Chemical evidence for the existence of activated G-actin

Wei Ping SHU, Dalton WANG* and Alfred STRACHER

Department of Biochemistry, SUNY Health Science Center at Brooklyn, 450 Clarkson Avenue, Brooklyn, New York, NY 11203, U.S.A.

Globular actin (G-actin) will polymerize to form filamentous actin (F-actin) under physiological ionic conditions, and is known to be regulated by univalent and bivalent cations, such as K^+ and Mg^{2+} . The current concept of this process involves four steps: activation, nucleation, elongation and annealing. Evidence for the existence of activated G-protein has been suggested by changes in the resistance to proteolysis [Rich & Estes (1976) *J. Mol. Biol.* **104**, 777–792] and u.v.-light absorption [Rouayrenc & Travers (1981) *Eur. J. Biochem.* **116**, 73–77]. More recently we [Liu *et al.* (1990) *Biochem. J.* **266**, 453–459] have provided direct chemical evidence for extensive conformational changes during the transformation of G-actin into F-actin. In this study we now present direct chemical evidence for the existence of a short-lived species, an activated form of G-actin, which can be detected by changes in the accessibility of the free thiol groups on the G-actin molecule when modified by a specific thiol-group-targeted reagent, 7-dimethylamino-4-methyl-3-*N*-maleimidylcoumarin (DACM). The presence of K^+ and/or Mg^{2+} ions caused a large increase in the accessibility of the thiol groups of Cys-217 and Cys-374, but not those of Cys-10 and Cys-257. Mg^{2+} effected relatively faster changes than did K^+ ions. The results suggest that the function of these ions is to convert G-actin into an activated form, and further suggest that the change in conformation is mainly confined to the large domain. Such changes at least involve certain portions of the G-actin molecule that contain Cys-217 and Cys-374. On the other hand, little or no significant change could be observed in the small domain of G-actin as reflected by the accessibility of Cys-10. The bound nucleotide remained as ATP during the activation of G-actin and was hydrolysed to ADP on polymerization. The activated G-actin had a life-time of about 8 min or less depending on the concentration of G-actin. At higher protein concentration, its life-time was much shorter, probably owing to the earlier onset of polymerization, which apparently is governed by the concentration of the activated form. The life-time of this new species can be extended by lowering the temperature and is less affected by actin concentration. This new species is considered to be an activated form of G-actin, since polymerization renders all the thiol groups on actin inaccessible to the reagent DACM.

INTRODUCTION

Actin is one of the principal components of contractile and motile systems of muscle and non-muscle cells respectively. Because actin filaments are structural components of cells, it is essential to understand their behaviour in terms of assembly and disassembly. It has been known that, in non-muscle cells, the transformation of monomeric actin to polymerized actin involves several reversible reactions, including monomeric activation, nucleation, elongation and annealing.

An enormous amount of information concerning the transformation of G-actin into F-actin exists in the literature (Korn, 1982; Pollard, 1990). However, direct evidence on the molecular arrangement of G-actin and its activated monomer as well as evidence for nucleation are still lacking. This is, in part, because the self-assembly property of G-actin and the transient nature of both activated actin and its oligomer render the crystallization of this protein almost impossible, although the actin–DNAase I complex has now been crystallized (Mannherz *et al.*, 1977; Sugino *et al.*, 1979) and the atomic structure of the actin component of such a complex has been elucidated (Suck *et al.*, 1981; Kabsch *et al.*, 1985, 1990). In addition, questions regarding whether cellular actin polymerization can be accomplished only by self-assembly or if a requirement exists for regulation by other proteins also need clarification. Thus a knowledge of the activated G-actin, its oligomer and the regulatory factors involved could lead towards a better understanding of the mechanism of actin polymerization and other fundamental biological processes.

In the polymerization reaction, activation of G-actin has been predicted and the existence of such activated monomers has been

suggested by the change in resistance to proteolysis (Rich & Estes, 1976) and change in u.v. absorption (Rouayrenc & Travers, 1981) or in proton-n.m.r. spectrum (Barden & Remedios, 1985) when G-actin is exposed to K^+ or Mg^{2+} . More recently we (Liu *et al.*, 1990) have presented evidence for a conformational change in the monomers, during the transformation of G-actin into F-actin, as detected by a complete loss of the accessibility of the free thiol groups to modification by 7-dimethylamino-4-methyl-3-*N*-maleimidylcoumarin (DACM). However, there is a lack of direct information to show to what extent conformational changes occur within the G-actin molecule or as a consequence of the formation of F-actin. Rich & Estes (1976) demonstrated that the addition of KCl to actin at concentrations below critical concentration decreased the rate of proteolysis by trypsin. On the basis of the similarity in the rates of proteolysis between G-actin treated with KCl and F-actin, these authors postulated a new species of actin referred to as 'F-actin monomer'. A number of investigators (Rich & Estes, 1976; Frieden *et al.*, 1980; Rouayrenc & Travers, 1981; Pardee & Spudich, 1982; Carlier *et al.*, 1986*a,b*; Zimmerle & Frieden, 1986; Selden *et al.*, 1986, 1989), using fluorescent labelled G-actin, provided evidence for conformational change on binding of cations, although the nature of the conformational change is unknown. The atomic structure of an actin–DNAase complex yielded an enormous amount of three-dimensional structural information on the actin component of this complex (Kabsch *et al.*, 1990). However, such a three-dimensional structure of actin may or may not completely represent the three-dimensional structure of free G-actin or the activated form of G-actin. We believe that chemical modifications for selective labelling of specific groups on G-actin can be

Abbreviation used: DACM, 7-dimethylamino-4-methyl-3-*N*-maleimidylcoumarin.

* To whom correspondence should be addressed.

successfully employed to detect and define the activated form of G-actin.

In the present paper we provide direct chemical evidence for the existence of such a new species.

EXPERIMENTAL

Materials

The chemicals used were commercial products: DACM, CNBr, formic acid, ATP, 2-mercaptoethanol, Nonidet P-40 and N-acetylcysteine were from Sigma Chemical Co., St. Louis, MO, U.S.A.; Ampholines, pH 3–10 and pH 4–7, were from FMC, Rockland, ME, U.S.A.

Methods

Preparation of actin. Actin was extracted from acetone-dried powder of leg and back skeletal muscles from a New Zealand White rabbit according to the procedure described by Spudich & Watt (1971). The purity of each preparation was checked by SDS/PAGE.

Protein concentration. The molecular concentration of G-actin was determined spectrophotometrically and calculated on the basis of a molecular mass of 42 kDa (Collins & Elzinga, 1975), using an absorbance of 1 mg of pure actin/ml (light-path 1 cm) at 290 nm as 0.62 and 0.64 for G-actin and F-actin respectively (Gordon *et al.*, 1976).

Chemical modification of thiol groups. The modification of thiol groups of actin with DACM was performed according to the procedures of Sutoh (1982) as modified by Liu *et al.* (1990) or as specified in each experiment.

CNBr cleavage of protein. The dialysed DACM-labelled product was freeze-dried and then treated with an equal volume of 1 M-CNBr in 70% (v/v) formic acid for 18 h at room temperature in the dark. It was dried under a stream of N₂ gas.

Gel isoelectric focusing. The products of the CNBr reaction were separated on tube gels using a mixture of Ampholines, pH 3–10 and pH 4–7, at a ratio of 1:4 according to the method of O'Farrell *et al.* (1977) as modified by Liu *et al.* (1990).

Estimation of DACM-containing CNBr fragments. The DACM-containing peptide bands were excised under the illumination of u.v. light. The fluorescent material in each excised gel section was soaked in 0.5 ml of a solution containing 6 M-urea, 2% (v/v) Nonidet P-40 and 5% (v/v) 2-mercaptoethanol at 4 °C for 2 weeks. The extent of fluorescent labelling in each sample after appropriate dilution with 0.05 M-Tris buffer, pH 9, was estimated with a Perkin-Elmer model 204-A fluorescence spectrophotometer with settings of wavelength at 385 nm and 476 nm for excitation and emission respectively.

Determination of ATP on activated G-actin. The status of ATP on the activated G-actin was estimated by determining the amount of inorganic phosphate formed as described by Itaya & Ui (1966).

Preparation of Ca²⁺-free G-actin. The G-actin was depleted of bound Ca²⁺ by dialysis for 2 days against 2 mM-Tris/HCl buffer, pH 8.0, containing 0.1 mM-ATP, 0.05 mM-MgSO₄, 1.5 mM-Na₂SO₄ and 0.2 mM-dithiothreitol at 4 °C by the method of Frieden (1983). The Ca²⁺-free G-actin was dialysed against the same buffer with no dithiothreitol for 3 h at 0 °C before reaction with DACM.

RESULTS

Effect of K⁺ or Mg²⁺ on the accessibility of free thiol groups on G-actin

The conformation of G-actin is known to be sensitive to certain concentrations of Mg²⁺ or K⁺ (Korn, 1982; Pollard,

1990). We have examined the accessibility of the free thiols of G-actin, of which Cys-10 is located in the small domain, whereas the other four thiol groups are distributed throughout that portion of the polypeptide chain which constitutes the large domain. Changes in the accessibility of these thiol groups were monitored in two ways: (i) the addition of DACM to a solution of G-actin was immediately followed by the addition of Mg²⁺ or K⁺, and sampling was made at selected time intervals, and (ii) the order of addition of DACM and Mg²⁺ or K⁺ was reversed and the addition of DACM was made at selected time intervals.

When the reactions were carried out in the presence of Mg²⁺ according to the first method, there was a gradual increase in the labelling of Cys-374 and Cys-217. This increase reached a maximum at the end of 2 min and remained at this level for up to 8 min (Figs. 1a and 1b). In contrast, the thiol groups of Cys-10 and Cys-257 remained more or less unaffected by Mg²⁺. As shown previously, Cys-285 did not react under these conditions. A similar increase in labelling of Cys-374 and Cys-217 was observed when the reactions were monitored by the second method (Fig. 1c). In both cases the thiol groups of Cys-374 and Cys-217 became increasingly more accessible as a function of time, whereas those of Cys-10 and Cys-257 remained more or less unchanged. The addition of KCl yielded practically identical results, i.e. Cys-217 and Cys-374 became increasingly more accessible to DACM with time in the presence of KCl whereas Cys-10 and Cys-257 again were essentially unaffected (Fig. 1d). It is apparent that the two cations exert similar effects on the accessibility of these thiol groups. These results suggest that the altered accessibility of these thiol groups is probably a consequence of a change in the conformation of the G-actin molecule, which is time-dependent. It should be noted that the levelling off of labelling was not due to a lack of the reagent, DACM, in the reaction mixture, as demonstrated by the much greater extent of labelling of G-actin under denaturing conditions (Lui *et al.*, 1990).

Effect of the concentration of Mg²⁺ or K⁺ on the accessibility of free thiol groups on G-actin

Fig. 2(a) shows changes in accessibility of the thiol groups of G-actin to DACM as a function of Mg²⁺ concentration. Apparently, 0.5 mM was sufficient to exert a rather large effect on Cys-217 and Cys-374 of G-actin. The greatest enhancement of accessibility was attained with 2 mM-Mg²⁺. Further increases in concentration produced no additional change.

K⁺ produced similar results (Fig. 2b). The induced change in accessibility of these thiol groups was approximately as rapid as that induced by Mg²⁺. The maximum effect was not attained until the K⁺ concentration reached 0.2 M.

K⁺- plus Mg²⁺-induced enhancement of accessibility of free thiol groups on G-actin to DACM

Fig. 3(a) shows results for the addition of 2 mM-Mg²⁺ plus 0.2 M-K⁺ to G-actin. The addition of both cations to G-actin results in a rapid change in the accessibility of Cys-374 and Cys-217 on G-actin, which is similar to the one induced by either cation alone. Under the conditions used, Mg²⁺ and K⁺ showed no synergistic effect, and therefore all subsequent experiments were performed in the presence of both cations.

Since it has been shown earlier by us (Liu *et al.*, 1990) that the accessibility of all free thiol groups on G-actin molecules diminishes once the G-actin polymerizes to form F-actin, a question inevitably raised is whether the cation-induced accessibility of these thiol groups will remain at the higher level or decline as the exposure of G-actin to these cations is extended. Fig. 3(b) clearly shows that when the time of exposure of G-actin to the cations was increased there was a sharp decline in accessibility of Cys-217 and Cys-374. Within 30 min, it fell to a

Fig. 1. Changes in accessibility of the thiol groups of G-actin to modification by DACM after addition of Mg^{2+} or K^+

(a) Separation of DACM-labelled CNBr-cleaved polypeptide fragments on isoelectric focusing gels; C, control (G-actin with no $MgCl_2$ added). (b) Estimated relative fluorescence intensity in each band (details are given in the Experimental section). The addition of DACM ($4.76 \mu M$) to a solution of G-actin ($4.76 \mu M$) was immediately followed by an addition of Mg^{2+} (2 mM) as indicated. A sample was taken at each prescribed time point. $\circ$, Cys-374; $\diamond$, Cys-217; $\bullet$, Cys-10; $\blacktriangle$, Cys-257. (c) Addition of Mg^{2+} (2 mM) was made to a solution of G-actin ($4.76 \mu M$) as indicated. A sample was taken at each prescribed time point and mixed with a solution of DACM (final concentration was $4.76 \mu M$). $\circ$, Cys-374; $\diamond$, Cys-217; $\bullet$, Cys-10; $\blacktriangle$, Cys-257. (d) The order of additions of KCl (0.2 M) and DACM was the same as described in (b). $\circ$, Cys-374; $\diamond$, Cys-217; $\bullet$, Cys-10; $\blacktriangle$, Cys-257. The reactions were performed at $24^\circ C$. The detailed procedures regarding the processing and analysis of the reaction products are given in the Experimental section.

Fig. 2. Effect of Mg^{2+} or K^+ concentration on the accessibility of the free thiol groups of G-actin

Changes in the accessibility of the thiol groups to modification by DACM as a function of (a) Mg^{2+} concentration and (b) K^+ concentration. Actin concentration was $4.76 \mu M$. $\circ$, Cys-374; $\diamond$, Cys-217; $\bullet$, Cys-10; $\blacktriangle$, Cys-257.

Fig. 3. Effect of Mg^{2+} and K^{+} on the accessibility of the thiol groups of G-actin to modification by DACM

The addition of Mg^{2+} (2 mM) and K^{+} (0.2 M) to a solution of G-actin ($4.76 \mu M$) was made at the time indicated. A sample was taken at each prescribed time point and mixed with a solution of DACM (final concentration $4.76 \mu M$). Exposure of G-actin to Mg^{2+} and K^{+} was for (a) 8 min or (b) 180 min. $\circ$, Cys-374; $\diamond$, Cys-217; $\bullet$, Cys-10; $\blacktriangle$, Cys-257. The inset shows the separation of the DACM-labelled CNBr-cleaved polypeptide fragments on isoelectric focusing gels; C, control (G-actin with no $MgCl_2/KCl$ added).

value sometimes lower than that of the control (in which G-actin was not exposed to either cation), suggesting that polymerization had occurred within 30 min.

Effect of G-actin concentration on the accessibility of free thiol groups on G-actin

The critical concentration of muscle actin (the minimal concentration necessary for polymerization to proceed under physiological ionic conditions) has been shown to be around 0.05 mg/ml of solution. Since the time required for the initiation of polymerization has an inverse relationship with the concentration of G-actin, we expected that the accessibility of these free thiol groups would be correlated closely with the polymerization reaction, and would exhibit a similar inverse relationship with actin concentration. Indeed, such an inverse relationship was observed, as shown in Fig. 4(a). For both Cys-374 and Cys-217, the accessibility of these thiol groups declined more rapidly as a function of G-actin concentration. However, the rate of this decline is greater in the case of Cys-374 than that for Cys-217, although in both cases the decline was inversely proportional to the concentration of G-actin up to 0.5 mg/ml of solution. With

further increases in actin concentration, cloudiness developed in the reaction solution that made further correlation unfeasible. The cloudiness of the reaction solution is presumably due to an onset of polymerization. This inverse relationship with actin concentration seems to precede, and is parallel to, the polymerization reaction.

Fig. 4. Effect of G-actin concentration on the accessibility of free thiol groups to DACM

Changes in the accessibility of Cys-217 and Cys-374 in the presence of Mg^{2+} (2 mM) and K^{+} (0.2 M) as a function of actin concentration at (a) 24 °C and (b) 4 °C. $\bullet$, Cys-374; $\diamond$, Cys-217. The value of each point represents the fraction of maximum accessible thiol groups on G-actin at the end of 8 min. The reactions were performed at 24 °C under conditions similar to those described for Fig. 3(a).

Fig. 5. Effect of temperature on the life-time of the enhanced accessible thiol groups

(a) The life-time of Cys-217 and Cys-374 (the length of time each thiol group remained maximally accessible to DACM) as a function of actin concentration. $\circ$, Cys-374; $\diamond$, Cys-217. (b) Changes in the accessibility of the thiol groups during the extended exposure of G-actin to Mg^{2+} and K^{+} at 4 °C; $\circ$, Cys-374; $\diamond$, Cys-217; $\bullet$, Cys-10; $\blacktriangle$, Cys-257. The inset shows the separation of DACM-labelled CNBr-cleaved polypeptide fragments by isoelectric focusing. C, control (G-actin with no $MgCl_2/KCl$ added).

Table 1. Hydrolysis of G-actin-bound ATP during the addition of K⁺ and Mg²⁺ ionsKCl and MgCl₂ concentrations were 0.2 M and 2 mM respectively.

G-actin (μ M)	Time after addition of cations (min)	Bound ATP hydrolysed (nmol)	
		25 °C	4 °C
4.76	2	0	0
	4	0	0
	8	0.98	0
	12	2.14	0
	16	2.79	0
11.9	2	1.0	0
	4	4.9	0
	8	9.5	0
	12	10.1	0
	16	10.6	0.25

Effect of temperature on the accessibility of free thiol groups on G-actin

Since there exists a parallel between the effect of G-actin concentration on polymerization and on the accessibility of Cys-374 and Cys-217, and the fact that the transformation of G-actin into F-actin is sensitive to temperature (i.e. the rate of polymerization of G-actin is faster at higher than at lower temperature), a similar effect of temperature on the accessibility of the thiol groups on G-actin could be expected. As shown in Fig. 4(b), at 4 °C, unlike at 24 °C, the increased accessibility induced by the cations remained at a much higher level at the end of 8 min. Furthermore, the rates of decline in the reaction for Cys-374 and Cys-217 were approximately identical at 4 °C. Both curves shifted towards the higher actin concentration. These results suggest that, at lower temperatures, these thiol groups largely remain

accessible to DACM and are much less dependent on the concentration of G-actin.

Fig. 5(a) shows that the life-time of these two accessible thiol groups at 24 °C decreased as the concentration of G-actin increased. Such a decrease in life-time is also inversely proportional to actin concentration, i.e. the length of time each thiol group remains maximally accessible is shortened relative to an increase in actin concentration. The effect of temperature on the life-time of the accessibility of these thiols is most striking if the exposure of G-actin to the cations is extended as shown in Fig. 5(b) (compare with Fig. 3b). The thiol groups of Cys-374 and Cys-217 remained fully accessible even after 30 min at 4 °C (Fig. 5b), compared with approx. 5 min at 24 °C. These results again indicate that the formation of such concentration-dependent short-lived species of G-actin precedes the polymerization reaction.

The ATP state on G-actin after the addition of cations

It is known that bound ATP on G-actin is converted into ADP on polymerization during the formation of F-actin (Straub & Feuer, 1950; Oosawa & Kasai, 1971). The question of whether ATP is hydrolysed to ADP during activation, nucleation or elongation remains to be answered, although elongation of G-actin into actin filaments has been reported to be accompanied by ATP hydrolysis (Carlier *et al.*, 1986b, 1987). We have examined the state of the bound ATP during activation of G-actin by cations and the results are given in Table 1. At 25 °C and a G-actin concentration of 4.76 μ M, there was no hydrolysis of the bound ATP to ADP within 4 min of the addition of KCl and MgCl₂. This result is in agreement with the observation made by Rouayrenc & Travers (1981). Hydrolysis became evident after 8 min, presumably as a result of the onset of polymerization. Hydrolysis of bound ATP at 25 °C occurred much earlier when the concentration of G-actin was increased from 4.76 μ M to 11.9 μ M. By lowering the temperature to 4 °C, this hydrolytic reaction could be effectively suppressed up to at least 16 min at the lower actin concentration and up to 12 min at the higher actin concentration.

Fig. 6. Effect of Mg²⁺ on the accessibility of thiol groups on Ca²⁺-free G-actin to DACM

The reactions were performed at 24 °C. The concentrations of G-actin and DACM were 4.76 μ M each with or without Mg²⁺ as indicated. The resulting modified protein was subjected to CNBr cleavage and the CNBr-cleaved fragments were separated on isoelectric focusing gels. The relative fluorescence intensity of each DACM-labelled fragment was determined.

Effect on the removal of Ca^{2+} on the accessibility of thiol groups on G-actin

We have examined the effect of the removal of Ca^{2+} on the accessibility of the thiol groups on G-actin. The results are presented in Fig. 6. In the presence of 0.4 mM- Mg^{2+} , there was practically no effect on the accessibility of any of the thiol groups on Ca^{2+} -G-actin. However, with the removal of Ca^{2+} from G-actin, 0.05 mM- Mg^{2+} caused a considerable increase in the accessibility of both Cys-217 and Cys-374. In contrast, the removal of Ca^{2+} resulted in a decrease in the accessibility of Cys-257 nearly to a point of completion. The accessibility of Cys-217 and Cys-374 of such Ca^{2+} -free G-actin became much more sensitive to Mg^{2+} ; 0.4 mM- Mg^{2+} caused a maximum increase in accessibility with no further increase when the concentration was raised to 2 mM. On the other hand, the reaction of Cys-10 and Cys-257 remained unchanged at the low level of accessibility. These results suggest that the conformation of Ca^{2+} -actin is different from that of Mg^{2+} -actin and are in agreement with the findings by Selden *et al.* (1986) that the properties of actin monomers are different between Mg^{2+} -actin and Ca^{2+} -actin, as the former has a lower critical concentration, a larger relative forward rate constant of polymerization and a smaller relative rate constant of depolymerization than the latter.

DISCUSSION

The initial step of actin polymerization has long been predicted to involve an activation of monomeric G-actin. Ample indirect evidence suggests its existence (Korn, 1982; Pollard, 1990), but direct evidence is lacking. This is largely due to the transient nature of the activated form of G-actin. Evidence has been provided in this study that either Mg^{2+} or K^{+} , under the experimental conditions, can induce rapid conformational change in G-actin as reflected by an enhanced accessibility of its thiol groups without any evidence of polymerization. Actin has been reported to contain a small *N*-terminal domain and a large *C*-terminal mass that contains amino acid residues 69–375 (Jacobson & Rosenbusch, 1976; Mornet & Ue, 1984). On the other hand, the three-dimensional structure of the actin component in the actin-DNAase I complex shows two domains that appear to be equal in size, and each domain is made up of two subdomains. The subdomains 1 and 2 correspond to the small domain of the proteolytic product whereas subdomains 3 and 4 correspond to the large domain (Kabsch *et al.*, 1990). In addition, both the *N*-terminal and *C*-terminal residues are found in subdomain 1. Whether this three-dimensional structure of the actin component of the complex can be equated exactly to that of G-actin or the activated form of G-actin remains to be determined. As pointed out by Holmes *et al.* (1990), the binding of actin and DNAase I is very tight and probably causes distortion in the region of subdomain 2 that is in close contact with DNAase I. Nevertheless, on the basis of our findings, the change in conformation is largely confined to the large domain and little or no significant change occurs in the small domain of G-actin. On the other hand, both domains are affected in the three-dimensional structure proposed by Kabsch *et al.* (1990), since subdomains 1 and 4 contain Cys-374 and Cys-257 respectively. The lack of change in the locale of Cys-10 cannot rule out possible changes in other locales within the small domain. Furthermore, whether the isomerization reflected by the increased accessibility of the thiol groups is a result of a localized re-arrangement of the polypeptide chain or an overall change in the large domain of the actin molecule remains to be determined. Nevertheless the data suggest that the function of these ions is to convert the G-actin into a new species of G-actin that retains its bound nucleotide in the form of triphosphate. The life-time of this new species is sensitive to

changes of temperature as well as of actin concentration. At lower temperature or around the critical concentration of actin, its life-time is prolonged considerably. Under these conditions, polymerization apparently proceeds relatively slowly. The extent of polymerization can be monitored by the decrease in accessibility of these thiol groups on actin, since the thiol groups of actin units of F-actin are totally inaccessible to the reagent DACM (Liu *et al.*, 1990).

The effect of temperature on the life-time of this new species is most pronounced when the exposure of G-actin to $\text{Mg}^{2+}/\text{K}^{+}$ is extended at 4 °C. During the early part of the exposure when the new species of G-actin are predominant, there is no evidence of polymerization. This conclusion is also supported by the fact that this new species contains ATP and not ADP. As polymerization starts to proceed, there is a concomitant decrease in the new species of G-actin with a corresponding increase in the extent of conversion of the bound ATP into ADP. Thus the formation of this new species of G-actin apparently precedes the polymerization.

It is known that G-actin, as isolated, exists in a Ca^{2+} -bound form whereas *in vivo* the monomeric actin is presumably in a Mg^{2+} -bound form (Weber *et al.*, 1969; Kitazawa *et al.*, 1982). At low ionic strength, Ca^{2+} (0.05–0.15 mM) retarded, whereas Mg^{2+} accelerated, the polymerization of G-actin (Mihash & Ooi, 1965; Dancker & Low, 1977). The removal of trace amounts of Ca^{2+} enhanced the polymerization (Maruyama, 1981). Our results suggest that these two bivalent cations play somewhat different roles, which elicit different conformations of G-actin. This conclusion is in agreement with the findings that Mg^{2+} can exchange with Ca^{2+} , with a resultant conformational change (Frieden *et al.*, 1980; Frieden, 1982; Carlier *et al.*, 1986a), and with the proposal that conformational differences in monomeric actin are related to the specific site occupied and the species of metal cation bound (Frieden *et al.*, 1980; Cooper *et al.*, 1983; Barden & Remedios, 1985; Carlier *et al.*, 1986b; Estes *et al.*, 1987; Zimmerle *et al.*, 1987; Zimmerle & Frieden, 1988). The interplay of these cations may be important in the process of polymerization or depolymerization as reported by Selden *et al.* (1986).

We have provided direct chemical evidence for the existence of a new species which can be considered an activated form of actin. Since all thiol groups of this new species became inaccessible to DACM during the formation of F-actin, further change in the configuration of this activated G-actin must take place. We suggest that this change occurs during nucleation, and propose the following sequence of reactions:

where ATP-G-actin is G-actin with bound ATP, ATP-G^{*}-actin is the activated form of G-actin, nuclea-G^{*}-actin is the oligomeric actin formed during nucleation and actin polymer is F-actin. Although direct evidence for the existence of the activated form of G-actin has now been demonstrated, additional studies are needed to define further this new G-actin species and its role as an intermediate in the transformation of G-actin into F-actin.

This research was supported by National Institutes of Health Grant HL14020 to A. S.

REFERENCES

- Barden, J. A. & Remedios, C. G. (1985) *Eur. J. Biochem.* **146**, 5–8
- Carlier, M.-F., Pantaloni, D. & Korn, E. D. (1986a) *J. Biol. Chem.* **261**, 10778–10784

- Carlier, M.-F., Pantaloni, D. & Korn, E. D. (1986b) *J. Biol. Chem.* **261**, 10785–10792
- Carlier, M.-F., Pantaloni, D. & Korn, E. D. (1987) *J. Biol. Chem.* **262**, 3052–3059
- Collins, J. H. & Elzinga, M. (1975) *J. Biol. Chem.* **250**, 5915–5920
- Cooper, J. A., Buhle, E. L., Jr., Walker, S. B., Tsong, T. Y. & Pollard, T. D. (1983) *Biochemistry* **22**, 2193–2202
- Dancker, P. & Low, I. (1977) *Biochim. Biophys. Acta* **484**, 169–176
- Estes, J. E., Selden, L. A. & Gershman, L. C. (1987) *J. Biol. Chem.* **262**, 4952–4957
- Frieden, C. (1982) *J. Biol. Chem.* **257**, 2882–2886
- Frieden, C. (1983) *Proc. Natl. Acad. Sci. U.S.A.* **80**, 6513–6517
- Frieden, C., Lieberman, D. & Gilbert, H. R. (1980) *J. Biol. Chem.* **255**, 8991–8993
- Gordon, D. J., Yang, Y. Z. & Korn, E. D. (1976) *J. Biol. Chem.* **251**, 7474–7479
- Holmes, K. C., Popp, D., Gebhard, W. & Kabsch, W. (1990) *Nature (London)* **347**, 44–49
- Itaya, K. & Ui, M. (1966) *Clin. Chim. Acta* **14**, 361–366
- Jacobson, G. R. & Rosenbusch, J. P. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 2742–2746
- Kabsch, W., Mannherz, H. G. & Such, D. (1985) *EMBO J.* **4**, 2113–2118
- Kabsch, W., Mannherz, H. G., Suck, D., Pai, E. F. & Holmes, K. (1990) *Nature (London)* **347**, 37–44
- Kitazawa, T., Schuman, H. & Somlyo, A. P. (1982) *J. Muscle Res. Cell Motil.* **3**, 437–454
- Korn, E. D. (1982) *Physiol. Rev.* **62**, 672–737
- Liu, D. F., Wang, D. & Stracher, A. (1990) *Biochem. J.* **266**, 453–459
- Mannherz, H. G., Kabsch, W. & Leberman, R. (1977) *FEBS Lett.* **73**, 141–143
- Maruyama, K. (1981) *Biochim. Biophys. Acta* **667**, 139–142
- Mihashi, K. & Ooi, T. (1965) in *Molecular Biology of Muscle Contraction* (Ebashi, S., Oosawa, F., Sekine, T. & Tonomura, Y., eds.), pp. 77–89, Igaku Shoin, Tokyo
- Mornet, D. & Ue, K. (1984) *Proc. Natl. Acad. Sci. U.S.A.* **81**, 3680–3684
- O'Farrell, P. Z., Goodman, H. M. & O'Farrell, P. H. (1977) *Cell* **12**, 1133–1142
- Oosawa, F. & Kasai, M. (1971) in *Subunits in Biological Systems* (Timasheff, S. N. & Fasman, G. D., eds.), pp. 261–322, Marcel Dekker, New York
- Pardee, J. D. & Spudich, J. A. (1982) *J. Cell Biol.* **98**, 648–654
- Pollard, T. D. (1990) *Curr. Opin. Cell Biol.* **2**, 33–40
- Rich, S. A. & Estes, J. E. (1976) *J. Mol. Biol.* **104**, 777–792
- Rouayrenc, J. F. & Travers, F. (1981) *Eur. J. Biochem.* **116**, 73–77
- Selden, L. A., Gershman, L. C. & Estes, J. E. (1986) *J. Muscle Res. Cell Motil.* **7**, 215–224
- Selden, L. A., Estes, J. E. & Gershman, L. C. (1989) *J. Biol. Chem.* **264**, 9271–9277
- Spudich, J. A. & Watt, S. (1971) *J. Biol. Chem.* **246**, 4866–4871
- Straub, F. B. & Feuer, G. (1950) *Biochim. Biophys. Acta* **4**, 455–470
- Suck, D., Kabsch, W. & Mannherz, H. G. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 4319–4323
- Sugino, H., Sakabe, N., Sakabe, K., Hatano, S., Oosawa, F., Mikawa, T. & Ebashi, S. (1979) *J. Biochem. (Tokyo)* **86**, 257–260
- Sutoh, K. (1982) *Biochemistry* **21**, 3654–3661
- Weber, A., Herz, R. & Reiss, I. (1969) *Biochemistry* **8**, 2266–2271
- Zimmerle, C. T. & Frieden, C. (1986) *Biochemistry* **25**, 6432–6438
- Zimmerle, C. T. & Frieden, C. (1988) *Biochemistry* **27**, 7759–7765
- Zimmerle, C., Patane, K. & Frieden, C. (1987) *Biochemistry* **26**, 6545–6552

Received 31 July 1991/2 October 1991; accepted 11 October 1991