Binding of Myosin A to F-Actin*

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The studies of Szent-Györgyi (1) have clearly established the formation of actomyosin from F-actin and myosin A. Szent-Györgyi (1), Portzehl, Schramm, and Weber (2), Jaisle (3), Snellman and Gelotte (4), and Spicer and Gergely (5) have suggested from their viscometric and centrifugal studies that the maximal amount of myosin A which can be bound by F-actin is in the ratio of 2 to 4:1, by weight. Laki, Spicer, and Carroll (6) and Portzehl et al. (2) have reported that the binding reaction is a reversible one which depends on temperature and ionic strength. However, it seems difficult to determine, by viscometric or ultracentrifugal experiments, the binding ratio of myosin A to F-actin, since such experiments must usually be performed under conditions involving large intermolecular interactions of fibrous proteins. Gergely and Kohler (7) have recently applied the method of light scattering, which can be used with protein solutions of much lower concentration. It may, however, be pointed out that under their experimental conditions the dissociation constant for the binding reaction is so small that its accurate measurement may be very difficult.

So far, no kinetic study has been made of the binding reaction of myosin A to F-actin. In the present study the velocity and equilibrium constants for the binding of myosin A to F-actin have been determined, under various experimental conditions, by use of the light scattering method. Furthermore, we have found that the optical rotatory dispersion changes slightly with the formation of actomyosin, and that actomyosin formation is a reversible one which depends on temperature and ionic strength. However, it seems difficult to determine, by viscometric or ultracentrifugal experiments, the binding ratio of myosin A to F-actin, since such experiments must usually be performed under conditions involving large intermolecular interactions of fibrous proteins. Gergely and Kohler (7) have recently applied the method of light scattering, which can be used with protein solutions of much lower concentration. It may, however, be pointed out that under their experimental conditions the dissociation constant for the binding reaction is so small that its accurate measurement may be very difficult.

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Experimental

Materials—Myosin A solution was obtained by the method of Perry (8), with slight modifications (9). This solution was used for measurements within 2 or 3 days after decapitation of the rabbit. Crude G-actin was prepared from acetone powder of rabbit skeletal muscle (10), according to the procedure of Mommaerts (11). The extract was adjusted to pH 8 and brought to 0.1 M KCl. F-actin was precipitated by ultracentrifugation at 7 × 10^4 g for 2 hours. The precipitate was dissolved in 0.1 M KCl and 0.4 mM ATP at pH 8.2. The solution was dialyzed for a week at 2° against a solution of 0.1 mM ATP at pH 8.2 (adjusted by Tris). The G-actin solution was then clarified by ultracentrifugation at 7 × 10^4 g for 2 hours. In the equilibrium studies, G-actin was transformed into the F form in 0.1 M KCl. In the kinetic studies, G-actin was transformed into the F form after removal of free ATP from the G-actin solution by passage through a column (1 × 6 to 1 × 7 cm) of Dowex 1, 200- to 400-mesh, which had been equilibrated with a buffer of 0.08 M NH&l-NH40H at pH 8.2 (12). Under the experimental conditions of the kinetic studies the concentration of free ATP was much lower than 10^-8 M.

Methods—Measurements of the scattering of light were made at an angle of 90° from the incident beam with a Brice-Phoenix photometer, or with the apparatus described previously (13). If necessary, the temperature of the solution was kept constant by circulation of water around the cell from an ultrathermostat (type NBS, Haake, Germany).

As indicated by Gergely and Kohler (7), the light scattering increment due to the combination of a myosin A molecule (M) with an actin site (A_A) depends on Step j (1 ≤ j ≤ n) of the equation, 

\[
M + \sum_{j=1}^{n} A_n M_{j-1} - A_n M_j
\]

where n is the number of binding sites of an F-actin molecule. Therefore, the extent of combination of myosin A with the actin site, a, was determined as follows: The light scattering increment due to the jth step depends on j, and the increment of a fixed jth step is assumed to be constant under the various experimental conditions, provided the size and shape of both the myosin A and the F-actin molecules do not vary throughout the experiments. Then, a is given by the equation,

\[
a = \frac{[AM]}{[A]} = f \times \frac{\Delta}{\Delta_m}
\]

where [A] is the total molar concentration of the binding site of F-actin, [AM] is the molar concentration of the actin site combined by myosin A, Δ is the light scattering increment resulting from the binding of myosin A at varying concentrations with F-actin at a constant concentration, and Δ_m is the maximum Δ resulting from the addition of sufficient myosin A to saturate the binding site of F-actin. The factor, f, is determined by measuring Δ/Δ_m under conditions wherein the dissociation constant is so small that all myosin A molecules bind to the F-actin site until saturation is attained. When Δ/Δ_m is plotted against the total molar concentration of myosin A [ΣM], f at each Δ/Δ_m is determined as the ratio of the value on the straight line connecting the origin and the saturation point (a, Fig. 1) to the observed value of Δ/Δ_m (a/b, Fig. 1), since f depends only on the extent of occupation of the F-actin site by myosin A, as mentioned above. As will be described below, the binding strength decreased with increasing combination of myosin A, even under favorable conditions (pH 7.5 and 25°). Therefore,
where a is determined as \( f X A/A,,\), as mentioned above. and K is independent of j, from the equation (14), interactions between bound myosin A molecules are negligible A,m+r + m, can be calculated, in the range of low j where the Therefore, the dissociation constant, K, of the reaction A,mj =

The binding of myosin A to F-actin is a reversible reaction. The final concentration of F-actin was about 0.03 mg per ml, and that of myosin A varied from 0 to 0.5 mg per ml. The dissociation constant, K, of the reaction A,mj =

The equilibrium studies were conducted with the use of 0.6 M KCl adjusted to pH 7.5, unless otherwise stated. In these experiments 0.3 ml of a fixed concentration of F-actin was added to each of a series of 10-ml portions containing varying amounts of myosin A. The final concentration of F-actin was about 0.03 mg per ml, and that of myosin A varied from 0 to 0.5 mg per ml. The binding of myosin A to F-actin is a reversible reaction. Therefore, the dissociation constant, K, of the reaction A,mj = A, Mj−1 + M, can be calculated, in the range of low j where the interactions between bound myosin A molecules are negligible and K is independent of j, from the equation (14),

where a is determined as \( f \times \Delta A,\) as mentioned above.

The kinetic studies were usually performed at pH 7.0 and 5-10°C in a 0.6 M KCl medium. Two milliliters of myosin A solution were blown into 8 ml of F-actin solution with a pipet with cut tip, and the increase in \( \Delta A,\) with time was recorded by a micrograph (type BD2, Kipp and Sonnen, Holland). The velocity constant was determined by multiplying the initial velocity of the increase of \( \Delta A,\) by the factor, f. The final constants of myosin A and F-actin were usually 0.04 and 0.01 mg per ml, respectively.

Optical rotation was measured by means of a model 200S-80Q photodensitometer with an oscillating polarizer prism (O. C. Rudolph and Sons). A xenon compact arc lamp was used as the source for continuous spectra (250 to 600 mp). The polarimeter could be set to about 0.002°. Water-jacketed, 10-cm polarimeter tubes, maintained at 25°C by circulation of water from an ultrathermostat, were used for the measurement. The concentration of actomyosin was about 0.6 mg per ml, because actomyosin solution showed a strong birefringence in the tube at concentrations higher than 1 mg per ml. The angle of rotation of actomyosin varied from −0.05° to −0.2° in the range of 450 to 320 mp. All results were expressed in terms of the equation of Moffitt and Yang (15),

\[
[m'] = \frac{3}{2} a + \frac{9}{100} [\alpha] = a + \frac{9b}{(2a - 3)}
\]

in which \([m']\) is the so-called effective residue rotation at any wave length, \( \lambda,\) this being the observed specific rotation, \([\alpha],\) corrected for the effects of the refractive index, \( n,\) and the average molecular weight of the single residue, \( M.\) The \( M.\) was calculated from the amino acid analyses of actin and of myosin A (16). The adjustable parameter, \( \lambda,\) was taken as constant and equal to 2140 A.

Modification of F-actin by 2, 4, 6-trinitrobenzenesulfonate was accomplished as follows. Trinitrobenzenesulfonate solution, adjusted to pH 8.2, was added to G-actin at pH 8.2 and at 2°C. The molar ratio of trinitrobenzenesulfonate to G-actin (molecular weight, 6.1 × 10⁵) (17) was 30. The pH of the mixture was maintained in the range of 8.2 to 8.4 by adding 0.2 M Tris. For observation of the reaction spectrophotometrically, aliquots of the reaction mixture were taken out at appropriate times and their pH values were adjusted to 7.0 to slow down the velocity of binding of trinitrobenzenesulfonate to G-actin. G-actin was transformed into the F form by the addition of 0.1 M KCl. The amount of bound trinitrophenyl groups was calculated from the absorption maximum of 345 mp with a molar extinction coefficient of 1.45 × 10⁴ (18).

The concentrations of the proteins were calculated by multiplying the nitrogen contents, determined by the micro-Kjeldahl method, by a factor of 6; or were determined by the biuret reaction (19). In the latter case the standard curves for myosin A and F-actin were constructed on the basis of their nitrogen content.

RESULTS

Equilibrium Studies

Binding Ratio—At 25°C and pH 7.5, various concentrations of myosin A were added to 0.08 mg per ml of F-actin (Fig. 1). Here, the value of K was too low to be measured, but it was estimated to be less than 10⁻⁴ M (see the next paragraph). If 10⁻⁴ M is taken as K, almost all myosin A molecules should be bound to F-actin in a range of a lower than 0.8. By extrapolating the curve \( \Delta A/m \) against \( [M],\) the weight of F-actin which can be bound by 1 mole of myosin A (molecular weight taken as 594 × 10⁵) was estimated to be 1.59 × 10⁴ g for five preparations. The binding ratio (F-actin to myosin A = 1:37 by weight) agrees completely with that obtained by Gergely and Kohler (7) from determination of the molecular weight of acto-
As already suggested by Laki et al. (6), the binding was fortified by elevation of temperature. For example, in the case of myosin A No. 57 and F-actin No. 15, the values of $K$ at 17° and 6° were 1.07 and $62.5 \times 10^{-8}$, respectively. When 0.06 mg per ml of myosin A No. 60 and 0.03 mg per ml of F-actin No. 21 were allowed to stand at 24°, $\Delta$ was 116. When the cell was cooled to 10.6°, $\Delta$ decreased to 71. When the cell was then returned to 24°, $\Delta$ increased gradually to the original value (Fig. 3). These results indicate that the reaction between myosin A and F-actin is reversible. In Fig. 4, log $K$ is plotted against the reciprocal of absolute temperature, $1/T$. The enthalpy change, $\Delta H$, calculated from the slope was very large and positive, $+57.5$ kg cal per mole. The thermodynamic and kinetic constants of the reaction are listed in Table I. This reaction shows a comparatively large negative free energy change as the net result of the very large $\Delta H$ and entropy change ($\Delta S = +234$ cal mole$^{-1}$ deg$^{-1}$).

**Effect of Ionic Medium**—The intrinsic dissociation constant, $K$, depends on pH, as shown in Fig. 5. The value of $K$ was reduced 3.2-fold with an increase in pH from 6.3 to 7.3, both at

**TABLE I**

| $K$ (at 9°) | $\Delta S$ | $\Delta S$ | $k$ (at 9°) | $\Delta H$ | $\Delta S$
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>mol/l</td>
<td>$k$ cal/mol</td>
<td>mol deg$^{-1}$ mol$^{-1}$</td>
<td>mol</td>
<td>$k$ cal/mol</td>
<td>mol deg$^{-1}$ mol$^{-1}$</td>
</tr>
<tr>
<td>$4.6 \times 10^{-7}$</td>
<td>$+57.5$</td>
<td>$+234$</td>
<td>$1.9 \times 10^8$</td>
<td>$+11.5$</td>
<td>$+6.2$</td>
</tr>
</tbody>
</table>
As is well known, the extent of the binding was reduced by an increase in concentration of KCl. In Fig. 6, \( \log K \) was plotted against the square root of the ionic strength, \( \sqrt{\mu} \). \( K \) increased 4.8-fold with increase in KCl concentration from 0.8 to 1.5 M, and \( \log K \) against \( \sqrt{\mu} \) was linear within this range of KCl concentration. On the other hand, the addition of 10% ethanol decreased \( K \) to 0.45 of the control value.

Optical Rotatory Dispersion—In Fig. 7 are shown the Moffitt-Yang plots of the optical rotatory dispersion curves of the myosin A-F-actin system (myosin A to F-actin = 4:1) in 0.6 M KCl and at pH 7.5 and 25°C. The rotary dispersion of reconstituted actomyosin and F-actin could not be determined with high accuracy, because they could be measured only at low protein concentration. It is seen in the figure that the \(-a_0\) term in

\[
\begin{align*}
\text{Fig. 5.} & \quad \text{Dependence of dissociation constant on pH.} \\
\text{The dissociation constant, } K, \text{ was determined by the procedure shown in the text and in Fig. 2 from measurements of } f_{\Delta A/\Delta m} \text{ at } 0.029 \text{ mg per ml of F-actin No. 14 and various concentrations (0.05, 0.1, 0.15, 0.2, 0.3, 0.4 and 0.6 mg per ml) of myosin A No. 54; 0.6 M KCl. } \circ, 6.5^\circ; , 16.5^\circ.
\end{align*}
\]

\[
\begin{align*}
\text{Fig. 6.} & \quad \text{Effect of KCl concentration on dissociation and rate constants.} \\
\text{The dissociation constant, } K, \text{ was determined by measuring } f_{\Delta A/\Delta m} \text{ at a fixed concentration of F-actin and various myosin A concentrations (0.03, 0.05, 0.075, 0.11, 0.15, 0.2, 0.3 and 0.6 mg per ml). The rate constant, } k, \text{ was determined from initial rate of increase in } f_{\Delta A/\Delta m} \text{ at fixed concentration of myosin A and of F-actin. } \circ, pK \text{ versus } \sqrt{\mu}, \text{ pH 7.5, 20°C; myosin A No. 58, 0.03 mg per ml of F-actin No. 16; } X, \text{ pK versus } \sqrt{\mu}, \text{ pH 7.5, 17°C, myosin A No. 57, 0.037 mg per ml of F-actin No. 15; } \bullet, \log k \text{ versus } \sqrt{\mu}, \text{ pH 7.0, 10°C, 0.04 mg per ml of myosin A No. 59, 0.01 mg per ml of F-actin No. 17. Changes in refractive index of solvent and in refractive index increment of protein by increase in KCl concentration were corrected in calculating } [\Delta M]/[\Sigma A] \text{ from } \Delta A/m.
\end{align*}
\]

\[
\begin{align*}
\text{Fig. 7.} & \quad \text{Moffitt-Yang plots of optical rotatory dispersions of actomyosin and myosin A.} \quad \text{0.6 M KCl, pH 7.5, 25°C.} \quad \text{Actomyosin (ratio, myosin A No. 67 to F-actin No. 28 = 4:1): } \circ, \text{ before mixing; } X, \text{ after mixing; } \bullet, \text{ in 9.15 M urea. Myosin A No. 62; } \Delta, \text{ 0.6 M KCl; } \Delta, \text{ in 8.5 M urea. Vertical bars indicate limits of accuracies of values of actomyosin. Accuracies of values of myosin A and F-actin are higher than those of actomyosin.}
\end{align*}
\]

creases and the \(-b_0\) term decreases slightly with formation of actomyosin. Even when the F-actin or actomyosin solution (0.5 to 0.6 mg per ml) showed birefringence immediately after being inserted into the tube, the birefringence decreased with time, and several hours later it did not interfere with the optical rotatory measurement. In such a case, the \(a_0\) term appeared to change remarkably with the formation of actomyosin, although the decrease of the \(-b_0\) term by several per cent was observed without fail for all six preparations tested. This may be due to the circumstance that slight orientation of the helical polypeptide chain conspicuously changes the \(a_0\) term and slightly changes the \(b_0\) term (20). In Table II are listed several typical results showing optical rotatory dispersion in the myosin A-F-actin system. The excess right-handed helical content, which was estimated by the Doty method (21), decreased by several per cent (2% from the \(a_0\) or 5% from the \(b_0\) term) on the formation of actomyosin. The helical content of myosin A was 57 to 60% or 56 to 62%; and of F-actin, 44 to 58% or 30 to 43%. The value of myosin A agrees with that estimated previously by Cohen and Szent-Györgyi (22) from the \(b_0\) term, but the value of actin is much higher than that reported by Kay (17).

Modification of Actin by Trinitrobenzenesulfonate—In Fig. 8 is shown the time course for the combination of trinitrobenzenesulfonate with G-actin. The binding of 1 mole of the reagent to 1 mole of G-actin occurred rapidly; thereafter, the rate of increase was gradual. The increment of absorption spectrum due to the combination of trinitrobenzenesulfonate (0.7 and 1.6 mole per mole of G-actin) agreed well with the absorption spectrum of the \(\varepsilon\)-trinitrophenyllysine, in the range of 340 to 500 mp. Furthermore, no N-terminal amino acid can be detected in G-actin by the fluorodinitrobenzene method (23). Accordingly, it is concluded that one amino group, probably of a lysine residue, in the G-actin molecule is attacked specifically by trinitrobenzenesul-
TABLE II

Excess right-handed helical contents of muscle proteins

<table>
<thead>
<tr>
<th>Protein</th>
<th>Concentration (mg/ml)</th>
<th>Medium</th>
<th>$a_0 - b_0$</th>
<th>Helical content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myosin A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. 62</td>
<td>6.2</td>
<td>0.6 M KCl</td>
<td>103</td>
<td>58.4</td>
</tr>
<tr>
<td>No. 68</td>
<td>6.1</td>
<td>0.6 M KCl</td>
<td>110</td>
<td>57.4</td>
</tr>
<tr>
<td>No. 69</td>
<td>0.97</td>
<td>0.6 M KCl</td>
<td>106</td>
<td>59.8</td>
</tr>
<tr>
<td>F-actin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. 29</td>
<td>0.5</td>
<td>0.6 M KCl</td>
<td>174</td>
<td>57.9</td>
</tr>
<tr>
<td>No. 31</td>
<td>0.5</td>
<td>0.6 M KCl</td>
<td>236</td>
<td>48.8</td>
</tr>
<tr>
<td>No. 32</td>
<td>0.5</td>
<td>0.6 M KCl</td>
<td>179</td>
<td>57.5</td>
</tr>
<tr>
<td>Sum of values of Myosin A No. 67 and F-actin No. 29</td>
<td>$M = 1.0^*$</td>
<td>0.6 M KCl</td>
<td>137</td>
<td>55.3</td>
</tr>
<tr>
<td>Actomyosin (myosin A No. 67, F-actin No. 29)</td>
<td>$A = 0.25$</td>
<td>8.5 M urea</td>
<td>513</td>
<td>0.0</td>
</tr>
</tbody>
</table>

* Specific optical rotation of myosin A did not depend appreciably on concentration in the range of 0.5 to 1.0 mg per ml.

Fig. 8. Time course of binding of trinitrobenzenesulfonate with G-actin. Ordinate, moles of bound trinitrophenyl group per mole of G-actin. pH 8.2, 3°. 52.1 μM G-actin No. 29 (molecular weight, 6.1 X 10^6), 1.56 mM trinitrobenzenesulfonate.

Fig. 9. Effects of binding of trinitrobenzenesulfonate with G-actin on G-F transformation of actin and binding of F-actin with myosin A. Degree of polymerization; X, G-actin No. 29, 0.1 M KCl, 5 mM MgCl₂; Δ, G-actin No. 31, 0.1 M KCl; pH 6.7, room temperature. Light scattering increment after mixing actin and myosin A; ○, actin No. 29, myosin A No. 67; ●, actin No. 30, myosin A No. 68; ●, actin No. 31, myosin A No. 69; 0.6 M KCl, pH 7.0, 10°, 0.04 mg per ml of myosin A, 0.01 mg per ml of F-actin.

Fig. 10. Relation between initial velocity of actomyosin formation and concentration of myosin A. Initial velocity is calculated from rate of actomyosin formation which is shown in inserted figure. Extent of occupation of active site with myosin A, [AM]/[ΣA], was determined by multiplying measured value of Δ/Δ₀, by Factor f, which was determined by the procedure shown in Fig. 1. The initial velocity of the increase of [AM]/[ΣA] is equal to f[ΣM]. 0.6 M KCl, pH 7.0, 9°. 0.01 mg per ml of F-actin No. 16. Concentration of myosin A No. 58, ○, 0.01; ●, 0.02; X, 0.04; Δ, 0.08 mg per ml.
formation after the addition of various concentrations of myosin A (0.01 to 0.08 mg per ml) to F-actin (0.01 mg per ml), and the dependence of the initial velocity on the concentration of myosin A. The initial velocity of the binding was proportional to the concentration of myosin A. From observations with increasing F-actin concentration, with myosin A held constant, it was also shown that the initial velocity is proportional to the concentration of F-actin. Therefore, the reaction is second order. The velocity constant, $k$, at $9^\circ$ was found to be $1.9 \times 10^4$ M$^{-1}$ sec$^{-1}$ for myosin A No. 58 and F-actin No. 16.

Effect of Temperature—As shown in Fig. 4, the plot of log $k$ against $1/T$ was approximately linear. The calculated energy of activation, $E$, from this line was 12.1 kg calories per mole. Accordingly, the enthalpy of activation at room temperature, $\Delta H^\circ = E - RT$ ($R$ is the gas constant), is calculated to be 11.5 kg calories per mole.

Effect of Ionic Medium—As indicated in Fig. 11, the value of $k$ increased 5-fold with an increase in pH from 6.2 to 7.4. $k$ decreased with increasing KCl concentration in the medium. The plot of log $k$ against $\sqrt{\mu}$ is seen in Fig. 6 to be a straight line in the range of KCl concentrations of 0.5 to 1.5 M. As shown in Fig. 12, Mg$^{++}$ enhanced the reaction velocity significantly and Ca$^{++}$ did so moderately. For instance, 0.5 mM Ca$^{++}$ and Mg$^{++}$ increased $k$ 2.4- and 4-fold, respectively. The divalent cation which may be contained in myosin A and F-actin preparations does not, however, seem to be an essential factor for the binding reaction, because 10 mM EDTA did not significantly decrease the reaction velocity of the system to which no divalent cation was added.

Discussion

The binding of myosin A to F-actin is a completely reversible reaction. By viscometric and light scattering methods, the size and shape of the myosin A molecule was observed to be only slightly dependent on temperature (5-25$^\circ$), on pH (6.3 to 8.2), or on KCl concentration (0.6 to 1.5 M). Furthermore, the reduced viscosity of F-actin, which was measured 30 or 40 minutes after each change of medium, was decreased by only approximately 4% when the KCl concentration was changed from 0.6 to 1.5 M, and by only 3% when the temperature was lowered from 20$^\circ$ to 5$^\circ$; it did not change at all when pH was increased from 6.3 to 7.5. Therefore, the effects of temperature and ionic media on the equilibrium and the rate constants for actomyosin formation probably are not significantly influenced by changes in gross properties of both proteins.

The standard entropy of activation, $\Delta S^\circ$, may be evaluated from the equation (24):$^3$

$$k = \frac{RT}{Nh} \exp(T\Delta S^\circ - \Delta H^\circ)/RT$$

where $N$ is the Avogadro constant and $h$ is the Planck constant. Utilizing the value of $\Delta H^\circ$ (11.5 kg calories per mole) and the measured velocity constant for binding, $k$ (at $9^\circ$, $k \sim 2 \times 10^4$ M$^{-1}$ see$^{-1}$), $\Delta S^\circ$ is found to be slightly positive and is approximately 6.2 cal deg$^{-1}$ mole$^{-1}$. It can be seen in Table I that the binding reaction of myosin A to F-actin is accompanied by extremely large positive entropy and enthalpy changes, and that the entropy and enthalpy changes are much larger than the entropy of activation and the enthalpy of activation.

The thermodynamic constants have already been measured for some antigen-antibody reactions. For the rabbit antibody complex with sulfanyl ovalbumin, $\Delta H = -2.85$ kg calories per mole and $\Delta S = +21$ cal deg$^{-1}$ mole$^{-1}$ (25); and for rabbit antibody complex with human serum albumin, $\Delta H = -3.66$ kg calories per mole and $\Delta S = +14$ cal deg$^{-1}$ mole$^{-1}$ (26). In the case of the dimerization of insulin molecules, $\Delta H$ and $\Delta S$ were found to be $-8.1$ kg calories per mole and $-12.1$ cal deg$^{-1}$ mole$^{-1}$, respectively (27). Furthermore, von Hippel, Gollert, and Morales (28) observed that some components of myosin B dissociate reversibly when the temperature is lowered. As noted by Doty and Myers (27), these positive or small negative values of $\Delta S$ seem to be quite unexpected as those of binding reactions. The entropy change of binding of myosin A to a structureless F-actin fiber based upon the loss of translational and rotational freedom.

$^3$ The transmission coefficient was assumed to be 1. In the case of a complicated reaction such as the present one, the coefficient is expected to be smaller than 1, and the legitimate value of $\Delta S^\circ$ may be smaller than the figure calculated here.
Taking as models for myosin A a cylinder 2000 Å in height and with a molecular weight of 5.94 × 10^5, and for F-actin a cylinder 12,000 Å in height and with a molecular weight of 3 × 10^6, -105 cal deg⁻¹ mole⁻¹ is calculated as the decrease in entropy. Thus, the observed value of the entropy change (+234 cal deg⁻¹ mole⁻¹) is 339 cal deg⁻¹ mole⁻¹, higher than the value expected for the binding.

As pointed out by Kauzmann (30), both electrostatic bonds and hydrophobic bonds are stabilized predominantly by entropy effects rather than by energy effects. In actomyosin formation, electrostatic effects seem to play a dominant role, because it was found that KCl is able to dissociate the complex. The observation that ethanol decreases the dissociation constant also supports this view. The mechanism of the ethanol effect may, however, be very complicated, because 10% ethanol reduced the viscosity of F-actin by 10% and promoted polymerization of myosin A (9).

The binding of myosin A to F-actin was strengthened by a shift of pH to the alkaline side of the isoelectric points of both proteins. This suggests that the reaction depends on the dissociation of specific groups on the protein molecule rather than on the total charge of the molecule. Bailey and Perry (31) reported that the binding of myosin A to F-actin is prevented by modifications of the sulfhydryl groups of myosin A, but not of actin. As described above, when one amino group, probably of a lysine residue, of actin was modified by trinitrobenzenesulfonate, the transformation of G-actin to F-actin was not inhibited but trinitrophenyl-F-actin could not bind with myosin A; trinitrophenyl-myosin A can be bound to intact F-actin (33).

Accordingly, it is plausible to conclude that the salt linkage between a sulfhydryl group of myosin A and an amino group of F-actin, -SH...H₂N⁺, is one of the bonds essential to actomyosin formation, although the present results cannot exclude the possibility that the salt linkage between a hydroxyl group and an amino group is essential to the binding. The dependence of K on pH may be partly attributed to the dissociation of a sulfhydryl group, -SH = -S⁻ + H⁺, and the promoting effect of divalent metal ions on the binding may be due to a decrease in nonspecific electrostatic repulsion between myosin A and F-actin, the net charges of both proteins being negative at pH 7.0.

It hardly seems reasonable, however, to attribute the large positive ∆S and ∆H values for this reaction merely to electrostatic effects (cf. Ref. (30), p. 47). Two possible explanations may be suggested for the extraordinarily large and positive ∆S (34): (a) Protein molecules may be unable to combine in their native states, and a partial unfolding in the neighborhood of the reaction centers may be a necessary prelude to the linkage. (b) The binding of protein molecules involves a large surface of contact between them, with an accompanying dehydration of charged groups on each molecule over this portion of the molecular surface. Since the hydration of charged groups is accompanied by a large decrease in entropy, a dehydration process will yield a corresponding increase (27). As mentioned above, the excess right-handed helical content seemed to decrease by several per cent with the formation of actomyosin, although this result was not conclusive because of the orientation effect of fibrous proteins. Thus, the second mechanism may be adequate.

According to Satake (18, 32), trinitrobenzenesulfonate reacts strictly with primary amino group. Furthermore, the binding of myosin A to F-actin is not inhibited by modification of sulfhydryl groups of actin (31).

in the present case, to explain the observed large increase in entropy.

The changes in optical rotatory dispersion of myosin A caused by adding pyrophosphate and by adding F-actin were observed to be of the same order. It is well known that pyrophosphate binds to myosin A (35, 36) and breaks the bond between myosin A and actin, in which an amino group has a share (7, 13, 37). Furthermore, comparison of the dependence of myosin A ATPase activity on divalent cations with that of actomyosin suggests that the conformation around the ATPase-active site of myosin A becomes loose as the latter combines with F-actin (38). Therefore, it may be concluded that the decrease in the helical content with actomyosin formation occurs mainly in the myosin A moiety rather than in actin.

**SUMMARY**

1. The dissociation constant, K, for the binding of myosin A to F-actin was measured under a wide range of experimental conditions, and the following results were obtained. (a) The binding ratio of myosin A and F-actin is 3.7:1, by weight. (b) The reaction is an endothermic equilibrium reaction. K is 4.6 × 10⁻⁴ M in 0.6 M KCl and at pH 7.5 and 9. The entropy and enthalpy changes of the binding are +234 cal deg⁻¹ mole⁻¹ and +57.5 kg calories per mole, respectively. (c) K decreases when pH is increased from 6.3 to 7.6, and increases with increased ionic strength. It decreases on addition of ethanol.

2. The following results were obtained from measurement of the rate of the binding. (a) Velocity of binding is proportional to the concentrations of both myosin A and F-actin. The rate constant, k, is 1.9 × 10⁸ m⁻¹ sec⁻¹ in 0.6 M KCl and at pH 7.0 and 9. (b) The rate constant, k, increases with a rise in temperature. The enthalpy of activation is estimated to be +11.5 kg calories per mole. (c) The rate constant, k, increases with an increase in pH from 6.3 to 8.0 and decreases with increased ionic strength. It increases on addition of Ca²⁺ and especially of Mg²⁺.

3. The optical rotatory power of the myosin A-F-actin system changes slightly on formation of actomyosin. The excess right-handed helical content estimated by the Doty method decreases by several per cent on formation of the complex.

4. When one amino group, probably of a lysine residue, of G-actin is modified by 2,4,6-trinitrobenzenesulfonate, the transformation of G-actin to F-actin is not inhibited, but the actomyosin formation is inhibited.

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**REFERENCES**


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Binding of Myosin A to F-Actin
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