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Enzymatic Hydrolysis of ATP and e-ATP by F-Actin

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Abstract

Enzymatic hydrolysis of e-ATP by F-actin with and without application of sonic vibration at various pHs was investigated and compared with that of ATP. There was no significant difference on enzymatic activity between F-actin-bound e-ADP and F-actin-bound ADP. The hydrolysis rate of e-ATP under sonic vibration decreases monotonically with decreasing pH, similar to that of ATP. The magnitude of e-ATP hydrolysis rate was, however, about one third of that of ATP hydrolysis rate in the pH range between 6.3 and 8.5. Enzymatic hydrolysis of e-ATP without sonic vibration at room or higher temperatures decreases monotonically with increasing pH and becomes almost negligible at pH 8.5. The pH profile and the magnitude of enzymatic hydrolysis without sonic vibration were similar with ATP. Since the fluorescence intensity of e-ATP at 410 nm is enhanced by the binding with G-actin, the exchange binding affinity of e-ATP to G-actin which can be measured fluorophotometrically was about one third of that of ATP.

Introduction

It is well known that ATP bound to G-actin is dephosphorylated into ADP and orthophosphate during the polymerization of G-actin molecules to F-actin filaments in the presence of neutral salts [1]. Furthermore, F-actin is known to catalyze also the hydrolysis of ATP at

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high temperature [2] or under sonic vibration [3]. The ATPase activity at acid pH range between 4.0 and 6.0, where F-actin forms paracrystalline aggregates, is especially high [4]. The complex of actin molecule with the substrate product (ADP) in a neutral salt solution is much more stable than a usual enzyme–substrate complex [2]. Since ATP or ADP bound to G-actin exchanges freely with ATP and ADP in the medium and also the hydrolysis of ATP requires the formation of a bond between actin molecules, it has been supposed that structural changes such as the breaking or loosening and the reformation of bonds between actin molecules must take place during the enzymatic behavior of F-actin [2]. However, the structural and functional properties of the active site in the actin molecule for nucleotide binding or splitting are entirely unknown. For their investigation, a comparative study using ATP analogs may be valuable.

Recently, synthesis of a strong fluorescent analog of ATP, 1-N^6-ethenoadenosine triphosphate (e-ATP) was reported [5]. Therefore, with a special reference to ATP hydrolysis, it was attempted to investigate the profile of e-ATP hydrolysis by F-actin with and without application of sonic vibration and at various pHs. The catalytic properties of myosin and heavy meromyosin for e-ATP were similar to those for ATP [6]. Stoichiometric binding of e-ATP to G-actin was investigated by Miki et al. [7] and also independently by Thames et al. [8]. Martonosi [9] studied the binding of various nucleoside phosphates (ATP, ITP, UTP, and d-ATP) to the ATP-binding site of G-actin. His results indicated that the base ring and especially the ribose moieties play an important role for the binding affinity of such nucleoside triphosphates. On the other hand, it was reported by Kuroda and Maruyama [4] that the enzymatic hydrolysis of these ATP analogs by F-actin at acid pH is similar to that of ATP; nevertheless at pH 8.3, 57° C, such triphosphates are hydrolyzed to a much smaller extent than ATP.

In this paper, it is shown that e-ATP hydrolysis by F-actin is similar to that of ATP over a wide pH range, and therefore it is suggested that e-ATP or e-ADP can be used as excellent fluorescent probes for structural and functional studies of G- and F-actin under various conditions.

Materials and Methods

e-ATP was synthesized from ATP and purified by DEAE-cellulose chromatography according to a slight modification of the method of Onishi et al. [10]. The ultraviolet absorption spectrum of purified e-ATP as compared with that of ATP is shown in Fig. 1. ATP has no absorption at the wavelengths near 300 nm, so various spectral studies of e-ATP plus actin solutions can be performed in the presence of ATP. As seen in

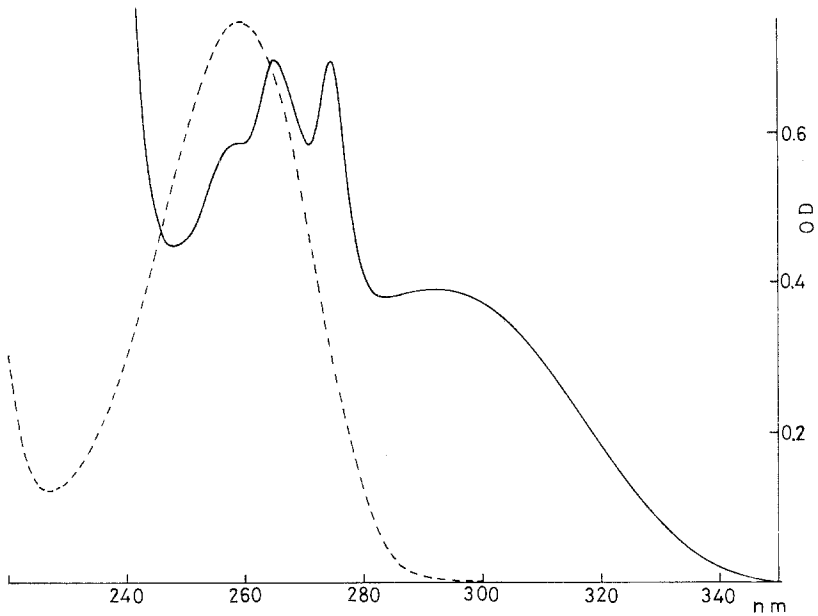

Figure 1. The absorption spectra of e-ATP and ATP: solid line, e-ATP; broken line, ATP. An absorption maximum is seen clearly near 295 nm where there is practically no absorption for ATP.

Fig. 1, e-ATP has a clear absorption maximum at the wavelength of 295 nm. In order to obtain pure e-ATP which is eluted by a salt and HCl solution, one must first exhaustively wash the synthesized product of e-ATP adsorbed on DEAE-cellulose by a slightly lower salt and HCl solution. If e-ATP is not highly purified, then the e-ATP solution has no absorption maximum at 295 nm, but rather the absorption pattern is a plateau or decreases gradually with increasing the wavelength at or near 295 nm [see ref. 5, 11].

Acetone powder of rabbit skeletal muscle was prepared with a method similar to that of Straub and Feuer [1]. G-Actin is extracted in cold water and polymerized in the presence of 30 mM KCl. A sufficient amount of e-ATP is added to pellets of the centrifuged F-actin and depolymerized. The same cycle of polymerization, centrifugation, and dissolution for actin purification is repeated once more. If necessary, the excess e-ATP and ADP not bound to G-actin are eliminated by the addition of 1/20 vol. of Dowex-1 suspension. Then, a solution of e-ATP, all of which is bound to G-actin, is obtained by paper filtration of the above solution. To eliminate actin from a solution containing e-ATP or e-ADP, the solution was heated for 10 min at 70° C in the presence of 3

or 5 mM $MgCl_2$, leading to the precipitation of denatured actin [12]. G-Actin which binds ATP instead of e-ATP was also prepared in a similar way. The F-actin preparation which originally binds e-ADP but not ADP was used for enzymatic study, unless otherwise indicated.

For measurements of fluorescence spectra, a Shimazu RF-501 spectrofluorometer was used. A Tominaga TI-100 sonic vibrator was used for sonication of F-actin solutions. ATP was obtained from Kyowa Hakko Kogyo Co. The other chemicals used were reagent grade.

Results and Discussion

When a solution of G-actin-bound e-ATP is polymerized to F-actin by the addition of 0.1 M KCl, the fluorescence intensity decreases considerably (see the top broken line and the solid line in Fig. 2). The qualitative features of the excitation and emission spectrum are,

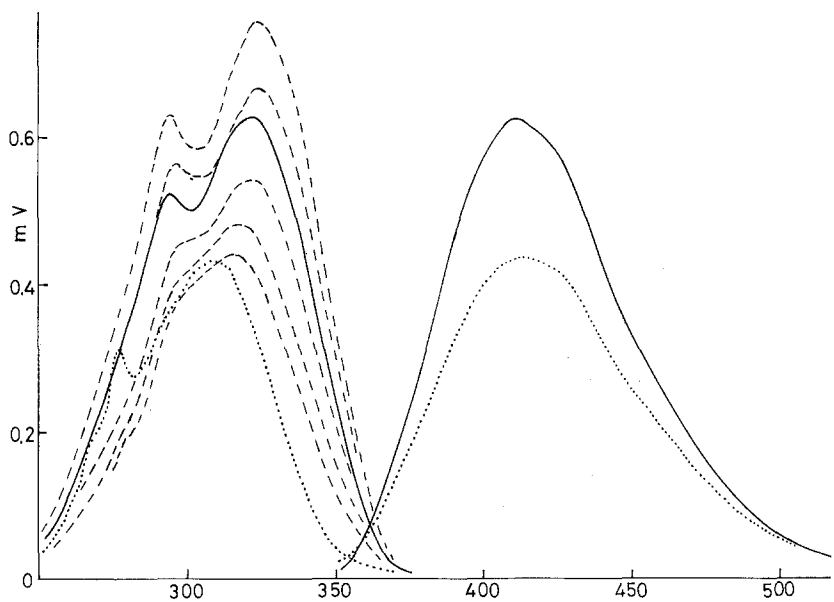

Figure 2. The excitation spectra at fluorescence wavelength of 410 nm and the emission spectra at the excitation wavelength for maximum fluorescence: The top broken line indicates e-ATP bound to G-actin. The lower broken lines are the excitation spectra of e-ATP plus G-actin solutions to which ATP is successively added. The solid lines are the excitation and emission spectra of e-ADP bound to F-actin. The dotted lines are the excitation and emission spectra of free e-ATP. The concentrations of e-ATP or e-ADP in the above solutions are all the same.

however, not changed by the polymerization. The broken lines in Fig. 2 show the decreases of fluorescence intensity by successive addition of ATP in a G-actin plus e-ATP solution. Such decreases in fluorescence intensity are, of course, due to the replacement of originally bound e-ATP with added ATP. Thus, the amounts of the replaced ATP at various concentrations of added ATP can be estimated by measuring the extent of fluorescence decreases. Since the concentrations of G-actin and e-ATP are originally known, the relative affinity constant of e-ATP to G-actin is calculated easily. The affinity constant was about one third that of ATP. Of course, the addition of ATP to G-actin plus e-ATP solution does not disturb the fluorescence measurements at the wavelengths longer than 300 nm, as demonstrated in Fig. 1. Thames et al. [8] have also studied the binding constant of e-ATP to G-actin by following the inactivation of e-ATP–G-actin complex [12]. They have found that the affinity of e-ATP for G-actin is about half of that of ATP in the absence of EDTA.

The excitation and emission spectra of free e-ATP at a concentration identical to that of e-ATP bound to G-actin are also indicated by the dotted lines in Fig. 2. The emission maximum of free e-ATP at 410 nm is also observed in e-ATP bound to G-actin and in e-ADP bound to F-actin. On the other hand, the excitation maxima of e-ATP bound to G-actin and to free e-ATP are 325 and 310 nm, respectively (Fig. 2). The fact

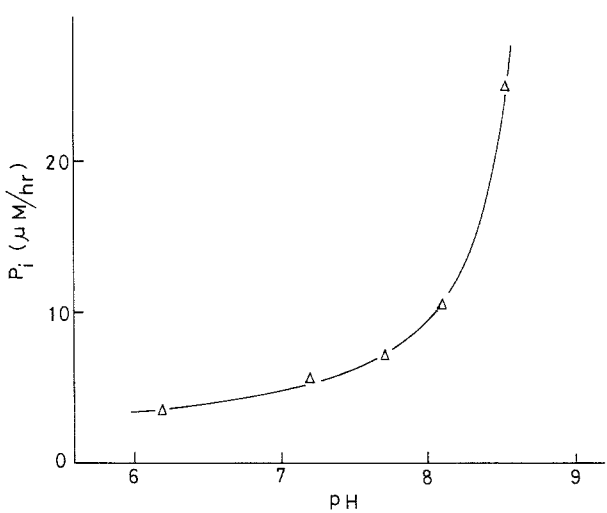

Figure 3. pH dependence on sonically activated e-ATP hydrolysis by F-actin in which preparation e-ADP but not ADP originally binds. Medium conditions: 2.9 mg/ml F-actin, 60 mM KCl, 20 mM Tris–HCl or Tris–maleate, 24° C, 600 μM e-ATP, 1 hr sonication.

that the binding of e-ATP to G-actin causes an enhancement of fluorescence intensity and a red shift in the excitation maxima has been also observed independently by Miki et al. [7] and by Thames et al. [8]. They also reported the experimental results on the stoichiometry of e-ATP binding to G-actin and its binding affinity.

Figure 3 shows the pH dependence of sonically activated hydrolysis of e-ATP by F-actin which binds e-ADP but not ADP. With decreasing pH from 8.5 to 6.3, the activity is monotonically decreased. A similar pH dependence of sonically activated hydrolysis of ATP by F-actin was obtained. However, its absolute value at each pH was about three times that of e-ATP hydrolysis. The replacement of e-ADP with ADP in original F-actin preparation and the use of F-actin which originally binds ADP did not cause any significant change in activity of ATP hydrolysis. This fact suggests that the structure and dynamic features of double-helical F-actin filament (breaking and reformation of bonds between actin molecules), is not altered by the replacement of ADP with e-ADP.

The e-ATPase activity of F-actin without application of a sonic field was measured at various pHs and a temperature of 24.6° C. As seen in Fig. 4, the activity decreases with increasing pH and becomes very small at a pH higher than 8.0. At a pH lower than 6.3, F-actin forms aggregates, and turbidity of the solution increases abruptly. However, there is no abrupt change in the hydrolysis rate of e-ATP in the vicinity of such critical pH, as indicated by the solid triangles in Fig. 4. The pH

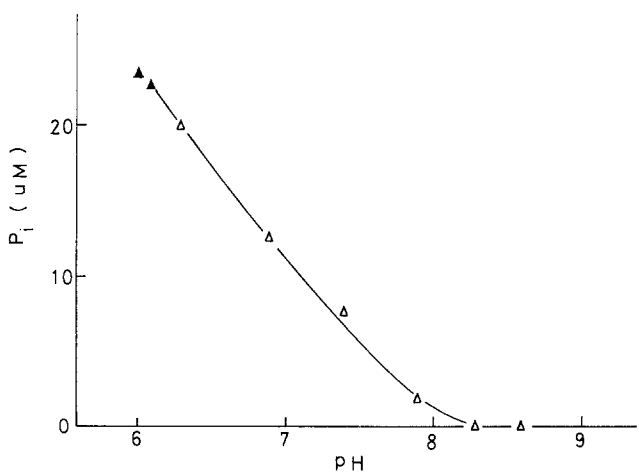

Figure 4. pH dependence on enzymatic hydrolysis of e-ATP by F-actin at 24.6° C. Medium conditions: 0.9 mg/ml F-actin, 60 mM KCl, 20 mM Tris–HCl or Tris–maleate, 300 mM e-ATP, and 2.5 hr incubation at 24.6° C. The solid triangles mean that at these pHs F-actin formed a turbid aggregate.

dependence and the magnitude of ATP hydrolysis by F-actin at the same temperature were similar, as in the case of e-ATP hydrolysis. Kuroda and Maruyama [4] observed very high activity of ATP hydrolysis by F-actin in the pH range between 4.0 and 6.0, where F-actin forms paracrystal aggregates, and pointed out that such high enzymatic splitting of ATP is a common property of paracrystalline F-actin aggregates exposed to acid pH. However, this interpretation is unlikely, and it seems rather that, not the aggregated state, but the rates of breaking and reformation of bonds in single F-actin filament, which reflects kinetic constants of the ATPase activity, is specifically dependent on pH.

It was reported in the previous paper [2] that, unlike the sonically activated hydrolysis of ATP, the ATPase activity at high temperature in the absence of free divalent cations is greatly dependent on the substrate concentration. This is also true for e-ATP as seen in Fig. 5. The rates of e-ATP and ATP hydrolysis measured at 37°C, pH 6.9, in the presence of free magnesium ions are almost constant during 7 hr. On the other hand, the rates of both e-ATP and ATP hydrolysis in the absence of free magnesium ions decrease greatly with decreasing the substrate concentration. This means that, contrary to those in the presence of free divalent cations, the V_{max} and the Michaelis constants for both e-ATP

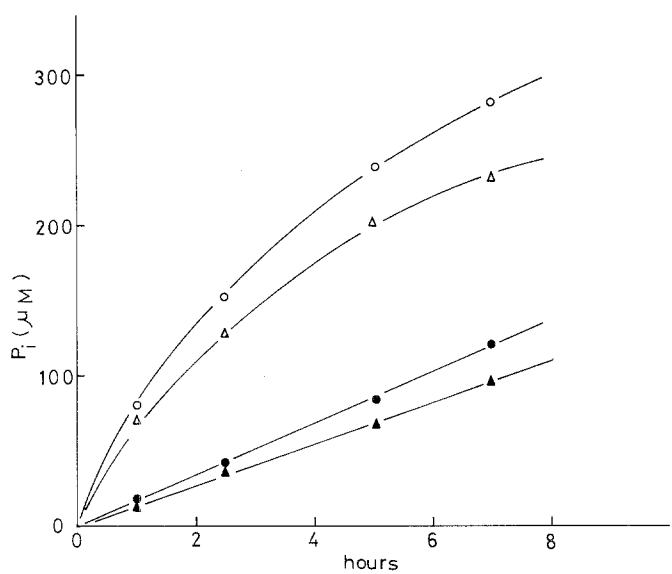

Figure 5. Demonstration of the time courses of e-ATP and ATP hydrolysis in the presence and absence of 1 mM $MgCl_2$. The concentration of e-ATP or ATP was 300 μM. The solutions of 3.4 mg/ml F-actin in 60 mM KCl, 20 mM Tris-maleate (pH 6.9) were incubated at 37°C. The circles indicate e-ATP; the triangles, ATP; solid marks, in the presence of 1 mM $MgCl_2$; open marks, in the absence of $MgCl_2$.

and ATP in the absence of free divalent cations are very large under the pH condition employed, as reported in the previous paper [2]. The straight line of the solid circles in Fig. 5 suggests again that the replacement of e-ADP bound originally to F-actin with ADP during ATP hydrolysis does not alter the rate of subsequent ATP hydrolysis.

From the above experiments, it is obvious that the structural and functional property of F-actin is not altered significantly by the use of the fluorescent ADP analog, e-ADP, as a prosthetic group of F-actin, and that such prosthetic group can be used as a fluorescent probe for a variety of in vitro investigations on the molecular mechanisms of muscular contraction, e.g., the interaction of F-actin with heavy meromyosin. Such research is now in progress and will be reported soon elsewhere.

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