Studies on Actin

VI. THE INTERACTION OF NUCLEOSIDE TRIPHOSPHATES WITH ACTIN*

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(Received for publication, October 13, 1960)

Although about 10 years have elapsed since the presence of adenosine 5'-triphosphate in G-actin solution and its participation in the polymerization process were demonstrated (2), very little is known about the nature of this interaction. It has been shown that in F-actin about 1 mole of adenosine 5'-diphosphate is bound per 60,000 g of protein (3, 4), and about 1 mole of inorganic phosphate is liberated per mole of G-actin during the G-F transformation.

Removal of ATP from G-actin solutions by dialysis (2), by precipitation at pH 4.75 (2), apyrase (2), hexokinase (5), or H-meromyosin treatment (1) leads to the irreversible loss of polymerizability. The presence in G-actin of stabilizing factors different from ATP has been postulated (5, 6). In a recent paper, Pragy described a G-actin fraction which polymerizes without the splitting of ATP (7).

We have studied the binding of ATP and various nucleoside phosphates to G- and F-actin by ultracentrifugal and Mg precipitation methods. Under identical experimental conditions, considerably more adenosine and inosine 5'-triphosphate than uridine, cytidine, guanosine 5'-triphosphate or adenosine or inosine 5'-diphosphate is bound to G-actin. ATP can be replaced with ITP and the polymerizability of the resulting actin remains unaltered. Substitution of UTP, CTP, GTP, ADP, or IDP, for ATP results in the loss of the polymerizability. In our experience about 0.6 mole of ATP is bound to a mole of G-actin at a free ATP concentration of 10^{-4} M. In accordance with this, the liberation of about 0.5 mole of inorganic phosphate was found to accompany the polymerization of each mole of actin.

A number of different substances including mercurials and Ca-chelating agents cause the displacement of ATP from the binding site with the concomitant loss of the polymerizability of actin. The participation of SH groups directly or indirectly in the ATP binding is implied in the effect of mercurials. The inhibitory effect of ethylenediaminetetraacetic acid and various other Ca complexing agents on the ATP binding and polymerizability of G-actin is compatible with the view that the bound Ca of actin (8) is involved in these processes. It was found that H-meromyosin accelerates the polymerization of actin, and this effect is strongly dependent on the ionic strength of the medium.

EXPERIMENTAL PROCEDURE

Actin was prepared as described earlier (9); however, no MgCl₂ was added during the course of the preparation. Heavy meromyosin fraction was isolated from a chymotrypsin digest of myosin as described earlier (9), with a digestion time of 3 minutes.

Hexokinase, grade III, was purchased from the Sigma Chemical Company.

Creatine kinase was prepared according to Noda et al. (10) by Dr. Heinz Kohler in our laboratory.

Binding of ATP—The binding of ATP to actin was studied by two methods. 1. Actin was precipitated with 0.05 M MgCl₂ (11) in the presence of 10^{-4} M Tris buffer, pH 7.8, and the decrease in the nucleotide and protein concentration of the solution after the removal of the Mg-actin precipitate by centrifugation for 15 minutes at 16,000 × g was measured. Generally, about 90% of the protein originally present was removed by this procedure. In the presence of salyrgan about 40% of the protein was not precipitated by Mg. Both the initial solution and the supernatant obtained after Mg precipitation were deproteinized with 2.5% perchloric acid, and the absorption of nucleotides was measured in a Beckman model DU spectrophotometer in the 240 to 300 nm wavelength range. The nucleotide concentration was calculated with the following molar absorption coefficients: ATP 14.9 × 10³ (260 nm), ITP 12 × 10³ (250 nm), UTP 9.8 × 10³ (260 nm), CTP 13.7 × 10³ (280 nm), GTP 13.4 × 10³ (255 nm), determined in the presence of 2.5% perchloric acid. In the presence of salyrgan, ATP was determined by measuring the amount of inorganic phosphate after hydrolysis in 1 M HCl for 8 minutes at 100°C. Protein was determined by measuring the absorption at 280 nm and applying proper corrections for the absorption of nucleotides or by the use of the biuret method.

The amount of bound nucleotides was calculated by the following formula: moles of bound nucleotides per mole of actin = (N₀ - Nₘₐ₆)/(P₀ - Pₘₐ₆), where N₀ is the original nucleotide concentration; Nₘ₆, the nucleotide concentration in the supernatant after Mg⁺⁺ precipitation; P₀, protein concentration in the original solution; Pₘ₆, protein concentration in the supernatant after Mg⁺⁺ precipitation. The molecular weight of actin was assumed to be 60,000 (12).

2. When the ultracentrifugal method of Chanutin et al. (13)
was used. G-actin solutions containing varying concentrations of ATP were centrifuged in the Spinco model L preparative ultracentrifuge for 6 to 9 hours at 100,000 X g. The contents of the centrifuge tubes were carefully removed in three or four successive layers and the nucleotide and protein contents were determined. The nucleotide concentration of each layer was plotted against the protein concentration, and the apparent free ATP concentration of the system was determined by extrapolation to zero protein concentration. Unless otherwise stated the pH of the medium was maintained between 7.2 and 7.8.

We observed that in the presence of salts there was an appreciable redistribution of nucleotides in the gravitational fields used for studying the binding of ATP to G-actin. Since the sedimentation of nucleotides causes an increase in the calculated amount of bound nucleotides, appropriate corrections were applied wherever the salt concentration of the system made this necessary.

Acid precipitation was carried out by adding 0.05 ml of 1 M acetate buffer, pH 4.75, to 5 ml of a G-actin solution (protein concentration about 5.0 mg per ml). After 5 minutes at room temperature, the precipitate was centrifuged at 2100 X g for 5 minutes. The supernatant was discarded, the sediment was taken up in 10 ml of a 10⁻⁴ M solution of the appropriate nucleotide, and the pH was adjusted to 7.3 to 7.4 with 10 to 20 drops of 10⁻⁴ M NaOH. To the clear solution of actin, 0.1 ml of 1 M acetate buffer, pH 4.75, was added and the precipitate was removed by centrifugation at 2100 X g for 5 minutes. The sediment was dissolved as before, and the precipitation and redissolution steps were repeated once more. In what follows, acid-precipitated actin refers to actin prepared by this method. Actin precipitated at pH 4.75 in the presence of ATP or ITP forms a loose, voluminous sediment, whereas a densely packed pellet appears in the absence of nucleotides or in the presence of UTP, ADP, IDP, CTP, or GTP. Viscosity measurements were carried out in an Ostwald viscometer at 29°. Unless otherwise stated the pH of the system was maintained between 7.2 and 7.8.

In some experiments the polymerization of actin was measured by the light scattering technique with a Brice-Speiser instrument (Phoenix Precision Instrument Company) coupled to a Brown recorder with a cylindrical glass cell of circular cross section with a total volume of 10 ml at 90° to the incident beam. Photooxidation of actin was carried out as described in an accompanying paper of this series (14).

Analytical Procedures—Protein was determined by a micro-Kjeldahl or a biuret procedure. For paper chromatographic separation of nucleotides, Whatman No. 4 paper and isobutyric acid-concentrated NH₄OH-H₂O (66:1:33) solvent were used. Sodium dodecyl sulfate was determined according to Karush et al. (15). Determination of inorganic phosphate was carried out by the method of Fiske and SubbaRow (16) or of Horwitt (17).

Ethylenediaminetetraacetic acid and other chelating compounds were obtained from Dojin Yakukagaku Kenkyusho, 38 Kamidōri-Machi, Kumamoto-Shi Kumamoto Pref., Japan.

RESULTS

ATP Binding of G-Actin—The ATP binding of G-actin as determined by the Mg precipitation and ultracentrifugal techniques is shown in Fig. 1. The apparent number of moles of bound ATP is 0.5 to 0.7 at a free ATP concentration of 10⁻⁴ M and approaches 1 mole per mole of actin at higher ATP concentrations. The ATP binding of actin precipitated with 5 X 10⁻² M CaCl₂ is the same as with Mg. The slightly lower amounts of the bound ATP observed in the ultracentrifugation experiments are probably due to the Donnan effect.¹ The amount of inorganic phosphate liberated during the polymerization is about 0.5 to 0.6 mole per mole of actin. This value is completely independent of the ATP concentration in the range of 10⁻⁴ to 5 X 10⁻³ M (Fig. 2). The inorganic phosphate liberated during the polymerization process apparently is not bound to the protein, since after ultracentrifugal removal of the F-actin at least 90% of the inorganic phosphate formed is present in the protein free supernatant (Table I).

pH Dependence of Binding of ATP to Actin.—The binding of ATP by G-actin considerably increases at lower pH values (Fig. 3). Similar correlation between pH and ATP binding was observed in the case of serum albumin.

¹ The results of the ultracentrifugal ATP binding studies have not been corrected for Donnan effect since the actual net charge of the actin is not known. It is reasonably expected that the corrected value of ATP binding is about 0.7 mole of ATP per mole of actin at a free ATP concentration of 10⁻⁴ M.
Inhibitors of ATP Binding and Polymerization of Actin

1. Effect of Chelating Agents—Actin solutions contain a small amount of Ca and Mg in a firmly bound form. It was assumed by Straub (8) that the bound Mg plays an important role in the polymerization process. In a series of experiments bearing on this problem we have found that chelating agents, like ethylenediaminetetraacetate, 1,2-cyclohexanediaminetetraacetate, diethylenetriaminepentacetate, and ethyl ether diaminetetraacetate, are effective inhibitors of the polymerization and ATP binding of actin at concentrations close to a 1:1 molar ratio of inhibitor to actin. The inhibition of polymerization and ATP binding occurs in a parallel fashion at about the same inhibitor concentration. Nitrilotriacetate, iminodiacetate, dihydroxyethylglycine, and Ca-ethylenediaminetetraacetate were found to be ineffective even at much higher concentrations (Figs. 4 and 5). Hexametaphosphate completely inhibited at low concentration the ATP binding of G-actin without affecting, however, the ADP binding of F-actin (Fig. 6). No inhibition of the ATP binding was observed in the presence of $5 \times 10^{-5}$ M sodium citrate or potassium oxalate or $10^{-3}$ M inorganic pyrophosphate.

2. Effect of Mercurials—It was first shown by Kuschinsky and Turba (18) and later confirmed by other workers (19) that mercurials inhibit the polymerization of actin. They concluded that SH groups are involved in the polymerization process. This inhibition of the polymerizability by salyrgan is completely reversed on the addition of excess glutathione. As shown in Fig. 7, HgCl$_2$ and salyrgan inhibited the ATP binding of G-actin in

![Fig. 4. The effect of chelating agents on the binding of ATP by G-actin. The amount of bound ATP was measured by the ultracentrifugal technique described under “Experimental Procedure.” Protein concentration: 4.25 to 5.80 mg per ml; ATP concentration: 1.3 to 1.6 $\times 10^{-4}$ M; pH 7.5 to 7.8.]

![Fig. 5. The effect of chelating agents on the polymerization of actin. The polymerization of actin solutions (protein concentration: 1.84 mg per ml) containing increasing concentrations of chelating agents was followed, after the addition of KCl and Tris buffer (pH 7.8) to a final concentration of 0.1 M and 0.01 M, respectively, in an Ostwald-type viscometer at 20°C. The data represent specific viscosity values obtained 30 minutes after the addition of KCl.]

![Fig. 3. The effect of pH on the binding of ATP by G-actin. The amount of bound nucleotides was determined by the ultracentrifugal technique. The pH of G-actin solutions (protein concentration 4 to 6 mg per ml) was adjusted by the addition of dilute HCl or NaOH solutions to the values indicated on the abscissa. Total nucleotide concentration, $1.5 \times 10^{-4}$ M.]

### Table I

<table>
<thead>
<tr>
<th>Concentration of P$_i$</th>
<th>Mole of P$_i$ liberated /Mole of actin</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-Actin</td>
<td>0.025</td>
</tr>
<tr>
<td>F-Actin</td>
<td>0.058</td>
</tr>
<tr>
<td>Supernatant</td>
<td>0.055</td>
</tr>
</tbody>
</table>
concentrations exceeding the 5:1 molar ratio of mercurial to actin. Salyrgan had no effect on the ADP binding of F-actin, since no radioactivity appeared in the supernatant of an F-actin solution containing bound C14-ADP (20) after ultracentrifugation in the presence of 10 moles of salyrgan per mole of actin.

3. Effect of Sodium Dodecyl Sulfate—As shown in Fig. 8, the amount of bound ATP sharply decreases with increasing concentrations of sodium dodecyl sulfate if the number of moles of bound sodium dodecyl sulfate exceeds 15 per 60,000 g of actin.

4. Precipitation of Actin at pH 4.75—Actin repeatedly precipitated at pH 4.75 in the absence of ATP or ITP loses its polymerizability (2). The ATP binding of this denatured actin was studied by the Mg precipitation procedure. It was found that the loss of polymerizability was accompanied by an irreversible loss of the ability to bind ATP. No ATP binding was observed at pH 7 in actin preparations that had been kept for 30 minutes at pH 3.0 or pH 11.0 or incubated at 75° for 15 minutes at neutral pH.

“Protective” Action of Nucleoside Phosphates—The results presented above, showing a correlation between loss of bound ATP and loss of polymerizability, are consistent with the early observations of Straub and Feuer (2) that isoelectrically precipitated and redissolved actin did not polymerize on addition of KCl unless the precipitate had been dissolved in an ATP solution. Straub and Feuer have also shown (2) that the presence of ATP during the dialysis of G-actin is necessary in order that its polymerizability may be preserved. They found ADP to be ineffective in “protecting” G-actin, whereas in Szent-Györgyi’s similar experiments, ATP could be replaced by ADP or ITP (21). To obtain information about the structural specificity of the nucleoside triphosphate interaction with actin, the polymerizability of actin precipitated three times at pH 4.75 and redissolved each time in the presence of ATP, ADP, IDP, UTP, CTP, or GTP has been investigated. Polymerization of acid-precipitated actin on addition of KCl took place only if 10^{-4} M ATP or ITP was present during the precipitation procedure outlined under “Experimental Procedure.” When the precipitate was dissolved in H2O or 10^{-4} M UTP, CTP, GTP, ADP, or IDP, there was a drop in viscosity on addition of KCl, rather than the usual increase indicating polymerization (Fig. 9). 

Liberation of Inorganic Phosphate by Acid-precipitated Actin

The depolymerization of an ultracentrifugally sedimented F-actin pellet in the presence of UTP, CTP, GTP, or ADP yields a partially polymerizable G-actin. This can be explained by the formation of ATP in all these cases presumably because of the presence of contaminating transphosphorylating enzymes. The use of the acid precipitation procedure eliminates this complication.
Solutions after Addition of 0.1 M KCl—The polymerization of actin containing about $10^{-4}$ M ATP is accompanied by the liberation of inorganic phosphate (2). If the ATP is replaced by ITP during repeated acid precipitations, no significant change can be observed in the rate of the liberation of inorganic phosphate during the polymerization process. No phosphate is liberated, after the addition of KCl, in actin preparations that were precipitated in the presence of $10^{-4}$ M KCl and 10$^{-2}$ M Tris buffer, pH 7.0. The solution was deproteinized with 5% perchloric acid and the nucleotide in the supernatant was characterized by absorption spectrophotometry and paper chromatography. It had a characteristic absorption maximum at 250 nm and on paper chromatography a single ultraviolet-absorbing spot in a position identical with that of the reference IDP was obtained. Therefore, the firmly bound nucleotide of F-actin obtained by polymerizing a G-ITP actin solution with 0.1 M KCl is IDP.

**Preparation of F-Actin which contains IDP as Bound Nucleotide**—Actin precipitated three times at pH 4.7 in the presence of $10^{-4}$ M ITP was polymerized with 0.1 M KCl and dialyzed for 2 days against 20 volumes of 0.1 M KCl and 10$^{-2}$ M Tris buffer, pH 7.0. The solution was deproteinized with 5% perchloric acid and the nucleotide in the supernatant is characterized by absorption spectrophotometry and paper chromatography. It had a characteristic absorption maximum at 250 nm and on paper chromatography a single ultraviolet-absorbing spot in a position identical with that of the reference IDP was obtained. Therefore, the firmly bound nucleotide of F-actin obtained by polymerizing a G-ITP actin solution with 0.1 M KCl is IDP.

**Effect of H-Meromyosin on Polymerization of Actin as Studied by Light Scattering Technique**—Addition of 0.4 mg per ml of H-meromyosin to a dilute G-actin solution (0.1 mg per ml) immediately after the addition of 0.1 M KCl caused an instantaneous increase in turbidity nearly equal to that observed after the addition of a corresponding amount of H-meromyosin to F-actin. (Table III). After the addition of ATP the scattering returned to a value corresponding to the sum of the scattering of F-actin and H-meromyosin. No similar increase in turbidity was observed if meromyosin was added to a G-actin solution in the absence of salts or in 0.6 M KCl. The increase in light scattering on the addition of H-meromyosin to F-actin was essentially the same in 0.1 and in 0.6 M KCl solution. No detectable polymerization of actin took place in the first 10 minutes after the addition of 0.1 M KCl in the absence of H-meromyosin, provided the protein concentration did not exceed 0.1 mg per ml.

Short photooxidation of G-actin in the presence of methylene blue causes the loss of polymerizability of actin with maintained actomyosin-forming and myosin-ATPase-activating effects (14). When H-meromyosin is added to photooxidized actin under the usual experimental conditions, no light scattering increase takes place, suggesting that the sudden increase in turbidity on mixing H-meromyosin with G-actin in 0.1 M KCl solution is somehow related to the polymerization of actin. G-Actin solutions incubated with H-meromyosin in salt-free medium or in 0.6 M KCl, at room temperature, progressively lose their ability to form a complex characterized by high turbidity with H-meromyosin when the KCl concentration is readjusted to 0.1 M (Figs. 11 and 12).

**Fig. 9.** The effect of various nucleotides on the polymerizability of actin. The viscosity of actin precipitated three times at pH 4.7 and dissolved each time in $10^{-4}$ M solutions of various nucleotides was measured in an Ostwald viscometer at 29°. At zero time KCl and Tris buffer, pH 7.8, were added to a final concentration of 0.1 M and 0.01 M, respectively. •, ATP; +, ITP; Δ, UTP; ○, CTP; □, GTP; ▲, ADP; ◊, IDP; ○, without nucleotide. Protein concentration, 1.0 to 2.4 mg per ml.

**Fig. 10.** Liberation of inorganic phosphate in actin solutions on the addition of KCl in the presence of various nucleotides. To solutions of actin precipitated three times at pH 4.7, with or without added nucleotides, KCl and Tris buffer, pH 7.8, were added to a final concentration of 0.1 M and 0.01 M, respectively. Samples were taken at intervals, and Pi was determined by the method of Horwitt (17). •, ATP; +, ITP; Δ, UTP; ○, CTP; □, GTP; ▲, ADP; ◊, IDP; ○, without nucleotide. Protein concentrations, 2.0 to 2.6 mg per ml.

<table>
<thead>
<tr>
<th>Table II</th>
<th>Binding of various nucleotides to actin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleotide</td>
<td>Average moles of nucleotide bound / 60,000 g actin</td>
</tr>
<tr>
<td>ATP</td>
<td>0.73</td>
</tr>
<tr>
<td>ITP</td>
<td>0.72</td>
</tr>
<tr>
<td>UTP</td>
<td>0.39</td>
</tr>
<tr>
<td>CTP</td>
<td>0.24</td>
</tr>
<tr>
<td>GTP</td>
<td>0.34</td>
</tr>
<tr>
<td>ADP</td>
<td>0.32</td>
</tr>
<tr>
<td>IDP</td>
<td>0.25</td>
</tr>
</tbody>
</table>
TABLE III

Effect of H-meromyosin on light scattering of G- and F-actin solutions of varying ionic strength

In the experiments with G-actin the actin stock solution containing 5 to 6 mg of protein per ml was diluted with H2O to such an extent that the subsequent addition of KCl, 10^{-4} M Tris buffer, pH 7.5, 10^{-4} M MgCl2 brought it to the protein concentrations indicated in the table. H-Meromyosin was added 1 minute after the addition of KCl and the readings were taken 2 minutes later.

A similar procedure was followed in the experiments with previously polymerized F-actin, except that the dilution of the stock F-actin solution was carried out with KCl solutions of varying ionic strength. The intensity of the scattered light was measured at 90° to the incident beam and the values are expressed in arbitrary units.

<table>
<thead>
<tr>
<th>Composition of the system</th>
<th>Concentration (mg/ml)</th>
<th>Light scattering in arbitrary units</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.1 M KCl</td>
</tr>
<tr>
<td>G-Actin</td>
<td>0.1</td>
<td>7</td>
</tr>
<tr>
<td>G-Actin</td>
<td>0.1</td>
<td>59</td>
</tr>
<tr>
<td>H-Meromyosin</td>
<td>0.4</td>
<td>17</td>
</tr>
<tr>
<td>G-Actin</td>
<td>0.1</td>
<td>10</td>
</tr>
<tr>
<td>H-Meromyosin</td>
<td>0.4</td>
<td>68</td>
</tr>
<tr>
<td>F-Actin</td>
<td>0.1</td>
<td>15</td>
</tr>
<tr>
<td>H-Meromyosin</td>
<td>0.4</td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

The results show that the amount of ATP bound to G-actin depends on the concentration of ATP present, and in the free ATP concentration range studied it approaches 1 mole per 60,000 g of protein. Estimation of the binding constant is hindered by the fact that the ATP-binding ability of actin molecules, deprived of ATP at low free ATP concentrations, is lost. The exact rate of this "denaturation" process is unknown, but it is certainly too fast to prevent the use of any of the conventional equilibrium methods for the determination of the binding constant.

The amount of inorganic phosphate liberated during the polymerization process in the presence of increasing ATP concentrations remains remarkably constant and corresponds to about 0.5 mole of inorganic phosphate per mole of actin. This value is about half of that obtained by Mommaerts (12). It cannot be attributed to the presence of inactive actin, since the nonpolymerizable protein content of our G-actin preparation, as characterized by the amount of protein which remains in the supernatant of an F-actin solution after centrifugation at 100,000 × g for 3 hours, is 10% or less. Clearly, further experiments are necessary to explain the reasons for this discrepancy. It should be mentioned that a complete lack of ATP splitting during the polymerization of actin has been reported from two laboratories (7, 22).

The interaction of nucleotides with G-actin depends on certain structural features of both the protein and the ligand. The ATP binding and polymerization of G-actin were inhibited by mercury, suggesting the direct or indirect participation of SH groups in these processes. The similar effect of various chelating agents like hexametaphosphate, ethylenediaminetetraacetic acid, 1,2-cyclohexanediaminetetraacetic acid, and diethylenetriaminopentaacetic acid indicates the involvement of the bound Ca or Mg in the processes of the ATP binding and polymerization. This interpretation is supported by the fact that hydroxyethylendiaminetetraacetic acid, nitrolotriacetic acid, iminodiacetic acid, dihydroxyethylglycine, and Ca-ethylenediamine tetraacetic acid which are forming less stable complexes with Ca or Mg (23) are ineffective. An alternative explanation, however, might be sought in an anion displacement mechanism which is perhaps more likely in the case of the SDS.

Incubation for 30 minutes at pH 3.0 or pH 11.0 completely destroys the ability of G-actin to bind ATP at neutral pH. Since at pH 3 considerable ATP binding persists over at least a 6-hour period, it is probable that the binding of ATP to actin at acid pH involves a different mechanism, possibly similar to the ATP binding of serum albumin. This is supported by the observation of Drabikowski that the ATP binding of heat-inactivated actin is not different from that of the control at around pH 3.0, although no ATP binding occurs at pH 7.0+ Removal of ATP from an actin solution by repeated precipitation of the protein

4 W. Drabikowski, personal communication.
at pH 4.7 leads to the loss of polymerizability; the ability of this
actin to bind ATP at neutral pH is irreversibly lost. It would
seem that ATP when bound to G-actin prevents an irreversible
configurational change, which, if it occurs, is usually manifested
in a slightly increased viscosity accompanied by the loss of
polymerizability.

This stabilizing effect was studied with the pH 4.7 precipita-
tion procedure by substituting ITP, CTP, GTP, UTP, ADP, or
IDP for ATP. Only ATP and ITP protected actin from the
loss of polymerizability during precipitation, whereas ADP, IDP,
UTP, CTP, and GTP were ineffective. Under identical experi-
mental conditions, the binding of ATP and ITP to actin was
about twice as great as that of UTP, CTP, GTP, ADP, and
IDP. Thus, the loss of polymerizability of actin containing
solely UTP, CTP, GTP, ADP, or IDP might be ascribed to its
inability to bind those nucleotides. The interaction of G-actin
with various nucleotides disclosed two important specificity
requirements: (a) first, the triphosphate chain and second, adeno-
ine-like configuration in the 1, 2, 3 positions of the ring. Actin
polymerized in the presence of ITP contains IDP as firmly
bound nucleotide. Study of the IDP-F-actin might provide
further information regarding the role of the actin-bound nu-
cleoside diphosphates in the formation of actomyosin.

In view of the parallelism between the splitting of ATP and
the polymerization process, the question arises whether an in-
crease in the rate of ATP splitting would result in an acceleration
of the polymerization. It was found, with the use of H-mero-
myosin as ATPase, that addition of H-meromyosin to a dilute
actin solution immediately after the addition of 0.1 M KCl causes
an instantaneous increase in light scattering which is nearly
as large as that observed after the addition of a corresponding
amount of H-meromyosin to F-actin.

The large increase in light scattering observed on mixing F-
actin and H-meromyosin is generally interpreted as being due to
the formation of F-actomeromyosin. The decrease of light
scattering on the addition of ATP most probably represents the
dissociation of actomeromyosin into F-actin and H-meromyosin.

The magnitude of the instantaneous light scattering increase
after the combination of G-actin with H-meromyosin seems to
indicate the formation of a complex similar to F-actomeromyos-
in. This requires the rapid transformation of the globular actin
into fibrous actin. Since the polymerization of actin at low
protein concentration is a relatively slow process, it is assumed
that the G-F transformation is considerably accelerated in the
presence of H-meromyosin. This interpretation is supported by
the following facts. (a) On the addition of ATP to a G-acto-
meromyosin system in 0.1 M KCl, the light scattering drops to
about the same level as observed with the F-actomeromyosin.
(b) No light scattering increase takes place with photooxidized
actin that has lost its polymerizability but is still able to com-
bine with myosin. (c) No light scattering increase is observed
on the addition of H-meromyosin to G-actin in salt-free medium
or at high ionic strength (0.6 M KCl) at which no polymerization
of actin takes place. Incubation of G-actin with H-meromyosin
in salt-free medium or in 0.6 M KCl solution leads to a gradual
loss of the turbidity increase on readjusting the ionic strength
to 0.1, indicating that under these conditions the H-meromyosin,
instead of promoting, actually inhibits the polymerization of
actin.

The increased rate of polymerization in the presence of H-
eromyosin in 0.1 M KCl is most probably due to a specific
interaction between G-actin and H-meromyosin accompanied by
the cleavage of ATP, and followed by the aggregation of the
G-H units into what appears to be F-acto-H-meromyosin.

The results can hardly be explained by the splitting of ATP
alone, since splitting of ATP by H-meromyosin also occurs in
salt-free medium or in 0.6 M KCl, but without resulting in in-
creased light scattering indicative of the formation of F-actin.
Furthermore, other ATP dephosphorylating systems—hexo-
kinase-glucose, creatine-creatine kinase, apyrase—seem to
inhibit rather than promote the polymerization of actin. These
results support and extend the earlier observations of Laki and
Clark (24) regarding the acceleration of the polymerization of
actin by myosin.

SUMMARY

1. The nucleoside phosphate binding of G- and F-actin was
studied by equilibrium dialysis, ultracentrifugal sedimentation,
and Mg precipitation methods.
2. It was found that the binding of various nucleoside phos-
phates to actin has two specificity requirements—a triphosphate
chain and an adenine-like configuration in the 1, 2, 3 position
of the ring—satisted only by adenosine 5'-triphosphate and
inosine 5'-triphosphate, but not by uridine 5'-triphosphate,
cytidine 5'-triphosphate, guanosine 5'-triphosphate, adenosine
5'-diphosphate, and inosine 5'-diphosphate.
3. The polymerizability of actin is lost if adenosine 5'-tripho-
phate or inosine 5'-triphosphate is replaced by uridine 5'-tri-
phosphate, guanosine 5'-triphosphate, cytidine 5'-triphosphate,
adenosine 5'-diophosphate, or inosine 5'-diophosphate.
4. The dependence of the adenosine 5'-triphosphate binding of
G-actin on pH and free adenosine triphosphate concentration was
determined.
5. The inhibitory action of mercurials and Ca-complexing
agents on the binding of adenosine 5'-triphosphate and poly-
erization of actin suggests the involvement of SH groups and
the bound Ca or Mg of actin in these processes.
6. Depending on the ionic strength of the medium, H-
eromyosin promotes (0.1 M KCl) or inhibits (salt-free medium
or 0.6 M KCl) the polymerization of actin.

Acknowledgments—The authors wish to express their gratitude
to John Gergely for his interest in this work and for his help in
the preparation of the manuscript, and to W. M. Kuehl for
carrying out some of the light scattering experiments.

Addendum—The results presented in this paper together with
our earlier experiments (Martonosi, A., Gouvea, M. A., and
Chem., 235, 1700 (1960)) clearly establish the role of ATP as
the prosthetic group of G-actin. The references made in con-
nection with our report by Ulbrecht et al. (Biochem. et Biophys.
Acta, 45, 443 (1960)), attributing to us the view that the pro-
thetic group of G-actin is ADP, are erroneous.

It should be mentioned that there is a good agreement between
our results and those of Ulbrecht et al. regarding the fact that
both the amount of ATP bound in the usual G-actin prepara-
tions and the amount of inorganic phosphate liberated in an ap-
narently complete polymerization process are considerably less than 1
mole per mole of actin.
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A. Martonosi and M. A. Gouvea


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