent origin. Transition probabilities of the random walkers are also shown.

t, i.e.,

$$n_A = s_A t \; (n_D = s_D t). \tag{10}$$

Here we have assumed that once a labeled subunit leaves the polymer it cannot be returned to the polymer with the label; newly assembling subunits are all unlabeled. This assumption is quite reasonable if exchange of a labeled ligand with an unlabeled one takes place very rapidly, as is usually the case, when a subunit is in the monomer state in the solution. The quantity of interest is therefore the sum

$$R(t) = L_{n_A}{}^{-} + L_{n_D}{}^{+}. \tag{11}$$

Nagai et al. (7) showed in general that the number L_n of once-visted sites after n steps by a one-dimensional random walker with asymmetric transition probabilities p and q can be expressed as

$$\begin{aligned} L_n &= n - \sum_{r=1}^{[n/2]} \frac{(n-2r+1)}{(2r-1)} \, {}_{2r}C_r (pq)^r \qquad (n \geq 2) \\ L_1 &= 1 \end{aligned} \tag{12}$$

where

$$\begin{aligned} [n/2] &= n/2 && n, \text{even} \\ &= (n-1)/2 && n, \text{odd} \end{aligned} \tag{13}$$

and ${}_{2r}C_r$ is the binomial coefficient. Furthermore, they showed that L_n can be decomposed into the number of once-visited sites in the plus direction from the origin $L^{+}{}_n$, and in the minus direction $L^{-}{}_n$, as

$$L_n{}^{\pm} = (1/2)L_n \pm (1/2)(p-q)n \tag{14}$$

(See Eqs. 14 and 49 of reference 7.)

Using Eq. 14, we can express the number of released labeled units in time t as

$$R(t) = (1/2)[(p_D - q_D)n_D - (p_A - q_A)n_A + L_{n_A} + L_{n_D}] \tag{15}$$

where L_{n_A} and L_{n_D} are the numbers of once-visited sites by random walkers at the A- and D-end, respectively. If the steady-state condition Eq. 8 holds, using Eq. 10, we have

$$R_{ss}(t) = (1/2)(L_{n_A} + L_{n_D}). \tag{16}$$

For large t the asymptotic form of $R(t)$ becomes

$$R(t) = (1/2)[(p_D - q_D)s_D t - (p_A - q_A)s_A t + L_{n_A}(\infty) + L_{n_D}(\infty)] \tag{17}$$

where

$$L_{n_A}(\infty) = \begin{cases} |p_A - q_A| s_A t + (1/|p_A - q_A|) - 1 & \text{for } p_A \neq q_A \\ \sqrt{8 s_A t/\pi} - 1 & \text{for } p_A = q_A \end{cases} \tag{18}$$

and

$$L_{n_D}(\infty) = \begin{cases} |p_D - q_D| s_D t + (1/|p_D - q_D|) - 1 & \text{for } p_D \neq q_D \\ \sqrt{8 s_D t/\pi} - 1 & \text{for } p_D = q_D. \end{cases} \tag{19}$$

(See Eqs. 21 and 28 of reference 7.) Therefore, the expressions for the asymptotes vary as the relations between p_A and q_A and between p_D and q_D change. For example, when $p_A > q_A$ and $p_D > q_D$, we obtain a linear function in t as the asymptote for $R(t)$, for $p_A > q_A$, and $p_D = q_D$; however, the asymptote becomes proportional to $\sqrt{t}$. Exhaustive results are tabulated in Table I. From this table we see that difference between the assembling end and the disassembling end is not definite, because, for example, for $p_A < q_A$ and $p_D < q_D$ which is referred to as the region (3, 3) in Table I, it is the D-end that is actually growing and the A-end is receding. By adjusting the monomer concentration c, we can realize various situations, in principle. It is obvious that not all nine regions in Table I can be realized for a particular system. If $k_A/K_A > k_D/K_D$ the regions (1, 3), (2, 3), (3, 1), (3, 2), and (3, 3) are realized, for the case $k_A/K_A = k_D/K_D$ only the regions (1, 3), (2, 2), and (3, 1) are possible, and for $k_A/K_A < k_D/K_D$ the regions (1, 1), (1, 2), (1, 3), (2, 1), and (3, 1) can be realized. In particular, for steady state, only the regions (1, 1), (2, 2), and (3, 3) are permitted due to the restriction imposed by Eq. 7.

Experimentally, it must be almost impossible to maintain the monomer concentration c to realize the regions (1, 2), (2, 1), (2, 2), (2, 3), and (3, 2). Therefore, practically tractable regions are (1, 1), (1, 3), (3, 1), and (3, 3), and as we mentioned above, steady state is realized somewhere in the region (1, 1) or (3, 3).

In Fig. 2 we show exact plots for the released labeled subunits $R(t)$ (Eq. 15), using hypothetical values for the rate constants, i.e., $K_A = 4$, $k_A = 1$, $K_D = 2$, and $k_D = 1$. We also show the asymptotes for five realizable cases for this set of the rate constants. It is interesting to observe that only for the steady-state case does it take a long time for the exact plot to approach its asymptotic values, for other cases the exact values almost coincide with the asymptotes in <10 units of time.

INITIALLY UNLABELED POLYMER CASE

Uptake of Labeled Subunits

Although calculation of the amount of uptake of labeled subunits abundantly available in the solution by initially unlabeled F-actin or a microtubule looks more complicated than the case treated in the last section, it turns out to be essentially equivalent, as we show in the following. We start from the situation shown in Fig. 1. The only difference is that the subunits in the polymer are initially

TABLE I
ASYMPTOTIC FORMS OF $R(t)$

	$p_D > q_D \left(c < \frac{k_D}{K_D}\right)$	$p_D = q_D \left(c = \frac{k_D}{K_D}\right)$	$p_D < q_D \left(c > \frac{k_D}{K_D}\right)$
$p_A > q_A$ $\left(c < \frac{k_A}{K_A}\right)$	$(p_D - q_D)s_D t + \frac{1}{2(p_A - q_A)} + \frac{1}{2(p_D - q_D)} - 1$ (1,1)	$\sqrt{\frac{2s_D t}{\pi}} + \frac{1}{2(p_A - q_A)} - 1$ (2,1)	$\frac{1}{2p_A - q_A)} - \frac{1}{2(p_D - q_D)} - 1$ (3,1)
$p_A = q_A$ $\left(c = \frac{k_A}{K_A}\right)$	$(p_D - q_D)s_D t + \sqrt{\frac{2s_A t}{\pi}} + \frac{1}{2(p_D - q_D)} - 1$ (2,1)	$\sqrt{\frac{2s_A t}{\pi}} + \sqrt{\frac{2s_D t}{\pi}} - 1$ (2,2)	$\sqrt{\frac{2s_A t}{\pi}} - \frac{1}{2(p_D - q_D)} - 1$ (3,2)
$p_A < q_A$ $\left(c > \frac{k_A}{K_A}\right)$	$(p_D - q_D)s_D t - (p_A - q_A)s_D t - \frac{1}{2(p_A - q_A)} + \frac{1}{2(p_D - q_D)} - 1$ (3,1)	$-(p_A - q_A)s_A t + \sqrt{\frac{2s_D t}{\pi}} - \frac{1}{2(p_A - q_A)} - 1$ (3,2)	$-(p_A - q_A)s_A t - \frac{1}{2(p_A - q_A)} - \frac{1}{2(p_D - q_D)} - 1$ (3,3)

unlabeled this time and newly acquired subunits from the solution are labeled. At the A-end the average number of labeled units taken up after n_A steps can be expressed as the sum of the mean displacement of the random walker $\langle x_A \rangle$ and the number of once-visited sites in the minus direction $L^-_{n_A}$. At the D-end the average number of units taken up after n_D steps can be expressed as the number of once-visited sites by the random walker in the plus direction $L^+_{n_D}$ minus the mean displacement $\langle x_D \rangle$. Therefore, the total number of uptakes of labeled subunits after time t is

$$\begin{aligned} U(t) &= \langle x_A \rangle + L_{n_A}{}^- + L_{n_D}{}^+ - \langle x_D \rangle \\ &= (p_A - q_A)n_A + (1/2)[1 - (p_A - q_A)]n_A \\ &\quad - \frac{1}{2}\sum_{r=1}^{[n_A/2]} \frac{(n_A - 2r + 1)}{(2r - 1)}\, {}_{2r}C_r(p_A q_A)^r \\ &\quad + (1/2)[1 + (p_D - q_D)]n_D \\ &\quad - \frac{1}{2}\sum_{r=1}^{[n_D/2]} \frac{(n_D - 2r + 1)}{(2r - 1)}\, {}_{2r}C_r(p_D q_D)^r \\ &\quad - (p_D - q_D) \end{aligned} \tag{20}$$

the last expression in Eq. 20 holds for n_A, $n_D \geq 2$. If we use Eq. 12 for the number of once-visited sites, $U(t)$ can be written as

$$U(t) = (1/2)[(p_A - q_A)n_A - (p_D - q_D)n_D + L_{n_A} + L_{n_D}] \tag{21}$$

including the n_A, $n_D = 1$ case. Eq. 21 shows that we have essentially the same expression for $U(t)$ as we had for the number of released labeled subunits in Eq. 15. At steady state, incorporating the conditions of Eq. 8, we have the same equation as Eq. 16 for $U_{ss}(t)$. Asymptotic forms of

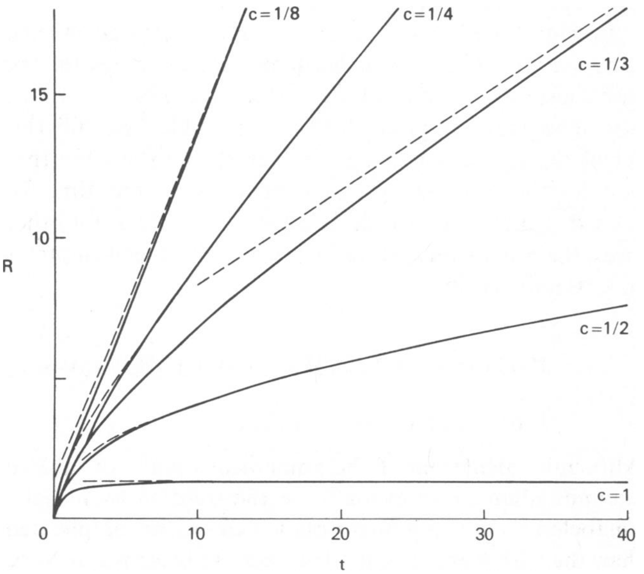

FIGURE 2 The number of released labeled subunits $R(t)$ is plotted for hypothetical set of rate constants, $K_A = 4$, $k_A = 1$, $K_D = 2$ and $k_D = 1$ using Eq. 15. Broken lines are the corresponding asymptotes plotted by using the expressions given in Table I.

TABLE II
ASYMPTOTIC FORMS OF $U(t)$

	$p_D > q_D \left(c < \frac{k_D}{K_D}\right)$	$p_D = q_D \left(c = \frac{k_D}{K_D}\right)$	$p_D < q_D \left(c > \frac{k_D}{K_D}\right)$
$p_A > q_A$ $\left(c < \frac{k_A}{K_A}\right)$	$(p_A - q_A)s_At$ $+ \frac{1}{2(p_A - q_A} + \frac{1}{2(p_D - q_D)} - 1$ (1,1)	$(p_A - q_A)s_At + \sqrt{\frac{2s_Dt}{\pi}}$ $+ \frac{1}{2(p_A - q_A)} - 1$ (1,2)	$(p_A - q_A)s_At - (p_D - q_D)s_Dt$ $+ \frac{1}{2(p_A - q_A)} - \frac{1}{2(p_D - q_D)} - 1$ (1,3)
$p_A = q_A$ $\left(c = \frac{k_A}{K_A}\right)$	$\sqrt{\frac{2s_At}{\pi}}$ $+ \frac{1}{2(p_D - q_D)} - 1$ (2,1)	$\sqrt{\frac{2s_At}{\pi}} + \sqrt{\frac{2s_Dt}{\pi}}$ -1 (2,2)	$-(p_D - q_D)s_Dt + \sqrt{\frac{2s_At}{\pi}}$ $- \frac{1}{2(p_D - q_D)} - 1$ (2,3)
$p_A < q_A$ $\left(c > \frac{k_D}{K_D}\right)$	$- \frac{1}{2(p_A - q_A)} + \frac{1}{2(p_D - q_D)} - 1$ (3,1)	$\sqrt{\frac{2s_Dt}{\pi}}$ $- \frac{1}{2(p_A - q_A)} - 1$ (3,2)	$-(p_D - q_D)s_Dt$ $- \frac{1}{2(p_A - q_A)} - \frac{1}{2(p_s - q_D)} - 1$ (3,3)

$U(t)$ for large t can be obtained by the same procedure for $R(t)$ case and the results are tabulated in Table II. The expression given in the region (1, 1) is the same as the one reported by Zeeberg et al. by a different method (Eq. 18 of reference 4).

Hill and Kirschner (8), in their recent review on microtubules, calculated the amount of uptake of unlabeled subunit into initially labeled polymer. This is essentially the same problem as the one presented in this section. They focused their attention on the steady state and gave results identical to ours for the asymptotic case, e.g., the average length of an unlabeled "cap" at D-end (Eq. 296 of reference 8). In our notation this quantity can be expressed by the last two terms of the first line of Eq. 20, since they considered the case in which the D-end is really a disassembling end. In the large t limit this becomes

$$U^D(t \to \infty) \sim q_D/(p_D - q_D) = K_Dc/(k_D - K_Dc). \qquad (24)$$

In their notation k_D is written as β_2 and K_D as β_1. They also considered the case in which the rates of polymerization and depolymerization are equal as a symmetric random walk problem and carried out formal calculation to obtain the mean number of labeled subunits $\overline{m}$ taken up at the D-end of a polymer for a large number of steps as (Eq. 301 of reference 8)

$$\overline{m} \sim 2(\beta_2 t/\pi)^{1/2} = 2(k_Dt/\pi)^{1/2}. \qquad (25)$$

This result was also given by Zeeberg et al. by a different method (Eq. 13 of reference 4). From our point of view this corresponds to the case presented in the region (2, 2) of Table II with steady-state condition. Because the symmetric random walk case $p_D = k_D/s_D = 1/2$, we have $k_D = s_D/2$. Therefore,

$$\overline{m} = 2(s_Dt/2\pi)^{1/2} = \sqrt{2s_Dt/\pi} \qquad (26)$$

which is exactly the contribution by the D-end for the region (2, 2) of Table II, neglecting the small constant value contribution.

What we emphasize here is that once we realize that the amount of release or uptake of labeled subunits from a polymer can be written in terms of well-studied quantities such as the mean displacement and the number of once-visited sites of a random walker, results for the amount of release or uptake can readily be obtained without serious calculations.

PULSE-LABELED POLYMER CASE

Remaining Labeled Subunits

One can carry out a so-called pulse label experiment in which one allows an unlabeled polymer to uptake labeled subunits for a certain time called a pulse time, then to uptake unlabeled units thereafter. This pulse-labeled case can be described essentially as we did in the last two sections because it is a combination of uptake and release of labeled subunits.

Let n_A^0 be the number of steps taken by the random walker at the A-end during the pulse time t_0. Then at the A-end the number of initially uptaken labeled subunits l_A^0 is

$$l_A^0 = \langle x^0{}_A \rangle + L_{n_A}{}^- \qquad (27)$$

where the first term is the mean displacement and the second is the number of once-visited sites in the minus direction, both during the pulse time. The remaining amount of labeled subunits after n_A steps becomes

$$l_{n_A} = l_A^0 - L_{n_A}{}^-. \qquad (28)$$

The measurement of the time t should start after the pulse time t_0 is completed. If we use Eq. 14, the remaining amount of labeled subunits in the polymer at the A-end can be written as

$$l_{n_A} = (1/2)[(p_A - q_A)n_A^0 + L_{n_A^0} + (p_A - q_A)n_A - L_{n_A}]. \qquad (29)$$

At the D-end the number of initially uptaken labeled subunits $l^o{}_D$ is given by

$$l_D^0 = L_{n_D^0}{}^+ - \langle x_D^0 \rangle \qquad (30)$$

where the first term is the number of once-visited sites in the plus direction and the second is the mean displacement after the pulse time t_0 has passed. The remaining amount of labeled subunits after time t is then

$$l_{n_D} = l_D^0 - L_{n_D}{}^+ \qquad (31)$$

or

$$l_{n_D} = (1/2)[-(p_D - q_D)\, n_D^0 + L_{n_D^0} - (p_D - q_D)n_D - L_{n_D}]. \qquad (32)$$

The total amount of remaining labeled subunit l which is a measurable quantitity is given by

$$l = l_{n_A} + l_{n_D}. \qquad (33)$$

When Eq. 32, or under different conditions Eq. 29, gives negative values, l_{n_D} or l_{n_A} should be set equal to zero, because the negative values mean that the pulse at the D- or A-end has been exhausted. The number of steps $n_D{}^{ex}$ or $n_A{}^{ex}$ at which the pulse at the D- or A-end is exhausted is given by setting Eq. 32 or 20 ≤ 0 as

$$L_{n_D} + (p_D - q_D)n_D^{ex} \geq L_{n_D^0} - (p_D - q_D)n_D^0 \qquad (34)$$

or

$$L_{n_A} - (p_A - q_A)n_A^{ex} \geq L_{n_A^0} + (p_A - q_A)n_A^0. \qquad (35)$$

In Fig. 3 we show how the remaining number of labeled subunits taken up as a pulse into a polymer decreases during time elapses under the steady-state condition, Eq. 8. It is meaningless to carry out pulse chase experiments under a condition that is far from steady state. Therefore, we impose the steady-state condition. Once again we use the same hypothetical rate constants, namely $K_A = 4$, $k_A = 1$, $K_D = 2$, and $k_D = 1$. For this system the steady state is realized at $c = 1/3$, and the A-end is actually growing and the D-end is receding. For the pulse time $t_0 = 30$, Eq. 34 (an inequality) holds as soon as $t = 3$, which means the pulse taken in at the D-end survives for only 2 units of time. For the case of $t_0 = 60$, the pulse at the D-end can be retained for >3 units of time but <6 units. An almost flat portion of each graph corresponds to the amount of the remaining pulse running down in the polymer from the A- to the D-end.

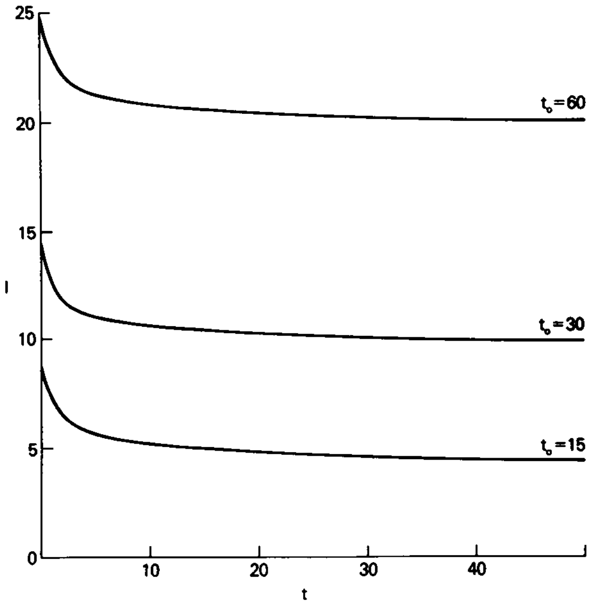

FIGURE 3 The remaining labeled subunits (Eq. 33) taken up as pulses of length t_0 = 15, 30, and 60 are plotted for the same set of rate constants used in Fig. 2 under the steady-state condition.

Our treatment of the pulse label case is a discrete-time version of the theory given by Zeeberg et al. (4), who considered a continuous time description using the Fokker-Planck equation.

ON POLLARD AND MOOSEKER'S RATE CONSTANTS

Recently Pollard and Mooseker measured actin polymerization rate constants directly by electron microscopy (8). For the solution condition of 20 mM KCl, they obtained the values $K_A = 5.91\ \mu M^{-1}$, $k_A = 6.0\ s^{-1}$, $k_D = 0.8$, and $k_D = 0.7$ (set 1), and for the other condition that 75 mM KCl and 5 mM $MgSO_4$ $K_A = 8.8$, $k_A = 2.0$, $k_D = 2.2$, and $K_D = 1.4$ (set 2).

For set 1 they claim that there is no treadmilling because the critical concentrations are the same at the two ends. The critical concentrations are given by setting Eqs. 1 and

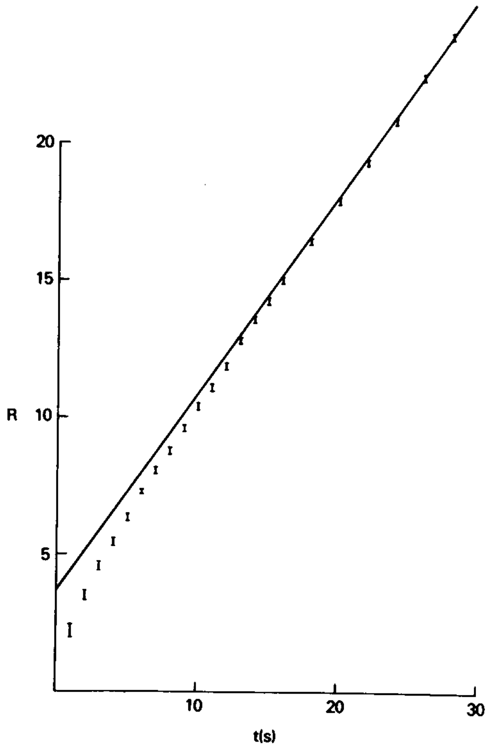

FIGURE 4 The number of released unlabeled subunits $R(t)$ is plotted for experimentally measured set of rate constants, $K_A = 8.8$ ($\mu M^{-1} \cdot s^{-1}$), $k_A = 2.0$ (s^{-1}), $K_D = 2.2$ and $k_D = 1.4$ (8). Each error bar represents the upper and the lower bound of the exact result due to the fact that we utilized the discrete time random walk theory. The straight line is the asymptote given by the equation derived in the region (1, 1) of Table I.

2 equal to zero. For set 1 we see that the two results are quite close to each other but not exactly identical; the one at the A-end is slightly larger than 1 μM and the one at the D-end is slightly smaller than 1 μM. The steady-state condition is given by Eq. 7 and it yields the monomer concentration at steady state, $c_{ss} = 1$ μM. According to Table I and II the steady state for the set 1 is realized in the region (3, 3), which means that actually it is the D-end that is growing and the A-end that is decreasing. If Pollard and Mooseker's experimental results for set 1 is accurate, "tail-to-head polymerization" should be observed at the monomer concentration 1 μM.

For set 2, the steady state is realized in the region (1, 1), which means the ordinary head-to-tail polymerization is seen. We calculated the number of released labeled subunits $R(t)$ for the set 2 as we did in Fig. 2 for a set of hypothetical rate constants. We show the result for only the steady state in Fig. 4. For the values given in set 2 quantities s_A and s_D defined in Eq. 9 become 4.72 and 2.08, respectively. Therefore, it is impossible to have integer values for n_A and n_D, which were defined in Eq. 10, simultaneously at any value of t. Because we utilized the notion of once-visited sites in discrete time random walk theory the number of steps n_A and n_D must be integers. However, for practical purposes it is not a drawback, because it is always possible to calculate the upper bound and the lower bound values for the number of once-visted sites for arbitrary values of s_A and s_D by choosing the closest integer values for s_At and s_Dt. In Fig. 4 the error bars show this fact. The exact results for set 2 lie somewhere on the bars, not necessarily in the middle. The straight line is the asymptote given by the equation shown in the region (1, 1) of Table I. We see after 25 s the exact values for $R(t)$ for set 2 can be approximated by the asymptote fairly well.

For the other four possible cases, the ones corresponding to the regions (1, 2), (1, 3), (2, 3), and (3, 3) of Table I, we do not show the graphs here. For these cases the exact values converge to the asymptotes very fast, in <5 s. Therefore, the asymptotic forms that can be plotted easily are sufficient to describe these cases. The fact that the steady-state case is the slowest to approach the asymptote is the same as we observed for the hypothetical values of the rate constants in Fig. 2.

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