Biophysical Chemistry 20 (1984) 319–331
Elsevier

BPC 00887

POLYDISPERSITY EFFECTS ON HEAD-TO-TAIL POLYMERIZATION OF F-ACTIN

A THEORETICAL TREATMENT

Yoshinori NAGAI [a],*, Hiroshi ASAI [b] and Takashi TSUCHIYA [c],**

Departments of [a] Applied Physics and [b] Physics, Waseda University, Tokyo 160, Japan and [c] Laboratory of Molecular Biology, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20205, U.S.A.

Received 28th February 1984
Revised manuscript received 20th June 1984
Accepted 22nd June 1984

Key words: Polydispersity; F-Actin; Head-to-tail polymerization

A theoretical description of the release of labeled subunits of F-actin that undergoes head-to-tail polymerization utilizing random-walk theory is extended to the case of a finite-length polymer. An exact expression is given for the number of released subunits in a suitable form for numerical calculation. By comparing the result for a finite-length polymer with that for an infinite-length case, the limitation of the infinite-length approximation is readily known. The results for finite-length polymers are applied to systems with polydispersity. It is shown that the number of labeled subunits released from F-actin depends strongly on the distribution functions of the polymer lengths and the values of the transition probabilities of the random walkers.

1. Introduction

A theoretical treatment of head-to-tail polymerization or treadmilling of F-actin or a microtubule has been carried out by Wegner [1], Hill [2,3], Zeeberg et al. [4], Hill and Kirschner [5] and Tsuchiya and Nagai [6]. All of these authors *** assumed that the polymers are infinitely long in order to avoid mathematical complication. In reality, however, the lengths of the polymers are finite and distributed over a certain range. Therefore, it is reasonable to imagine that there exist experimental conditions in which the results obtained with the theories cited above cannot be applied straightforwardly. In the present paper we consider the effects of finiteness of polymer length and polydispersity on the release of labeled subunits of F-actin that undergoes head-to-tail polymerization. Precise analysis such as this is unavoidable when one is interested in a fundamental problem such as determination of the mechanism of sustainment of the critical concentration, i.e., whether F-actin really undergoes treadmilling. One of the most important results reported by the above authors [2–6] is that the number of labeled subunits released by an infinitely long polymer increases linearly as a function of time t when the polymer is treadmilling, whereas the same quantity follows a $\sqrt{t}$ function when each polymer end is just fluctuating. Thus, one is tempted to look at the curvature of the quantity under consideration as a function of time to determine the main mech-

---

* Present address: Laboratory of Molecular Biology, Azabu University School of Veterinary Medicine, Fuchinobe, Sagamihara 229, Japan.

** Present address: Biological Information Division, CSK Research Institute, 4-39-5 Tamagawa Setagaya-Ku Tokyo 158, Japan.

*** Hill [2] considered briefly the steady-state polymer length distribution.

anism of polymerization. We show in this paper that there are realizable experimental conditions under which the number of labeled subunits released from the whole system with polydispersity behaves quite misleadingly, if an observer sticks to the analysis based on the assumption that the polymer length is infinite or that the whole system can be represented by a polymer of the average length.

In section 2 we briefly review our two-random-walker description of head-to-tail polymerization of F-actin. In section 3 we calculate the average number of labeled subunits released from a finite-length F-actin which undergoes head-to-tail polymerization in the solution and compare the results with that of the infinite-length case. This treatment of a finite-length chain is a prerequisite for studies of polydispersity effects. Also, this is important to determine the applicability of the infinite-length approximation to a system that can be represented by the average polymer length. In section 4 the effect of polydispersity is shown. We assume hypothetical but probable distribution functions of polymer lengths and calculate the average number of labeled subunits released from the polymers. We observe cases in which analysis using a finite-length polymer, in addition to the infinite-length approximation, fails due to a strong polydispersity effect as well as cases where the analysis for a finite-length polymer is valid. It is straightforward to deal with a real distribution of polymer lengths once it is measured.

2. Two-random-walker description

When head-to-tail polymerzation of F-actin takes place in a solution in which ATP molecules are present, the following reaction equations describe the situation [7]:

$$G + ATP \rightleftarrows G \cdot ATP \qquad (1)$$

$$G \cdot ATP + F_n \cdot ADP \rightarrow F_{n+1} \cdot ADP + P_i \qquad (2)$$

$$F_{n+1} \cdot ADP \rightleftarrows F_n \cdot ADP + G \cdot ADP \qquad (3)$$

$$G \cdot ADP + ATP \rightleftarrows G \cdot ATP + ADP \qquad (4)$$

where G denotes G-actin, F_n F-actin of n subunits and P_i orthophosphate.

We now regard each end of F-actin as a random walker: random walker 1 for end 1 and walker 2 for end 2 with asymmetric transition probabilities p_1, q_1 and p_2, q_2, respectively. (In ref. 6 we termed each end A- and D-ends denoting assembling and disassembling ends, respectively. However, we also showed that there exist situations in which the A-end is disassembling and the D-end assembling. Hence, we consider it appropriate to call each end simply end 1 and end 2.) These transition probabilities are related to four rate constants (see fig. 1), namely, α_1 and β_1 which are the rate constants for binding and release of G-actin at end 1 and α_2 and β_2 at end 2, as:

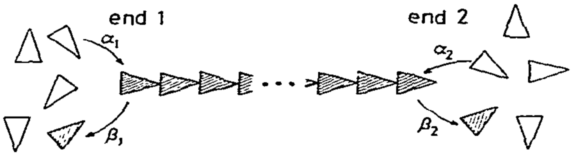


Fig. 1. Schematic representation of polymerization and depolymerization at each end of F-actin. Wedges represent the polarity of a subunit of F-actin or G-actin. The rate constant for polymerization at end 1 is denoted by α_1 and that at end 2 by α_2. The rate constants for depolymerization are β_1 and β_2 at respective ends.

$$p_1 = \frac{\beta_1}{\alpha_1 c + \beta_1}, \qquad q_1 = 1 - p_1 = \frac{\alpha_1 c}{\alpha_1 c + \beta_1} \qquad (5)$$

$$p_2 = \frac{\alpha_2 c}{\alpha_2 c + \beta_2}, \qquad q_2 = 1 - p_2 = \frac{\beta_2}{\alpha_2 c + \beta_2} \qquad (6)$$

where c is the concentration of G-actin in the solution. In other words, p_1 is the probability that the actin polymer is shortened by the length of a monomer at end 1, q_1 the probability that the polymer is elongated by the same length at end 1 during a unit time Δt_1, whereas p_2 is the probability that the polymer becomes longer by the monomer length and q_2 the probability that the polymer becomes shorter by the same length at end 2 during a unit time Δt_2. The unit times Δt_1 and Δt_2 are related to the rate constants as

$$\Delta t_1 = 1/(\alpha_1 c + \beta_1) \qquad (7)$$

$$\Delta t_2 = 1/(\alpha_2 c + \beta_2) \qquad (8)$$

respectively. *

---

* In our treatment association or dissociation of a subunit at each end occurs exactly once during a respective unit time, which means that we ignore fluctuations in the number of such events during a unit time.

3. Release of labeled subunits from a finite-length F-actin

In this section we calculate the average number of labeled subunits released from a finite-length F-actin which undergoes treadmilling in the solution. In order to treat the effects of polydispersity it is necessary first to establish rigorous understanding of a finite-length polymer case. For an infinitely long polymer, the one-random-walker description is sufficient because the number of once-visited sites by the walker at end 1 can be calculated independently of that by the walker at end 2 [6]. (For a detailed discussion of the quantity called once-visited sites, see ref. 8.) However, for a finite-length polymer this is no longer the case, and the treatment inevitably becomes complicated.

Let l be the degree of polymerization of an initially labeled F-actin. * We denote the probability that m_1 labeled subunits are released from end 1 during time t by $B_1(m_1,t)$ and that of m_2 labeled subunits from end 2 by $B_2(m_2,t)$. Polymerization or depolymerization itself at end 1 takes place independently of that at end 2 even for a finite-polymer case provided the polymer is longer than a monomer. 'Depolymerization' of a dimer to two monomers requires special consideration, so we depict the situation in fig. 2. For the case shown in fig. 2a it is possible to distinguish from which end the labeled subunit has been released by means of the polarity of the subunit. Hence, we can take p_1 and q_2 values different from those of longer polymers. However, for fig. 2b, the decomposition process is governed by a unique rate constant or transition probability. We interpret this as shown in fig. 2c for consistency with other cases, namely, we assume that we still can use different values for p_1 and q_2. **

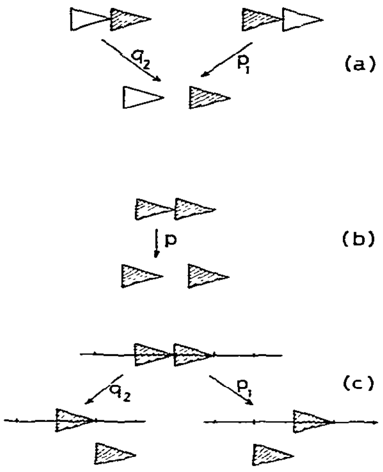


Fig. 2. Breakdown of a dimer into two monomers relevant to release of labeled subunits. (a) Both processes can be distinguished and treated in exactly the same manner as longer polymers. (b) For this process the decomposition rate is unique (represented by p). (c) Our interpretation of the event shown in b. We assume that different values for p_1 and q_2 can be used to make the event consistent with other cases.

The probability $R(k,t)$ that k labeled subunits are released during time t can be written as

$$R(k,t) = \sum_{m_1=0}^{k} B_1(m_1,t) B_2(k-m_1,t) \tag{9}$$

$0 \le k \le l-1$

It is possible to normalize the probability $R(k,t)$ such that the relation

$$R(l,t) = 1 - \sum_{k=0}^{l-1} R(k,t) \tag{10}$$

holds. Here, $R(l,t)$ is the probability that all the labeled subunits are released during time t. The average number $\langle k \rangle_t$ of labeled subunits released during time t is given by

$$\langle k \rangle_t = \sum_{k=0}^{l} kR(k,t)$$

---

* See fig. 1 where labeled subunits are denoted by hatched figures. We assume as before [6] that once a labeled subunit leaves the polymer it cannot return to the polymer in the labeled state. This assumption is reasonable if exchange of a labeled ligand with an unlabeled one takes place very rapidly when a subunit is in the solution.

** Here, we have ignored so-called nucleation effects, i.e., the rate constants (or equivalently the transition probabilities) for association or dissociation of a subunit are independent of the length of a polymer with which the subunit is interacting. In other words we are dealing with noncooperative polymerization.

$$= \sum_{k=0}^{l-1} k \sum_{m_1=0}^{k} B_1(m_1,t) B_2(k-m_1,t) + lR(l,t) \tag{11}$$

Substituting eq. 10 into the last expression, we have

$$\langle k \rangle_t = l - \sum_{k=0}^{l-1} (l-k) \sum_{m_1=0}^{k} B_1(m_1,t) B(k-m_1,t) \tag{12}$$

The probabilities $B_1(m_1,t)$ and $B_2(m_2,t)$ can be expressed by the first passage time probabilities as shown below. The probability $B_1(m_1,t)$ implies that the random walker reaches position m_1 for the first time after a certain number of steps and then never goes beyond m_1 during time t, or

$$B_1(m_1,t) = \sum_{N=0}^{[\frac{t}{\Delta t_1}]} f^{(1)}_{m_1}(N) \left\{ 1 - \sum_{N'=N}^{[\frac{t}{\Delta t_1}]} f^{(1)}_{1}(N'-N) \right\}$$

$$= \sum_{N=0}^{[\frac{t}{\Delta t_1}]} f^{(1)}_{m_1}(N) - \sum_{N=0}^{[\frac{t}{\Delta t_1}]} f^{(1)}_{m_1+1}(N) \tag{13}$$

where $f^{(1)}_{m_1}(N)$ is the first passage time probability for walker 1 to reach position m_1 after N steps and the square brackets mean that the maximum integer part of the value in the brackets should be taken, e.g., [3.52] = 3. Similarly, $B_2(m_2,t)$ can be expressed as

$$B_2(m_2,t) = \sum_{N=0}^{[\frac{t}{\Delta t_2}]} f^{(2)}_{-m_2}(N) - \sum_{N=0}^{[\frac{t}{\Delta t_2}]} f^{(2)}_{-m_2-1}(N) \tag{14}$$

Now we have to obtain an explicit expression for the first passage time probabilities. For the problem of two random walkers a finite distance apart, like the one we are now dealing with, one must usually consider interaction between the walkers, which makes the treatment quite complicated or frequently impossible in an analytical sense. However, in our case we can avoid explicit treatment of the interaction between the two walkers because we are interested only in the number of released labeled subunits or the once-visited sites by the walkers. Consider the last expression of eq. 11, where the summation with respect to k is carried out from 0 to $l-1$. This means that a region of the once-visited sites by walker 1 is always separated from that by walker 2 by at least one site length. When the two regions collide (interact) which corresponds to the case of release of all the labeled subunits, it is taken care of by the last term of eq. 11 and the process we can observe terminates there. Therefore, we do not need to take into account the interaction between walkers 1 and 2 to derive the explicit expressions of B_1 and B_2. Hence, we can employ a well-known form for the first passage time probability $f^{(1)}_{m_1}(N)$ for the free-boundary condition which is available in, e.g., the work of Feller [9]:

$$f^{(1)}_{m_1}(N) = \frac{m_1}{N} \cdot N^{C}(N+m_1)/2 \cdot p_1^{(N+m_1)/2} \cdot q_1^{(N-m_1)/2} \tag{15}$$

where $N^{C}(N+m_1)/2$ is the binomial coefficient which is valid only for integer values of $(N+m_1)/2$. We also have

$$f^{(2)}_{-m_2}(N) = \frac{m_2}{N} \cdot N^{C}(N+m_2)/2 \cdot p_2^{(N-m_2)/2} \cdot q_2^{(N+m_2)/2} \tag{16}$$

As we have shown before [6] it is clear that for an infinitely long polymer the simple formula

$$\langle k \rangle_t = \langle m_1 \rangle_t + \langle m_2 \rangle_t \tag{17}$$

holds, where $\langle m_1 \rangle_t$ is the average number of labeled subunits released exclusively from end 1 and $\langle m_2 \rangle_t$ is that from end 2. Now we have the exact expression eq. 12 (along with eqs. 13–16) for a finite-length actin polymer. Eq. 12 is written in a suitable form for numerical calculation. In figs. 3–5 we compare the results for eq. 12 with those for eq. 17 under various conditions to examine the finite-length effect. The abscissa is mesaured by the shorter unit time of the two, i.e., Δt_1 or Δt_2 in all these figures. Provided a graph for a finite-length case coincides with that for an infinite-length case, one may safely use the infinite-length approximation. In the present paper we use the relation

$$(p_1 - q_1)(1/\Delta t_1) = (p_2 - q_2)(1/\Delta t_2) \tag{18}$$

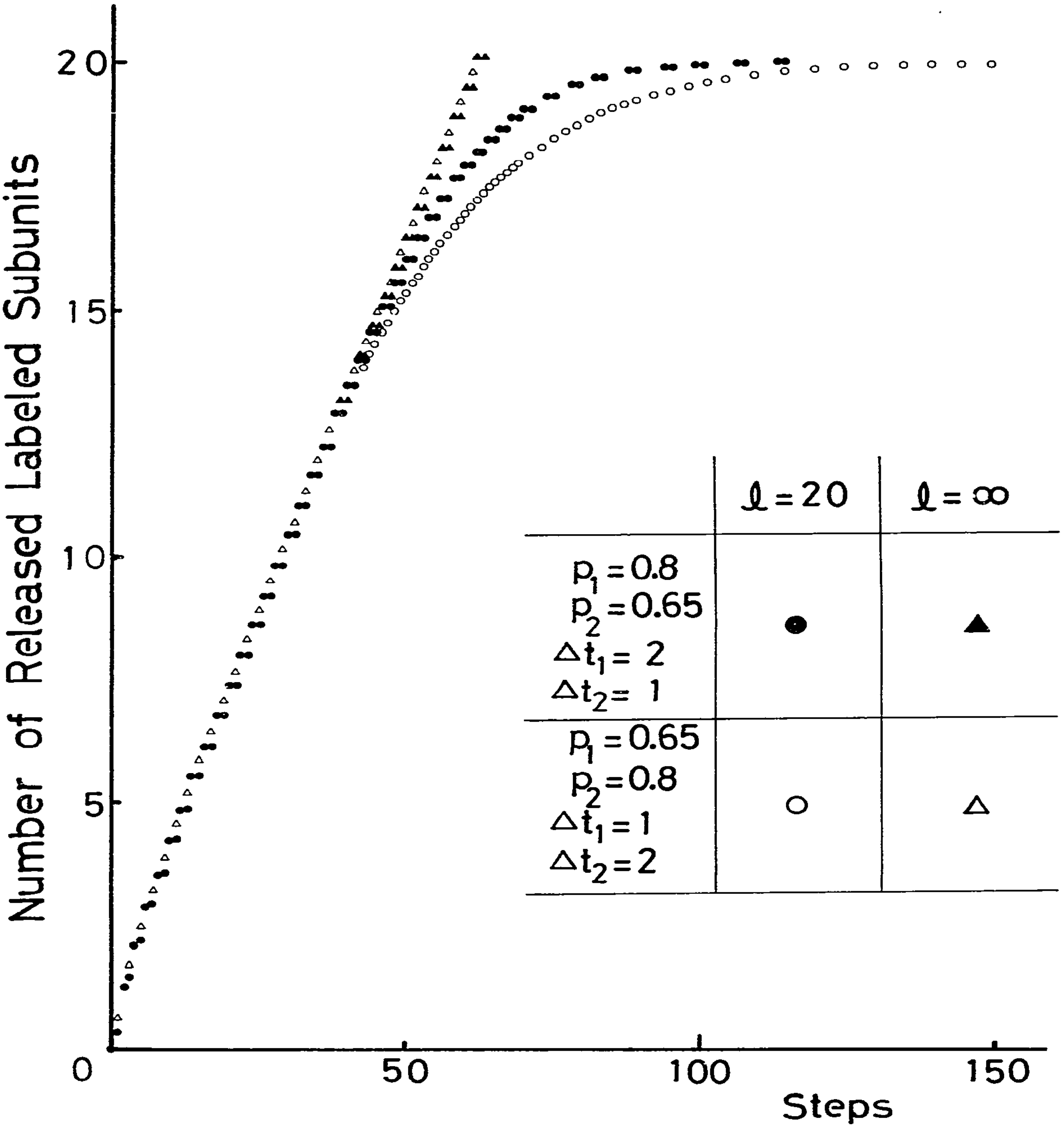


Fig. 3. The number of released labeled subunits for a finite-length case ($l = 20$) calculated from eq. 12 and the infinite-length polymers for two sets of parameters which satisfy the steady-state condition.

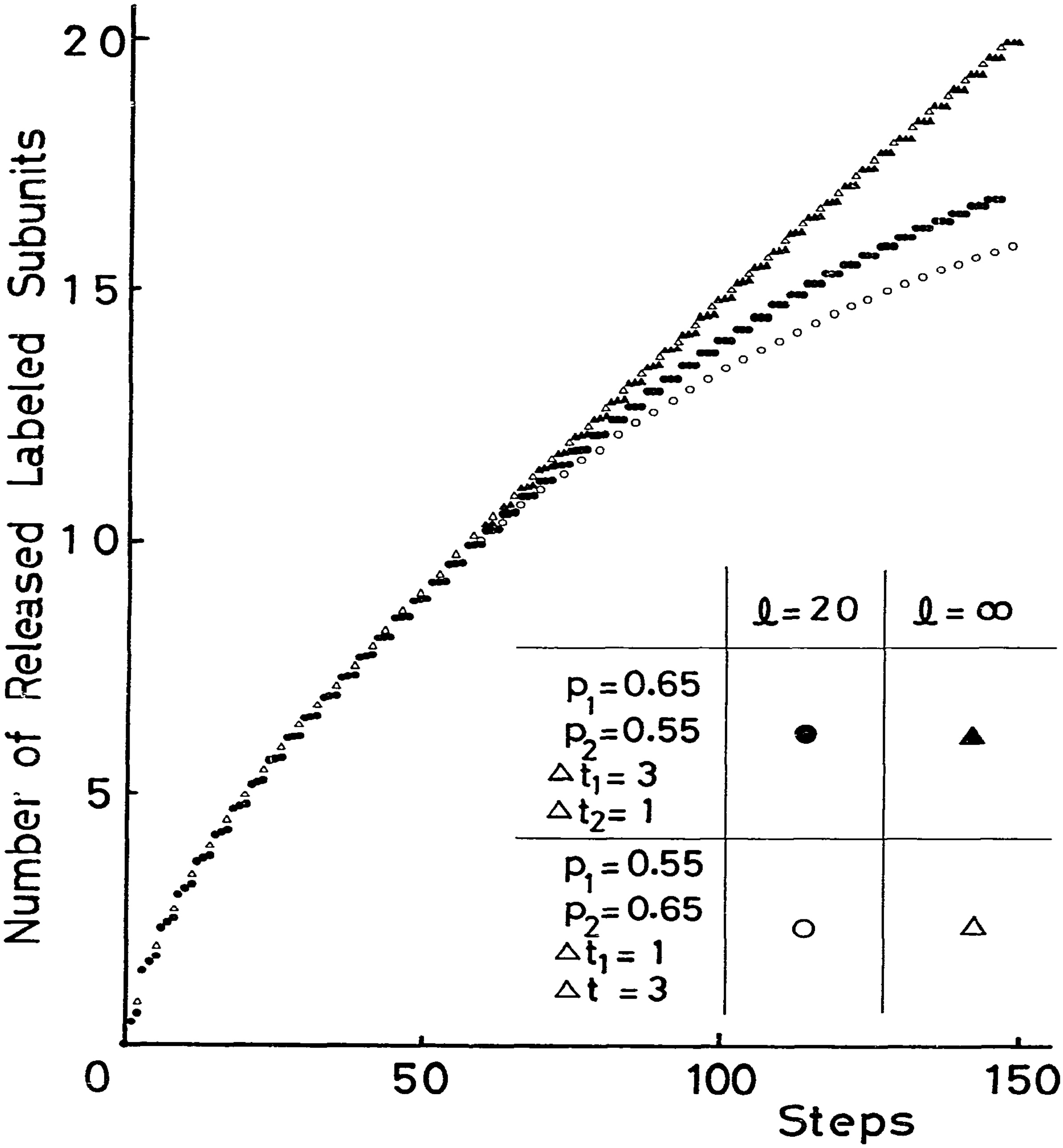


Fig. 4 The number of released labeled subunits for a finite-length case ($l = 20$) and the infinite-length polymers for two sets of parameters which satisfy the steady-state condition.

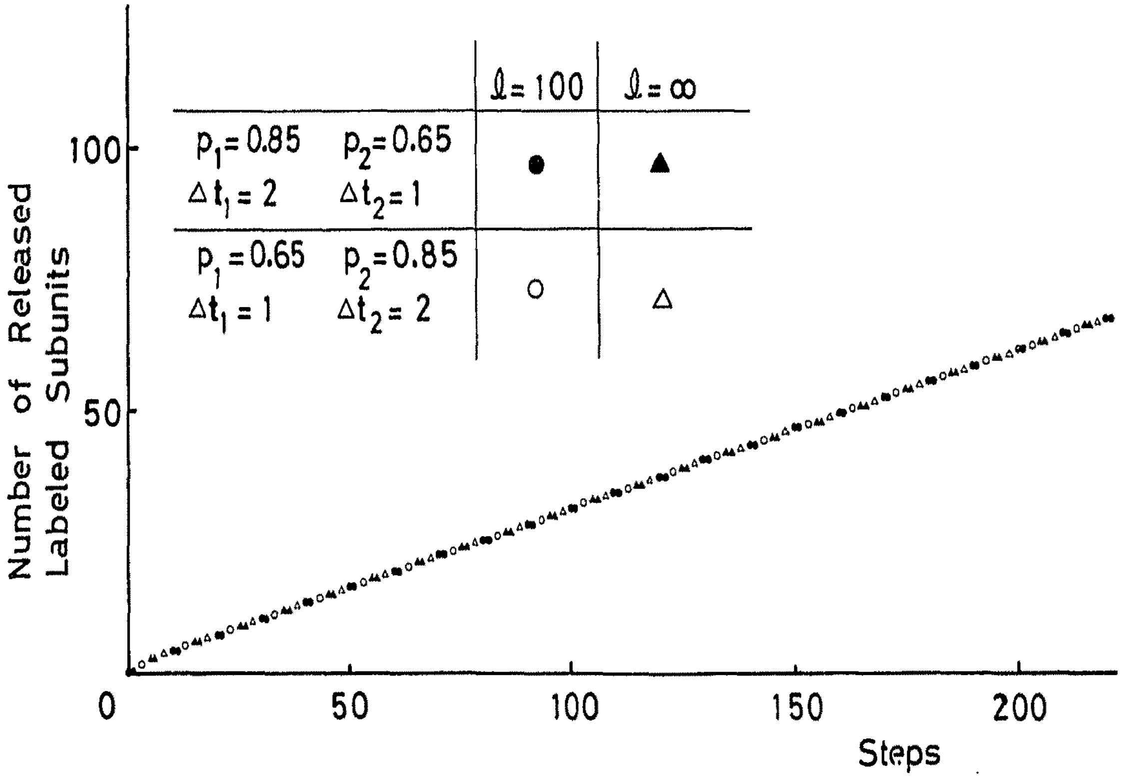
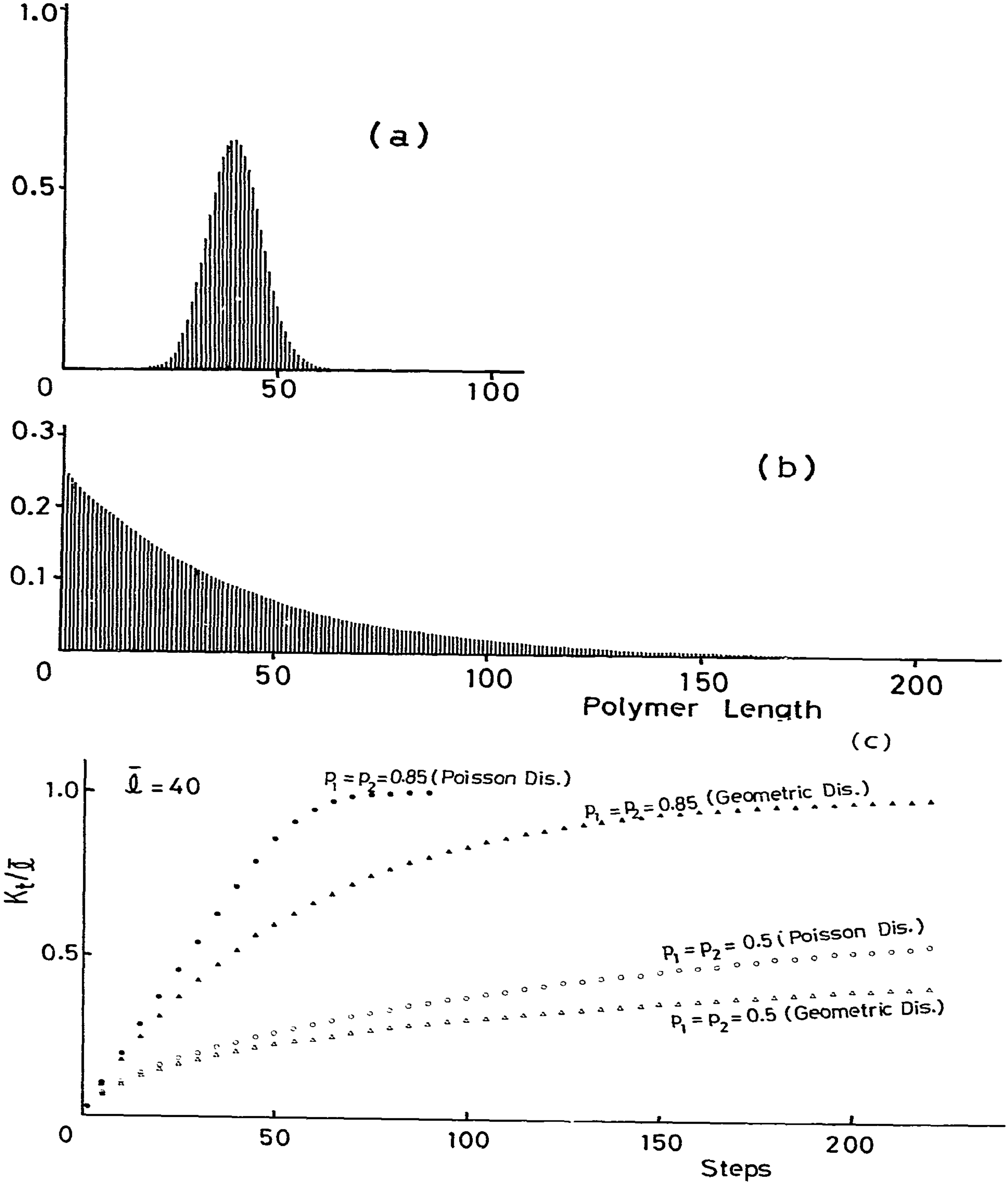


Fig. 5. The number of released labeled subunits for a finite-length case ($l = 100$) and the infinite-length polymers for the same sets of parameters as in fig. 3. No substantial difference can be observed within the number of steps studied.


(a)
1.0
0.5
0
50
100
(b)
0.3
0.2
0.1
0
50
100
150
200
Polymer Length
(c)
$\bar{l}$ = 40
$K_t/\bar{l}$
1.0
0.5
0
$P_1 = P_2 = 0.85$ (Poisson Dis.)
$P_1 = P_2 = 0.85$ (Geometric Dis.)
$P_1 = P_2 = 0.5$ (Poisson Dis.)
$P_1 = P_2 = 0.5$ (Geometric Dis.)
50
100
150
200
Steps

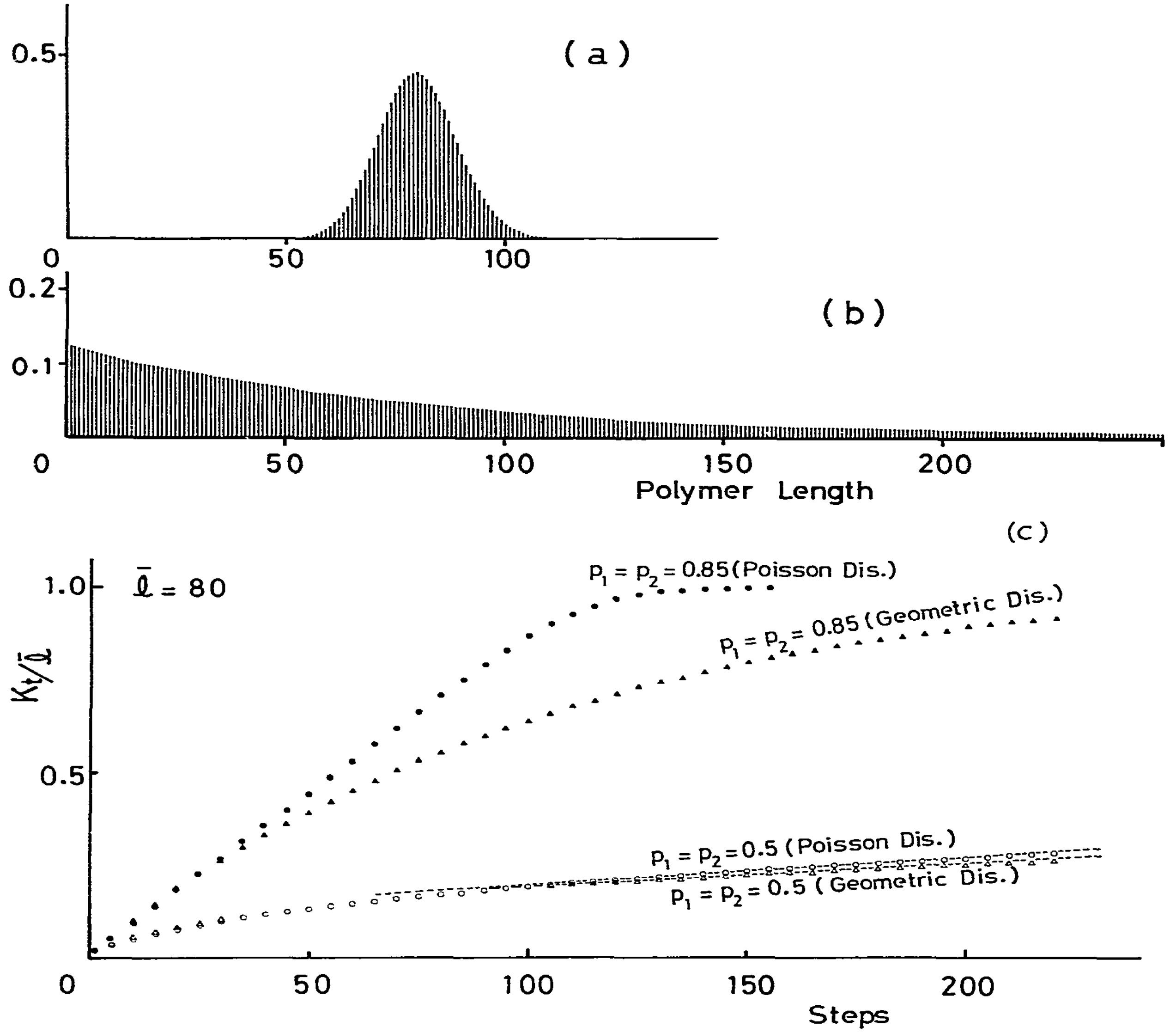


Fig. 7. (a) Histogram of polymer-length distribution given by the Poisson distribution (eq. 22) with average length $\bar{l} = 80$. (b) Histogram of polymer-length distribution given by the geometric distribution (eq. 23) with average length $\bar{l} = 80$. (c) Number of labeled subunits released from the whole system K_t divided by the average length $\bar{l} = 80$ for the Poisson distribution and the geometric distribution with the transition probabilities $p_1 = p_2 = 0.85$ (treadmilling case) and $p_1 = p_2 = 0.5$ (fluctuating case).

Fig. 6. (a) Histogram of polymer-length distribution given by the Poisson distribution (eq. 22) with average length $\bar{l} = 40$. (b) Histogram of polymer-length distribution of the geometric distribution (eq. 23) with average length $\bar{l} = 40$. (c) Number of labeled subunits released from the whole system K_t divided by the average length $\bar{l} = 40$ for the Poisson distribution and the geometric distribution with the transition probabilities $p_1 = p_2 = 0.85$ (treadmilling case) and $p_1 = p_2 = 0.5$ (fluctuating case).

as the steady-state condition which is valid as long as the assumption that all the rate constants in eqs. 5 and 6 are independent of the polymer length. It is interesting to observe in figs. 3 and 4 that, at the steady state, if we exchange the characteristic of end 1 with that of end 2 the difference in the number of released labeled subunits becomes apparent for a finite-length case (cf. ● and ○), whereas for the infinite-length case no substantial difference can be detected (▲ and △).

4. Polydispersity effects

As we have obtained the exact formula for the number of labeled subunits released from F-actin of arbitrary length, we are now in a position to study the effects of polydispersity.

When we have an arbitrary distribution of the polymer length $W(l)$, the average number of labeled subunits K_t released from the whole system is given as

$$K_t = \sum_{l=1}^{\infty} \langle k \rangle_{t,l} W(l) \tag{19}$$

where $\langle k \rangle_{t,l}$ is given by eq. 12. Here, the subscript l is added to emphasize length dependence explicitly. Since

$$\langle k \rangle_{t,l} \le l \tag{20}$$

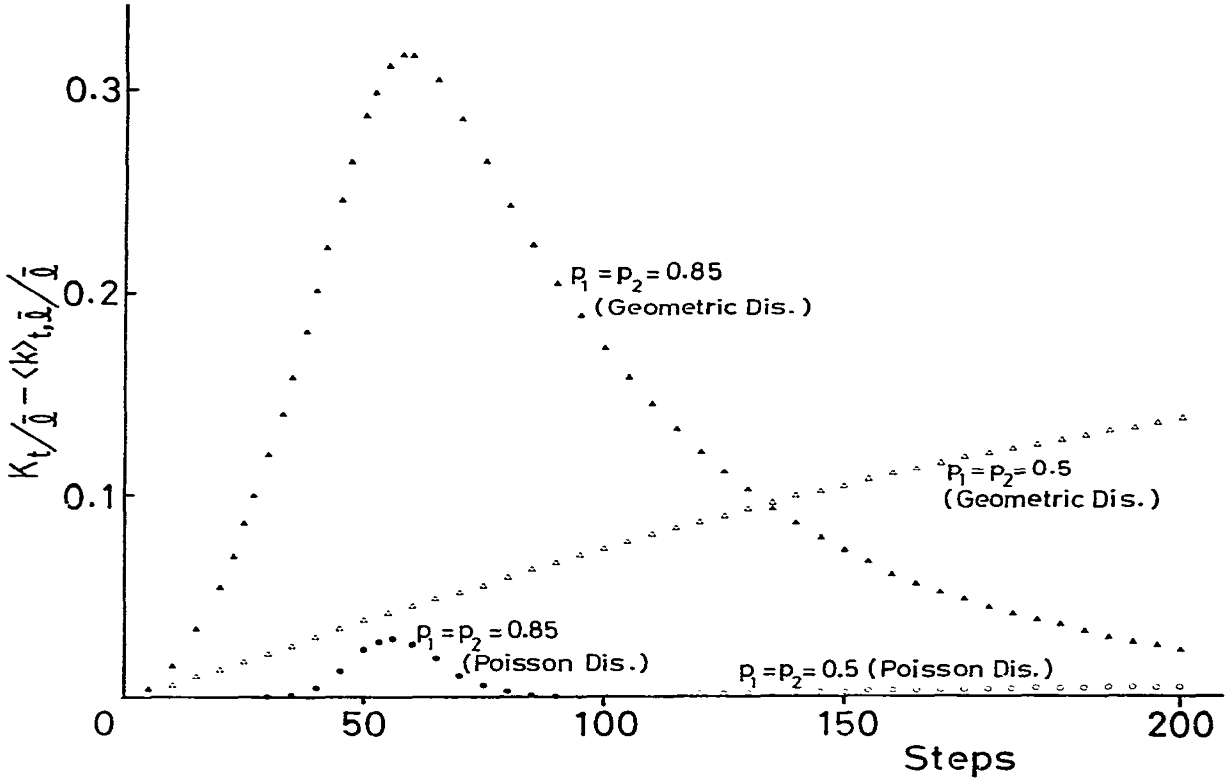


Fig. 8. Difference in number of released labeled subunits between the systems with and without polymer-length distribution. The ordinate represents the quantity $K_t/\bar{l} - \langle k \rangle_{t,\bar{l}}/\bar{l}$ for $\bar{l} = 40$. For the treadmilling case ($p_1 = p_2 = 0.85$) the difference is substantially negligible for the Poisson distribution whereas it is enhanced for the geometric distribution. For the fluctuating case ($p_1 = p_2 = 0.5$) the difference is visible for the geometric distribution and almost vanishes for the Poisson distribution.

we have

$$\sum_l \langle k \rangle_{t,l} W(l) \le \sum_l l W(l) = \bar{l} \tag{21}$$

where $\bar{l}$ is the average length of the polymer. Therefore, we see that the quantity $K_t/\bar{l}$ never exceeds unity. This quantity $K_t/\bar{l}$ is calculated here for two hypothetical but quite probable distributions, namely, the Poisson distribution given as

$$W_{\text{p}}(l) = \frac{(\bar{l}-1)^{l-1}}{(l-1)!} \exp[-(\bar{l}-1)] \tag{22}$$

and the geometric distribution expressed as

$$W_{\text{G}}(l) = \frac{1}{\bar{l}} \left(\frac{\bar{l}-1}{\bar{l}} \right)^{l-1} \quad l \ge 1 \tag{23}$$

In figs. 6 and 7 we show the results for $\bar{l} = 40$ and 80, respectively; in figs. 6a and 7a histograms of the Poisson distribution, in figs. 6b and 7b histograms of the geometric distribution and in figs. 6c and 7c the quantity of interest, namely, $K_t/\bar{l}$ for a typical treadmilling case ($p_1 = p_2 = 0.85$) and the fluctuating case ($p_1 = p_2 = 0.5$).

In figs. 6c and 7c for the treadmilling case ($p_1 = p_2 = 0.85$) the plots of $K_t/\bar{l}$ increase linearly until they reach saturation points as long as the length distribution is of the Poisson type. This result suggests that in the treadmilling case with the Poisson distribution, the number of labeled subunits released from the system increases as if the system has no polydispersity effect. This is confirmed in figs. 8 and 9 where the differences

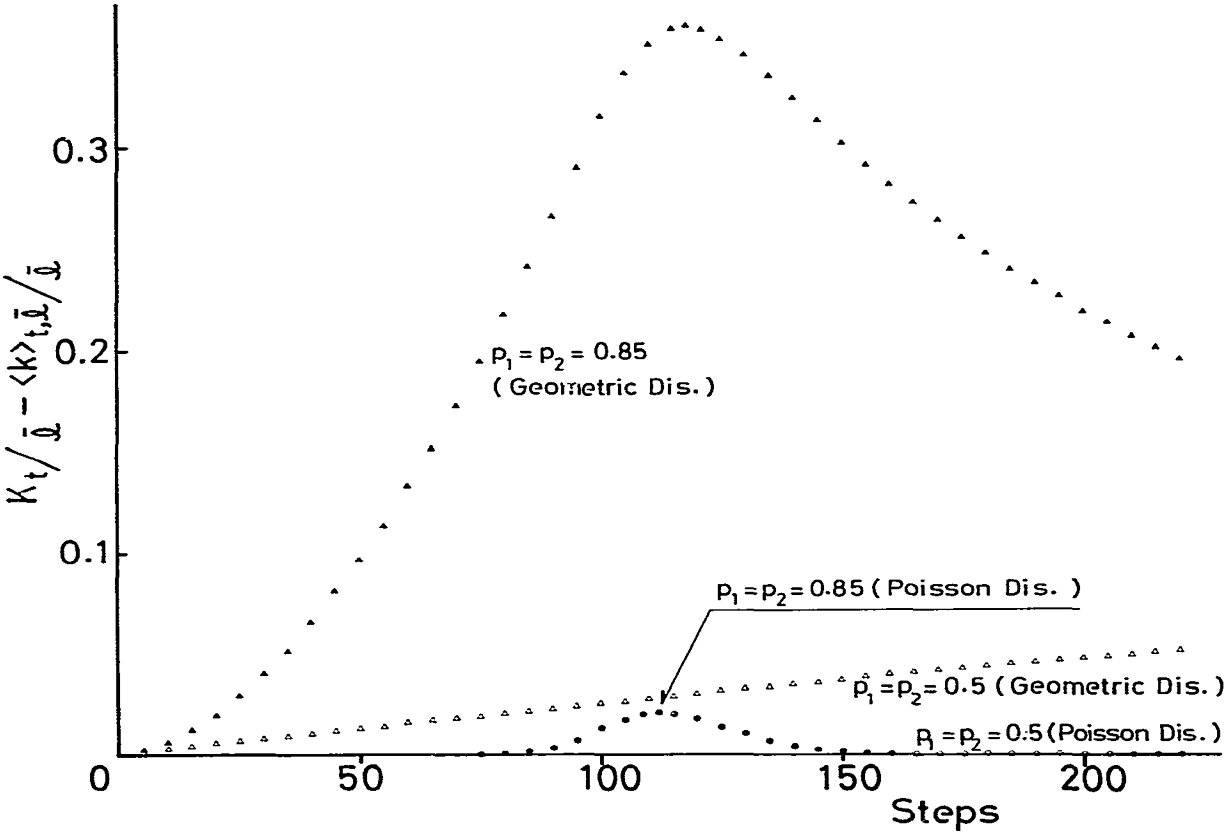


Fig. 9. The quantity $K_t/\bar{l} - \langle k \rangle_{t,\bar{l}}/\bar{l}$ for $\bar{l} = 80$. The overall tendency is the same as that of fig. 8.

between the quantity $K_t/\bar{l}$ for systems with polydispersity and the quantity $\langle k \rangle_{t,l}/l$ for polymers with a fixed finite length $l = \bar{l}$ are shown.

On the other hand, even for the treadmilling case ($p_1 = p_2 = 0.85$) the plots of $K_t/\bar{l}$ are no longer linear for systems with the geometric distribution. For these cases we observe in figs. 8 and 9 that the finite-length-polymer approximation fails to represent the systems, or in other words, the polydispersity effect is strongly manifested.

For the fluctuating case ($p_1 = p_2 = 0.5$) the effect due to the difference in distribution function on the quantity $K_t/\bar{l}$ disappears as the average length $\bar{l}$ increases (figs. 6c and 7c).

Though we have considered here only hypothetical distribution functions which have neat mathematical expressions, it is straightforward to apply our method to a realistic system once the polymer-length distribution is given.

5. Discussion

5.1. On polydispersity effects

We have discussed in section 4 typical polydispersity effects on the number of labeled subunits released from initially labeled systems of F-actin. We see from the figures therein that caution should be exercised in analyzing experimental data. We now explain this point using typical examples in fig. 10, in which the abscissa is set proportional to $\sqrt{t}$ and the ordinate represents the quantity K_t itself. In this plane the asymptote for the number of labeled subunits released from an infinitely long F-actin becomes a straight line. In ordinary experiments one measures some physical quantity proportional to K_t. The two straight lines in fig. 10 show that it is quite misleading to interpret the straight portions of the measured data as a manifestation of fluctuating ends when the systems have special forms of polydispersity. In these examples, neglecting the polydispersity effect leads to a conclusion that is contrary to actuality even qualitatively, i.e., that the polymer ends are just fluctuating while they are actually treadmilling. We can also imagine the opposite case: for a certain distribution and for a limited time range, a curve for a finite-length polymer with its ends treadmilling may be fitted to actual data for a system with polydispersity of polymers with fluctuating ends. Examples of this case are given in fig. 7c; the lower two curves appear to have straight-line asymptotes while they actually represent fluctuating cases with polydispersity.

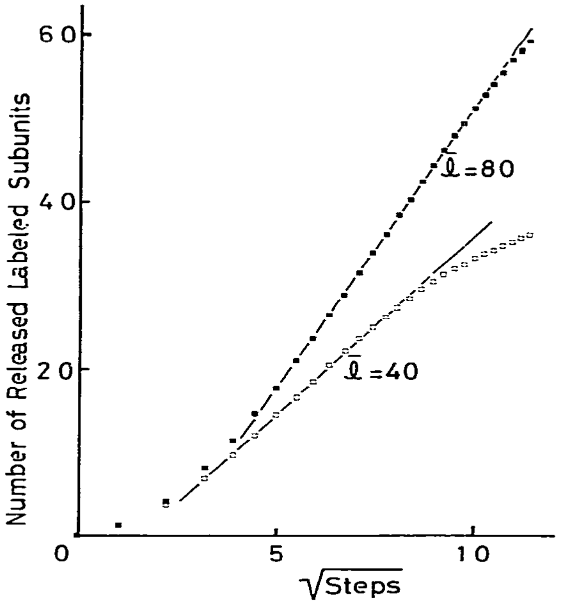


Fig. 10. The data for $p_1 = p_2 = 0.85$ (geometric distribution) shown in fig. 6c replotted. The abscissa is proportional to $\sqrt{t}$ and the ordinate represents K_t.

5.2. On uptake of labeled subunits

In ref. 6 we calculated the average number of uptake and pulse-chase of labeled subunits as well as the number of release for an infinite-length polymer. The reason we have not included the discussion of uptake in this paper is its tremendous complexity when it is extended to a finite-length case. For the infinite case the expression for uptake is essentially identical to that for release. However, this is not always the case for a finite-length polymer. Brenner and Korn [10] reported results of uptake experiments of F-actin and discussed the mechanism of sustainment of the critical concentration or steady polymer lengths using the theoretical results for an infinitely long polymer. Although we cannot discuss their data directly due to the reason mentioned above, it is quite probable that there are also situations where polydispersity effects cannot be ignored for the case of uptake.

References

1 A. Wegner, J. Mol. Biol. 108 (1976) 139.
2 T.L. Hill, Proc. Natl. Acad. Sci. U.S.A. 77 (1980) 4803.
3 T.L. Hill, Biophys. J. 33 (1981) 353.
4 B. Zeeberg, R. Reid and M. Caplow, J. Biol. Chem. 255 (1980) 9891.
5 T.L. Hill and M.W. Kirschner, Int. Rev. Cytol. 78 (1982) 1.
6 T. Tsuchiya and Y. Nagai, Biophys. J. 41 (1983) 275.
7 A. Martonosi, M.A. Gouvea and J. Gergely, J. Biol. Chem. 235 (1960) 1700.
8 Y. Nagai, H. Asai and T. Tsuchiya, Biophys. Chem. 13 (1981) 213.
9 W. Feller, An introduction to probability theory and its applications, vol. 1, 3rd edn. (John Wiley & Sons, New York, 1968) p. 349.
10 S.L. Brenner and E.D. Korn, J. Biol. Chem. 258 (1983) 5013.