Actin Polymerization and Its Relationship to Adenosine Triphosphatase Activity

By Veeraraghavan K. Murthy, Ph.D., Lamar E. Crevasse, M.D., and Joseph C. Shipp, M.D.

ABSTRACT

F-actin was prepared from pig heart myofibrils without the use of organic solvents. G-actin, prepared from F-actin by sonication, had adenosine triphosphatase activity. Dephosphorylation of added ATP by G-actin had a broad pH optimum between pH 7.5 and pH 9.0, specifically required Mg2+, and was activated by EGTA. G-actin prepared from skeletal muscle of rabbits by similar methods also showed ATPase activity. Cardiac G-actin and G-actin from skeletal muscle had equimolar nucleotide bound to them. Polymerization of cardiac G-actin was greatest at a pH of about 6.5 and declined above pH 6.8. KCl was necessary for polymerization, and EGTA inhibited the conversion to F-actin. By selectively acetylating G-actin, using acetic anhydride, a preparation of G-actin was obtained that did not have adenosine triphosphatase activity but did polymerize under optimal conditions. It is concluded that dephosphorylation of ATP and the polymerization process in cardiac G-actin are two different reactions occurring at different sites on the actin molecule.

ADDITIONAL KEY WORDS

myofibrils F-actin G-actin sonication selective acetylation adenosine triphosphatase polymerization pig heart

The myofibrils of skeletal muscle and cardiac muscle are the contractile elements. Two proteins, F-actin and myosin, can be isolated separately by chemical methods from the myofibrils. These proteins can react, under suitable conditions, to form the complex actomyosin which, in the presence of adenosine triphosphate and in a specific ionic environment, contracts. Thus, the actomyosin complex can be looked upon, to a certain extent, as a much simpler model of the in vivo contraction process. It is also known that F-actin can undergo reversible depolymerization to G-actin.

Polymerization of G-actin to F-actin has been the subject of several studies (1-3). Straub and Feuer (4), Laki et al. (5), and Mommaerts (6) showed that the ATP molecule bound to G-actin was degraded with the liberation of Pi during the polymerization of G-actin to F-actin; a quantitative relationship between the polymerization process and the liberation of Pi in the medium was reported. However, more recent experiments have shown that the dephosphorylation reaction is not essential for polymerization of G-actin and that polymerization of G-ADP to F-actin occurs without the liberation of Pi (7, 8). Kasai et al. (9) demonstrated that G-actin which was free of nucleotide and divalent cation could polymerize to F-actin. Oosawa et al. (10) have suggested a cyclic process to explain the liberation of Pi during the polymerization of G-actin. There is a general

Abbreviations: G-ADP, G-ATP, G-UTP = G-actin containing bound adenosine diphosphate, adenosine triphosphate, and uridine triphosphate, respectively; ATPase = adenosine triphosphatase; EGTA = ethylene glycol bis (B-aminoethyl ether) — N,N'-tetracetic acid.
belief that G-actin does not possess ATPase activity (11). In all of the above studies, G-actin was prepared by extracting an acetone-dried powder of skeletal muscle with suitable aqueous solvents.

A method by which "natural F-actin" can be prepared from muscle without using organic solvents was reported recently (12). The purpose of the present study was to use this method of preparation to characterize the polymerizing property of G-actin of pig heart and to define its relationship to Pi liberation. The results showed that cardiac G-actin, prepared as described, has a distinct ATPase activity, and that G-actin, in which the ATPase activity was completely blocked by chemical acetylation, could polymerize to F-actin. These results suggest that the ATPase activity and the polymerization reaction occurred at two different sites of the G-actin molecule. A preliminary report of this study has appeared (13).

Materials and Methods

Materials.—ATP, UTP, and GTP were purchased from Sigma Chemical Company; trypsin from Behringer; histidine from Calbiochem; EGTA from K & K Laboratories; acetic anhydride from Merck and succinic anhydride from Eastman Organic Chemicals. All other chemicals were of Analar quality.

Methods.—Fresh pig hearts were frozen and used within a fortnight. "Purified natural F-actin" was prepared from left ventricles as described by Hama et al. (12). G-actin was obtained by sonicating a solution of F-actin in 4 mM tris buffer, pH 8.0, in an MSE sonicator for 2 minutes at 0° and centrifuging at 2g for 120 minutes at 105,000 × g (14). The supernatant fluid was labeled "natural G-actin." In experiments in which excess nucleotides were removed, G-actin solution, pH 8.0, was stirred gently at 0° with Dowex-I-Cl and centrifuged to deposit the resin (15). The supernatant fluid was termed "treated G-actin." Myosin from the ventricles of pigs was prepared according to Brahms and Kay (16). Protein was determined according to Hussein et al. (17).

The procedure described by Martonosi and Gouvea (18) was adopted with slight alterations in experiments in which G-actin was precipitated in the presence of various nucleotides. All operations were carried out at 0°. The desired nucleotide was added to natural G-actin to a final concentration of 0.2 mM. After 10 minutes, cold 0.2 M acetate buffer, pH 4.5, was added to the solution to bring the pH to 4.8. At the end of 5 minutes, the suspension was centrifuged at 3000 × g for 10 minutes. The precipitate was taken up in 4 mM tris buffer, pH 7.6, containing 0.2 mM nucleotide. The procedure of precipitation and dissolution was repeated once. Finally the precipitate was dissolved in 4 mM tris buffer at

**FIGURE 1**

Sedimentation pattern of purified natural F-actin from cardiac muscle. From left to right, 12 minutes and 19 minutes after full speed (20,410 rpm) was reached. 4 mM tris buffer, pH 7.6; 0.2 mM ATP; 25°; 2.8 mg F-actin/ml.
pH 7.6. The solution was centrifuged at $10^5 \times g$ for 90 minutes and the supernatant fluid used.

**Acetylation of G-actin.**—To 10.0 ml natural G-actin (0.7 to 0.9 mg protein/ml, pH 7.8) at 0°, 15 $\mu$l of acetic anhydride were added, and the pH was quickly adjusted to 7.6 with 1 N NaOH. The clear solution was left in ice for 3 minutes and occasionally stirred. ATP (final concentration, 0.5 mM) was added and the pH was adjusted to 4.8 using cold 0.2 M acetate buffer, pH 4.5. After 15 minutes, the suspension was centrifuged in cold at $10,400 \times g$ for 10 minutes. The sediment was washed once with 0.01 M acetate buffer, pH 4.8 containing 0.5 mM ATP. The precipitate was dissolved in 4 mM bis buffer, pH 8.0.

**Succinylation of G-actin.**—0.5 ml of a solution of succinic anhydride (15 mg/ml) was added to G-actin solution (15 mg protein) in tris buffer at pH 7.8. pH adjustment, isoelectric precipitation, washing and dissolution were the same as for acetylation.

Polymerization was measured by changes in turbidity at 660 nm, (18, 19). The suspension was centrifuged at $10^5 \times g$ for 90 minutes, and the sediment was confirmed as F-actin, as described in Results.

Inorganic phosphate was determined according to the procedure of Sumner (20). Viscosity was measured with a viscometer of the Ostwald type. The flow time for water at 25° was 70 seconds.

In experiments in which nucleotides bound to G-actin were measured, the procedure described by Asakura (15) was adopted to determine the amount of nucleotide bound to several different aliquots of treated G-actin solution containing known amounts of protein. The molecular weight of G-actin was assumed to be 60,000 (1, 21).

**Results**

Since Hama et al. (12) used skeletal muscle as the source of F-actin, it was necessary to confirm the purity of the cardiac F-actin obtained by their procedure. The sedimentation pattern of cardiac F-actin (Fig. 1) obtained in a Model E Spinco analytical centrifuge showed a single peak, and the sedimentation coefficient was 38.4, which is in good

<table>
<thead>
<tr>
<th>Group</th>
<th>Reaction mixture</th>
<th>No. of observations</th>
<th>amole P$_i$/mg protein (SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Myosin alone</td>
<td>2</td>
<td>0.78 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>F-actin alone</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Myosin + F-actin</td>
<td>2</td>
<td>1.12 ± 0.07</td>
</tr>
<tr>
<td>B</td>
<td>Complete System (natural G-actin)</td>
<td>7</td>
<td>0.76 ± 0.025</td>
</tr>
<tr>
<td></td>
<td>- ATP</td>
<td>7</td>
<td>0.02 ± 0.004</td>
</tr>
<tr>
<td></td>
<td>- Mg$^{2+}$</td>
<td>7</td>
<td>0.14 ± 0.016</td>
</tr>
<tr>
<td></td>
<td>- G-actin</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Ca$^{2+}$ replaced Mg$^{2+}$</td>
<td>7</td>
<td>0.20 ± 0.015</td>
</tr>
<tr>
<td></td>
<td>+ Ca$^{2+}$</td>
<td>7</td>
<td>0.75 ± 0.042</td>
</tr>
<tr>
<td></td>
<td>UTP replaced ATP</td>
<td>7</td>
<td>0.19 ± 0.008</td>
</tr>
<tr>
<td></td>
<td>GTP replaced ATP</td>
<td>7</td>
<td>0.14 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>Heated G-actin replaced natural G-actin</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>+ 0.1 M KCl</td>
<td>3</td>
<td>0.62 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>+ 0.2 M KCl</td>
<td>3</td>
<td>0.38 ± 0.02</td>
</tr>
<tr>
<td>C</td>
<td>Complete System (treated G-actin)</td>
<td>5</td>
<td>0.87 ± 0.022</td>
</tr>
<tr>
<td></td>
<td>- ATP</td>
<td>5</td>
<td>0.19 ± 0.011</td>
</tr>
<tr>
<td></td>
<td>- Mg$^{2+}$</td>
<td>5</td>
<td>0.22 ± 0.013</td>
</tr>
<tr>
<td></td>
<td>UTP replaced ATP</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

A. The incubation system contained: 0.1 M tris buffer, pH 7.8; 2 mM ATP, 0.15 M KCl; 1 mM Mg$^{2+}$; 1 mM Ca$^{2+}$. Myosin and F-actin were added in amounts of 0.9 mg protein and 0.1 mg protein, respectively; incubation for 15 minutes at 37°.

B and C. Complete system contained: 20 mM tris buffer, pH 7.8; 0.8 mM ATP; 1.0 mM Mg$^{2+}$; G-actin, 0.7-0.9 mg protein/flask. Amounts of added materials were: 1.0 mM Ca$^{2+}$, 1.0 mM UTP or GTP, 0.1-0.2 M KCl. Total volume, 2.0 ml; incubation for 15 minutes at 37°.
agreement with that reported for F-actin from skeletal muscle (12) obtained by the same extraction procedure. The single most important property of F-actin has been its activation of myosin ATPase. Results shown in Table 1, A, demonstrate that F-actin activated the cardiac myosin ATPase.

F-actin from skeletal muscle depolymerizes on dialysis against water containing ATP (4). In the present experiments attempts to depolymerize cardiac F-actin by dialysis against an ATP solution yielded small amounts of G-actin. However, preparation of G-actin by subjecting F-actin to sonication was satisfactory (14). About 70 to 80% of the F-actin was depolymerized, as measured by the protein content of sonicates after centrifugation at 10^5 \times g for 120 minutes. The intrinsic viscosity of a typical preparation of cardiac natural G-actin was 0.14 deciliter/g, which is in agreement with the value reported by Katz and Hall (21).

CARDIAC G-ACTIN ATPase

The results (Table 1, B) demonstrate that G-actin catalyzed dephosphorylation of added ATP. Mg^{2+} was required for optimal activity, whereas replacement of Mg^{2+} by Ca^{2+} in equal concentration resulted in decreased liberation of P_i. The presence of 1 mM Ca^{2+} had no effect on the magnesium activation of ATPase of G-actin. It is also clear from the results (Table 1, B) that when ATP was replaced by UTP or GTP, less P_i was liberated. There was no liberation of P_i in the absence of added nucleoside triphosphate. Heating G-actin for 10 minutes at 80° resulted in complete loss of activity.

Removal of excess nucleotide from a G-actin solution results in loss of polymerizing capacity of actin (4, 5); Dowex-treated G-actin did not lose its ATPase activity (Table 1, C). Adding 0.1 mM KCl and 0.2 mM KCI to the reaction mixture inhibited P_i liberation by 18% and 50%, respectively (Table 1, B). The dephosphorylation reaction showed a broad pH optimum between pH 7.8 and pH 9.0 (Fig. 2), and the total amounts of P_i formed approached a maximum after about 40 minutes of incubation (Fig. 3).

Substitution of ATP bound to the G-actin molecule by UTP or GTP has been shown to result in loss of polymerizing capacity (18). It was of interest to study the effect of substituting bound ATP by UTP on the ATPase activity of G-actin. Natural G-actin was isoelectrically precipitated twice, in both the absence and the presence of ATP or UTP. ATPase activity of the resulting various G-actins was studied. The results are shown in Table 2, A. G-actin precipitated in presence of ATP actively liberated P_i from added ATP. Ca^{2+} poorly activated the ATPase compared to Mg^{2+}. Adding 0.1 mM KCl inhibited the reaction. These results are in agreement with those obtained with natural G-actin. EGTA (0.13 mM and 0.25 mM) activated the ATPase of G-actin (Table 2, A).

Further results (Table 2, A) demonstrated that the presence or absence of any type of nucleoside triphosphate during precipitation did not affect ATPase activity. The activity obtained was comparable to that observed with G-ATP as enzyme. The liberation of P_i from UTP as substrate by G-UTP was the same as that when natural G-actin acted on UTP.

The polymerizing activity of G-actin was...
TABLE 2
Effect of Substitution of Nucleoside Phosphate on Actin ATPase and Polymerization

<table>
<thead>
<tr>
<th>Nucleoside phosphate added during precipitation</th>
<th>Substrate Condition</th>
<th>ATPase (umole Pi/mg protein)</th>
<th>Polymerization (± O.D. units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td></td>
<td></td>
<td>(Mean ± SEM)</td>
</tr>
<tr>
<td>A</td>
<td>Acetylated G-actin (complete system)</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Acetylated G-actin (− KC1)</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Natural G-actin (complete system)</td>
<td>4</td>
<td>0.810 ± 0.061</td>
</tr>
<tr>
<td></td>
<td>Natural G-actin (− KC1)</td>
<td>4</td>
<td>0.220 ± 0.033</td>
</tr>
</tbody>
</table>

Actin ATPase system was the same as described for Table 1. Polymerizing system: 20 mM maleate buffer, pH 6.8; 1.0 mM Mg^2+; 0.4 mM ATP; 0.1 mM KC1; natural G-actin, 2.8 mg protein. Total volume, 6.0 ml; incubation for 180 minutes at 25°. The type of G-actin added to the incubation mixture is indicated in parentheses.

quite labile compared to ATPase activity. If the two processes differ, it should be possible to separate one from the other. Also the possibility that ATP may be degraded in the ATPase site and the ADP transferred to the site of polymerization was considered. To obtain G-actin which could be polymerized in the complete absence of its ATPase activity, chemical acetylation or succinylation of the G-actin molecule was attempted. The results are shown in Table 2, B. Treatment with acetic anhydride resulted in complete loss of
ATPase activity of G-actin. Acetylated G-actin, however, retained polymerizing capacity. The specific loss of ATPase activity of G-actin through acetylation appeared to be selective under the controlled experimental conditions.

It is well known that each molecule of G-actin has one molecule of nucleotide, generally ATP, bound to its molecule (1-3). Preparations of treated G-actin obtained from pig heart and from rabbit skeletal muscle were tested for the fulfillment of this relationship. The results are shown in Figure 4. A direct 1:1 correlation existed between the amount of bound nucleotide and the amount of G-actin.

G-Actin ATPase from Skeletal Muscle

It was desirable to learn if the G-actin from skeletal muscle showed ATPase activity, since Hama et al. (12) have well characterized their natural F-actin obtained from skeletal muscle. Natural F-actin from the back and hind legs of rabbits was prepared according to Hama et al. (12). Natural G-actin was obtained by subjecting natural F-actin to sonication followed by high speed centrifugation, as described for cardiac G-actin.

Results shown in Table 3 demonstrate that natural G-actin obtained from skeletal muscle also exhibited ATPase activity. No Pi was liberated when either ATP or G-actin was omitted from the incubation system. Adding Mg2+ was necessary for optimal activity, and substituting Mg2+ by Ca2+ in equal concentration resulted in significantly less formation of P1 from added ATP. These results are in complete agreement with those obtained with cardiac G-actin.

Polymerization

Results presented in Figure 2 show that the optimum pH for polymerization was around 6.7. Polymerization appeared near maximal after incubation for about 140 minutes at 25° (Fig. 3).

The results of adding ATP, Mg2+ or Ca2+ on the polymerization are shown in Table 4. Adding 0.4 mM ATP to the system increased

---

### TABLE 3

ATPase Activity of G-actin from Skeletal Muscle

<table>
<thead>
<tr>
<th>Reaction mixture</th>
<th>No. of observations</th>
<th>umole Pi/mg protein (Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete system (natural G-actin)</td>
<td>3</td>
<td>0.6 ± 0.03</td>
</tr>
<tr>
<td>- Mg2+</td>
<td>3</td>
<td>0.27 ± 0.06</td>
</tr>
<tr>
<td>- Ca2+ (1.0 mM) replaced Mg2+</td>
<td>3</td>
<td>0.16 ± 0.02</td>
</tr>
<tr>
<td>- ATP</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>- G-actin</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

Complete system contained: 20 mM tris buffer, pH 7.8; 1.0 mM Mg2+; 1.0 mM ATP; G-actin, 0.7-0.9 mg protein/flask. Total volume, 2.0 ml; incubation for 15 minutes at 37°.

### TABLE 4

Requirements for Actin Polymerization

<table>
<thead>
<tr>
<th>Reaction mixture</th>
<th>No. of observations</th>
<th>Polymerization O.D. units (SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete system</td>
<td>4</td>
<td>82 ± 8</td>
</tr>
<tr>
<td>- Mg2+</td>
<td>4</td>
<td>50 ± 5</td>
</tr>
<tr>
<td>- ATP</td>
<td>4</td>
<td>42 ± 5</td>
</tr>
<tr>
<td>+ 1 mM Ca2+</td>
<td>4</td>
<td>65 ± 3</td>
</tr>
<tr>
<td>- KCl</td>
<td>4</td>
<td>10 ± 3</td>
</tr>
<tr>
<td>+ 0.25 mM EGTA</td>
<td>4</td>
<td>15 ± 2</td>
</tr>
<tr>
<td>+ 0.25 mM EGTA + 11 mM Ca2+</td>
<td>4</td>
<td>45 ± 4</td>
</tr>
</tbody>
</table>

Incubation system as in Table 2.
G-ACTIN POLYMERIZATION AND ATPase

the polymerization, and even in the absence of added ATP, significant amounts of F-actin were formed. Adding 1 mM Mg$^{2+}$ increased the polymerization, and adding 1 mM Ca$^{2+}$ to the Mg$^{2+}$-containing system inhibited it by 20%.

When 0.25 mM EGTA was added to the reaction mixture about 80% of the polymerization (Table 4) was inhibited. The inhibition could be partly reversed by adding excess (11 mM) Ca$^{2+}$. Excess Mg$^{2+}$ had no effect. These findings indicated that adequate Ca$^{2+}$ was bound to the G-acrin, and the bound Ca$^{2+}$ was necessary for polymerization. For optimal polymerization, the presence of both Ca$^{2+}$ and Mg$^{2+}$ seemed to be necessary. Adding KCl to the system was essential for the polymerization of G-acrin to F-acrin.

Concurrent with studies of ATPase activity, the effect of substituting nucleoside phosphate bound to G-acrin on the polymerization of actin was studied (Table 2). Polymerization occurred only when the isoelectric precipitation was carried out in the presence of ATP; in the presence of UTP or GTP the actin so prepared did not polymerize. Acetylation of G-acrin molecule with acetic anhydride affected the polymerizing capacity of actin. Polymerization of acetylated G-acrin resulted in decreased amounts of F-acrin as compared to control (Figure 3).

Succinylation of G-acrin, under the experimental conditions, resulted in loss of both ATPase activity and polymerizing capacity.

Discussion

Our results demonstrate that G-acrin prepared from left ventricles of pigs has ATPase activity. The low viscosity of the actin preparation suggests that it was free of tropomyosin (22). Moreover, Hama et al. (12), whose procedure was used in the present studies, compared the amino acid composition of their natural F-acrin with that of an F-acrin free of myosin and tropomyosin and found them essentially the same. The sedimentation constant of cardiac F-acrin and its ability to activate myosin ATPase compare well with similar properties of the purified F-acrin obtained from skeletal muscle by Hama et al. (12). G-actins obtained from skeletal muscle of rabbits as well as cardiac muscle from pigs were tested for the ratio of bound nucleotide to protein content. There was a 1:1 ratio of bound nucleotide to amount of G-acrin. Furthermore, G-acrin was always subjected to high speed centrifugation ($10^5 \times g$) before its ATPase activity (in the supernatant fraction) was tested. These results and the fact that G-acrin, which was precipitated twice isoelectrically, still showed characteristic ATPase activity argue against the possibility that contaminating myosin or tropomyosin or microsomal particles were responsible for the ATPase activity.

The results also demonstrate that cardiac G-acrin possessed both ATPase activity and polymerizing capacity. Cardiac G-acrin isoelectrically precipitated in the absence of nucleoside triphosphate lost its polymerizing capacity, but the ATPase activity was unaffected.

G-acrin prepared from F-acrin from skeletal muscle had typical ATPase activity confirming the results observed with G-acrin from cardiac muscle.

Yagi et al. (23) observed low but continuous liberation of $\text{P}_1$ from ATP in non-polymerizing G-acrin prepared from skeletal muscle acetone-dried powder. In the light of the results obtained in the present studies, it is conceivable that treatment of muscle with acetone or other organic solvents may have partially destroyed the ATPase activity of G-acrin. Recent studies by Maruyama and his colleagues (24) pointed out that acetone treatment caused many changes in the properties of actin.

The fact that through acetylation the ATPase activity could be selectively destroyed but the polymerizing capacity of G-acrin was preserved demonstrated that cardiac G-acrin showed an ATPase activity that was not involved in polymerization. This finding suggested that the two reactions occurred at two different sites on the actin molecule.

The results of polymerization of cardiac G-acrin reported in this paper agree closely
with the observations of others on the polymerization of actin from skeletal muscle. Thus, the loss of polymerizing capacity of actin precipitated in the absence of ATP (4) or in the presence of UTP or GTP (18) were in agreement. It was reported that UTP or GTP, unlike ATP, was weakly bound to G-actin molecule (25). The acceleration of polymerization of cardiac G-actin by Mg$^{2+}$ and deceleration by Ca$^{2+}$ (26), the binding of Ca$^{2+}$ to G-actin (27, 28) and its possible role in polymerization (3) are in agreement with the results obtained using actin from skeletal muscle.

Kasai et al. (26) reported that full polymerization of actin from rabbit skeletal muscle showed a plateau between pH 7.0 and pH 9.0. Cardiac G-actin polymerized optimally at pH values between 6.2 and 6.8 and rather poorly at pH 9.0. Mommaerts (19), as well as Tsuboi et al. (29) have observed optimal polymerization of skeletal G-actin to F-actin at pH values around 6.0, in agreement with results of the present study.

Martonosi and Gouvea (18) reported that actin from skeletal muscle, precipitated isoelectrically in the presence of UTP or GTP, released no P$_i$ during attempted polymerization; in the present study, cardiac G-actin liberated comparatively small amounts of P$_i$ under these conditions. The presence of 0.1 M KC$_1$ in the reaction mixture inhibited the ATPase activity of G-actin (Table 1, B). Since Martonosi and Gouvea (18) measured the liberation of P$_i$ from UTP or GTP in the presence of KC$_1$, it is possible that the liberation could have been inhibited by KC$_1$.

Evidence from the present study to support the view that the observed ATPase activity and polymerizing property of G-actin were two different and unrelated reactions included: (1) the two reactions had different pH optima (Fig. 2); (2) it was possible to obtain G-actin devoid of ATPase activity but possessing polymerizing capacity, and vice versa; (3) addition of EGTA activated the ATPase but inhibited the polymerization; and (4) adding KC$_1$ inhibited ATPase activity but was indispensable for polymerization.

Further experiments have been carried out in which the F-actins, obtained by separately polymerizing control and acetylated G-actins, were subjected to sonication in the presence of ATP (30). The results showed that control F-actin actively dephosphorylated ATP, whereas no P$_i$ was liberated with F-actin obtained from acetylated G-actin. Natural F-actin of heart muscle of pigs showed slight Mg$^{2+}$-activated ATPase, confirming similar results reported by Hama et al. (12) with skeletal muscle natural F-actin. The ATPase activity of natural F-actin was less than that of natural G-actin. These results and the fact that adding 0.1 M KC$_1$ and 0.2 M KC$_1$ (conditions favoring polymerization) decreased the ATPase activity of G-actin suggested that the ATPase site of G-actin was partially masked during polymerization to F-actin.

Acknowledgment

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References

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