BBA 45762

BEHAVIOR OF DIVALENT CATIONS AND NUCLEOTIDES BOUND TO F-ACTIN*

MICHIKI KASAI AND FUMIO OOSAWA

Department of Physics and Institute of Molecular Biology Faculty of Science, Nagoya University, Nagoya (Japan)

(Received September 16th, 1968)

SUMMARY

1. Nucleotides and divalent cations bound to F-actin were found to be slowly exchanged in the absence of any agitation such as sonic vibration; the half-life time, $\tau_{1/2}$, was about 5 h at 37°.

2. The rate of exchange of divalent cation (Ca^{2+}) was always a little faster than that of nucleotide (ADP); however, the general manner of the exchange of divalent cations was similar to that of nucleotides.

3. The rate of exchange was almost independent of protein concentration. High temperature or high pH increased the rate of exchange. Activation enthalpies for the exchange of ADP and Ca^{2+} were both about 25 kcal/mole at neutral pH.

4. The release of divalent cations or nucleotides occurred in divalent-cation- or nucleotide-free solvent. The incorporation of divalent cations and nucleotides into divalent-cation- and nucleotide-free F-actin was examined, and their binding constants were estimated.

5. In the absence of ATP, the rate of exchange was decreased by myosin, H-meromyosin and tropomyosin. A large increase of Ca^{2+} and ADP exchange was observed with superprecipitation.

6. The mechanism of exchange or release is discussed on the basis of two models: the cycle of partial destruction of the F-actin structure, and the G–F equilibrium cycle at the peripheral region of F-actin.

INTRODUCTION

Studies made on nucleotides bound to actin over the past few years have shown that ATP bound to G-actin is exchangeable with ATP in the solvent[3,4], but ADP bound to F-actin is not exchangeable[3]. Similar studies on divalent cations bound to actin have shown a rapid exchange in G-actin[5,6] but no exchange in F-actin[5,6]. However, the discovery that with sonic vibration F-actin catalyses the dephosphorylation of added ATP[7] led to the experiment in which nucleotides and divalent cations bound to F-actin were made exchangeable by sonic vibration[6,8,9]. On the other hand, it has been confirmed that bound nucleotides and cations are slowly released from

* A part of this work was reported in the Conference of Biological Movement (1965, Tokyo)[1] and the 7th International Congress of Biochemistry (1967, Tokyo)[2].

F-actin by prolonged dialysis[10,11]. This suggested that in F-actin, slow exchange of nucleotides and cations can take place even without sonic vibration.

The purpose of this paper is to present results of extensive studies on the exchange, release and incorporation of divalent cations and nucleotides bound to F-actin under various solvent conditions and without sonic vibration[1,2,12]. Effects of salt concentration, pH and temperature are reported first, and the effects of myosin, H-meromyosin and other muscle proteins are then described.

EXPERIMENTAL

Crude G-actin extracted from the acetone-dried powder of rabbit skeletal muscle into cold water was purified by polymerization by 30 mM KCl[13–15]. The ultracentrifuged F-actin pellet was dissolved in water containing 500 μM ATP and dialysed for 3–4 days against a solvent of 5 mM Tris–HCl buffer (pH 8.0) in ice water. The G-actin solution obtained was clarified by ultracentrifugation at 100 000 $\times$ *g* for 90 min.

F-actin having bound $^{45}Ca^{2+}$ and/or [^{14}C]ATP was obtained by polymerization of labelled G-actin in KCl. Labelling of G-actin with $^{45}Ca^{2+}$ was carried out by incubation of a G-actin solution in a low concentration of $^{45}CaCl_2$ and subsequent treatment with 0.1 vol. of Dowex 50-X8(mesh, 200–400; K^+ type). Resin was removed by filtering or centrifuging a few minutes after mixing. Labelling of G-actin with [^{14}C]ATP was carried out by incubation in a low concentration of [^{14}C]ATP and subsequent treatment with Dowex 1-X2 (mesh, 200–300; Cl^- type).

The amount of Ca^{2+} bound to actin was determined by the method of YANAGISAWA[16] and also by radioactivity counting. Both methods showed that it was 1.5 $\pm$ 0.3 moles per 60 000 $\times$ *g* of G-actin[17–19]. The amount of ATP bound to G-actin, which was determined by absorption at 260 nm after deproteinization by $HClO_4$ (15%), was 1.3 $\pm$ 0.3 moles per 60 000 $\times$ *g* (refs. 10, 17, 20). This value means 1.0 mole per 40 000–50 000 g of actin, which is the molecular weight reported recently[21,47]. In the case of F-actin the amount of bound Ca^{2+} fluctuated from 1.0 mole to 1.5 moles, but the amount of ADP bound was the same as that for G-actin. This fluctuation of Ca^{2+} content might be due to the release of bound Ca^{2+} from F-actin, since F-actin just after polymerization was always found to bind about 1.5 moles of Ca^{2+}.

After storage in various solvents for various periods, the solutions of labelled F-actin were centrifuged at 100 000 $\times$ *g* for 90 min. Amounts of protein and the respective radioactivities of the supernatant and the pellet were measured. As another method for separating free divalent cations and nucleotides released from F-actin, Dowex 50 (K^+-type) or Dowex 1 (Cl^--type) was used, as described above. Thus, the exchange or the release of the Ca^{2+} and/or ADP bound to F-actin in the incubation period was followed. Gentle stirring of the solution during incubation was confirmed to have no effect on the exchange rate.

F-actin having no Ca^{2+} and/or no ADP prepared by prolonged dialysis[11] was used in the experiments to test for the incorporation of Ca^{2+} and ADP. F-actin having no Ca^{2+} was also prepared by sonication of F-actin in the presence of EDTA[9].

Myosin was prepared by the method described by PERRY[22] with slight modifications. H-meromyosin was prepared from myosin by the method of SZENT-GYÖRGYI[20].

Tropomyosin was prepared by the method of BAILEY[24]. Native tropomyosin and α-actinin were prepared by the method of EBASHI AND EBASHI[25,26].

Randomly labelled [^{14}C]ATP was purchased as a solution from Schwarz Bio Research, Orangeburg, N.Y. $^{45}Ca^{2+}$ was purchased as a solution of $^{45}CaCl_2 \cdot HCl$ from The Radiochemical Centre, Amersham, Great Britain.

The concentration of F-actin was determined from the flow birefringence measured by a Rao-type home-made apparatus at a shear rate of 10 sec^{-1} (ref. 27). Radioactivity was counted in a 2π gas-flow counter with duplication.

Sonic vibration of F-actin was performed in a Kubota sonic generator at 10 kcycles.

RESULTS

Exchange of bound Ca^{2+}

F-actin labelled with $^{45}Ca^{2+}$ was dissolved in a salt solution containing cold Ca^{2+}. After standing for various periods at different temperatures, the solution was centrifuged and $^{45}Ca^{2+}$ released from F-actin or $^{45}Ca^{2+}$ remaining in F-actin was measured. The results are shown in Fig. 1. During this experiment the amount of F-actin in the solution did not change, according to the flow birefringence measurements. The rate of exchange increases with increasing temperature and becomes so high that at the highest temperature used in this experiment (37°), the half-life time of exchange is about 5 h, and the exchange is almost completed in 24 h.

In the same figure the exchange rates in two solutions at the same temperature (4°) with and without dialysis are shown[1,12,28]. The dialysis was carried out against a large volume of solvent of the same ionic strength as the F-actin solution. Nevertheless, the faster exchange was found with dialysis. The reason is not explained yet.

The time course of exchange was not expressed by a simple exponential curve. Here, the rate constant, k, of the exchange is estimated from the half-life time of exchange, $\tau_{1/2}$, in Fig. 1 by the relation $k = 0.693/\tau_{1/2}$ (see APPENDIX). The relation

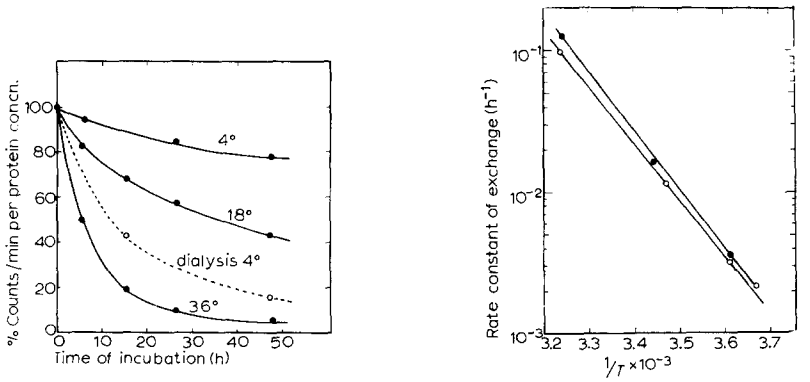


Fig. 1. Exchange of Ca^{2+} bound to F-actin. $^{45}Ca^{2+}$-labelled F-actin was incubated in a temperature-controlled bath and $^{45}Ca^{2+}$ in F-actin was counted after ultracentrifugation. pH was adjusted to 8.0 at different temperatures. Tris–HCl (pH 8.0), 10 mM; KCl, 50 mM; ATP, 500 μM; and actin concentration, 1.5 mg/ml. ●, allowed to stand; ○, dialysed against the same solvent.

Fig. 2. Relation between the exchange rate of Ca^{2+} and the absolute temperature. The rate of exchange is given in the log scale against the reciprocal of the absolute temperature (Arrhenius plot). ●, data from Fig. 1 ($\Delta H^{\ddagger} = 25$ kcal/mole); ○, a different preparation ($\Delta H^{\ddagger} = 24$ kcal/mole). Dowex 50 was used to remove the free Ca^{2+} (see EXPERIMENTAL). Tris–HCl (pH 8.1), 10 mM; $CaCl_2$, 1 mM; ATP, 500 μM; and actin, 3.2 mg/ml.

between the rate constant and the temperature in the Arrhenius plot shows that the activation enthalpy of the rate determining process of exchange is about 25 kcal per mole (see Fig. 2).

The rate of exchange was followed by changing the protein concentration. The half-life time, $\tau_{1/2}$, of exchange is almost constant and slightly increases with increasing protein concentration.

The dependence of the exchange rate on pH was examined at an ionic strength similar to the above case. The pH was adjusted by Tris–HCl buffer. The result is shown in Fig. 3. The amount of F-actin measured by flow birefringence, after standing at various pH's, changed little with pH or with time in the pH range studied here. The half-life time of $^{45}Ca^{2+}$ has a maximum; that is, the rate has a minimum at a pH between 7 and 8 and increases with increasing pH. Such an effect of high pH is very remarkable at low temperatures. The activation enthalpy for the exchange seems to decrease at high pH. At the highest pH (9.5), the slow depolymerization of F-actin took place during incubation. Another point to be noted is that the initial content of radioactive $^{45}Ca^{2+}$ in F-actin decreased at higher pH's; that is, when pH was raised, some exchange or release occurred immediately, as shown in Fig. 3. The half-life given in the figure is for the slow exchange after the initial immediate exchange.

The half-life of the exchange of Ca^{2+} decreases slightly with increasing concentration of Ca^{2+}. In the presence of Mg^{2+}, the exchange rate of Ca^{2+} with Mg^{2+} was almost the same as that with Ca^{2+}.

As in the case of high pH, at high KCl concentrations the exchange took place in two steps. The half time of exchange of the second step increases with increasing KCl concentration.

The exchange or release of Ca^{2+} was accelerated by ATP. This effect was saturated at about 100 μM ATP.

Exchange of bound ADP

The exchange of ADP bound to F-actin with ATP or ADP in the solvent was examined in a way similar to bound Ca^{2+}. Bound ADP is released slowly, and ATP

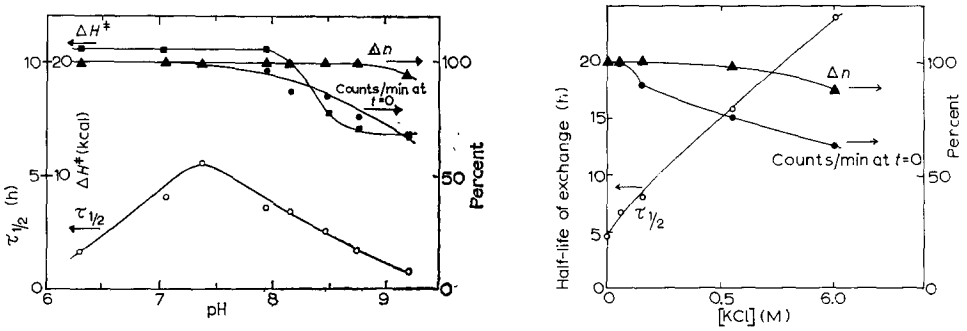


Fig. 3. Effect of pH on exchange of Ca^{2+}. ○, half-life time of exchange ($\tau_{1/2}$); ■, activation enthalpy; ▲, flow birefringence after 20 h incubation; ●, counts/min of $^{45}Ca^{2+}$ just after changing solvent conditions. Dowex 50 was used to remove free divalent cations. Tris–HCl, 10 mM; KCl, 50 mM; $CaCl_2$, 1 mM; ATP, 500 μM; actin, 1.4 mg/ml; and temperature, 37°.

Fig. 4. Effect of KCl concentration on exchange of bound ADP. Dowex 1 was used to remove free nucleotides. Symbols are the same as in Fig. 3. Tris–HCl (pH 8.0), 10 mM; ATP, 500 μM; actin, 1.4 mM; and temperature, 37°.

in the solvent is incorporated into F-actin; then it is dephosphorylated into ADP. The results are similar to those found in the case of Ca^{2+}. The high temperature makes bound ADP exchangeable, and dialysis accelerates the exchange. The high pH is favourable to the exchange, although the effect of pH on the exchange of ADP is smaller than that on Ca^{2+}. The effect of KCl is similar to the case of Ca^{2+} (see Fig. 4).

Divalent cation has no effect. The effect of ATP (to increase the exchange rate) is saturated at a low ATP concentration.

A solution of G-actin containing no free nucleotides or divalent cations was separated into two parts. To one part was added $^{45}CaCl_2$ and to the other [^{14}C]ATP. Two F-actin solutions labelled with $^{45}Ca^{2+}$ and [^{14}C]ADP were obtained by polymerization in KCl after treatment by Dowex 50 and Dowex 1, respectively. They were kept in the same conditions of ionic strength, pH and temperature, and the exchange rates of Ca^{2+} and ADP were compared. The results are shown in Fig. 5. Both in the presence and absence of ATP in the solvent, the rate of release or exchange of bound Ca^{2+} increases with increasing concentration of Ca^{2+} in the solvent. However, the rate of release or exchange of bound ADP does not change with the concentration of Ca^{2+} either in the presence or absence of ATP. The exchange of bound ADP with ATP in the solvent is much faster than the release of bound ADP in the absence of ATP, independently of the concentration of Ca^{2+}. The exchange of bound Ca^{2+} is also accelerated by ATP in the solvent. In the absence of ATP the exchange rate of Ca^{2+} is much faster than the rate of release of ADP, and in the presence of ATP the exchange rate of Ca^{2+} is a little faster than that of ADP.

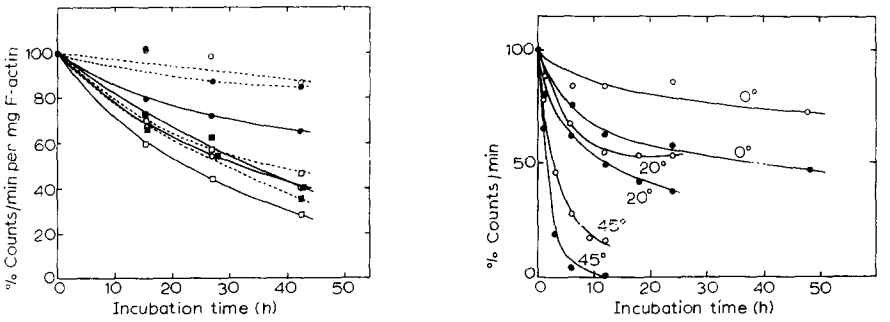


Fig. 5. Comparison of exchange rates of Ca^{2+} and ADP. Free nucleotides and divalent cations were removed by ultracentrifugation. Tris–HCl (pH 8.2), 12 mM; KCl ,60 mM; actin, 2.2 mg/ml; and temperature, 16°. —, exchange of Ca^{2+}; ----, exchange of ADP. ●, ATP, 0 mM; $CaCl_2$, 0 mM; ○, ATP, 0 mM; $CaCl_2$, 1 mM; ■, ATP, 1 mM; $CaCl_2$, 0 mM; □, ATP, 1 mM; $CaCl_2$, 1 mM.

Fig. 6. Comparison between release and exchange of bound Ca^{2+}. Release of radioactivity of F-actin was examined after treatment with Dowex 50. Tris–HCl (pH 8.0), 10 mM; KCl, 50 mM; ATP, 500 μM; and actin, 1 mg/ml. ●, 1 mM $CaCl_2$; ○, no $CaCl_2$.

Release and rebinding

In Fig. 6, the release and exchange of Ca^{2+} are compared. The release is generally similar to the exchange, although the rate of release is somewhat slower. The release of Ca^{2+} is accelerated by increasing the temperature. The activation enthalpy is about 25 kcal/mole. During the release of Ca^{2+} no denaturation of F-actin was observed in the presence of ATP.

The release of Ca^{2+} occurs readily, but the release of ADP does not always occur.

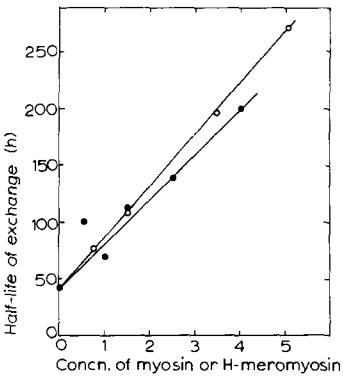
This difference is related to the fact that the Ca^{2+}-free F-actin can easily be prepared, *e.g.*, by sonic vibration in the presence of EDTA[9], while the ADP-free F-actin cannot be obtained easily[29].

Rebinding of Ca^{2+} was examined in the Ca^{2+}-free F-actin obtained by sonic vibration of F-actin in the presence of EDTA or by prolonged dialysis. The rate of incorporation of $^{45}Ca^{2+}$ in the solvent to this F-actin increased with increasing concentration of Ca^{2+} and was faster in the presence of ATP than in the absence of ATP.

Sonic vibration causes incorporation of Ca^{2+} to speed up. The final level of binding is higher in the presence of ATP. The apparent binding constant of Ca^{2+} estimated from the experimental data was about $2 \cdot 10^4$–$5 \cdot 10^4$ M^{-1} in the absence of ATP and $8 \cdot 10^4$–$15 \cdot 10^4$ M^{-1} in the presence of 100 μM ATP.

Effect of muscle proteins

Various concentrations of H-meromyosin and myosin were added to solutions of labelled F-actin and the exchange of bound ADP and Ca^{2+} was investigated. After standing with various solvent conditions, solutions were ultracentrifuged, and the radioactivity and the protein concentration of supernatants and pellets were measured. As shown in Fig. 7, in the absence of ATP the exchange rate of Ca^{2+} decreased with increasing concentration of H-meromyosin or myosin. This effect was not saturated up to a weight ratio of H-meromyosin or myosin to F-actin of 4.



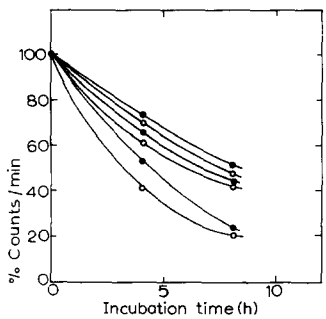


Fig. 7. Effect of myosin or H-meromyosin on exchange of bound Ca^{2+}. Concentration of myosin or H-meromyosin are given in a weight ratio to actin. Free Ca^{2+} was removed by ultracentrifugation. Tris–HCl (pH 8.0), 10 mM; KCl, 50 mM; $CaCl_2$, 0.5 mM; actin, 1.5 mg/ml; and temperature, 0°. ○, myosin; ●, H-meromyosin.

Fig. 8. Effect of tropomyosin on exchange of bound Ca^{2+}. Free Ca^{2+} was removed by Dowex 50. Tris–HCl (pH 8.0), 5 mM; KCl, 50 mM; $CaCl_2$, 1 mM; and temperature, 37°. ●, for Straub-type F-actin, 1.5 mg/ml. ●, for Ebashi-type F-actin, 1.5 mg/ml. ○, no addition of tropomyosin; ●, 0.5 mg/ml tropomyosin; ○, 1.5 mg/ml tropomyosin (symbols from top to bottom).

In the presence of ATP, at low temperatures where superprecipitation or dissociation does not occur[6,30–33], the rate of exchange of Ca^{2+} decreases with increasing concentration of H-meromyosin or myosin. The exchange of ADP is, in general, similar to Ca^{2+}.

In the presence of H-meromyosin or myosin, the absolute value of the exchange rate is increased by raising the temperature.

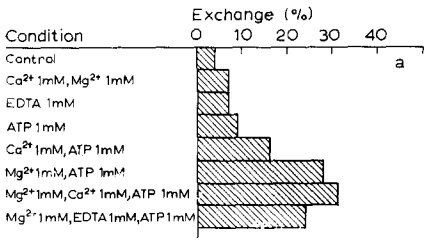
As shown in Fig. 8, in the presence of tropomyosin, the rate of exchange and

release of Ca^{2+} bound to F-actin decreased; the release of bound Ca^{2+} from Straub-type F-actin[13,14] was especially affected by tropomyosin. Under all conditions, Ca^{2+} bound to Ebashi-type F-actin[3,4] was more exchangeable, but the difference was not large. These phenomena may be understood as being due to the binding of tropomyosin to F-actin. The presence of 1 mg/ml of α-actinin in 1 mg/ml of F-actin solution increased the rate by about 10–15% at 37°.

Exchange or release during superprecipitation

To examine the possibility of conformational change in actin polymers during the *in vitro* concentration or the superprecipitation of actomyosin by ATP, the exchange or release of nucleotides and divalent cations bound to F-actin can be a good indicator, if such a change has occurred. This kind of work has been carried out by several researchers[1,5,6,31–33,35,36,46]. The first positive result on the increased exchange of bound Ca^{2+} during superprecipitation was shown in a preliminary study by the present authors several years ago[6,12]. The experiments of SZENT-GYÖRGYI AND PRIOR[31] showed clearly that some (at most 50%) of the ADP bound to F-actin was released or exchanged during superprecipitation. Similar positive data are presented here again.

Labelled F-actin was mixed with myosin and the complex was washed by repeated centrifugation–dissolution. Under the conditions shown in Fig. 9, the additional washing was confirmed not to increase the release of radioactivity. ATP was then added, and the release of radioactivity was observed. The maximum exchange (or release) was observed under conditions favourable to superprecipitation, namely when the solution contains Mg^{2+} and Ca^{2+}, or Mg^{2+} only. Figs. 9a and b show that the release of Ca^{2+} under optimal conditions is 30%, while that of ADP is 20%. The incorporation of ADP into F-actin was also examined when superprecipitation was induced by the creatine phosphate–creatine kinase system with a very small amount of radioactive ADP. As seen in Fig. 10, the incorporation of ADP occurred instantaneously after the addition of creatine phosphate. In this experiment, the effect of Mg^{2+} was very remarkable, and occasionally almost 100% incorporation was observed.



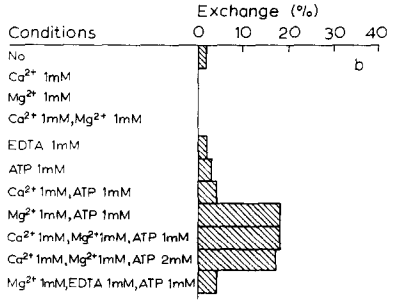


Fig. 9a. Release of $^{45}Ca^{2+}$ from F-actin during superprecipitation. The mixture of F-actin and myosin was washed 3 times by repetition of dilution to 0.05 M KCl and dissolution in 0.5 M KCl. After the addition of ATP, sample solutions were left standing for 2 h and ultracentrifuged at 100000 × *g* for 2 h. $^{45}Ca^{2+}$ was counted and protein concentration was measured in the supernatant. Conditions: KCl, 50 mM; Tris–maleate (pH 7.0), 10 mM; actin, 0.15 mg/ml; and ratio actin/myosin = 1 : 4, at 25°. b. Release of [^{14}C]ADP from F-actin during superprecipitation. Experimental procedures were the same as in a: KCl, 50 mM; Tris–maleate (pH 7.0), 10 mM; actin, 0.25 mg/ml; and actin/myosin = 1 : 5, at 25°.

The incorporation was larger when (partially) nucleotide-free F-actin was used. After instantaneous incorporation, gradual release of radioactivity was observed.

At high salt concentrations such as 0.6 M KCl, the exchange of Ca^{2+} or ADP after the addition of ATP was very small.

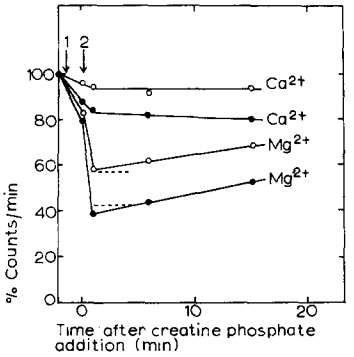


Fig. 10. Incorporation of [^{14}C]ADP into F-actin during superprecipitation. F-actin and myosin were mixed in a ratio of 1 : 4 and 50 μg/ml of creatine kinase was added. The experiment was carried out as follows. Arrow 1, addition of 5.6 μM [^{14}C]ATP; Arrow 2, addition of 85 μM creatine phosphate. At indicated times after the addition of creatine phosphate, the sample solution was centrifuged at 10000 rev./min for 1 min. ^{14}C in the supernatant solution was counted. ○, F-actin 0.179 mg/ml with bound ADP 4.6 μM; ●, partially ADP-free F-actin 0.173 mg/ml with bound ADP 1.2 μM. Tris–maleate (pH 7.1), 10 mM; KCl, 50 mM; and $MgCl_2$, 4.2 mM, or $CaCl_2$, 4.2 mM at 20°. Creatine kinase was prepared according to the method of KUBY, NODA AND LARDY[45].

In the case of H-meromyosin instead of myosin, the fast exchange of Ca^{2+} or ADP bound to F-actin was also observed after the addition of ATP, but the amount of exchange did not exceed 15%[3,6]. The slow exchange of Ca^{2+} or ADP bound to F-actin in the presence of H-meromyosin or myosin, reported in the preceding section, took place after the fast exchange.

DISCUSSION

The slow exchange of Ca^{2+} or ADP in F-actin without any agitation was observed and analysed under various solvent conditions[1,12,32]. The activation enthalpy of exchange is almost the same as that of the ATPase of F-actin at high temperatures found by ASAI AND TAWADA[37]. Although ATPase was not measured in the temperature range of the present experiment, the extrapolation of their data to lower temperatures shows the rate of the ATP hydrolysis to be about 0.01 mole/min per mole of actin at 37°; that is, 1 mole per 2 h per actin monomer. This value roughly corresponds to the half-life of the exchange, $\tau_{1/2} = 5$ h. It is reasonable to consider that the exchange and the ATP hydrolysis are both coupled with the same elementary process in the polymer structure.

Two mechanisms can be postulated concerning the ATPase and the exchange; these are the G–F transformation cycle at the peripheral region of F-actin and the spontaneous partial interruption of F-actin polymers in the middle region[38–40]. The present experiment is not quantitative enough to distinguish between these two mechanisms (see APPENDIX).

The apparent binding constant of Ca^{2+} to F-actin obtained from the results of the release with sonic vibration was about 10^5 M^{-1}. This binding constant is of the same order as that in the case of G-actin (10^5 M^{-1})[5,42]. For the binding constant of nucleotide, SEIDEL, CHAK AND WEBER[43] obtained almost the same values for G- and F-actin, although the absolute value was higher than that of Ca^{2+}.

A structural change in F-actin associated with the interaction with myosin in the presence of ATP had been expected for various reasons. Since our preliminary reports[6], observations suggesting such structural changes were reported from several laboratories, but the results differ widely[1,5,6,12,31–33,35,36,46]. The fluctuation of data may be due to the heterogeneous interaction of myosin and actin during and after super-precipitation. The sliding mechanism of muscular contraction suggests that the contraction is a result of some change in the higher-order arrangement of actin and myosin filaments, and after the contraction, the molecular and polymer structure of each filament is restored to the initial state[44]. The most important point is thus not to compare the states before and after the contraction but to see what happened during contraction. Therefore, in spite of the experimental difficulty, exchange or release of ADP and Ca^{2+} is a good indicator of the change of polymer structure during contraction.

APPENDIX

Time course of exchange

We assume that exchange of bound Ca^{2+} or ADP occurs only in the monomeric state, G-actin, or only at the end of F-actin when the cyclic reaction such as F–G–F occurs. The time required for the exchange of the y-th monomer from the end of a polymer at time zero is approximately given by ay^2, according to analogy to the Brownian motion or random stochastic process, where a is the time required for one cyclic reaction. Therefore, after time t the exchange can reach the $\sqrt{t/a}$-th monomer from the end, on the average.

According to the polymerization theory, the length distribution of n-mer of F-actin is given by $A\,e^{-bn}$ in equilibrium, where A and b are constants[39,48]. Then the probability of the exchange per monomer in F-actin after time t is given approximately by

$$E(t) = \frac{A \sum_{0}^{2\sqrt{t/a}} n\,e^{-bn} + A \sum_{2\sqrt{t/a}}^{\infty} \frac{2\sqrt{t/a}}{n}\,n\,e^{-bn}}{A \sum_{0}^{\infty} n\,e^{-bn}} = 1 - e^{-2b\sqrt{t/a}}$$

The exchange occurs quickly at first and gradually slows down. This relation shows good agreement with the experimental data.

At the same time, ATP splitting occurs at the end of polymers. The total amount of the splitting at time t per monomer in F-actin is given by

$$\frac{2\frac{t}{a}A \sum_{0}^{\infty} e^{-bn}}{A \sum_{0}^{\infty} n\,e^{-bn}} = 2\frac{t}{a}(1 - e^{-b}) = 2b\frac{t}{a}$$

because b is very small since the average length of F-actin is very long.

However if, contrary to the above assumption, the exchange occurs at every position in the polymer at random, then the probability of the exchange can be given by

$$E(t) = 1 - e^{-2b\frac{t}{a}}$$

In this case the initial velocity of the exchange agrees with that of the ATP splitting. In the case of sonically activated ATPase, such an agreement was obtained[8].

ACKNOWLEDGEMENTS

The authors are indebted to Dr. EIZO NAKANO of the Department of Biology, Faculty of Science, Nagoya University, for valuable advice and for providing facilities for the isotope treatments. The work was supported by a grant from the Matsunaga Science Foundation.

REFERENCES

1 M. KASAI AND F. OOSAWA, *Abstr. Conf. Biol. Movement, Tokyo, 1965*, p. 11.
2 F. OOSAWA, S. HATANO, M. KASAI, K. TAWADA AND T. TOTSUKA, *7th Intern. Congr. Biochem., Tokyo*, 1967, p. 325.
3 A. MARTONOSI, M. A. GOUVEA AND J. GERGELY, *J. Biol. Chem.*, 235 (1960) 1700.
4 R. C. STROHMAN AND J. J. SAMOTODIN, *J. Biol. Chem.*, 237 (1962) 363.
5 M. BARANY, F. FINKELMAN AND T. THERATTIL-ANTONY, *Arch. Biochem. Biophys.*, 98 (1962) 28.
6 F. OOSAWA, S. ASAKURA, H. ASAI, M. KASAI, S. KOBAYASHI, K. MIHASHI, T. OOI, M. TANIGUCHI AND E. NAKANO, in J. GERGELY, *Biochemistry of Muscle Contraction*, Little Brown, Boston, 1964, p. 158.
7 S. ASAKURA, *Biochim. Biophys. Acta*, 52 (1961) 65.
8 S. ASAKURA, M. TANIGUCHI AND F. OOSAWA, *Biochim. Biophys. Acta*, 74 (1963) 140.
9 M. BARANY AND F. FINKELMAN, *Biochim. Biophys. Acta*, 63 (1962) 98.
10 W. F. H. M. MOMMAERTS, *J. Biol. Chem.*, 198 (1952) 469.
11 M. KASAI AND F. OOSAWA, *Biochim. Biophys. Acta*, 75 (1963) 223.
12 M. KASAI AND F. OOSAWA, *Ann. Rep. Res. Group Biophys. Japan*, 5 (1965) 51.
13 F. B. STRAUB, *Studies Inst. Med. Chem. Univ. Szeged*, 1 (1941) 5.
14 W. F. H. M. MOMMAERTS, *J. Biol. Chem.*, 188 (1951) 599.
15 W. DRABIKOWSKY AND J. GERGELY, *J. Biol. Chem.*, 237 (1962) 3418.
16 F. YANAGISAWA, *J. Biochem. Tokyo*, 42 (1955) 3.
17 F. B. STRAUB AND G. FEUER, *Biochim. Biophys. Acta*, 4 (1950) 455.
18 Y. TONOMURA AND J. YOSHIMURA, *J. Biochem. Tokyo*, 50 (1961) 79.
19 A. CHARAMBACH, M. BARANY AND F. FINKELMAN, *Arch. Biochem. Biophys.*, 93 (1961) 456.
20 A. G. SZENT-GYÖRGYI, *Arch. Biochem. Biophys.*, 31 (1951) 97.
21 M. REES AND M. YOUNG, *J. Biol. Chem.*, 242 (1967) 4449.
22 S. V. PERRY, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. 2, Academic Press, New York, 1955, p. 582.
23 A. G. SZENT-GYÖRGYI, *Arch. Biochem. Biophys.*, 42 (1952) 305.
24 K. BAILEY, *Biochem. J.*, 43 (1948) 271.
25 S. EBASHI AND F. EBASHI, *J. Biochem. Tokyo*, 55 (1964) 604.
26 S. EBASHI AND F. EBASHI, *J. Biochem. Tokyo*, 58 (1956) 7.
27 M. KASAI, H. KAWASHIMA AND F. OOSAWA, *J. Poly. Sci.*, 44 (1960) 51.
28 F. OOSAWA, S. ASAKURA, S. HIGASHI, M. KASAI, S. KOBAYASHI, E. NAKANO, T. OHNISHI AND M. TANIGUCHI, *Molecular Biology of Muscular Contraction*, Igakushoin and Elsevier, Tokyo and Amsterdam, 1965, p. 56.
29 M. BARANY, A. F. TUCCI AND T. E. CONVER, *J. Mol. Biol.*, 19 (1966) 483.
30 M. KASAI, E. NAKANO AND F. OOSAWA, *Biochim. Biophys. Acta*, 94 (1965) 494.
31 A. G. SZENT-GYÖRGYI AND G. PRIOR, *J. Mol. Biol.*, 15 (1966) 515.
32 C. M. MOOS, J. E. ETES AND E. EISENBERG, *Biochem. Biophys. Res. Commun.*, 23 (1966) 347.
33 S. KITAGAWA, A. MARTONOSI AND J. GERGELY, *Federation Proc.*, 24 (1965) 598.
34 S. EBASHI AND K. MARUYAMA, *J. Biochem. Tokyo*, 58 (1965) 20.
35 A. MARTONOSI, M. A. GOUREA AND J. GERGELY, *J. Biol. Chem.*, 235 (1960) 1707.

36 K. SEKIYA, K. TAKEUCHI AND Y. TONOMURA, *J. Biochem. Tokyo*, 61 (1967) 567.
37 H. ASAI AND K. TAWADA, *J. Mol. Biol.*, 20 (1966) 403.
38 S. ASAKURA, M. TANIGUCHI AND F. OOSAWA, *J. Mol. Biol.*, 7 (1963) 55.
39 F. OOSAWA AND M. KASAI, *J. Mol. Biol.*, 4 (1962) 10.
40 F. OOSAWA, S. ASAKURA AND T. OOI, *Progr. Theoret. Phys. Kyoto Suppl.*, 17 (1961) 14.
41 Y. NAKAOKA AND M. KASAI, *J. Mol. Biol.*, to be published.
42 A. MARTONOSI, C. M. MOLINO AND J. GERGELY, *J. Biol. Chem.*, 239 (1964) 1057.
43 D. T. SEIDEL, D. V. CHAK AND H. H. WEBER, *Biochim. Biophys. Acta*, 140 (1967) 93.
44 A. HUXLEY, *Progr. Biophys. Biophys. Chem.*, 7 (1957) 257.
45 S. A. KUBY, L. H. NODA AND H. A. LARDY, *J. Biol. Chem.*, 210 (1954) 65.
46 S. KITAGAWA, W. DRABIKOWSKI AND J. GERGELY, *Arch. Biochem. Biophys.*, 125 (1968) 706.
47 K. TSUBOI, *Biochim. Biophys. Acta*, 151 (1968) 420.
48 F. OOSAWA AND S. HIGASHI, *Progr. Theoret. Biol.*, 1 (1967) 79.