

Differences in G-Actin Containing Bound ATP or ADP: The Mg^{2+} -Induced Conformational Change Requires ATP[†]

Carl Frieden* and Kalliopi Patane

Department of Biological Chemistry, Division of Biology and Biomedical Sciences, Washington University School of Medicine, St. Louis, Missouri 63110

Received December 19, 1984

ABSTRACT: The role of adenosine 5'-triphosphate (ATP) in the Mg^{2+} -induced conformational change of rabbit skeletal muscle G-actin has been investigated by comparing actin containing bound ADP with actin containing bound ATP. As previously described [Frieden, C. (1982) *J. Biol. Chem.* 257, 2882-2886], *N*-acetyl-*N'*-(5-sulfo-1-naphthyl)ethylenediamine-labeled G-actin containing ATP undergoes a time-dependent Mg^{2+} -induced fluorescence change that reflects a conformational change in the actin. Addition of Mg^{2+} to labeled G-actin containing ADP gives no fluorescence change, suggesting that the conformational change does not occur. The fluorescence change can be restored on the addition of ATP. Examination of the time courses of these experiments suggests that ATP must replace ADP prior to the Mg^{2+} -induced change. The Mg^{2+} -induced polymerization of actin containing ADP is extraordinarily slow compared to that of actin containing ATP. The lack of the Mg^{2+} -induced conformational change, which is an essential step in the Mg^{2+} -induced polymerization, is probably the cause for the very slow polymerization of actin containing ADP. On the other hand, at 20 °C, at pH 8, and in 2 mM Mg^{2+} , the elongation rate from the slow growing end of an actin filament, measured by using the protein brevin to block growth at the fast growing end, is only 4 times slower for actin containing ADP than for actin containing ATP.

Monomeric G-actin contains one tightly bound ATP that, on polymerization, is hydrolyzed to ADP. The hydrolysis has been postulated as a mechanism for distinguishing the two growing ends of the filament (Wegner, 1976). However, a role for the ATP itself remains uncertain although there are data which show that the presence of this nucleotide stabilizes G-actin (Asakura, 1961; Oosawa & Kasai, 1971; Waechter & Engel, 1977). We report here the investigation of the role of ATP with respect to the Mg^{2+} -induced conformational change.

An activation step prior to polymerization of actin has been proposed by several groups (Rouayrenc & Travers, 1981; Cooper et al., 1983; Frieden, 1983; Tobacman & Korn, 1983). Frieden (1983) and Gershman et al. (1984) have shown that Mg^{2+} displacement of bound Ca^{2+} is important in defining the rate of the Mg^{2+} -induced polymerization reaction. In fact, the rate for the activation step in the Mg^{2+} -induced polymerization is consistent with a conformational change in G-actin occurring as a consequence of Mg^{2+} displacing Ca^{2+} from the divalent cation binding site (Frieden, 1983). The kinetic parameters that describe this conformational change, which is necessary for the polymerization, are essentially the same as those observed by a change in the fluorescence of actin labeled at cysteine-374 with the fluoroprobe *N*-(iodoacetyl)-*N'*-(5-sulfo-1-naphthyl)ethylenediamine (1,5-IAEDANS)¹ (Frieden et al., 1980; Frieden, 1982; Frieden, 1983), thus indicating that the fluorescence change monitors the conformational change. As will be shown, the presence of ADP rather than ATP in G-actin results in the inability of the G-actin to undergo the Mg^{2+} -induced conformational change as monitored by fluorescence.

The mechanism of polymerization of G-actin containing ADP instead of ATP and the properties of the filaments so formed are currently under intensive investigation (Lal et al.,

1984; Pantaloni et al., 1984; Pollard, 1984). The critical concentration as well as the rate constants from the filaments differs for actin containing ADP relative to the values observed for actin containing ATP. Lal et al. (1984) have used cross-linked trimers of actin to determine elongation rates while Pollard (1983) obtained nuclei by gentle mixing methods. These experiments measure primarily the elongation rate constant for the fast growing end of an actin filament, and Lal et al. (1984) have shown that this rate constant is 2.2-fold larger for actin containing ATP compared to that for actin containing ADP. In the results reported below, we use the actin binding protein brevin, which binds 2 mol of G-actin (Bryan & Kurth, 1984; Doi & Frieden, 1984; Lees et al., 1984). Brevin appears to block the fast growing end of an actin filament, allowing measurement of elongation rates from the slow growing end of the filament. Using this procedure, we find that the elongation rate constant is about 4 times slower for actin containing ADP compared to that for actin containing ATP.

MATERIALS AND METHODS

Materials. Yeast hexokinase and ATP (99-100% pure) were obtained from Sigma. *N*-(1-Pyrenyl)iodoacetamide was obtained from Molecule Probes and *N*-(iodoacetyl)-*N'*-(5-sulfo-1-naphthyl)ethylenediamine from Pierce Chemical Co. "Ultrapure" $MgSO_4$ was obtained from Ventron Corp., Alpha Division. All other materials were reagent grade, and all solutions were made with deionized distilled water.

¹ Abbreviations: 1,5-IAEDANS-labeled G-actin, actin labeled with *N*-(iodoacetyl)-*N'*-(5-sulfo-1-naphthyl)ethylenediamine; pyrene-labeled G-actin, actin labeled with *N*-(1-pyrenyl)iodoacetamide; Tris-HCl, tris-(hydroxymethyl)aminomethane hydrochloride; G buffer, 2 mM Tris-HCl, pH 8, containing 200 μ M ATP, 200 μ M Ca^{2+} , and 1.5 mM $Na_2S_2O_3$; EGTA, ethylene glycol bis(β -aminoethyl ether)-*N,N,N',N'*-tetraacetic acid.

[†] Supported by National Institutes of Health Grant AM 13332.

Preparation of Proteins. Rabbit skeletal muscle G-actin was isolated and purified according to the procedure of Spudis & Watt (1971), modified as noted elsewhere (Frieden et al., 1980), with a gel filtration step (Sephadex G-150) as described (MacLean-Fletcher & Pollard, 1980). Unless used immediately after gel filtration, actin was stored at -20°C after lyophilization in the presence of sucrose (2 mg of sucrose/mg of actin). Lyophilized actin was dissolved and dialyzed at 4°C for 1–2 days against 2 mM Tris-HCl, pH 8, containing 200 μM ATP, 200 μM Ca^{2+} , and 1.5 mM NaN_3 (G buffer). The protein concentration was determined spectrophotometrically by using $A_{1\text{mg/mL}}^{290\text{nm}} = 0.63$ at 290 nm (Houk & Ue, 1974) or with covalently modified actin by the method of Bradford (1976), using G-actin as a standard, or with 1,5-IAEDANS-labeled actin by correcting the 290-nm reading (Frieden et al., 1980) for the incorporated dye.

Modification of actin was performed as described elsewhere for either *N*-(1-pyrenyl)iodoacetamide (Tellam & Frieden, 1982) or *N*-(iodoacetyl)-*N'*-(5-sulfo-1-naphthyl)ethylenediamine (Frieden et al., 1980). The concentration of 1,5-IAEDANS label was determined from the absorbance at 337 nm by using an extinction coefficient of 6000 $\text{M}^{-1}\text{cm}^{-1}$ (Hudson & Weber, 1973). 1,5-IAEDANS-labeled actin contained 1.1 mol of fluorophore/mol of actin. Brevin was prepared from rabbit plasma according to the method of Harris & Weeds (1983) with modifications as described by Doi & Frieden (1984).

Preparation of ADP-Containing G-Actin. The procedure used is modified from that given by Pollard (1984). 1,5-IAEDANS-labeled actin was polymerized at a concentration of 2 mg/mL in G buffer by the addition of 2 mM Mg^{2+} . Yeast hexokinase (0.01 mg/mL), glucose (200 μM), and diadenosine 5'-pentaphosphate, a potent inhibitor of adenylate kinase (Lienhard & Secemski, 1973) (10 μM), were added, and the actin solution was left at room temperature for 10–15 min. As shown by Pollard (1984), this procedure effectively removes all ATP. The solution is then diluted 10-fold in buffer containing 2 mM Tris-HCl, pH 8, 50 μM ADP, 50 μM Ca^{2+} , and 1.5 mM NaN_3 , passed 2–3 times through a syringe needle, and allowed to stand for 10–15 min in order to effect complete depolymerization. The diluted actin solution is centrifuged at 100000g for 1 h and the supernatant solution (at 0.2 mg/mL actin) concentrated by centrifugation through Amicon centrifuge cones (CF25) to a concentration of 1–2 mg/mL. At this point, the actin solution contains 2 mM Tris-HCl, pH 8, ~ 50 μM ADP, ~ 20 μM glucose 6-phosphate, 0.2 mM Mg^{2+} , 50 μM Ca^{2+} , 1.5 mM NaN_3 , and some hexokinase. The procedure takes 4–5 h, and the material is used within a few hours of its preparation. The 1,5-IAEDANS-labeled actin prepared in this manner is monomeric as measured by the value of the sedimentation coefficient ($s_{20} = 2.9$ S at 1 mg/mL) in sedimentation velocity experiments and appears to contain no oligomers or higher molecular weight species. That the bound nucleotide in the monomeric G-actin is ADP as expected (Pollard, 1984) is also indicated by the fact that titration experiments show that the bound nucleotide can be completely displaced by stoichiometric amounts of 1,*N*⁶-ethanoadenosine 5'-triphosphate (data not shown). Since ATP binds about 5 times more tightly than the 1,*N*⁶-ethanoadenosine 5'-triphosphate (Wächter & Engel, 1975), it is unlikely that any residual ATP is present on the G-actin.

Fluorescence experiments using 1,5-IAEDANS-labeled G-actin, as described below, involve dilution of the ADP-containing actin by at least 20–50-fold, thus lowering the hexokinase and remaining glucose concentrations by at least

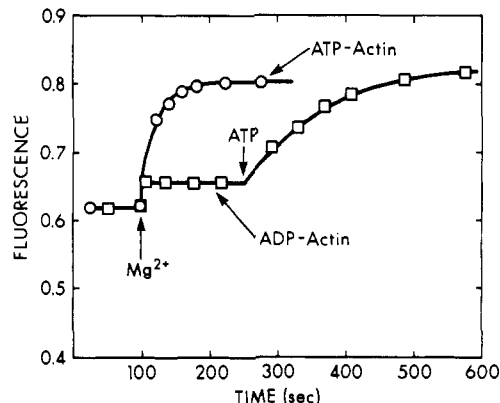


FIGURE 1: Changes in fluorescence of 1,5-IAEDANS-labeled G-actin on Mg^{2+} or ATP addition. At arrow labeled Mg^{2+} , 1 mM Mg^{2+} was added to G-actin containing ATP (O) or ADP (□). At arrow labeled ATP, 200 μM ATP was added to G-actin containing ADP. The experiments were performed in 2 mM Tris-HCl buffer, pH 8, 20°C , containing 1.5 mM NaN_3 at an actin concentration of 0.02 mg/mL (0.47 μM). For G-actin containing ATP, the solution also contained 200 μM ATP, 10 μM Ca^{2+} , and 10 μM ADP. For G-actin containing ADP, the solution contained 10 μM ADP and 10 μM Ca^{2+} . The excitation and emission wavelengths were 340 and 460 nm, respectively.

that amount and the concentration of Mg^{2+} to well below 10 μM . Thus, for any experiments involving the readdition of ATP, very little of the added ATP would be converted to ADP.

The preparation of G-actin containing ADP for polymerization experiments was performed in exactly the same way as with labeled actin. A trace amount of pyrene-labeled actin was added at the beginning of the procedure and was sufficient to measure polymerization. In this way it was assured that the pyrene-labeled actin contained only ADP. For these experiments, in order to remove Mg^{2+} , the concentrated actin was centrifuged through a small column of Sephadex G-25 previously equilibrated with 2 mM Tris-HCl, pH 8, containing 50 μM ADP, 50 μM Ca^{2+} , and 1.5 mM NaN_3 .

Fluorescence Experiments. Fluorescence experiments were performed with a Spex Fluorolog spectrofluorometer. With 1,5-IAEDANS-labeled actin, excitation and emission wavelengths were 340 and 460 nm, respectively, while for pyrene-labeled actin they were 365 and 386 nm. For measuring the conformational changes by the enhanced fluorescence of 1,5-IAEDANS-labeled G-actin, the concentration of actin was usually less than the critical concentration, and it is unlikely that any polymerization occurs. The polymerization reaction was measured by using trace amounts of pyrene-labeled actin ($<1\%$) as described elsewhere (Tellam & Frieden, 1982). All actin solutions were centrifuged at 100000g for 1 h or at 180000g for 30 min prior to use. Solutions were stirred with a magnetic stirrer located at the bottom of the cuvette for about 10 s after the addition of Mg^{2+} . All experiments were performed at 20°C . Data were collected continuously and stored in digital mode for later recall.

RESULTS

Mg^{2+} - and ATP-Induced Fluorescence Changes in 1,5-IAEDANS-Labeled G-Actin Containing ADP. Figure 1 shows results for the addition of 1 mM Mg^{2+} to 1,5-IAEDANS-labeled G-actin containing either ATP or ADP. As previously observed (Frieden et al., 1980; Frieden, 1982), Mg^{2+} addition to labeled G-actin containing ATP results in a time-dependent 25–30% change in fluorescence. Under the conditions used in this experiment (10 μM Ca^{2+} , 10 μM ADP), the half-time of the fluorescence change is about 10–15 s. In contrast, the addition of Mg^{2+} to G-actin containing ADP in the presence of 10 μM Ca^{2+} and 10 μM ADP resulted in a very small

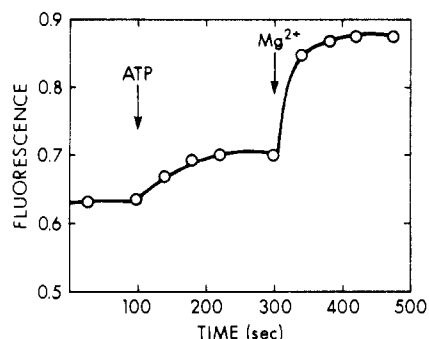


FIGURE 2: Changes in fluorescence of 1,5-IAEDANS-labeled G-actin containing ADP. At arrow labeled ATP, 200 μ M ATP was added to 0.05 mg/mL (1.18 μ M) 1,5-IAEDANS-labeled G-actin containing ADP. At arrow labeled Mg^{2+} , 1 mM Mg^{2+} was added. The experiments were performed in 2 mM Tris-HCl buffer, pH 8, 20 $^{\circ}$ C, containing 10 μ M ADP, 10 μ M Ca^{2+} , and 1.5 mM NaN_3 .

fluorescence increase (<5%) that occurred instantaneously. This change was independent of the Ca^{2+} concentration from 10–100 μ M. A control experiment (using Na_2SO_4) indicates that this change may be due to the change in ionic strength rather than the addition of the Mg^{2+} . Increasing the Mg^{2+} concentration added to 1,5-IAEDANS-labeled G-actin containing ADP gave a similar (somewhat larger) instantaneous change in fluorescence (data not shown). Thus, the time-dependent Mg^{2+} -induced fluorescence change that occurs in G-actin containing ATP does not appear to occur in G-actin containing ADP.

It is important to show that, under these conditions, G-actin containing ADP was not inactivated and thus made incapable of responding to Mg^{2+} . When, in the presence of 1 mM Mg^{2+} , ATP is added to G-actin containing ADP (at arrow labeled ATP in Figure 1), there is a time-dependent fluorescence increase. The same result is observed in the presence of sufficient EGTA to remove any residual Ca^{2+} in the solution. This fluorescence change is considerably slower ($t_{1/2} \sim 100$ s) than that observed for the Mg^{2+} -induced effect on actin containing ATP shown in Figure 1. The extent of the fluorescence change after addition of both Mg^{2+} and ATP is 35%, somewhat larger than that due to Mg^{2+} alone.

This result suggested that the addition of ATP to G-actin containing ADP in the absence of Mg^{2+} might also give a fluorescence change. Figure 2 shows that this is indeed the case. The addition of 200 μ M ATP to G-actin containing ADP shows a slow ($t_{1/2} = \sim 40$ s) fluorescence change with a small (11%) change in extent. The further addition of Mg^{2+} gives, as expected, a second change in fluorescence with a half-time similar to that of Mg^{2+} addition to G-actin containing ATP.

When ATP and Mg^{2+} are added simultaneously to ADP-containing G-actin, the time course of the fluorescence change is similar to that observed for the addition of ATP to G-actin containing ADP in the absence of Mg^{2+} (data not shown). As discussed later, this result suggests that the process occurs first by the displacement of ADP by ATP, followed by the addition of Mg^{2+} to G-actin that contains ATP.

Polymerization of ADP-Containing Actin. Figure 3 presents information about the polymerization of actin containing either ADP or ATP in the presence or absence of brevins. Brevins, a plasma protein similar to gelsolin (Yin & Cole, 1983), has been shown to bind G-actin (Bryan & Kurth, 1984; Doi & Frieden, 1984; Lees et al., 1984) and to serve as a nucleus for the growth of actin polymers in the presence of Ca^{2+} (Doi & Frieden, 1984). Elongation in the presence of brevins occurs at the slow growing end of the actin filament (Harris & Schwartz, 1981). In all the experiments shown in Figure 3,

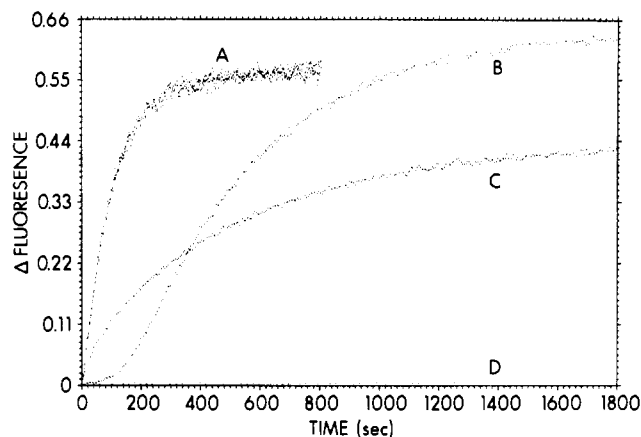


FIGURE 3: Polymerization of actin containing ATP or ADP in the presence and absence of brevins. Experiments were performed by following the fluorescence change of pyrene-labeled actin containing either ATP (A and B) or ADP (C and D). The actin concentration in all experiments was 1 mg/mL (23.5 μ M), and polymerization was induced by addition of 2 mM Mg^{2+} . Curves A and C also contained 0.23 μ M brevins (actin:brevins ratio = 100:1). The experiments were performed in 2 mM Tris-HCl buffer, pH 8, 20 $^{\circ}$ C, containing 200 μ M ATP, 200 μ M Ca^{2+} , and 1.5 mM NaN_3 (A and B) or 200 μ M ADP, 200 μ M Ca^{2+} , and 1.5 mM NaN_3 (C and D).

polymerization was induced by the addition of 2 mM Mg^{2+} .

In the absence of brevins, the polymerization of actin containing ADP proceeds very slowly. In the experiment shown in curve D of Figure 3, less than 2% of the actin is polymerized after 1800 s (30 min) while the polymerization of actin containing ATP is essentially complete within this time. In the presence of brevins (actin:brevins ratio = 100:1), polymerization of actin containing ATP is complete in less than 10 min, while polymerization of actin containing ADP takes somewhat over 30 min. For both cases, in the presence of brevins, polymerization proceeds without a noticeable lag. As discussed elsewhere (Doi & Frieden, 1984) and below, polymerization in the presence of brevins primarily represents elongation from a brevins-actin complex. Clearly, the rate of polymerization of actin containing ADP in the presence of brevins is considerably faster than that in the absence of brevins while, as expected from other studies (Pantaloni et al., 1984; Pollard, 1984), the final extent of polymerization of actin containing ADP is considerably less than that of actin containing ATP. Calculated from the fluorescence change, the critical concentration in the presence of brevins for actin containing ADP at 2 mM Mg^{2+} is about 7 μ M, at least 10 times larger than that for the actin containing ATP under the same conditions. Using a mechanism for simple elongation and a computer simulation system described elsewhere (Barshop et al., 1983; Frieden & Goddette, 1983), we find that the rate constants for elongation in the presence of brevins are 4×10^4 s $^{-1}$ M $^{-1}$ and 1×10^4 s $^{-1}$ M $^{-1}$ for actin containing ATP and ADP, respectively, i.e., a 4-fold difference.

DISCUSSION

The tightly bound ATP in G-actin may serve at least two roles, one related to the presence of the ATP itself and the other related to its hydrolysis to ADP. The latter has been proposed as a mechanism to distinguish between the two ends of an actin filament (Wegner, 1976) although hydrolysis per se appears not to be required for polymerization. The results of this paper deal primarily with the role of ATP itself and secondarily with the role of the nucleotide in the Mg^{2+} -induced polymerization reaction.

We have previously shown (Frieden et al., 1980; Frieden, 1982) that the addition of Mg^{2+} to 1,5-IAEDANS-labeled

G-actin containing ATP results in a time-dependent fluorescence change. This change was interpreted as a Mg^{2+} -dependent conformational change due to displacement of Ca^{2+} from the tight divalent cation binding site of G-actin. We later showed that, at pH 8 and 20 °C, this conformational change is a necessary step in the Mg^{2+} -induced polymerization process (Frieden, 1983). A corresponding activation step in the Ca^{2+} - or KCl-induced polymerization has not been defined.

The data presented here show that Mg^{2+} does not induce a fluorescence change in G-actin containing ADP. Since the fluorescence change seems clearly to represent a conformational change, we can conclude that Mg^{2+} cannot induce this change in G-actin containing ADP. It is of course possible that Mg^{2+} induces a conformational change that is not detected by using the labeled G-actin. However, in view of the requirement for a conformational change in the Mg^{2+} -induced polymerization process and the lack of polymerization of actin containing ADP, this seems unlikely. The addition of ATP to G-actin containing ADP results in the return in the ability of Mg^{2+} to induce the conformational change, but the change occurs at a slow rate. It is not clear what the rate-determining step in this process may be. Such a step could be the rate at which ADP dissociates from the G-actin or the rate of a conformational change induced subsequent to ATP binding. Recent experiments (C. Frieden, unpublished results) indicate that the latter explanation may be correct. In this regard, the time-dependent fluorescence change that occurs on ATP addition to G-actin containing ADP is of interest.

It is not clear whether Mg^{2+} can bind to G-actin containing ADP or whether the ADP must be displaced with ATP before the initial binding of Mg^{2+} can occur. However, in an experiment in which ATP and Mg^{2+} were added simultaneously to G-actin containing ADP, the time course of the fluorescence change was consistent with that observed for the addition of ATP to G-actin in the absence of Mg^{2+} . This result suggests that ADP is replaced by ATP and then Mg^{2+} binds to induce the conformational change. In this regard, we have shown elsewhere (Frieden, 1982) that Mg^{2+} binding to the tight divalent cation site of G-actin containing ATP is a two-step process: a relatively weak binding ($K_d = 900 \mu M$) followed by the conformational change leading to an overall dissociation constant of about $40 \mu M$. Since the conformational change does not occur in ADP-containing G-actin, it is certainly possible that Mg^{2+} is bound only poorly to this actin.

Computer analysis of the full time course of actin polymerization shows that, at pH 8 and 20 °C, the Mg^{2+} -induced conformational change detected by the fluorescence experiments is a step that precedes the Mg^{2+} -induced polymerization process (Frieden, 1983). Since G-actin containing ADP does not undergo the change on Mg^{2+} addition, we might expect that it would not polymerize. Figure 3 shows that the Mg^{2+} -induced polymerization is extraordinarily slow. Thus, in the time that the polymerization of actin containing ATP is complete, less than 2% of polymerization has occurred when actin containing ADP is used. In their study of the polymerization of ADP-containing actin, Lal et al. (1984) also find that polymerization of ADP-containing actin is very slow unless solutions were briefly sonicated (presumably to produce nuclei).

The next question is whether ATP is required for elongation. Lal et al. (1984), using cross-linked actin trimers as nuclei, have shown only a 2.2-fold slower elongation rate between actin containing ADP relative to actin containing ATP, by using 1 mM Mg^{2+} to induce polymerization. While the experiments of Lal et al. (1984) involve elongation from both ends of the

actin filament, the experiments in the presence of brevin (Figure 3) involve elongation from the slow growing end of the actin filament, since brevin blocks elongation from the fast growing end (Harris & Schwartz, 1981). With brevin, the elongation rates differ by about 4-fold for actin containing ADP relative to actin containing ATP.

From the extent of the fluorescence change, the critical concentration for actin containing ADP can be calculated (from Figure 3 in the presence of brevin) to be $7 \mu M$, in agreement with observations from others (Lal et al., 1984; Pantaloni et al., 1984; Pollard, 1984). Using this value and the elongation rate constant of $1 \times 10^4 M^{-1} s^{-1}$, we can calculate the disassembly rate from the slow growing end as $0.07 s^{-1}$. These rate constants are about 100-fold lower than those observed by Lal et al. (1984) for actin nucleated with cross-linked trimers, indicating a 100-fold difference in the rate constants for the fast and slow growing ends of an actin filament containing ADP to which G-actin containing ADP is added.

Since elongation is rapid, the very slow Mg^{2+} -induced polymerization of actin containing ADP probably reflects the lack of the Mg^{2+} -induced conformational change. This step, as shown previously (Frieden, 1983), precedes the nucleation step in the polymerization process. If nucleation occurs only poorly, this could explain the very slow polymerization of actin containing ADP.

In summary, the results of this paper explore the role of bound ATP in actin polymerization by comparing actin containing ADP with actin containing ATP. The observation that actin containing ADP does not undergo a conformational change on Mg^{2+} addition, which is required for the Mg^{2+} -induced polymerization, suggests that the presence of bound ATP is necessary for this change and the subsequent nucleation of actin prior to elongation. The very slow polymerization of actin containing ADP is consistent with this idea.

ACKNOWLEDGMENTS

We thank Drs. E. Korn and T. Pollard for sending preprints related to the polymerization of ADP-actin (Pollard, 1984; Lal et al., 1984).

Registry No. ATP, 56-65-5; ADP, 58-64-0; Mg, 7439-95-4.

REFERENCES

- Asakura, S. (1961) *Arch. Biochem. Biophys.* 92, 140-149.
- Barshop, B. A., Wrenn, R. F., & Frieden, C. (1983) *Anal. Biochem.* 130, 134-135.
- Bradford, M. (1976) *Anal. Biochem.* 72, 248-254.
- Bryan, J., & Kurth, M. C. (1984) *J. Biol. Chem.* 259, 7480-7487.
- Cooper, J. A., Buhle, E. C., Jr., Walker, S. B., Tsong, T. Y., & Pollard, T. D. (1983) *Biochemistry* 22, 2193-2202.
- Doi, Y., & Frieden, C. (1984) *J. Biol. Chem.* 259, 11868-11875.
- Frieden, C. (1982) *J. Biol. Chem.* 257, 2882-2886.
- Frieden, C. (1983) *Proc. Natl. Acad. Sci. U.S.A.* 80, 6513-6517.
- Frieden, C., & Goddette, D. W. (1983) *Biochemistry* 22, 5836-5843.
- Frieden, C., Lieberman, D., & Gilbert, H. R. (1980) *J. Biol. Chem.* 255, 8991-8993.
- Gershman, L. C., Newman, J., Selden, L. A., & Estes, J. E. (1984) *Biochemistry* 23, 2199-2203.
- Harris, D. A., & Schwartz, J. H. (1981) *Proc. Natl. Acad. Sci. U.S.A.* 78, 6798-6802.
- Harris, H. E., & Weeds, A. G. (1983) *Biochemistry* 22, 2728-2741.

- Houk, T. W., Jr., & Ue, K. (1974) *Anal. Biochem.* 62, 66-74.
- Hudson, E. N., & Weber, G. (1973) *Biochemistry* 12, 4154-4163.
- Lal, A. A., Brenner, S. L., & Korn, E. D. (1984) *J. Biol. Chem.* 259, 13061-13065.
- Lees, A., Haddad, J. G., & Lin, S. (1984) *Biochemistry* 23, 3038-3047.
- Lienhard, G. E., & Secemski, I. I. (1973) *J. Biol. Chem.* 248, 1121-1123.
- MacLean-Fletcher, S., & Pollard, T. D. (1980) *Biochem. Biophys. Res. Commun.* 96, 18-27.
- Oosawa, F., & Kasai, M. (1971) *Biol. Macromol.* 5, 261-322.
- Pantaloni, D., Cartier, M.-F., Coue, M., Lal, A. A., Brenner, S. L., & Korn, E. D. (1984) *J. Biol. Chem.* 259, 6274-6283.
- Pollard, T. D. (1983) *Anal. Biochem.* 134, 406-412.
- Pollard, T. D. (1984) *J. Cell Biol.* 99, 769-777.
- Rouayrenc, J., & Travers, F. (1981) *Eur. J. Biochem.* 116, 73-77.
- Spudich, J. A., & Watt, S. (1971) *J. Biol. Chem.* 246, 4866-4871.
- Tellam, R., & Frieden, C. (1982) *Biochemistry* 21, 3207-3214.
- Tobacman, L. S., & Korn, E. D. (1983) *J. Biol. Chem.* 258, 3207-3214.
- Waechter, F., & Engel, J. (1975) *Eur. J. Biochem.* 57, 453-459.
- Waechter, F., & Engel, J. (1977) *Eur. J. Biochem.* 74, 227-232.
- Wegner, A. (1976) *J. Mol. Biol.* 108, 139-150.
- Yin, H. L., & Cole, F. S. (1983) *J. Cell Biol.* 97, 374a.

Interaction of Lipoprotein Lipase and Apolipoprotein C-II with Sonicated Vesicles of 1,2-Ditetradecylphosphatidylcholine: Comparison of Binding Constants[†]

Larry R. McLean* and Richard L. Jackson

Division of Lipoprotein Research, Department of Pharmacology and Cell Biophysics, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267-0575, and Merrell Dow Research Institute, Cincinnati, Ohio 45215

Received November 5, 1984; Revised Manuscript Received February 6, 1985

ABSTRACT: The interaction of lipoprotein lipase (LpL) and its activator protein, apolipoprotein C-II (apoC-II), with a nonhydrolyzable phosphatidylcholine, 1,2-ditetradecyl-*rac*-glycero-3-phosphocholine (C14-ether-PC), was studied by fluorescence spectroscopy. A complex of 320 molecules of C14-ether-PC per LpL was isolated by density gradient ultracentrifugation in KBr. The intrinsic tryptophan fluorescence emission spectrum of LpL was shifted from 336 nm in the absence of lipid to 330 nm in the LpL-lipid complex; the shift was associated with a 40% increase in fluorescence intensity. Addition of C14-ether-PC vesicles to apoC-II caused a 2.5-fold increase in intrinsic tryptophan fluorescence and a shift in emission maximum from 340 to 317 nm. LpL and apoC-II/C14-ether-PC stoichiometries and binding constants were determined by measuring the increase in the intrinsic tryptophan fluorescence as a function of lipid and protein concentrations; for LpL the rate and magnitude of the fluorescence increases were relatively independent of temperature in the range 4-37 °C. A stoichiometry of 270 PC per LpL for the LpL-lipid complex compares favorably with the value obtained in the isolated complex. The dissociation constant (K_d) of the complex is 4.3×10^{-8} M. For apoC-II, the stoichiometry of the complex is 18 PC per apoprotein, and the K_d is 3.0×10^{-6} M. These data suggest that LpL binds more strongly than apoC-II to phosphatidylcholine interfaces.

Lipoprotein lipase (LpL)¹ (EC 3.1.1.34) catalyzes the hydrolysis of tri- and diacylglycerols, phosphatidylcholines (PC), and phosphatidylethanolamines in plasma triacylglycerol-rich lipoproteins [for reviews, see Cryer (1981), Quinn et al. (1983), and Hamosh & Hamosh (1983)]. The triacylglycerol-rich lipoprotein substrate consists of a central core of neutral lipid, primarily cholesteryl esters and triacylglycerols, and a surface monolayer of lipids and various apolipoproteins (Morrisett et al., 1977). The primary lipid components of the surface monolayer are PC and cholesterol. One of the proteins associated with the surface monolayer of the lipoprotein substrate, apolipoprotein C-II (apoC-II), is required for maximal rates of hydrolysis of triacylglycerols and long-chain PC molecules by LpL.

Four functional sites on the LpL molecule have been defined: (1) a glycosaminoglycan binding site that anchors LpL

to the endothelial cell surface, (2) an active (catalytic) site, (3) an apoC-II binding site, and (4) a lipid binding site that interacts with the surface of the lipoprotein substrate. Although the series of molecular events responsible for LpL catalysis remain speculative, several steps in the reaction pathway may be defined (Quinn et al., 1983). First, the enzyme binds to the substrate interface. Then, the active site of the enzyme binds a substrate molecule forming an interfacial Michaelis-Menten complex in which catalysis occurs. Finally, the products dissociate from the active site of the enzyme. One possible mechanism by which apoC-II enhances the activity of LpL is by promoting the binding of LpL to the substrate interface. Alternatively, LpL activation by apoC-II may occur subsequent to binding of LpL to the interface.

¹ Abbreviations: LpL, lipoprotein lipase; PC, phosphatidylcholine; C14-ether-PC, 1,2-ditetradecyl-*rac*-glycero-3-phosphocholine; [¹⁴C]DP-PC, L- α -dipalmitoyl[1-¹⁴C]phosphatidylcholine; DMPC, dimyristoylphosphatidylcholine; apoC-II, apolipoprotein C-II; T_m , lipid phase transition temperature; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

[†] This research was supported by U.S. Public Health Service Grants P01 HL 22619 and R01 HL 23019, by General Clinical Research Center and CLINFO Grant NIH RR 00068, and by Molecular and Cellular Biology Training Grant NIH HL 07527.