BBA 46305

LENGTH DISTRIBUTION OF F-ACTIN TRANSFORMED FROM Mg-POLYMER

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(Received December 13th, 1971)

SUMMARY

The length distribution of F-actin produced from Mg-polymer formed in the presence of β-actinin was investigated in detail by electron microscopy. When Mg-polymer was formed at a low $MgCl_2$ concentration (approx. 1 mM), where a fairly large amount of G-actin coexisted with Mg-polymer, and was transformed into F-actin by the addition of KCl at a fairly high temperature (45 °C), the length distribution of F-actin was of a Poisson type. The number average length was approximately 0.6 μm. On the other hand, the formation of Mg-polymer at a high $MgCl_2$ concentration (approx. 2 mM), where only a small amount of G-actin remained, resulted in a simple exponential distribution of particle length of the transformed F-actin and its average lengths became shorter. Moreover, when the amount of G-actin coexisting with Mg-polymer was increased by the addition of G-actin to the Mg-polymer solution and transformation then induced, the average length of the resultant F-actin was increased and the length distribution became more homogeneous. These results are explained by the theories of polymerization in systems with a fixed number of polymers.

The amount of G-actin coexisting with Mg-polymer also increased with increase of total actin concentration. However, in this case, the average lengths were not significantly changed after its transformation into F-actin.

When the transformed F-actin solution was left standing for a long time, the length distribution gradually changed from the Poisson type to the simple exponential type. This was probably due to the fact that a re-arrangement of the preformed F-actin took place. The relaxation time for such redistribution was about 25 h at 35 °C.

Both β-actinin and ATP were indispensable for the transformation of Mg-polymer into the F-actin particles having a homogeneous length distribution.

INTRODUCTION

It is well known that the I-filaments in myofibrils have a unit particle length of 1 μm in vertebrate skeletal muscle[1]. On the other hand, as reported previously, the length distribution of F-actin polymerized *in vitro* is of a simple exponential type[2]. Is there any special mechanism to regulate the particle length of F-actin *in vivo*?

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Several mechanisms have been proposed for the regulation of F-actin filaments to a definite length[2,3].

Recently, Oosawa[4] has claimed theoretically that a Poisson-type size distribution, which has a sharp maximum, should be obtained during polymerization of globular protein molecules, when spontaneous nucleation is practically inhibited and the number of polymers is maintained constant. The *in vitro* polymerization of bacterial flagellin into flagella has been shown to be an example of such a case[5].

Mg-polymer, a special form of F-actin, has been known to exist in the case of actin prepared from plasmodia of myxomycete, *Physarum polycephalum*[6,7] and recently, a similar form has been shown for rabbit actin in the presence of β-actinin[8]. The Mg-polymer can be transformed quickly to normal F-actin on the addition of KCl especially at a fairly high temperature, 45 °C[8,9]. While we measured the particle length of F-actin thus formed, we found a somewhat homogeneous distribution of the particle length which appeared to be of the Poisson type. It was considered that this transformation might be regarded as a polymerization with a fixed number of polymers. Therefore, the condition was sought for in which the length distribution was of the Poisson type. It was found that the formation of Mg-polymer at a low $MgCl_2$ concentration could lead to a Poisson distribution. Furthermore, the larger the amount of G-actin coexisting with the Mg-polymer, the more homogeneous the length distribution of the transformed F-actin became.

MATERIAL AND METHODS

Preparation of proteins

Actin extracted at 0 °C from acetone-dried skeletal muscle of the rabbit was purified by the method of Mommaerts[10] with a minor modification[5]. β-Actinin was prepared from rabbit skeletal muscle as described previously[11].

Formation of Mg-polymer and its transformation

Unless otherwise specified, Mg-polymer was formed as follows: G-actin (0.5 mg/ml) was pre-incubated with β-actinin, 5–10 % by weight to actin, in the presence of 1 mM $MgCl_2$, 0.5 mM ATP and 10 mM Tris–maleate buffer (pH 7.0) at 0 °C for 30 min. Subsequently the Mg-polymer solution was diluted five times by the addition of 0.125 M KCl containing 0.5 mM ATP and 10 mM Tris–maleate buffer at 45 °C. Upon dilution, the transformation into normal F-actin took place. The final concentrations of actin, $MgCl_2$ and KCl were 0.1 mg/ml, 0.2 mM and 0.1 M, respectively.

Electron microscopy

A Hitachi 11-B electron microscope was used at 75 kV acceleration voltage. Carbon-coated grids were used and negative staining was carried out with about 1 % uranyl acetate. Measurements of particle length of F-actin were carried out as described before[2].

Ultracentrifuge

Sedimentation measurements were performed in a Beckman Spinco Model E ultracentrifuge at 20410 rev./min and 22 °C using the synthetic boundary cell.

RESULTS

Length distribution of the transformed F-actin

It was found that the F-actin obtained at 45 °C after the addition of 0.1 M KCl to Mg-polymer, which had been incubated in the presence of 1 mM $MgCl_2$ at 0 °C, showed a fairly homogeneous length distribution in comparison with that of KCl-polymerized F-actin[2]. The distribution curve had a sharp maximum and appeared to be satisfied by the Poisson distribution. A typical result was shown in Figs 1 and 2. There was a peak at the length of approximately 0.6 μm. The mean values, $\langle l \rangle_n$ (number average) and $\langle l \rangle_w$ (weight average), were 0.57 μm and 0.74 μm, respectively and the ratio, $\langle l \rangle_w / \langle l \rangle_n$, was 1.30.

It should be mentioned at this point that the transformation to F-actin was accomplished within a few minutes after the addition of KCl judging from the change in the degree of flow birefringence.

Tropomyosin did not alter the length distribution even when added before or after the transformation.

Effect of $MgCl_2$ concentration

Kamiya *et al.*[8] have reported that the ratio of the weight average length to the number average length of F-actin transformed from Mg-polymer is nearly equal to 2.0, suggesting that the length distribution is exponential. On the other hand, the Poisson distribution was obtained in this paper as described above. The difference between the two experiments lay in the $MgCl_2$ concentrations used for the formation of Mg-polymer. To elucidate this difference, a series of experiments was performed on the samples formed at different $MgCl_2$ concentrations and the

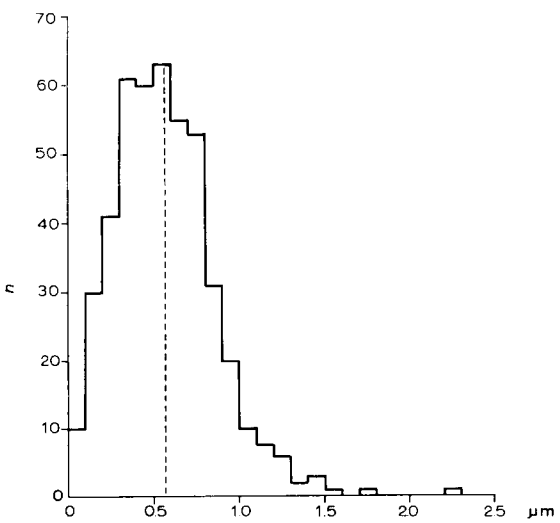

Fig. 1. Length distribution of the F-actin transformed from Mg-polymer. G-actin, 0.5 mg/ml, was pre-incubated with 6% β-actinin, by weight ratio to actin, in the presence of 1 mM $MgCl_2$, 0.5 mM ATP and 10 mM Tris–maleate buffer (pH 7.0) at 0 °C for 30 min. Subsequently Mg-polymer was transformed into F-actin by the addition of 0.1 M KCl containing 0.5 mM ATP at pH 7.0 and 45 °C. The broken line denotes the number average length and n denotes the number of particles measured (total $n = 458$).

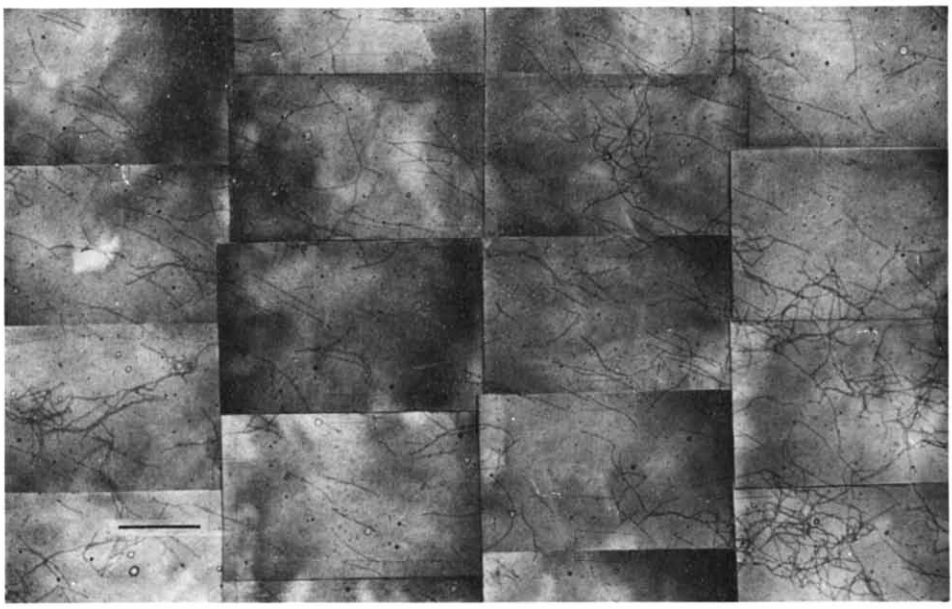

Fig. 2. Electron micrograph of the transformed F-actin particles which show a length distribution of the Poisson type. The result shown in Fig. 1 was obtained from this photograph. Scale, 1 μm.

TABLE I

EFFECT OF $MgCl_2$ CONCENTRATION ON THE PARTICLE LENGTH OF THE TRANSFORMED F-ACTIN

For conditions, see the legend for Fig. 2. n, the number of particles measured; $\langle l \rangle_n$, the number average length; $\langle l \rangle_w$, the weight average length; $\langle l \rangle_w / \langle l \rangle_n$, the ratio of the two lengths.

$MgCl_2$ concentration (mM)	n	$\langle l \rangle_n$ (μm)	$\langle l \rangle_w$ (μm)	$\langle l \rangle_w / \langle l \rangle_n$
0.5	583	0.69	0.99	1.43
1.0	577	0.58	0.76	1.31
2.0	482	0.31	0.51	1.65
3.0	470	0.28	0.47	1.68

results are presented in Table I and Fig. 3. It is evident that the length distributions are of the Poisson type, when Mg-polymer was formed at lower $MgCl_2$ concentrations, whereas at higher $MgCl_2$ concentrations they showed a simple exponential distribution. The ratio, $\langle l \rangle_w / \langle l \rangle_n$, was 1.31 for 1 mM $MgCl_2$, whereas it was 1.68 for 3 mM, also suggesting that the former was more homogeneous than the latter. However, the sharpness of the distribution curve decreased when the $MgCl_2$ concentration was lowered to 0.5 mM. The optimal $MgCl_2$ concentration was 1.0 mM. It is to be noted that the average lengths of the transformed F-actin became shorter as the Mg concentration was increased, and this fact will be discussed later.

It was thought that this phenomenon might be correlated with the G-actin concentration coexisting with Mg-polymer since at lower $MgCl_2$ concentrations

more G-actin coexisted with Mg-polymer, as already described by Kamiya *et al.*[8]. The effect of G-actin concentration coexisting with Mg-polymer was therefore examined, as described in the following section.

Effect of G-actin concentration coexisting with Mg-polymer

In this section we examined the dependence of the average lengths and the length distribution of fully transformed F-actin filaments on the G-actin concentration coexisting with Mg-polymer. Mg-polymer was formed at 1 mM $MgCl_2$ and varied amounts of G-actin were added to the Mg-polymer solutions. Soon after the addition of G-actin, each sample was incubated for 6 min at 45 °C in the presence of 0.1 M KCl and 0.5 mM ATP. The length distributions and the average lengths of F-actin thus obtained are summarized in Fig. 4 and Table II. As the amount of added G-actin was increased, the average length was increased, main-

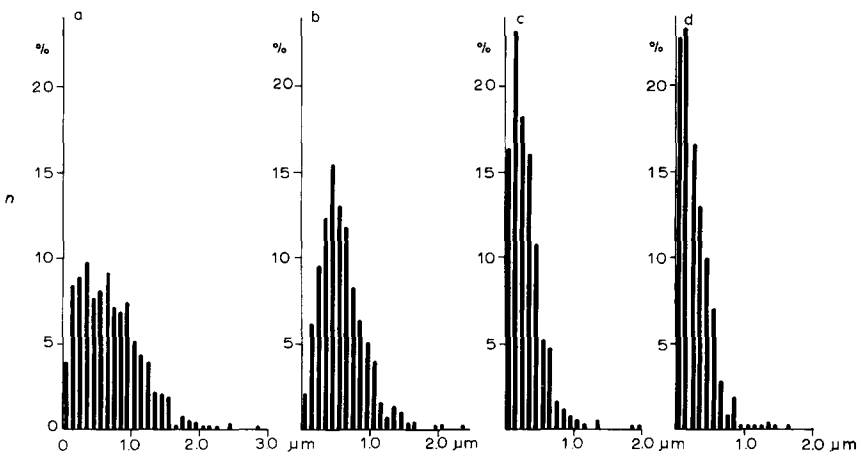

Fig. 3. Effect of $MgCl_2$ concentration on the particle length of the transformed F-actin. Mg-polymer was formed at varied $MgCl_2$ concentrations and transformed into F-actin as described in Fig. 1. (a) 0.5 mM, (b) 1.0 mM, (c) 2.0 mM, (d) 3.0 mM.

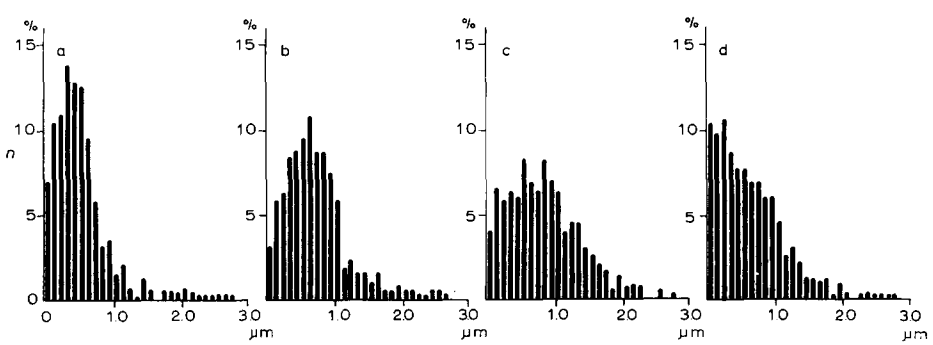

Fig. 4. Effect of adding varied G-actin concentrations to Mg-polymer on the particle length of the transformed F-actin. Mg-polymer (0.25 mg/5 ml) was prepared as described in Fig. 1. Then varied amounts of G-actin were added to 5 ml of the Mg-polymer solution. Immediately after the addition of G-actin, the Mg-polymer was transformed to F-actin. (a) control, (b) 0.08 mg, (c) 0.21 mg, (d) 0.42 mg.

TABLE II

EFFECT OF ADDING VARIED G-ACTIN CONCENTRATION TO Mg POLYMER ON THE PARTICLE LENGTH OF THE TRANSFORMED F-ACTIN

Conditions as in Fig. 3, and symbols as in Table I.

Added G-actin (mg)	*n*	$\langle l \rangle_n$ (μm)	$\langle l \rangle_w$ (μm)	$\langle l \rangle_w / \langle l \rangle_n$
0	541	0.54	0.87	1.61
0.08	376	0.77	1.14	1.48
0.13	414	0.82	1.13	1.39
0.21	423	0.85	1.20	1.41
0.42	583	0.63	1.00	1.59

TABLE III

EFFECT OF INCREASING AMOUNT OF β-ACTININ ON THE PARTICLE LENGTH OF THE TRANSFORMED F-ACTIN

For conditions and symbols, see the legend for Fig. 4 and Table I, respectively.

β-Actinin concentration (% by weight to actin)	*n*	$\langle l \rangle_n$ (μm)	$\langle l \rangle_w$ (μm)	$\langle l \rangle_w / \langle l \rangle_n$
0.0	360	1.05	2.14	2.04
2.3	623	0.60	0.89	1.48
4.7	653	0.37	0.52	1.41
7.0	556	0.30	0.40	1.33
14.0	408	0.23	0.33	1.43

taining a similar distribution curve. However, when a large amount of G-actin was added, the number of the shorter filaments was increased and the average lengths, consequently, became shorter as shown in Fig. 4d. We shall discuss this point later.

Effect of β-actinin concentration

As reported by Kamiya *et al.*[8], the presence of β-actinin is necessary for the formation of Mg-polymer, but the particle length of Mg-polymer itself is almost independent of the amount of β-actinin present ($\langle l \rangle_n = 0.22$ μm; $\langle l \rangle_w = 0.43$ μm). In the present study, we investigated the effect of increasing amount of β-actinin on the particle length of the transformed F-actin. The results are shown in Fig. 5 and Table III. In contrast with the case of Mg-polymer itself, the shortening activity of β-actinin, which was usually observed in KCl-polymerized F-actin[2], increased with the amount of β-actinin in the case of the transformed F-actin. For example, $\langle l \rangle_n$ was 1.05, 0.37 and 0.23 μm for concentration of β-actinin of 0, 4.7 and 14 % by weight ratio to actin. Yet the length distribution was of the Poisson type irrespective of the amount of β-actinin added. On the other hand, in the case of KCl-polymerized F-actin, β-actinin shortens the lengths of F-actin without changing the heterogeneous distribution[2].

G-actin coexisting with Mg-polymer

Oosawa and his collaborators[12,13] have reported that the polymerization

of G-actin can be regarded as a condensation phenomenon similar to the condensation of gas into liquid, and that the concentration of G-actin coexisting with F-actin is constant (critical concentration) for a given constant solvent condition, when

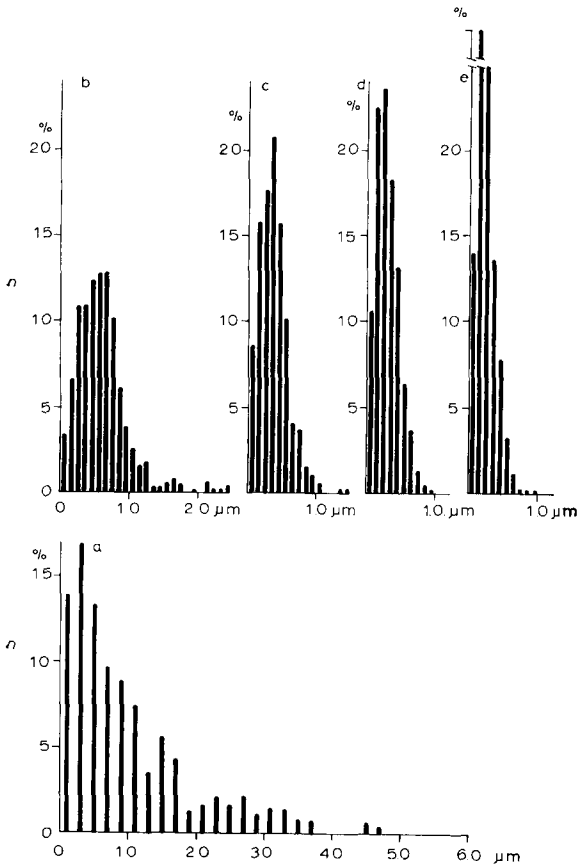

Fig. 5. Effect of increasing amounts of β-actinin on the particle length of the transformed F-actin. Mg-polymer was formed in the presence of varied amounts of β-actinin and transformed to F-actin. (a) None, (b) 2.3%, (c) 4.7%, (d) 7.0%, (e) 14.0%.

true equilibrium between G- and F-actin is established. In the present study we tried to examine the dependence of G-actin concentration coexisting with Mg-polymer on the initial G-actin concentration under a constant solvent condition by using the ultracentrifuge. G-actin was incubated at various protein concentrations in the presence of 1 mM $MgCl_2$ with 6 % β-actinin at 0 °C. Sedimentation patterns of each sample were photographed according to the Rayleigh optical system using synthetic boundary cell. From the data shown in Fig. 6, the concentrations of G-actin were estimated. They were 0.5, 1.0 and 1.4 mg/ml for initial G-actin concentrations of 1.0, 2.1 and 3.0 mg/ml, respectively. These results clearly suggest that the concentration of G-actin coexisting with Mg-polymer increased in proportion to the initial G-actin concentration. This result seemed to contradict with the theory of condensation phenomenon. This point will be discussed later. In

relation to this result, we performed the following experiment. Each Mg-polymer solution obtained as mentioned above was diluted so as to have identical protein concentration as each other by the addition of 0.1 M KCl. Immediately after

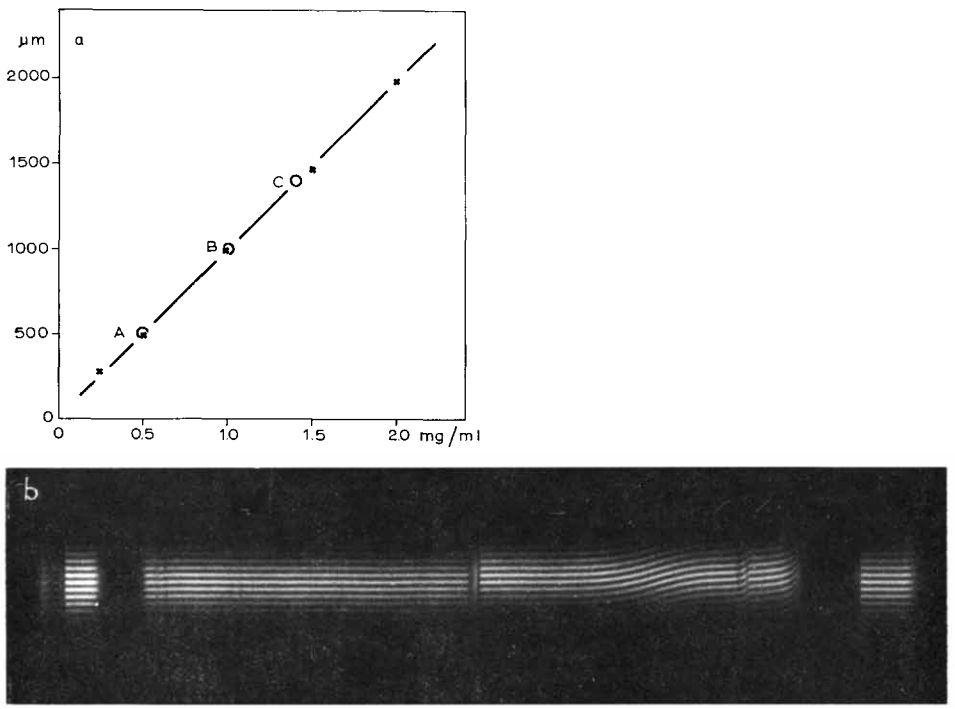

Fig. 6. The dependence of G-actin concentration coexisting with Mg-polymer on the initial G-actin concentration. G-actin was incubated at different protein concentrations in the presence of 1 mM $MgCl_2$ and 0.5 mM ATP with 6% β-actinin at pH 7.0 and 0 °C. The samples were dialyzed against the same solvent for 24 h. Sedimentation patterns were photographed according to the Rayleigh optical system by using synthetic boundary cell. (a) Plot of the lateral shift of the fringes across the boundary corresponding to G-actin *versus* G-actin concentration. ×, G-actin at the concentrations given as on the abscissa ○, G-actin coexisting with Mg-polymer which was formed at the initial G-actin concentration of 1.0 (A), 2.1 (B) and 3.0 (C) mg/ml. (b) Interference pattern of Mg-polymer–G-actin system. G-actin, 2 mg/ml. Centrifuged for 42 min at 20410 rev./min.

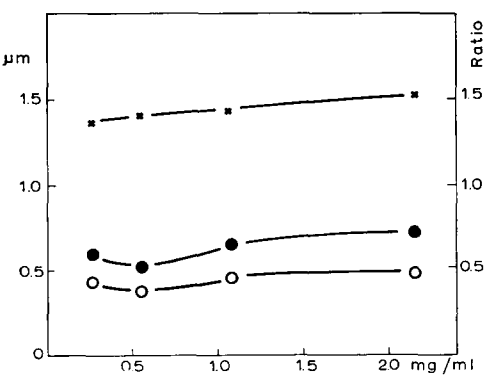

Fig. 7. Dependence of the particle length of the transformed F-actin on the initial G-actin concentration. For conditions, see text. ○—○, $\langle l \rangle_u$; ●—●, $\langle l \rangle_w$; ×—×, $\langle l \rangle_w / \langle l \rangle_n$.

dilution, they were incubated for 6 min at 45 °C. The particle length of the resultant F-actin was measured by electron microscopy. The result is shown in Fig. 7. It was found that the average lengths were almost independent of the initial G-actin concentration.

Effect of temperature and ATP

The transformation of Mg-polymer was performed in the above experiments at a fairly high temperature (45 °C), where rapid polymerization must have taken place. It was an interesting problem whether the length distribution of F-actin was of the Poisson type when the transformation was induced at low temperatures. As shown in Fig. 8, the distribution curves were almost the same in shape (Poisson type), although the average lengths were decreased as the temperature was lowered to 0 °C. When the sample transformed at 0 °C was further incubated at 0 °C for 1 h, neither the average lengths nor the length distribution were changed significantly, but the further incubation at 45 °C for 6 min brought about longer average lengths and a transient distribution curve (Fig. 8e). These results suggested that at a low temperature, there were a number of G-actin molecules remaining unpolymerized in the transformed F-actin solutions.

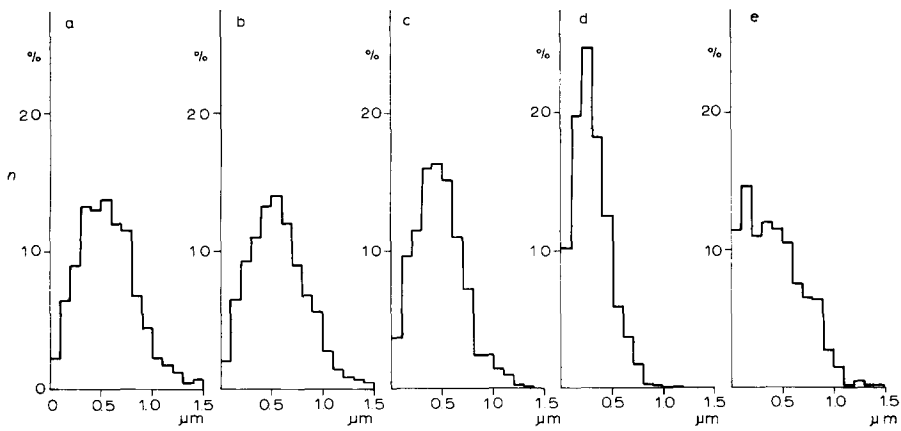

Fig. 8. Temperature dependence of the particle length of the transformed F-actin. Mg-polymer was prepared as described in Fig. 1, and the Mg-polymer was transformed at varied temperature. (a) 45 °C, (b) 35 °C, (c) 25 °C, (d) 0 °C, (e) after incubation at 0 °C for 6 min, further incubated at 45 °C for 6 min.

The effect of ATP on the length distribution of the transformed F-actin was investigated at 35 °C where the thermal inactivation of actin was considered to be negligible. It was found that the presence of ATP was absolutely required for the distribution to be of the Poisson type. Otherwise, F-actin or Mg-polymer was considerably inactivated to form random aggregates even at 35 °C. The average length of F-actin that then remained without deformation was shorter and the distribution curve appeared to be exponential. For example, $\langle l \rangle_n$ and $\langle l \rangle_w$ were 0.62 μm and 0.93 μm with a $\langle l \rangle_w / \langle l \rangle_n$ ratio of 1.50 in the presence of ATP, whereas in the absence of ATP these values were 0.35 μm, 0.57 μm and 1.63, respectively.

Redistribution

In this section we tried to examine the possibility that the Poisson distribution once established during the transformation could remain unchanged for a long time. An F-actin transformed under an optimal solvent condition at 45 °C was further incubated at 35 °C for a long time. The temperature was chosen to avoid the thermal inactivation as little as possible. As time elapsed, the distribution curve gradually became exponential. By 25 h, the curve almost completely changed to the simple exponential type as shown in Fig. 9. The ratio, $\langle l \rangle_w / \langle l \rangle_n$, was 1.68 at 6 min after initiation of incubation, whereas it was 1.88 at 25 h. This observation was in good agreement with Oosawa's[4] theoretical prediction.

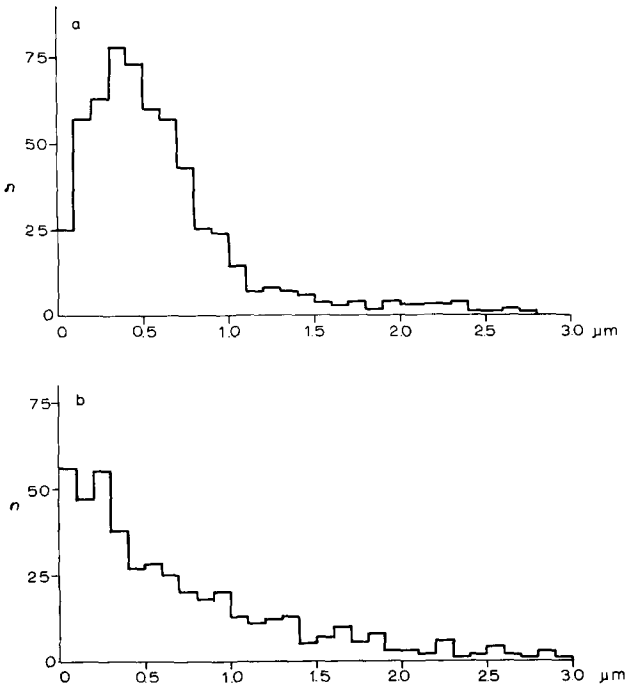

Fig. 9. Redistribution of particle length of the transformed F-actin. Mg-polymer was formed and transformed as described in Fig. 1, and the Mg-polymer was further incubated at 35 °C. (a) At 6 min after the initiation of incubation, (b) at 25 h after the initiation of incubation.

DISCUSSION

Recently, Oosawa has analyzed theoretically the size distribution of tubular or helical polymers of globular protein molecules, such as actin and flagellin, during reversible polymerization and has concluded that the polymerization process consists of three stages—nucleation, growth and redistribution of polymer size. He predicts that a Poisson-type size distribution should be established during polymerization when spontaneous nucleation, the first stage of polymerization, is virtually inhibited and the number of polymers is constant, although it tends towards an exponential type in the final equilibrium[4]. In the case of bacterial flagellin polymerizing onto added seeds, Asakura *et al.*[5] have found that the length

distribution of flagella is of a Poisson type. In the case of actin it has been difficult to inhibit the spontaneous nuclei formation. Consequently, the length distribution of F-actin usually becomes a simple exponential type[2].

In the present investigation, we could obtain a rather homogeneous length distribution of F-actin which was evidently different from a simple exponential type and appeared to be a Poisson type. In order to determine whether the Poisson type of distribution could be applied to this length distribution, we calculated the number of F-actin particles of each class from the number-average length obtained experimentally according to the Poisson distribution formula. In Fig. 10, the calculated and experimental results are shown in comparison. The agreement between the two was fairly satisfactory except for a slight difference. The number of F-actin particles around the mode was a little decreased and those in each side were increased slightly in the experimental data. This is probably due to the occurrence of the redistribution of F-actin particles even at an early stage after transformation (see Fig. 1). From these considerations, it can be concluded that the length distribution of F-actin transformed from Mg-polymer is actually of the Poisson type.

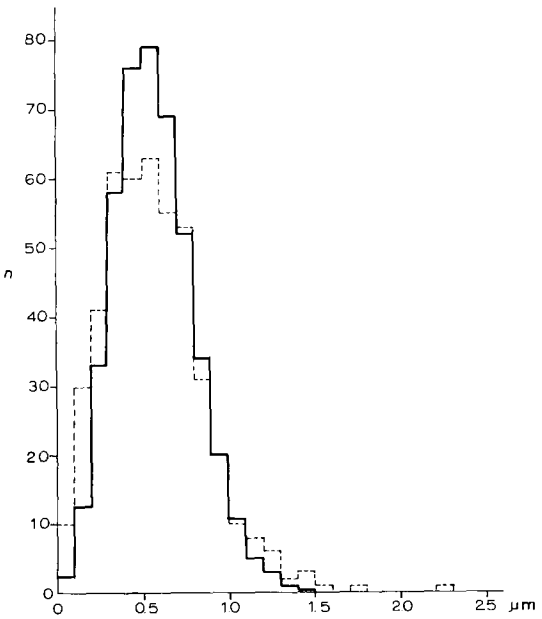

Fig. 10. Experimental and calculated length distributions of the transformed F-actin. The experimental values were obtained from Fig. 1. – – – –, experimental; ———, calculated.

Based on the above conclusion and Oosawa's theory, we can draw the following postulates about the transformation of Mg-polymer into F-actin: (i) Mg-polymer, which can be nuclei, coexists with an adequate amount of G-actin in the presence of a low $MgCl_2$ concentration; (ii) upon the addition of KCl, the pre-existing nuclei (Mg-polymer) grow so rapidly that most of the G-actin coexisting with Mg-polymer are used up by the growth of original nuclei; very few new nuclei

are formed; and (iii) as time elapses, redistribution of the preformed F-actin particles takes place. Based on these assumptions, we can explain all of the results obtained in this paper, In (ii), the length distribution of transformed F-actin becomes a Poisson type and in (iii) it tends towards an exponential type. The relaxation time for such a redistribution was observed to be about 25 h at 35 °C (Fig. 9), which was in good agreement with the theoretical value predicted by Oosawa (30 h)[4].

In (i) and (ii) of the assumptions mentioned above, we must take into account the observation that the concentration of G-actin coexisting with Mg-polymer affected the length distribution as well as the average lengths. When the G-actin concentration was lowered by raising the $MgCl_2$ concentration in a Mg-polymer solution, even the pre-existing nuclei could hardly grow. Therefore, both the length distribution and the average length of F-actin had to tend toward those of Mg-polymer itself as the G-actin concentration decreased (Table I). On the other hand, when $MgCl_2$ concentration was lowered, the concentration of G-actin was increased enough for the pre-existent nuclei to grow, which resulted in the Poisson type of length distribution of the transformed F-actin, However, when G-actin concentration was greatly raised, as was the case of the addition of G-actin to a Mg-polymer solution, it was possible that a number of new nuclei had been formed before G-actin was used up for the growth of pre-existent nuclei. It followed that the number of shorter particles increased. Consequently, the distribution curve approached an exponential type and the average length became shorter (Fig. 3).

As shown in Fig. 6, the G-actin concentration coexisting with Mg-polymer was increased by an increase of the initial G-actin concentration. From this result, we could assume that the formation of Mg-polymer might not be a condensation phenomenon. Because, if a condensation phenomenon was occurring, the G-actin concentration coexisting with Mg-polymer should be constant ("critical concentration"), irrespective of the initial actin concentration. The result that the average length did not depend on the initial G-actin concentration is interpreted as follows: The number of Mg-polymer as well as the amount of G-actin increased with increase of initial G-actin concentration, *e.g.* the ratio between the two was independent of the initial actin concentration. Therefore, when each sample was diluted to the same protein concentration, the number concentrations of G-actin and of Mg-polymer were identical to each other. Therefore, the length distributions of F-actin after transformation were the same at every actin concentration.

We should discuss the effect of β-actinin on the transformation process of Mg-polymer. As reported by Kamiya *et al.*[8], β-actinin binds to Mg-polymer more than to F-actin and the bound β-actinin makes the structure of Mg-polymer fragile. Upon the addition of KCl at a fairly high temperature, the structure of Mg-polymer may be converted into that of F-actin and at the same time some of β-actinin might be released from Mg-polymer. Such converted F-actin particles might be able to act as nuclei and to grow very rapidly. When a large amount of β-actinin was added, β-actinin might bind even to G-actin and consequently the polymerizability of G-actin might be retarded. Thus, Mg-polymer could not grow even when KCl was added at 45 °C (Fig. 5).

Finally, we should mention that a Poisson type of length distribution of

F-actin similar to that described in this paper was observed previously at a very early stage of polymerization of G-actin[2]. However, in that case, a large amount of G-actin still remained unpolymerized at such an early stage, so that F-actin which showed a Poisson type of length distribution was only a few per cent of total actin present. In the present study, however, polymerization of G-actin was completed within a few minutes. In both cases, the homogeneous length distribution could be explained by the manner of elongation of pre-existent nuclei, but there was a large difference in quantity between the two.

ACKNOWLEDGEMENTS

The authors thank Prof. H. Noda and Mrs M. Kikuchi for analytical ultracentrifuge runs, and Mr T. Ishii for his supplying β-actinin preparations. This work was supported by grants from Muscular Dystrophy Association of America, Inc., Ministry of Education and Ministry of Health and Welfare (Japan).

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