Werner, E. A. (1919) *J. Chem. Soc. 115*, 1093–1102.
White, E. H., & Woodcock, D. J. (1968) in *The Chemistry of the Amino Group* (Patai, S., Ed.) pp 440–483, Wiley-Interscience, New York.
White, E. H., Jelinski, L. W., Politzer, I. R., Branchini, B., & Roswell, D. F. (1981) *J. Am. Chem. Soc. 103*, 4231–4239.
Wilson, W. D., Wang, Y.-H., Kusuma, S., Chandrasekaran, S., Yang, N. C., & Boykin, D. W. (1985) *J. Am. Chem. Soc. 107*, 4989–4995.
Wurdeman, R. L., & Gold, B. (1988) *Chem. Res. Toxicol. 1*, 146–147.
Zimmermann, H. W. (1986) *Angew. Chem., Int. Ed. Engl. 25*, 115–130.

Kinetics of Adenosine 5′-Triphosphate and Adenosine 5′-Diphosphate Interaction with G-Actin

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ABSTRACT: Double mixing experiments using a three-syringe stopped-flow apparatus have given values of the second-order rate constants for association of ϵATP, ATP, and ADP to G-actin of 6.8×10^6 M^{-1} s^{-1}, 6.1×10^6 M^{-1} s^{-1}, and 6.3×10^6 M^{-1} s^{-1}, respectively, at pH 7.6, 20 °C, and 0.65 mM free Ca^{2+}. The previously established ca. 100-fold weaker binding of ADP than ATP to G-actin is due to a much faster dissociation rate of ADP than ATP, rather than to a slower association rate as was previously reported. This difference between ADP and ATP largely disappears under more nearly physiological conditions (0.8 mM Mg^{2+} and 100 mM KCl). Association rate constants for the three nucleotides under these conditions are 2.13×10^6 M^{-1} s^{-1}, 1.1×10^6 M^{-1} s^{-1}, and 1.2×10^6 M^{-1} s^{-1}, respectively, for ϵATP, ATP, and ADP. The rate constant for association of ϵATP is only slightly affected by reducing the Mg^{2+} concentration from 0.8 to 0.2 mM, whereas that for ADP association is reduced by a factor of ca. 3. This, together with the observed increase in the apparent association rate constant of ϵATP on increasing the Ca^{2+} concentration in the 10–100 μM range, suggests that G-actin recognizes and binds the metal–nucleotide complexes.

The rate constants for the interaction of ATP and ADP with G-actin are important determining factors in the behavior of the G-actin–F-actin system, particularly with respect to the treadmilling properties of actin filaments. This phenomenon has received much attention in recent years, but it appeared to us that some of the kinetic parameters had not been well characterized. We have therefore undertaken a more detailed investigation of the association and dissociation kinetics of of the G-actin–ATP and G-actin–ADP complexes. The results obtained indicate that previously reported values for the association and dissociation rate constants for ADP at low ionic strength are incorrect and that divalent metal ion complexes of nucleotides are recognized by actin in the binding reaction.

Materials and Methods

Protein Preparations. Actin preparations from rabbit skeletal muscle were obtained as described earlier (Drabikowski & Nowak, 1973). Final ATP–G-actin solutions in 0.2 mM ATP, 0.2 mM $CaCl_2$, and 2 mM Hepes buffer, pH 7.6, were kept at 0 °C for no longer than 1 week.

To obtain ϵATP–G-actin,[1] the ATP–G-actin was polymerized with 0.1 M KCl; F-actin was collected by ultracentrifugation and then depolymerized by homogenization in a Teflon/glass homogenizer in a solution of 0.2 mM ϵATP, 0.2 mM $CaCl_2$, and 2 mM Hepes buffer, pH 7.6, followed by a 24-h dialysis against the same buffer solution.

Fluorescence Measurements. These were carried out essentially as previously described (Nowak et al., 1988) with either an SLM 8000 fluorescence spectrophotometer or, where high time resolution was required, a Durrum D-132 three-syringe rapid-mixing apparatus equipped with a D-137 dual detector unit.

Other Procedures. Actin concentration was determined spectrophotometrically at 290 nm with an absorption coefficient of 0.63 $mg \cdot mL^{-1} \cdot cm^{-1}$ (Houk & Ue, 1974). Molar actin concentrations were calculated with the value of M_r 42 000 for G-actin (Elzinga, 1973).

The concentration of ϵATP was determined at 265 nm with an absorption coefficient of 5700 $M^{-1} \cdot cm^{-1}$ (Secrist et al., 1972).

Reagents. ϵATP was prepared by the method of Secrist et al. (1972) and separated from unreacted ATP by chromatography on a QEAE-Sephadex G-50 column. ATP (disodium salt) was purchased from Pharma-Waldhof (Düsseldorf). ADP (Pharma-Waldhof) was purified prior to use if necessary as previously described (Nowak et al., 1988). Hepes was a

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[1] Abbreviation: ϵATP, $1,N^6$-ethenoadenosine 5′-triphosphate.

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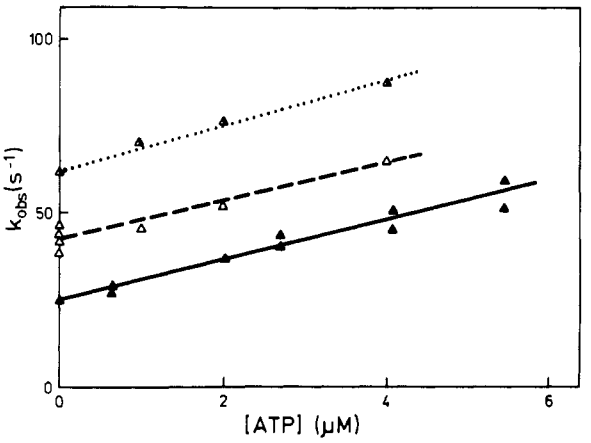

FIGURE 1: Dependence of the observed first-order rate constant for ϵATP binding to G-actin on the concentration of ATP. Conditions: 4 mM Hepes, pH 7.6, 0.5 mM EDTA, 1.15 mM $CaCl_2$ (ca. 0.65 mM free Ca^{2+}), 1.23 μM G-actin. [ϵATP] = 3.96 (▲), 6.31 (△), or 8.43 μM (▲). T = 20–22 °C.

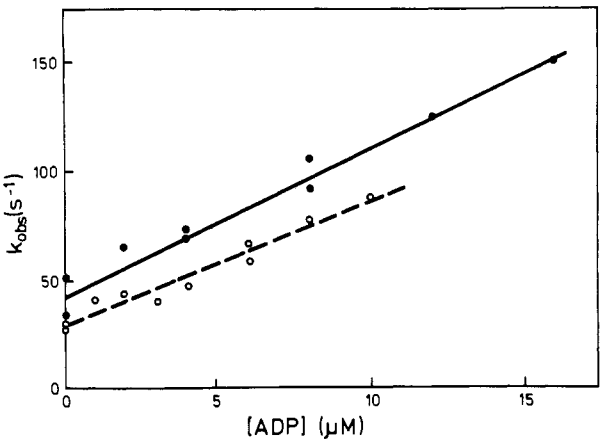

FIGURE 2: Dependence of the observed pseudo-first-order rate constant for ϵATP binding to G-actin on the concentration of ADP. Conditions as in Figure 1, except 1.40 μM G-actin. [ϵATP] = 4.75 (○) or 6.46 μM (●).

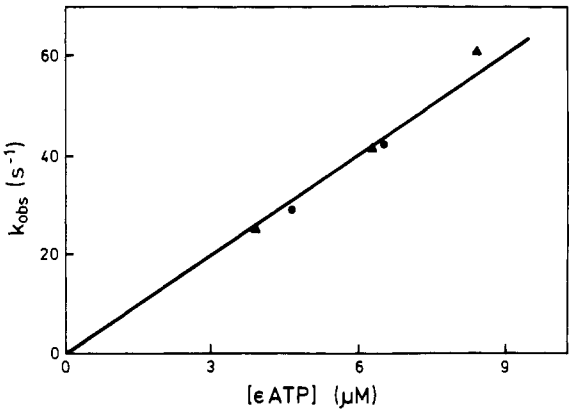

FIGURE 3: Ordinate intercepts from Figures 1 and 2 as a function of ϵATP concentration.

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Results and Discussion

Kinetics of ATP Association with G-Actin. The apparent second-order rate constant for ϵATP binding to G-actin was determined as previously described by transient removal of tightly bound metal ions and bound nucleotide using EDTA followed by remixing with an excess of divalent metal ions over EDTA in the presence of a variable concentration of ϵATP (Waechter & Engel, 1977; Nowak et al., 1988). The results reported in this section refer to association kinetics at pH 7.6 and 0.65 mM $CaCl_2$. At this Ca^{2+} concentration, nucleoside triphosphates are present mainly as their calcium complexes. As reported previously (Nowak et al., 1988), a value of (7.35 ± 0.44) × 10^6 M^{-1} s^{-1} was found for ϵATP.

The kinetics of ATP binding to G-actin were investigated in competition experiments with ϵATP. Inclusion of ATP together with ϵATP in the third syringe of the stopped-flow machine resulted, as expected, in an increased rate constant of the binding transient together with a decreased amplitude (only ϵATP binding is detected). In the competing reactions

$$A + \epsilon ATP \xrightarrow{k_\epsilon} A\text{–}\epsilon ATP$$

$$A + ATP \xrightarrow{k_t} A\text{–}ATP$$

it can be easily shown that the rate constant for ϵATP binding is given by

$$\lambda = k_\epsilon(\epsilon ATP) + k_t(ATP)$$

and the relative amplitude by

$$\Delta F_{ATP}/\Delta F = 1/[1 + k_t(ATP)/k_\epsilon(\epsilon ATP)]$$

where ΔF is the amplitude of the transient in the absence of ATP and ΔF_{ATP} is the amplitude in its presence. Since the amplitude measurement was not very accurate with the multimixing apparatus, only the rate constants were used to evaluate k (from plots of λ against ATP concentration with ϵATP at constant concentration). The slope of the line in such plots (Figure 1) gave an average value of (6.12 ± 0.05) × 10^6 M^{-1}, the rate constant for association of ATP with G-actin, and the y-axis intercept allowed calculation of the corresponding rate constant for ϵATP (ca. 7 × 10^6 M^{-1}), which was in good agreement with that obtained from experiments in the absence of ATP. Thus, there is no significant difference between the association rate constants for ATP and ϵATP, as already inferred from previous determinations of the relative binding constants and the dissociation rate constants (Waechter & Engel, 1977).

Kinetics of ADP Association with G-Actin. From the previously determined value of the association rate constant of ADP with G-actin (ca. 3 × 10^4 M^{-1} s^{-1}; Neidl & Engel, 1979), it was expected that relatively high concentrations of ADP would be needed to compete with the binding of ϵATP. Surprisingly, even at concentrations of ADP that were approximately equimolar with ϵATP, a marked effect on the binding transient was seen. As with ATP, the amplitude was decreased while the rate constant was increased. Analysis according to the equations given above led to a value of (6.33 ± 0.84) × 10^6 M^{-1} s^{-1} for the association rate constant (k_d) with G-actin (Figure 2), which is very similar to the values for ATP and ϵATP.

The intercepts from Figures 1 and 2 have been plotted against the ϵATP concentration in Figure 3, and the slope of the line obtained gives a value of (6.75 ± 53) × 10^6 M^{-1} s^{-1} for the association rate constant for ϵATP.

Similar results were obtained in the presence of 0.2 mM free Ca^{2+} (results not shown) except that the rate constant for ADP was a factor of 2–3 lower than for the two triphosphates. This point will be addressed in more detail in the case of Mg^{2+} as a divalent metal ion in a later section.

At first sight, it is difficult to reconcile this surprising result with the well-established observation that ADP binds about 2 orders of magnitude more weakly than ATP to G-actin. The explanation becomes apparent in experiments of the type

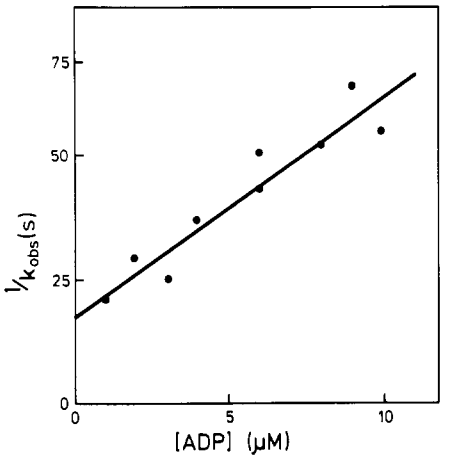

FIGURE 4: Dependence of the reciprocal of the rate constant for the second (slow) phase in the experiment described in Figure 2 on the ADP concentration.

described here at longer times after mixing than were investigated for the association transients. The ADP bound initially in competition with ATP is partially released again after a time range of seconds and minutes. Thus, it is apparent that the initial distribution (i.e., shortly after binding is complete) is not the equilibrium distribution and that ADP is released because it binds considerably more weakly than ATP. Since the association rate constants are similar in magnitude, this must be due to a more rapid effective rate of dissociation of ADP than εATP. The rate constant for the slow second phase, which behaved as a single exponential, was seen to vary with the relative concentrations of the two nucleotides, as expected on theoretical grounds, in the sense that it was slower at higher ADP concentrations. Since the second phase was so much slower than the first, the two processes can be regarded as uncoupled. It can be shown that the rate constant for the second phase in this situation is given by

$$\lambda = \frac{k_{-d}k_{\epsilon}[\epsilon ATP] + k_{-d}k_{-\epsilon} + k_{-\epsilon}k_d[ADP]}{k_{-d} + k_{-\epsilon} + k_{\epsilon}[\epsilon ATP] + k_d[ADP]}$$

where k_{ϵ} and k_d are the association rate constants, and $k_{-\epsilon}$ and k_{-d} are the dissociation rate constants for εATP and ADP, respectively. Since k_{-d} is apparently much larger than $k_{-\epsilon}$ and since under the conditions used $k_d[ADP]$ and $k_{\epsilon}[\epsilon ATP]$ are both much greater than k_{-d}, this can be simplified to

$$\lambda = k_{-d}k_{\epsilon}[\epsilon ATP]/(k_d[ADP] + k_{\epsilon}[\epsilon ATP])$$

or after rearrangement

$$1/\lambda = 1/k_{-d} + (k_d[ADP]/k_{-d}k_{\epsilon}[\epsilon ATP])$$

Thus, if [εATP] is held constant and [ADP] is varied, a plot of $1/l$ against [ADP] should be linear and $1/k_{-d}$ can be obtained by extrapolation of [ADP] to zero. The results obtained are plotted in this manner in Figure 4, and it can be seen that the relationship is linear within the limits of experimental error. The value of k_{-d} was determined from fits to the nonlinearized from of the equation. The average value obtained from this and two further experiments using different concentrations of εATP was $(5.7 \pm 0.38) \times 10^{-2}$ s^{-1} compared with the value of 1.04×10^{-3} s^{-1} for εATP under the same conditions (0.65 mM Ca^{2+}). The rate constant for ATP dissociation under these conditions is known to be severalfold lower than for εATP, and this was confirmed in competition binding experiments of the type discussed here for ADP/εATP. In this case, after establishment of the initial distribution on mixing actin with ATP and εATP, already bound εATP was displaced at a very slow rate to reach the final equilibrium. The kinetics of this phase were not studied in detail, since the rate was so slow and the rate constant for ATP release can be measured much more simply by direct displacement with εATP.

It is relevant to speculate on the reasons for the difference in the kinetic constants for interaction of ADP with G-actin determined here and in previous work. The values determined earlier were based on direct measurement of the dissociation rate of ADP from G-actin isolated as its complex with ADP (Neidl & Engel, 1979). It seems likely to us that a significant amount of the G-actin–ATP complex was produced in the procedure used for the preparation of G-actin–ADP, despite the checks on ATP contamination reported. We have examined many samples of commercially available ADP by a sensitive and quantitative HPLC assay and find that all samples contain of the order of 1% ATP impurity. Since the ratio of affinities (ATP/ADP) to G-actin is about 100, it is to be expected that similar amounts of the two species should be obtained on incubation of G-actin with an excess of this mixture. Thus, it is possible that in the earlier determinations of the dissociation rate constant the results obtained were dominated by the much slower release of ATP. We feel confident that our determination of the kinetic constants for ADP interaction with G-actin is reliable, especially since the on- and off-rate constants were determined independently of each other and under conditions where a 1% contamination with ATP would not affect the results, due to the low ADP concentrations used. In addition, we have used HPLC-purified ADP containing no detectable ATP (i.e., <0.1%) and found identical behavior.

Kinetics of Nucleotide Interaction with G-Actin under Physiological Conditions. It has been reported that under ionic conditions, which are more nearly similar to those in the cell, the affinities of ATP and ADP become very similar, in contrast to the situation at very low ionic strength and in the absence of Mg^{2+} ions (Wanger & Wegner, 1983). Double mixing experiments of the type described above are well suited to investigations under these conditions, since the rates of nucleotide and metal ion binding are much faster than the otherwise interfering polymerization reaction that occurs under these conditions.

Figure 5 shows the results of experiments on the rate of association of εATP and ADP with G-actin in the presence of 0.8 or 0.2 mM Mg^{2+} and 100 mM KCl. In the first mixing step, εATP–G-actin, prepared as for other experiments in the presence of Ca^{2+}, was mixed with a large excess of EDTA. After a delay of 7 s, this mixture was remixed with Mg^{2+} and KCl to give the concentrations mentioned. Thus, the rate of association of the nucleotides in the presence of Mg^{2+} and KCl was measured, although the starting actin contained Ca^{2+} and no KCl. The intercepts on the ordinate of Figure 5a can be used to calculate the rate constant for εATP association. These were found to be $(2.13 \pm 0.05) \times 10^6$ M^{-1} s^{-1} and $(2.03 \pm 0.06) \times 10^6$ M^{-1} s^{-1} at 0.8 and 0.2 mM Mg^{2+}, respectively. Thus, the association rate constant is a factor of ca. 3 lower than that measured at 0.65 mM [Ca^{2+}] but is relatively insensitive to Mg^{2+} in the range 0.2–0.8 mM.

Several interesting points arise from the experiments in the presence of ADP. First, as seen from the obvious difference in the slopes of the plots at 0.2 and 0.8 mM Mg^{2+} (Figure 5a), the association rate constant is strongly dependent on the Mg^{2+} ion concentration in this range, changing from $(4.15 \pm 0.49) \times 10^5$ M^{-1} s^{-1} at 0.2 mM Mg^{2+} to $(1.15 \pm 0.05) \times 10^6$ M^{-1} s^{-1} at 0.8 mM Mg^{2+}. Second, at the higher Mg^{2+} concentration, the rate constant is identical with that measured for ATP under the same conditions. This point is illustrated in

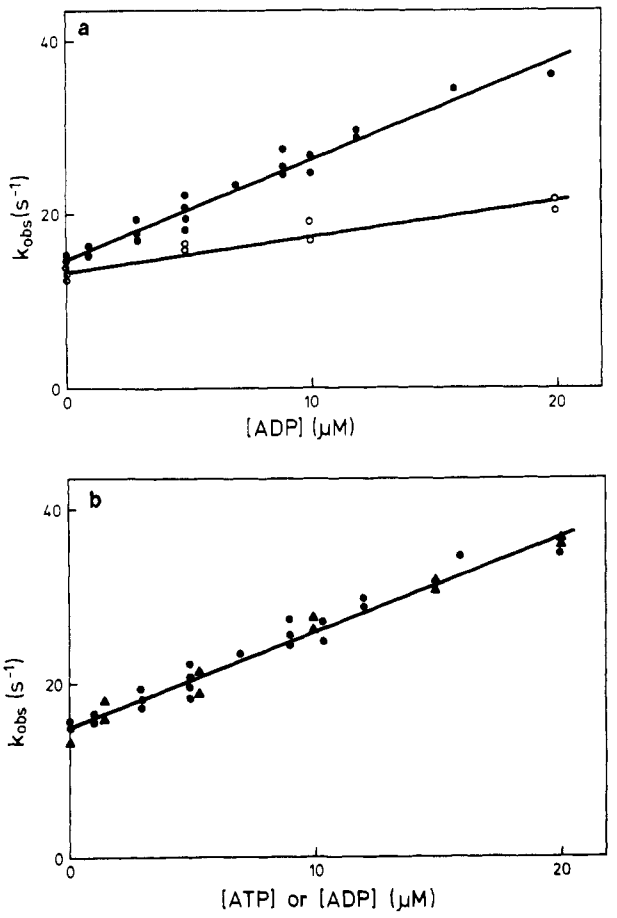

FIGURE 5: (a) Dependence of the observed pseudo-first-order rate constant for ϵATP binding to G-actin under physiological conditions on the concentration of ADP. Conditions: 4 mM Hepes, pH 7.6, 0.5 mM EDTA, 100 mM KCl, 0.7 mM $MgCl_2$ (○; free $[Mg^{2+}]$ = ca. 0.2 mM) or 1.3 mM $MgCl_2$ (●; free $[Mg^{2+}]$ = ca. 0.8 mM), 1.70 μM G-actin. [ϵATP] = 6.56 (○) or 6.76 μM (●). (b) The same as (a) but including points for ATP (▲) as well as ADP (●) with free $[Mg^{2+}]$ = 0.8 mM and [ϵATP] = 6.76 μM.

Figure 5b, where the results for ADP and ATP are plotted together. Third, no slow phase arising from dissociation of ADP bound initially was seen, in contrast with the results described above for the low ionic strength/Ca^{2+} conditions. This indicates that, after the initial binding transient, the equilibrium situation regarding ADP/ϵATP binding already pertains, suggesting that the ratio of the equilibrium constants for ϵATP and ADP binding is on the same order as the ratio of the association rate constants, i.e., ca. 2. This compares with ca. 3 from the work of Wanger and Wegner (1983), which is good agreement, and contrasts strongly with the factor of ca. 100 obtained under the low ionic strength/Ca^{2+} conditions. Surprisingly, ϵATP reproducibly displayed a 2-fold faster rate constant for association than either ATP or ADP.

The results presented here on the transient kinetics of ATP and ADP binding to G-actin under physiological conditions confirm the equilibrium results reported by Wanger and Wegner (1983) and give values for the rate constants for ATP and ADP association. Rate constants for dissociation have not yet been measured.

Influence of Divalent Metal Concentration of the Association Rate of Nucleotides with G-Actin. The observed dependence of the rate of ADP binding to G-actin is of interest with respect to the question of the location of the metal ion in G-actin–metal–nucleotide complexes. This point is controversial, with evidence both for (Loscalzo & Reed, 1976; Miki & Wahl, 1985; Brauer & Sykes, 1985; Jacobson &

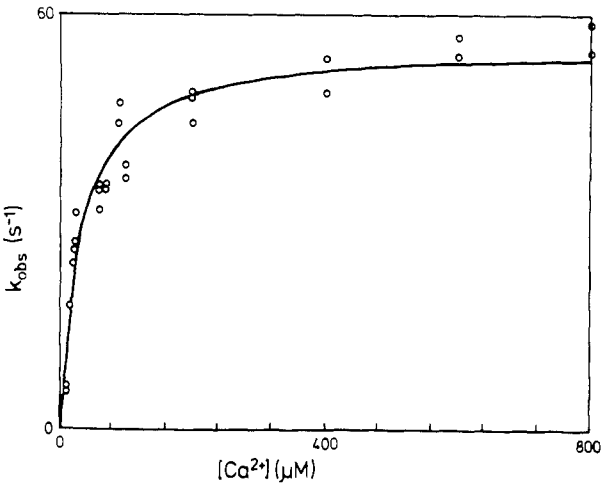

FIGURE 6: Dependence of the apparent pseudo-first-order rate constant for ϵATP binding to G-actin on the free Ca^{2+} concentration. Conditions as in Figure 1, except that the EDTA and added Ca^{2+} concentrations were varied to give free Ca^{2+} concentrations in the 10–800 μM range. [G-actin] = 1.81 μM; [ϵATP] = 6.42 μM.

Rosenbusch, 1976) and against (Barden et al., 1980; Barden & dos Remedios, 1984) spatial proximity of metal and nucleotide binding sites appearing in the literature. In principal, kinetic studies can help to answer this question. Three situations can be envisaged concerning the expected dependence of the rate of nucleotide binding on the divalent metal ion concentration. If only metal-free nucleotide is recognized in the association step, increasing the metal ion concentration at constant nucleotide concentration should lead to a hyperbolic decrease in the observed rate of binding, and the concentration dependence should be predictable from the known association constant of nucleotide and metal ion. If the nucleotide–metal ion complex is recognized, the rate of binding should increase hyperbolically with the metal ion concentration. Obviously, this effect will only be detectable at concentrations not too much above the metal–nucleotide dissociation constant. Finally, it is conceivable, though perhaps less likely, that both free nucleotide and its metal ion complex are equally well recognized, in which case no metal ion concentration dependence should be observed.

The results shown in Figure 5 indicate that on changing the Mg^{2+} concentration from 0.2 to 0.8 mM there is very little effect on the rate of ϵATP binding but a large increase in the rate of ADP binding. This is easily explainable if only metal–nucleotide complexes are recognized by G-actin. Since the affinity of nucleoside triphosphates for Mg^{2+} is about 1 order of magnitude greater than that of diphosphates, a larger effect is to be expected on the concentration of Mg–ADP than of Mg–ϵATP on making this change in concentration of Mg^{2+}. By use of literature values of the affinity constants (Phillips, 1966), changing Mg^{2+} from 0.2 to 0.8 mM should alter the relative proportions of Mg–ADP to total nucleotide from 24% to 55%, but of Mg–ATP to total nucleotide only from 95% to ca. 99%. This is in reasonable, if not exact, agreement with the changes seen in the apparent rate constants for association with G-actin. Thus, this evidence supports the hypothesis that binding of nucleotide to G-actin occurs via the metal–nucleotide complex.

Further support for this suggestion comes from the experimental results shown in Figure 6. Here the free calcium concentration in ϵATP association experiments was varied by varying the excess of Ca^{2+} over EDTA at the second mixing step in double mixing experiments. It can be seen that there is a marked dependence of the apparent rate of association

on the calcium concentration at concentrations up to about 200 μM and that these data can be fitted to a hyperbolic curve. The apparent Ca^{2+} dissociation constant derived from these experiments is ca. 26 μM. If the suggested mechanism of binding is correct, this should have the same value as the independently determined dissociation constant for the Ca–ϵATP complex. We have recently shown that this has a value of 12 μM at very low ionic strength (4 mM HEPES, pH 7.6) and is the same as the corresponding constant for the Ca–ATP complex (Nowak et al., 1988). However, literature values for the dissociation constant for Ca–ATP at an ionic strength of 0.1 M are in the range of 100 μM, so that there is apparently a relatively large dependence on ionic strength, or in particular on the concentration of monovalent metal ions. Thus, it is likely that the relevant constant under the conditions used in our experiments should be somewhat higher than 12 μM due to metal ions added with EDTA. In addition to this, the Ca^{2+} concentration is subject to relatively large errors, since EDTA is mixed with a very small excess of Ca^{2+} to obtain the points at the lower end of the curve. Bearing this in mind, the agreement between the two values is good enough to be taken as supporting evidence for the involvement of Ca–ϵATP in the association reaction. As discussed previously (Nowak et al., 1988), the Ca^{2+} ion involved in this reaction does not appear to be bound at the single high-affinity site for divalent cations on actin.

Conclusions

Second-order rate constants for the association of ATP and ADP with G-actin have been measured for the first time. We conclude from the experiments reported here that the ca. 100-fold difference in the affinity of ATP and ADP for G-actin at low ionic strength in the presence of Ca^{2+} is not due to a 100-fold slower rate of binding, as previously reported, but to a faster rate of ADP release, which could also be measured directly in the experiments reported. It should be pointed out that the measured rate constants of nucleotide release from G-actin in the presence of divalent metal ions is not a single step process. We have recently demonstrated that the rate of nucleotide release from G-actin devoid of metal ions is relatively fast, at least in the case of ϵATP and ATP (Nowak et al., 1988), and that it is the slow rate of metal ion release which is responsible for the slow effective dissociation rates (Gershman et al., 1986; Nowak et al., 1988). This is in contrast to earlier interpretations of the rates of nucleotide and metal ion exchange, which had suggested that metal ion release was a fast process followed by much slower nucleotide release. While it has not been demonstrated that the same situation holds when ADP is the bound nucleotide, it seems very likely to be the case. The much faster rate of ADP than ATP release could be due to a lower Ca^{2+} affinity of G-actin–ADP, to a higher rate constant for ADP release from this complex, or to a combination of both effects.

These differences are not apparent under more nearly physiological conditions of ionic strength and divalent metal ion concentrations. Since both the affinities of ATP and ADP for G-actin and their association rate constants in the presence of 100 mM KCl and 0.8 mM $MgCl_2$ are essentially identical, it follows that the rate constants for release of ATP and ADP are also identical. These factors should be born in mind in attempts to model the treadmilling phenomenon (Neuhaus et al., 1983).

It is not clear why the rate of ADP dissociation is so strongly affected by changing the ionic conditions, but one possible explanation is as follows. As pointed out previously (Nowak et al. 1988) and as was apparent from earlier work (Kuehl & Gergely, 1969; Strzelecka-Golaszewska, 1973), there is more than one route for dissociation of nucleotides from G-actin in the presence of divalent metal ions. In principle, the order of dissociation of metal ion and nucleotide can be reversed by manipulating the metal ion concentration, and this is reflected by the observation that on increasing the metal ion concentration, the observed rate constant for ϵATP dissociation decreases hyperbolically to a definite limit but not to zero (Nowak et al., 1988). This represents the rate of nucleotide dissociation without prior dissociation of the tightly bound metal ion. It is possible that under the conditions of high Mg^{2+} concentration and high ionic strength used here and by Wanger and Wegner (1983) this limit has been reached for both ATP and ADP and that this limiting rate is similar for both nucleotides. Since the association rates are also similar, the overall affinities for ATP and ADP would then be very similar, despite the fact that differences are seen under different ionic conditions.

Acknowledgments

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Registry No. ATP, 56-65-5; ADP, 58-64-0; ϵ-ATP, 37482-17-0; Mg, 7439-95-4.

References

Barden, J. A., & dos Remedios, C. G. (1984) *J. Biochem. (Tokyo) 96*, 913–921.

Barden, J. A., Cooke, R., Wright, P. E., & dos Remedios, C. G. (1980) *Biochemistry 19*, 5912–5916.

Drabikowski, W., & Nowak, E. (1973) *Biochim. Biophys. Acta 328*, 470–480.

Elzinga, M., Collins, J. H., Kuehl, W. M., & Adelstein, R. S. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70*, 2687–2691.

Houk, W. T., & Ue, K. (1974) *Anal. Biochem. 62*, 66–74.

Jacobson, G. R., & Rosenbusch, J. P. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 2742–2746.

Kuehl, W. M., & Gergely, J. (1969) *J. Biol. Chem. 244*, 4720–4729.

Loscalzo, J., & Reed, G. H. (1976) *Biochemistry 15*, 5407–5412.

Miki, M., & Wahl, P. (1985) *Biochim. Biophys. Acta 828*, 188–195.

Neidl, C., & Engel, J. (1979) *Eur. J. Biochem. 101*, 163–169.

Neuhaus, J.-M., Wanger, M., Keiser, T., & Wegner, A. (1983) *J. Muscle Res. Cell Motility 4*, 507–527.

Nowak, E., Strzelecka-Golaszewska, H., & Goody, R. S. (1988) *Biochemistry 27*, 1785–1792.

Philips, R. (1966) *Chem. Rev. 66*, 501–527.

Secrist, J. A., Barrio, J. R., Leonard, N. J., & Weber, G. (1972) *Biochemistry 11*, 3499–3506.

Strzelecka-Golaszewska, H. (1973) *Biochim. Biophys. Acta 310*, 60–69.

Waechter, F., & Engel, J. (1977) *Eur. J. Biochem. 74*, 227–232.

Wanger, M., & Wegner, A. (1983) *FEBS Lett. 162*, 112–116.