Dynamics of Linear Protein Polymer Disassembly*

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This report presents a kinetic model for endwise depolymerization of linear, self-assembling protein systems. We develop a reliable method for predicting the shape of depolymerization curves (remaining polymer weight versus time) on the basis of the initial polymer length distribution. Computer simulations are used to illustrate changes in the polymer length distribution and average polymer weight during disassembly. In addition, our method provides an accurate determination of the microscopic rate constant for subunit release. Application of this analysis to dilution-induced and cold-induced disassembly of microtubules is illustrated in the preceding paper (Karr, T. L., Kristofferson, D., and Purich, D. L. (1980) J. Biol. Chem. 255, 8560-8566). A survey of other possible applications of this treatment to microtubules, flagella, F-actin, and tobacco mosaic virus protein is included.

Linear protein polymers may undergo disassembly by a variety of mechanisms. Some models have been proposed for loss of monomeric units from the interior of the polymer (viz. Inoué’s model for microtubule action in mitosis (16)). Yet, such models require substantial quaternary rearrangements that are likely to be thermodynamically disfavored. In this respect, it is natural to assume that many protein polymers such as microtubules and actin disassemble by successive loss of subunits from the polymer ends. Disassembly of these polymers may be initiated by using a variety of methods: temperature jumps, solvent changes, addition or removal of salts or denaturants, pressure changes, and dilution. The course of the reaction has usually been monitored by observing changes in flow birefringence, viscosity, or turbidity and the data presented as plots of changes in these quantities with time. Very few investigators have attempted to explain the shape of the depolymerization curves obtained. The usual approach is to fit the data to a first order curve, but one may ask what feature of the process is really first order.

First order processes such as radioactive decay stem from a species which reacts once to form product. It is highly unlikely that the first order kinetics often seen for linear polymers would result from single step total disintegration of the polymers to monomers. Simultaneous dissociation of the thousands of subunits involved is very improbable. In addition, an explanation of a first order curve by this mechanism requires the same decay constant for polymers of vastly differing lengths. Oosawa and Asakura (17) explained the observed critical concentration behavior of protein polymers in terms of a reversible linear polymerization. This strongly suggests that the disassembly reaction occurs at the end of the polymer in the reverse manner of assembly.

Electron microscopic studies of polymer length distributions usually yield a broad range of lengths which might appear to make the explanation of first order kinetics even more difficult. However, earlier investigators (2, 7) recognized that the shape of the length distribution may provide an answer, and one group (2) used a graphical method to predict the shape of the depolymerization curve. In this paper, we provide a mathematical formulation that corrects and extends the previous attempts at explanation. We develop the equations for predicting the shapes of depolymerization curves and use the results to obtain the microscopic off rate constant. In addition, we are able to predict changes in distribution shape and average polymer length during endwise disassembly. The theory thereby enables an investigator to determine whether a system of polymers disassembles endwise by comparing a theoretical depolymerization curve calculated from a polymer length distribution with an experimental depolymerization curve (see preceding paper for the case of microtubules). The method is highly discriminatory in this regard because each length distribution yields a unique depolymerization curve. Changes in the distribution shape are, therefore, readily detectable in the calculated depolymerization curves.

THEORY

Our interest in explaining the shape of protein polymer depolymerization curves stemmed from our previous dilution experiments on microtubules at steady state (1). The curiously close fit of our data to a first order plot and the absence of an immediately obvious explanation led us to consider the problem in terms of the change in the length distribution during depolymerization. Previous investigators had also realized that the depolymerization curve was dependent on the length distribution of polymers. Johnson and Borisy (2), assuming that the rate of depolymerization for each microtubule was a constant independent of polymer length, went on to conclude that all of the microtubules depolymerize by the same length increment in a given time interval. Accordingly, the distribution function of polymer number concentration versus length would be seen to shift at a constant rate toward shorter lengths. The shape of the distribution function would be maintained exactly during this translation, i.e. 10 microtubules (MT’s) of length 10 μm would go to 10 MT’s of length 9 μm in time t and then to 10 MT’s of length 8 μm in time 2t with all other groups depolymerizing in unison, too. While Johnson and Borisy (2) mainly employed graphical methods, this intuitive approach can also be expressed in a simple mathematical form which can then be used to predict the experimental depolymerization curves. It will be helpful to examine this first to evaluate the theory better. Although we shall continue the discussion in

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reference to microtubules, it is obvious that the following may apply to any linear polymer. The tubulin dimer is the monomeric unit in depolymerization. If we define \( x \) to be the number of dimers in a microtubule and \( f(x) \) to be the number concentration of microtubules containing \( x \) dimers, then the following simple relationships result:

\[
N = \sum_{x=m}^{n} f(x), \quad D = \sum_{x=m}^{n} xf(x), \quad W_{0} = MW_{M}_{T_{0}} = \sum_{x=m}^{n} xf(x) \quad (1,2,3)
\]

where \( N \) is the total number concentration of microtubules, \( D \) is the total number of monomers in polymeric form per unit volume, \( W_{0} \) is the total polymer weight per volume, \( MW_{M}_{T_{0}} \) is the molecular weight of the tubulin dimer (110,000), and the indices \( m \) and \( n \) represent the number of dimers in the shortest and longest microtubules. The idea of a lower limit \( m \) is impracticable; however, for the purpose of the following derivations, any exact value for \( m \) is unnecessary provided \( n \gg m \). Berne (3) considered light scattering from rods of lengths much greater than the wavelength of incident light, and he has shown that the turbidity is directly proportional to the weight concentration. As the length drops below a minimal value, the contribution to light scattering becomes negligible. Experimentally then \( m \) may be viewed as an approximate indicator of minimal microtubule lengths necessary to contribute significantly to light scattering. This meaning for \( m \) also validates the inequality \( n \gg m \) as will be shown.

We now wish to find the equation for the total polymer weight remaining at time \( t \), \( W(t) \), because a plot of this versus time should match the depolymerization curve obtained from light-scattering experiments. Since we are assuming that the distribution is moving to the left along the \( x \) axis at a constant rate while maintaining its shape, let us call the average dissociation time per dimer \( t_{D} \). This represents the time it takes for the distribution to shift one place to the left on the \( x \) axis. Accordingly, after a \( t \) time, the distribution has shifted \( t/t_{D} \) places to the left. Therefore, if we want the total weight of polymers which contain \( x \) dimers, we must multiply the dimer weight by \( x \) dimers and also by the number of polymers containing \( x \) dimers. At \( t = 0 \), the number of these polymers was \( f(x) \), but at time \( t \), a little reflection will show that the number is \( f(x + t/t_{D}) \) since the value of the distribution function at position \( x \) at time \( t \) was originally \( t/t_{D} \) places farther to the right along the \( x \) axis. The polymer weight at time \( t \) is, therefore,

\[
W(t) = \sum_{x=m}^{n} MW_{M}_{T_{0}}f(x + t/t_{D}) \quad (4)
\]

where the upper limit is now \( n - t/t_{D} \) since the longest microtubules have lost \( t/t_{D} \) dimers in time \( t \). If \( f(x) \), the initial polymer length distribution function, is known, then Equation 4 can be used to predict experimental depolymerization curves as long as the quantity measured is directly proportional to polymer weight. Berne (3) has shown the validity of this proportionality for light-scattering measurements on microtubules.

Unfortunately, a flaw exists in the above reasoning. The assumption that the distribution will maintain its shape with time is in error. Depolymerization (and all other chemical reactions) must be described in statistical terms and must not be viewed as a mechanistic process whereby all of the polymers react in unison. As described below, an initially sharp ("spike") distribution will broaden with time and this broadening will affect the rate of polymer loss. The extent of this broadening and its impact on the depolymerization rate will be seen to be more pronounced in some distributions and smaller in others. Consequently, the theory described above is only approximately correct since it neglects the broadening of the distribution.

Let us now use a kinetic approach to derive the equation for \( W(t) \). If the environmental conditions (temperature, solution variables, etc.) are appropriately modified so that polymer assembly is negligible, then we may model the depolymerization reaction by a series of first order reactions:

\[
MT_{n} \stackrel{k_{1}}{\rightarrow} MT_{n-1} \stackrel{k_{2}}{\rightarrow} \ldots \stackrel{k_{n}}{\rightarrow} \text{small MT's and dimers}
\]

where \( MT_{n} \) is a microtubule of \( n \) dimers, etc. and \( n \) and \( m \) retain their former meanings. We assume that microtubules of less than \( m \) dimers do not contribute measurably to light scattering, and, consequently, we do not add them into the totals for polymer weight. An additional assumption is that all of the rate constants are equal to \( k \) since it seems reasonable to believe that dimers at the ends of microtubules have no "knowledge" of the polymer length.

If \( c_{i} \) represents the number concentration of \( MT_{i} \)s, then the following kinetic equation holds for all \( i \) not equal to \( n \):

\[
\frac{dc_{i}}{dt} = -kc_{i} + kc_{i+1} = k(c_{i+1} - c_{i}) \quad \text{for} \quad i \neq n \quad (5)
\]

For \( i \) equal to \( n \) we get simply

\[
\frac{dc_{n}}{dt} = -kc_{n} \quad \text{or} \quad c_{n} = c_{n}^{0}e^{-kt} \quad (6,7)
\]

where \( c_{n} = c_{n}^{0} \) at \( t = 0 \) in Equation 7. Equation 6 holds for the longest polymer since no larger species yields \( MT_{n} \) upon depolymerizing.

Equation 5 can now be solved for sequentially decreasing values of \( i \) beginning with \( i = n - 1, n - 2, \) etc. as follows. Substituting Equation 7 into Equation 5 with \( i = n - 1 \) yields

\[
\frac{dc_{n-1}}{dt} = k(c_{n} - c_{n-1}) = k(c_{n}^{0}e^{-kt} - c_{n-1})
\]

This equation can be written in the standard form for linear nonhomogeneous first order differential equations as follows:

\[
\frac{dc_{n-1}}{dt} + kc_{n-1} = kc_{n}^{0}e^{-kt}
\]

An equation of this form can be solved by finding an integrating factor which in this case is \( e^{kt} \). Multiplication of both sides of the above equation by this factor gives

\[
e^{kt}\frac{dc_{n-1}}{dt} + ke^{kt}c_{n-1} = kc_{n}^{0}e^{kt}
\]

Since the left side of this equation equals \( \frac{d}{dt}(e^{kt}c_{n-1}) \), we have

\[
\frac{d}{dt}(e^{kt}c_{n-1}) = kc_{n}^{0} \quad \text{or} \quad \int \frac{d}{dt}(e^{kt}c_{n-1}) = kc_{n}^{0} \int dt
\]

Integrating and solving for the constant of integration under the condition that \( c_{n-1} = c_{n-1}^{0} \) when \( t = 0 \) gives

\[
c_{n-1} = (kc_{n}^{0} + c_{n-1}^{0})e^{-kt} \quad (8)
\]

Equation 8 can then be substituted back into Equation 5 with \( i \) now equal to \( n - 2 \). The solution is then repeated using the same integrating factor as before, \( e^{kt} \). After a few trials, it becomes apparent that the solution for \( c_{n-j} \) is

\[
c_{n-j} = e^{-kt} \sum_{i=0}^{j} \frac{(kt)^{i}}{i!} c_{n-i}^{0} \quad (9)
\]

Since we can now determine the number of polymers with \( n-j \) subunits at time \( t \) for any value of \( j \) from zero to \( n-m \),
we can immediately write down the equation for polymer weight at time \( t \):

\[
W(t) = \sum_{j=0}^{\infty} M W_{n0}(n-j)c_{n-j} = M W_{n0} \sum_{j=0}^{\infty} \left( \frac{kt}{j} \right)^{n-j-i} c_{n-j} \tag{10}
\]

where we have used Equation 9 and have factored two quantities out of the sum to obtain the right side of Equation 10. Given the initial concentration values \( c_{n-j} \)’s, Equation 10 can be evaluated to yield the correct depolymerization curve. The sums can be easily handled on a computer or numerical solutions of the system of equations generated from Equation 5 may be calculated using standard procedures, e.g. the Runge-Kutta method.

We may now compare the depolymerization curves predicted by the two theories for three important types of initial length distributions: “spike,” exponential, and Poisson-like (by “spike” distribution we mean that all microtubules initially have the same length; for the term “Poisson-like,” see the “Discussion”). The spike distribution can be solved analytically. The exponential distribution can be handled by approximating sums with integrals using the Euler-Maclaurin formula.

The spike distribution can be simply defined as follows using the notation of the earlier theory:

\[
f(u) = \begin{cases} f(n), & u = n \\ 0, & u \neq n \end{cases}
\]

where \( u \) is \( x \) or \( x + t/t_0 \) depending on whether Equation 3 or 4 is utilized. Using Equations 3 and 11 for the initial weight of polymer we get

\[
W_0 = M W_{n0} f(n) \tag{12}
\]

Combining Equations 4 and 11 gives the polymer weight at time \( t \).

\[
W(t) = M W_{n0} (n - t/t_0) f(n) = M W_{n0} f(n) - \frac{M W_{n0} f(n)}{n} (t/t_0) \tag{13}
\]

Substituting Equation 12 into 13 yields:

\[
W(t) = W_0 - \frac{W_0}{n t_0} t \tag{14}
\]

Consequently, the old theory predicts that the depolymerization curve is linear for the full extent of the reaction if all of the microtubules were initially the same length.

Now consider the same distribution in light of our new theory. Repeating the equations we have

\[
c_{n-j} = e^{-kt} \sum_{i=0}^{\infty} \left( \frac{kt}{j} \right)^{n-j-i} c_{n-j} \tag{9,10}
\]

It should be re-emphasized that \( n \) is the number of dimers in the longest microtubule present initially, not at time \( t \), and \( m \) is the number of dimers in a microtubule at the “limit of detectability” by light scattering. Although this may be a rather nebulous concept, it is actually unnecessary in the following derivation except to the extent that \( n-m \) is assumed to be a large number. Our electron microscope observations give a maximum microtubule length of about 70 \( \mu \)m. Using the approximate figure of 1600 dimers/\( \mu \)m means that \( n \) is

\( ^2 \) Assuming 8 nm/turn of the microtubule helix and 13 dimers/turn, we obtain a value of 1600 dimers/\( \mu \)m of tubule length.

The lower limit \( m \) must obviously be smaller than the average microtubule length. The average length is rarely longer than 15 \( \mu \)m which gives 24,000 dimers as a very generous upper limit on \( m \). One can immediately see that \( n-m \) is a large number.

We now continue the derivation of \( W(t) \) for the “spike” distribution along the lines of the new theory. The distribution can be expressed as follows:

\[
c_{n-j} = \begin{cases} c_i & \text{if } i = 0 \\ 0 & \text{if } i \neq 0 \end{cases} \tag{15}
\]

From Equations 9 and 15 we immediately get

\[
c_{n-j} = e^{-kt} \frac{(kt)^{n-j}}{j!} \tag{16}
\]

since all of the other terms in the sum are zero. Substituting this result into Equation 10 gives

\[
W(t) = \sum_{j=0}^{\infty} M W_{n0}(n-j)e^{-kt} \frac{(kt)^{n-j}}{j!} = M W_{n0} e^{-kt} \sum_{j=0}^{\infty} \frac{(kt)^{n-j}}{j!} \tag{17}
\]

Simplifying the sum yields

\[
W(t) = M W_{n0} e^{-kt} \left[ \sum_{j=0}^{\infty} \frac{(kt)^{n-j}}{j!} \right] \tag{17}
\]

The first term in the brackets resembles the series

\[
e^x = \sum_{i=0}^{\infty} \frac{x^i}{i!}
\]

which converges for all values of \( x \). This result can be substituted in Equation 17 as long as the following limitations are realized. The sum in Equation 17 does not go to infinity. However, since \( n-m \) is a large quantity, there will normally be a large enough number of terms in the sum for it to converge to \( e^x \) to a very high degree of accuracy. Unfortunately, large values of \( t \) in Equation 17 do cause problems. Since the ratio of the \((j+1)\)th term to the \(j\)th term in the sum is \( kt/j \), each successive term in the series will actually increase until \( kt \) equals \( j \), the number of terms summed up. In Equation 17, once \( kt \) is comparable in size to the total number of terms in the series (\( n-m+1 \)), the series will not converge to \( e^x \).

The best way of doing these sums is to use a computer. However, it is still instructive to see the results in the time domain where the sum still converges to \( e^x \). Substituting this in Equation 17 gives

\[
W(t) = M W_{n0} e^{-kt} \left[ \sum_{j=0}^{\infty} \frac{(kt)^{n-j}}{j!} \right] \tag{17}
\]

where the lower limit on the remaining sum has been increased to 1 since the \( j = 0 \) term is obviously zero. Factoring out a \((kt)\) from the second sum and simplifying yields

\[
W(t) = M W_{n0} e^{-kt} \left[ \sum_{j=0}^{\infty} \frac{(kt)^{n-j}}{j!} \right] \tag{17}
\]

Letting \( u = j-1 \) gives

\[
W(t) = M W_{n0} e^{-kt} \left[ \sum_{u=0}^{\infty} \frac{(kt)^{n-u-1}}{u!} \right] \tag{17}
\]

The remaining series is now in the form where the \( e^x \) approximation may be employed.

\[
W(t) = M W_{n0} e^{-kt} \left[ \sum_{u=0}^{\infty} \frac{(kt)^{n-u}}{u!} \right] = M W_{n0} e^{-kt} \tag{17}
\]

Since the initial polymer weight for the spike distribution is \( W_0 = M W_{n0} c_0 \), the above equation reduces to
Interestingly, we come to the same conclusion as before with the old depolymerization theory (Equation 14) as long as the \( kt \ll n - m \) assumption is not violated. Comparison of Equations 14 and 18 shows that \( k = 1/t_p \), i.e. the average dissociation time \( t_p \) is the inverse of the rate constant. \( k \) can obviously be calculated from the slope of the line. However, due to the gradual broadening of the initial spike (Fig. 1, B to E), all of the microtubules will not disappear at the same time, and the depolymerization plot deviates from linearity toward the end of the reaction. Fig. 1A compares the results predicted by the old theory with our new theory of depolymerization kinetics. The units used in these and the following computer calculations are arbitrary, i.e. monomer weight equals 1, \( k \) equals 10 units\(^{-1} \), and the units for \( t \) are the reciprocal of those for \( k \).

Finally, let us examine the results for the exponential and Poisson-like distributions. Fig. 2A shows the depolymerization curve predicted by our theory for an exponential length distribution. A log plot of these data would give first order kinetics for approximately the first 85% of the reaction. The dotted tangent line is used to illustrate the deviation from zero order kinetics. The same distribution would result in a very similar curve if calculated on the basis of the old theory. However, it would be seen to dip very slightly below our curve during the final 40% of the reaction and, consequently, would go to zero more quickly. At 4 computer time units, the old theory curve is about 12% lower than our curve, and the old theory curve reaches zero at approximately 8 computer time units instead of 10 as in our case. Fig. 2, B to E, shows the length distribution as a function of time after initiation of depolymerization. The shape is fairly well maintained throughout the reaction, although rather small deviations do occur. The distribution appears to shift 10 places to the left \( (k = 10 = 1/t_p) \) for each unit of time. Table I lists the actual numerical frequencies corresponding to successive shifts. According to the old theory, the frequency values would be constant; by our theory, they slowly increase.

Fig. 3A shows the predicted depolymerization curve for a Poisson-like distribution. A log plot would show that the kinetics is approximately first order for the Poisson-like distribution since the peak occurs very quickly and leaves a long near exponential tail. The parameters \( (p \) and \( q \) where \( c_0 = A x^p e^{- q x} \)) for this distribution were approximately those obtained from a least squares fit to actual experimental data. For distributions possessing a peak farther to the right on the \( x \) axis, the kinetics deviates to an even greater extent from first order.

Table I

<table>
<thead>
<tr>
<th>Time</th>
<th>Polymer species (n-mer)</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>units</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>60</td>
<td>15.85</td>
</tr>
<tr>
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<td>50</td>
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<tr>
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<td>16.61</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>17.00</td>
</tr>
</tbody>
</table>

Fig. 2. Exponential distribution kinetics. A, depolymerization curve for an exponential distribution. The initial polymer concentrations were obtained from the equation \( c_0 = 1000 \exp(-0.0690775 x) \) which gives a value of 1000 for \( x = 0 \) and 1 for \( x = 100 \). The dashed line illustrates the deviation from zero order kinetics. B to E, plots of the length distribution at times 0, 1, 2, and 3, respectively. Each graduation on the frequency axis corresponds to 200 polymers.

Fig. 1. Spike distribution kinetics. A, depolymerization curve for a spike distribution. Computations were done starting with 1000 polymers each containing 100 subunits. The subunit weight is arbitrarily defined as 1. Units for time are also arbitrary, but must correlate with those used in the rate constant, \( k \). The value used for \( k \) was 10 units\(^{-1} \). The dashed line indicates the divergence of the old theory from our results. Prior to that point, the two are identical. B to E, plots of the length distribution at times 1, 3, 5, and 7, respectively. Each graduation on the frequency axis corresponds to 20 polymers.
Experimental length distribution and comparing it to the microscopic depolymerization rate constant. The theory presented now allows an investigator to segregating distributions on this basis. The fit of an experimental histogram to a Poisson distribution results from a series first order polymerization scheme. The solution of the rate equations for such a mechanism is almost identical with that shown in Equations 5 to 9 except that we begin with only monomer present and proceed in the opposite direction, again assuming that the \( k \) values are all equal.

The concentration of the \( j \)th species is given by

\[
c_j = e^{-\mu} \frac{(\mu t)^j}{j!} e^{-\mu t}
\]

where \( \mu \) is the mean of the distribution and \( f(x) \) is the relative frequency of \( x \). The fit of an experimental histogram to a Poisson distribution profoundly affects the disassembly kinetics, we have compared the shape of our microtubule length distribution histograms to a Poisson distribution. Many previous investigators have described the length distributions at steady state by using the term “Poisson.” A Poisson distribution is of the form

\[
f(x) = \frac{e^{-\mu} \mu^x}{x!}
\]

We have seen above that the apparent first order fit of depolymerization data for protein polymers may result from endwise dissociation of subunits from polymers initially comprising an exponential (or near exponential) distribution of lengths. The theory presented now allows an investigator to test a polymeric system for a successive endwise depolymerization mechanism by calculating a disassembly curve from an experimental length distribution and comparing it to the observed curve (see preceding paper for experimental and computational details). One also obtains an accurate value for the microscopic depolymerization rate constant.

Several systems other than microtubules may require further interpretation in light of these results. Gerber et al. (5) measured rates of disassembly of Salmonella flagella. Depolymerization was initiated by a temperature jump (to higher temperatures) and the reaction was followed by viscometry. Unfortunately, the dependence on the length distribution was not considered. Flagella are also susceptible to depolymerization by acid or acetone treatment (6). With our theory, one could determine whether these reagents cause endwise depolymerization or instantaneous total dissociation by extensive subunit loss along the flagella. Depolymerization of actin initiated by dialysis has been studied by electron microscopy and flow birefringence (7). The authors were aware of the importance of the length distribution but did not attempt to calculate a depolymerization curve from their data. The behavior of the average polymer length during their depolymerization experiments appears to correlate with our prediction for a Poisson-like distribution (see Fig. 4 above). Since their data show an exponential distribution of lengths, further study is needed here. Kasai (8) studied actin depolymerization by dilution at various temperatures. His data were not first order but no attempt at correlation with the length distribution was made. Lauffer and co-workers (9-13) have extensively characterized the assembly and disassembly reactions of nucleic acid-free tobacco mosaic virus protein. Depolymerization may be induced by temperature jumps, addition of urea, and pressurization. These investigators suggest that tobacco mosaic virus disassembly is first order (9). While the polymers are of “various lengths” (13), no distribution work has been reported.

Because the distribution profoundly affects the disassembly kinetics, we have compared the shape of our microtubule length distribution histograms to a Poisson distribution. Many previous investigators have described the length distributions at steady state by using the term “Poisson.” A Poisson distribution is of the form

\[
f(x) = \frac{e^{-\mu} \mu^x}{x!}
\]

where \( \mu \) is the mean of the distribution and \( f(x) \) is the relative frequency of \( x \). A distribution of lengths similar to a Poisson distribution results from a series first order polymerization scheme. The solution of the rate equations for such a mechanism is almost identical with that shown in Equations 5 to 9 except that we begin with only monomer present and proceed in the opposite direction, again assuming that the \( k \) values are all equal. The concentration of the \( j \)th species is given by

\[
c_j = e^{-\mu t} \frac{(\mu t)^j}{j!} e^{-\mu t}
\]

If only monomer is present initially (all \( c_i = 0 \) except \( c_0 \)), then Equation 20 simplifies to

\[
c_j = c_0 \frac{(\mu t)^j}{(j-1)!} e^{-\mu t}
\]

If \( \mu = \lambda \), then the resemblance between Equations 19 and 21 is almost complete with the exception that \( c_j \) is being calculated from the \( j-1 \) value, i.e. the \( c_j \) values would be precisely Poisson if the distribution was shifted back one place. The fit of an experimental histogram to a Poisson distribution can be done by calculating the mean, \( \mu \), of the experimental distribution and using its value in Equation 19 to find the relative frequencies \( f(x) \). Multiplying each \( f(x) \) by the total number of MT’s in the histogram gives the Poisson frequencies for each histogram interval. We have found that it is not correct to describe most MT histograms by the term Poisson since the MT distributions are usually skewed toward shorter lengths than those calculated for a true Poisson distribution of corresponding mean \( \mu \). Since we have shown above that a series first order polymerization yields a distrib-

![Figure 3](image-url) Poisson-like distribution kinetics. A, depolymerization curve for a Poisson-like distribution. Initial polymer concentrations were obtained from \( c_0 = 543.656 \exp(-0.2x) \). This equation gives a peak in the distribution of 1000 at \( x = 5 \). The first power of \( x \) and the factor of 0.2 are approximately those obtained from a least squares fit to an actual experimental MT histogram. The dashed tangent line is the initial rate of depolymerization. B to E, plots of the length distribution at times 0, 1, 2, and 3, respectively. Each graduation on the frequency axis corresponds to 200 polymers.

![Figure 4](image-url) A comparison of the behavior of the average weights (or lengths) of polymers for the exponential distribution (solid line) and the Poisson-like distribution (dashed line). Subunit weight is defined to be 1.
Microtubule Depolymerization

bution which is almost Poisson, a reasonable explanation of the skewness in MT distributions toward shorter lengths is the reversible nature of the actual polymerization process. We have, therefore, used the function \( A x^{-b} \) with \( A \) and \( b \) as constants to describe an experimental histogram rather than Equation 19. Equation 19 gives distribution shapes similar to those shown in Fig. 1, \( B \) to \( E \), as can be determined by referring once again to equation 16 and letting \( \mu = kt \) therein.

It should also be noted that we have made no attempt to compensate for the possibility that depolymerization may occur at both ends of a polymer or that the polymer may be a multiple start helix, etc. In this case, the shape of the calculated depolymerization curve would remain the same, but the value used for \( k \) would have to be corrected by an appropriate constant dependent upon the number of possible sites of depolymerization at each or either end. The work of Wegner (14) on actin and Margolis and Wilson (15) and our group (1) on microtubules emphasizes the necessity to think carefully about both ends. Should different rates of depolymerization exist at opposite ends, our present theory would be unable to distinguish them, yielding only a composite value for the depolymerization rate constant.

For the case of microtubules, it will also be interesting to see if calcium, GDP, and drug-induced disassembly follow the endwise depolymerization mechanism. Studies of these effects are currently under way in our laboratory.

To conclude, the mathematical presentation of the kinetics of endwise depolymerization contained in this paper offers a new means for a more rigorous experimental determination of the existence of this mechanism in protein polymer systems. Much additional work in this field is still needed as well as a re-examination of past depolymerization data. Since the initial stages of the depolymerization reaction are usually obscured by mechanistic complications, it would appear that the logical first step in the understanding of self-assembling systems is a thorough investigation of the mechanism of depolymerization.

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REFERENCES