INTERACTION OF ACTIN WATER ε-ATP

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1. Introduction

Since Straub found actin, one of the major proteins of the contractile system [1], the structural and functional relation of actin with bound nucleotide has been extensively studied. The essential role of the nucleotide in the biological activity of actin is however not yet clear [2,3]. As a new approach to this problem, we attempted to introduce ε-ATP, a fluorescent analog of ATP [4], and found that G-actin binds ε-ATP and the bound ε-ATP is hydrolysed in the course of polymerization of actin in a very similar way to ATP. Structure and function of F-actin having bound ε-ADP is almost the same as F-actin having bound ADP.

2. Materials and methods

Actin from rabbit skeletal muscle was prepared according to Straub [2] with a slight modification to remove native tropomyosin before acetone-treatment [5]. Actin was polymerized in 30 mM KCl in an iced-bath to avoid contamination of tropomyosin. Details of purification of actin is given in a previous paper [5]. G-actin having bound ADP (G-actin–ADP) was prepared according to Mihashi [6]. ε-ATP was synthesized from ATP (Sigma Co.) by the method of Secrist et al. [4]. The absorption spectrum of the synthesized ε-ATP was identical to that obtained by them. Optical absorption and fluorescence were measured with Zeiss PMQ II and Hitachi MPF-2A respectively.

3. Results

3.1. *Binding of ε-ATP to G-actin*

ε-ATP was added to a solution of G-actin–ADP of about 4 mg/ml at 0°C to give a final concentration of ε-ATP of 200 μM. The solution was dialysed against 500 ml of a cold buffer solution containing 20 μM ε-ATP and 1 mM bicarbonate overnight. An aliquot of the dialysed solution was subjected to column chromatography on Sephadex G-25 (1.5 × 30 cm) at 4°C. When eluted with 1 mM bicarbonate solution, a large amount of ε-ATP appeared at the position of G-actin as well as a delayed peak corresponding to free ε-ATP. The absorption spectrum of ε-ATP shifted to a longer wavelength upon the binding to G-actin (fig. 1). The change was quite similar to the spectrum shift of ε-ATP when transferred from an aqueous to a dioxane solution [4]. Despite of this change, the fluorescence emission maximum did not shift from 410 nm. The stoichiometry of the binding of ε-ATP was determined from the fluorescence intensity at 410 nm after

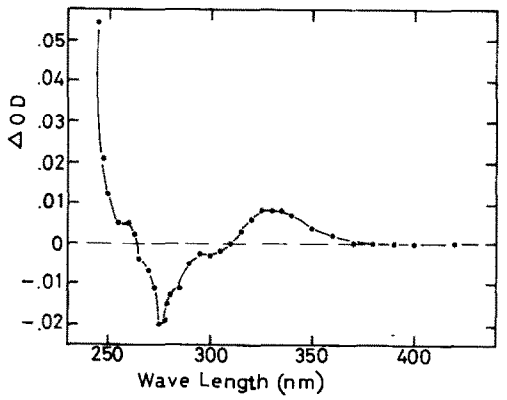

Fig. 1. Absorption change of ε-ATP caused by the binding to G-actin. Optical density was measured with doubled-cell difference spectrum technique. Content of cell; Reference-1/G-actin–ADP; Reference-2/ε-ATP; Sample/G-actin–ADP plus ε-ATP. G-actin–ADP 2.0 mg/ml, μM ε-ATP 100, solvent, 5 mM Tris–HCl (pH 7.0). path length of cells 2 mm.

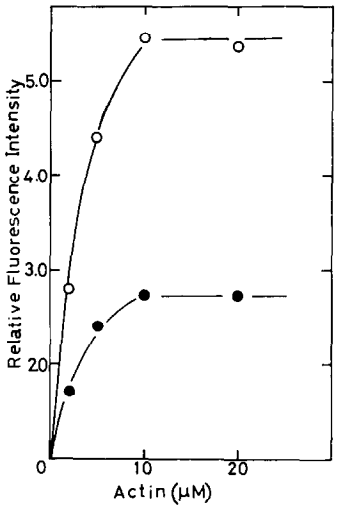

Fig. 2. Fluorescence intensity of ϵ-ATP bound to G-actin. The solutions contained ϵ-ATP 9.7 μM and various amount of G-actin–ADP. Solvent, 5 mM Tris–HCl (pH 7.6). Excitation 340 nm (○), and 330 nm (●), emission 410 nm.

excitation at 340 nm where absorption of free ϵ-ATP was almost negligible relative to the actin-bound ϵ-ATP. The binding saturated at the molar ratio of ϵ-ATP to actin equal to unity (fig. 2).

3.2. *Hydrolysis of the bound ϵ-ATP accompanying polymerization of actin and incorporation of the resultant ϵ-ADP in F-actin*

G-actin-ϵ–ATP thus obtained was polymerized by adding KCl (60 mM) and the time courses of liberation of inorganic phosphate and appearance of flow birefringence were followed. The hydrolysis of ϵ-ATP bound to G-actin occurred as polymerization proceeded (fig. 3). The total amount of inorganic phosphate liberated (11 μM) was slightly higher than the amount of actin (9.1 μM; taking the molecular weight of actin of 45×10^3). However if we adopt the molecular weight chemically determined recently by Elzynga et al. [7], the agreement becomes better. F-actin–ϵ-ADP thus obtained was dialysed against a large volume of cold solution containing 0.1 M KCl, 1 mM $MgCl_2$ and 20 mM phosphate buffer (pH 7.0) overnight. This F-actin–ϵ-ADP was diluted with the dialysate to give several concentrations of F-actin. The fluorescence intensity at 410 nm of these solutions was proportional to the amount of F-actin. This

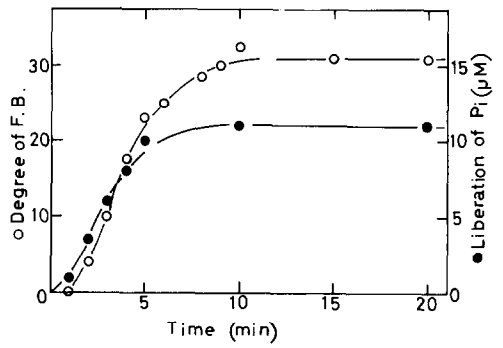

Fig. 3. Time course of polymerization (○) of G-actin-ϵ-ATP and liberation of inorganic phosphate (●). Polymerization of actin was determined from flow birefringence increase (F.B.). G-actin–ADP 0.4 mg/ml. 50 μM ϵ-ATP, 10 mM Tris–HCl (pH 7.6). Polymerization was initiated by the addition of KCl (final concentration 60 mM) at 28°C. The degree of flow birefringence of 1 mg/ml of F-actin is about 77°C with the flow birefringence apparatus used here.

verifies that the dilution did not cause removal of ϵ-ADP from F-actin. The specific flow birefringence of F-actin–ϵ-ADP was the same as purified F-action–ADP.

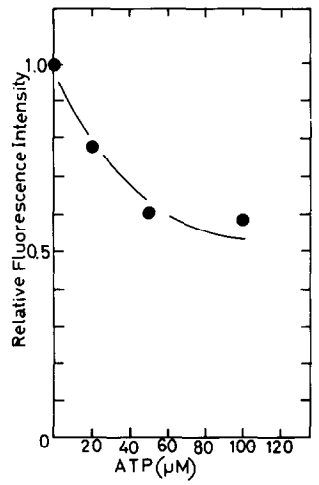

Fig. 4. Competitive binding of ϵ-ATP and ATP to G-actin. G-actin-ADP of 16 μM was incubated in the presence of ϵ-ATP 91 μM and various amount of ATP for 2 hr at 0°C and then polymerized by the addition of 100 mM KCl, 1 mM $MgCl_2$ (solvent, 20 mM Tris–HCl, pH 7.6). After free ϵ-ATP and ATP were dialysed out in the same buffer solution, fluorescence of ϵ-ADP bound to F-actin was measured. Excitation 330 nm, emission 410 nm.

When G-actin–ADP was incubated with ϵ-ATP in the presence of various amounts of ATP for 2 hr and polymerized in the same way as above, the amount of ϵ-ADP incorporated in F-actin was reduced as the amount of ATP increased (fig. 4). It is already known that ATP replaces actin-bound ADP [2,3], the above result indicates that ϵ-ATP and ATP competes for the nucleotide-binding site of G-actin.

3.3. *Structural and functional properties of F-actin–ϵ-ADP*

Difference absorption spectrum of F-actin–ϵ-ADP to G-actin–ϵ-ATP was essentially the same as that of F-actin–ADP to G-actin–ATP (fig. 5). It is therefore likely that the structure of protein moiety of F-actin–ϵ-ADP is quite similar, if not identical, to F-actin–ADP.

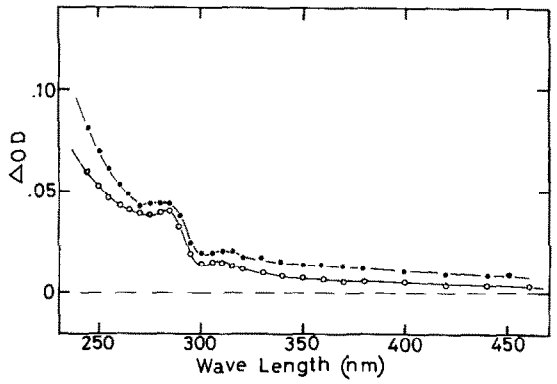

Fig. 5. Absorption change of G-actin–ϵ-ATP associated with polymerization of actin (○). Optical density was measured with doubled-cell technique. Content of cell; Reference-1/G-actin-ADP plus ϵ-ATP; Reference-2/KCl; Sample/G-actin-ADP, ϵ-ATP and KCl. G-actin–ADP 2.1 mg/ml, μM ϵ-ATP, 100 mM KCl. Solvent, 5 mM Tris–maleate (pH 7.0). Optical density was measured at room temperature. The absorption change of G-actin–ATP associated with polymerization is also shown (●). In the curve of G-actin–ATP, the zero level of the ordinate is shifted by +0.005 for a better comparison with G-actin–ϵ-ATP. The correction for the scattering of F-actin is not made.

Mg-activated ATPase of heavy meromyosin from rabbit skeletal muscle was enhanced 5-fold when F-actin–ϵ-ADP of 20 molar excess of heavy meromyosin was present. Similar measurement was made at several concentrations of F-actin–ϵ-ADP, and V_m determined from Lineweaver–Burk plot was 250 μmoles/min/gram of heavy meromyosin, which is very close to the activation of F-actin–ADP.

4. Discussion

So far as we have studied, ϵ-ATP replaces ATP in relation to G-actin without any serious perturbation on actin. Because of its high fluorescence power, ϵ-ATP opened a way to the dynamic study of F-actin; the structural alteration of F-actin associated with its interaction with myosin and regulatory proteins is investigated and the report will be given shortly.

Acknowledgements

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