FLUORESCENCE STUDY OF ε-ADP BOUND TO RABBIT F-ACTIN: STRUCTURAL CHANGE IN THE ADENINE SUBSITE OF F-ACTIN UNDER THE INFLUENCE OF HEAVY MEROMYOSIN

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1. Introduction

In a recent nanosecond pulse-fluorometric study of F-actin-ε-ADP [1], it was found that ε-ADP is tightly bound in F-actin and the macromolecular motion of F-actin is characterised by a correlation time as long as several microseconds. The study is extended in the present work so that the fluorescence of ε-ADP bound to F-actin was measured in the presence of an increasing amount of heavy meromyosin. Both static and time-dependent fluorescence measurements show that binding of heavy meromyosin to F-actin induces a cooperative structural change in the adenine subsite of F-actin. The cooperativity is enhanced by the combination of F-actin–ε-ADP with tropomyosin.

2. Experimentals

2.1. *Materials*

F-actin, heavy meromyosin, tropomyosin of rabbit skeletal muscle were prepared as described previously [2–4]. Incorporation of ε-ADP into F-actin was made according to the method of Miki et al. [5]. Saturation of F-actin with ε-ADP was accomplished by repeating G–F cycle of actin in the presence of a sufficient amount of ε-ATP.

2.2. *Fluorescence measurements*

Static fluorescence was measured with Hitachi MPF-2A. Precautions were taken to exclude exciting light from detection by the use of filters UV D1B and UV 39. Determination of fluorescence decay and anisotropy was performed with an instrument whose principal components are of ORTEC 9200 [6]. The exciting light was provided by a spark bursting in air at atmospheric pressure. Details of the instrument and measurements were described elsewhere [7]. The experimental decay curves $i_{||}(t)$ and $i_{\perp}(t)$ were measured separately and s(t) and d(t) were computed as follows [8],

$$s(t) = i_{||}(t) + 2\, i_{\perp}(t)$$
$$d(t) = i_{||}(t) - i_{\perp}(t)$$

As reported previously [1], fluorescence decay s(t) and d(t) of F-actin–ε-ADP are described by single exponential respectively and the decay time (τ_s, τ_d) can be simply determined by linear approximation of ln s(t) and ln d(t) as a function of t. Then, by extrapolation of the straight line to the time of maximal fluorescence intensity, r_0 the limiting anisotropy at zero time was determined as d(0)/s(0).

3. Results

3.1. *Fluorescence change of F-actin–ε-ADP under the influence of heavy meromyosin*

3.1.1. At low saturation of heavy meromyosin binding

When heavy meromyosin was added to F-actin–ε-ADP (0.16 mg/ml) at neutral pH, the turbidity of the solution increased in excess of the sum of contribution from both F-actin–ε-ADP and heavy

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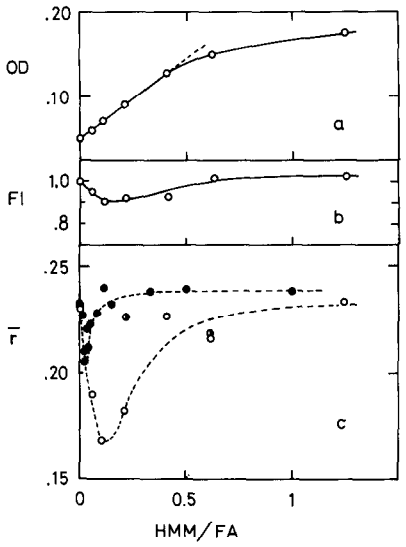

Fig.1. The influence of heavy meromyosin on F-actin-ϵ-ADP. (a) Optical density at 330 nm, (b) Static fluorescence intensity; excitation 340 nm, emission 440 nm. (c) Static fluorescence anisotropy; excitation 340 nm, emission 440 nm; (○) F-actin–ϵ-ADP; (◉) F-actin–ϵ-ADP + ATP (3.5 mM); (●) F-actin–ϵ-ADP combined with tropomyosin. F-actin–ϵ-ADP 0.16 mg/ml, KCl 100 mM, $MgCl_2$ 2 mM, phosphate buffer 10 mM (pH 7.0); at room temperature.

meromyosin. This turbidity increase was proportional to the amount of heavy meromyosin in the range of the molar ratio of heavy meromyosin to F-actin HMM/A less than 0.5 (fig.1a). Judging from the high value of binding constant of heavy meromyosin to F-actin–ADP so far reported ($2.6 \times 10^7\ M^{-1}$ [9]) and assuming the same binding constant to F-actin–ϵ-ADP, we find that almost all heavy meromyosin added is bound to F-actin–ϵ-ADP.

In spite of this stoichiometric relation of binding, the fluorescence of ϵ-ADP (both intensity and polarization) changed non-linearly with the amount of heavy meromyosin. As is shown in fig.1b, the static fluorescence intensity begins to decrease in the presence of a small amount of heavy meromyosin. The maximum desensitization (about 10%) is observed at HMM/A equal to 1/9. At this molar ratio, the fluorescence life time (τ_s) was slightly but distinctly smaller than F-actin–ϵ-ADP alone (table 1). The change in the fluorescence anisotropy is more

Table 1
Influence of heavy meromyosin on the fluorescence parameters of ϵ-ADP bound to F-actin

HMM/A	0[a]	1/9	1/1
τ_s	32.1 ± 0.2	31.7	31.9
τ_d	31.9 ± 0.2	32.4	32.8
r_o	0.23 ± 0.005	0.18	0.22

[a] The results obtained in the absence of heavy meromyosin were in very good agreement with the reported ones [1].

conspicuous. The static anisotropy decreased from 0.23 to 0.18 (fig.1c). On the other hand, it is found from time-dependent anisotropy measurement that the slope of d(t) curve is not affected by the presence of heavy meromyosin over the entire range of time measured but that the value $d(0)/s(0) = r_o$ is greatly reduced. This indicates that the fluorescence depolarization is not due to activation of Brownian motion of F-actin. Also, one can easily see, by taking consideration of the life time (26 nsec at room temperature) and $r_o = 0.23$ of free ϵ-ADP that the observed change can not be explained by release of ϵ-ADP from F-actin. As a matter of fact, we found that upon the addition of ATP (3.5 mM) to this F-actin–ϵ-ADP-HMM solution, the static anisotropy is reversed instantaneously to a level essentially the same as that of F-actin–ϵ-ADP alone (fig.1c). This is compatible with our knowledge

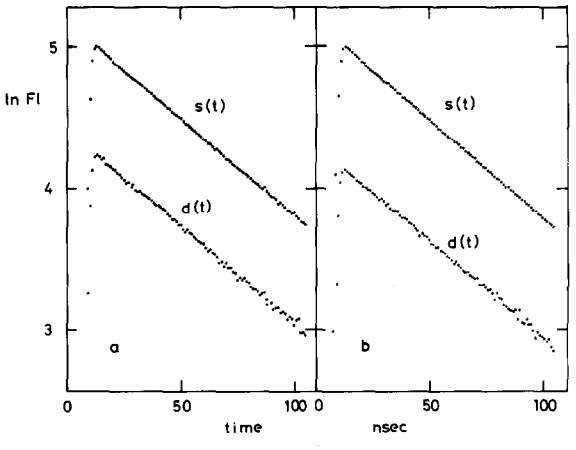

Fig.2. Fluorescence decay s(t) and d(t) of ϵ-ADP bound to F-actin. (a) F-actin–ϵ-ADP, (b) F-actin–ϵ-ADP + heavy meromyosin (HMM/A = 1/9). Room temperature.

that dissociation of heavy meromyosin from F-actin occurs upon the addition of ATP and actually indicates that the ϵ-ADP is still binding to F-actin. Therefore, it is reasonable to consider that a local structural change occurs in the adenine subsite of F-actin under the influence of heavy meromyosin and as a consequence the electronic state of ϵ-ADP is perturbed. A possibility of existence of 'forbidden character' in the sense of Albrecht [10] was pointed out previously [1].

3.1.2. At high saturation of heavy meromyosin binding

As the amount of heavy meromyosin increased, the static anisotropy is reversed to increase, and at a half saturation the static anisotropy is almost in the same order as F-actin–ϵ-ADP alone. Further addition of heavy meromyosin did not cause any appreciable change in fluorescence. However the turbidity of the solution continues to increase with an increment much smaller than the initial binding of heavy meromyosin (fig.1a). This may indicate that the mode of binding of heavy meromyosin to F-actin is different at higher saturation (e.g., HMM/A $>$ 0.5) or may be simply due to a lower binding constant.

3.2. *The effect of tropomyosin*

The above result clearly showed that the adenine subsite of F-actin is under the cooperative influence of binding of heavy meromyosin. The cooperativity will arise form the actin–actin interaction as well as interaction between actin and heavy meromyosin. With respect to this point, we found that the combination of tropomyosin to F-actin–ϵ-ADP affected its interaction with heavy meromysins in such a way that the maximal change occurs at the molar ratio of HMM/A as low as 1/40 (fig.1c). This is presumably because actin–actin interaction is intensified by tropomyosin, since the presence of tropomyosin seems to weaken the binding of heavy meromyosin to F-actin [11].

It should be added finally that the structural change at the adenine subsite of actin observed through the fluorescence of bound ϵ-ADP is also brought about by subfragment-1 of heavy meromyosin. The detailed analysis of this change in terms of cooperativity in F-actin is in progress and will be reported shortly elsewhere.

Conclusion

Fluorescence and its anisotropy of ϵ-ADP bound to F-actin changed in a biphasic way with the amount of heavy meromyosin bound to F-actin. The maximal change appeared at relatively low saturation of heavy meromyosin, indicating a strong cooperativity of this change. At the saturation of F-actin by heavy meromyosin, fluorescence and anisotropy became close to the initial value. However, this does not necessarily mean that the state of each monomeric unit of F-actin turns back to the initial one, since a previous study showed that heavy meromyosin induces a monotonous change in the fluorescence of fluorescein mercuric acetate bound to Cys-373 [12]. It is of interest that the fluorescence change observed in the present study is parallel to the change in the macromolecular flexibility of F-actin detected by quasielastic light scattering method [12,13]. Also, Loscalzo et al. found very recently a local structural change of F-actin under the influence of subfragment-1 [14], which is very similar to the present result.

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