FLUORESCENCE STUDIES OF 1,N^6-ETHENOADENOSINE TRIPHOSPHATE BOUND TO G-ACTIN: THE NUCLEOTIDE BASE IS INACCESSIBLE TO WATER

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SUMMARY: When 1,N^6-ethenoadenosine triphosphate (ε-ATP) is free in solution, its fluorescence is collisionally quenched by iodide ion, by methionine, by tryptophan, and by cysteine. None of these quenches the fluorescence of ε-ATP bound to G-actin. Thus, the ethenoadenine base is bound in a region of the protein which is inaccessible to collisions with these reagents. Since we have previously shown that the fluorescence of ε-ATP is quenched by water, the long lifetime of ε-ATP bound to G-actin (36 nsec, vs 27 nsec for ε-ATP in water) indicates that the bound nucleotide base is inaccessible to collisional quenching by water molecules.

In vertebrate skeletal muscle, the hydrolysis of ATP during the interaction of myosin and actin provides the energy for the work done by the contracting muscle. G-actin, the monomeric form of actin, binds one molecule of ATP. The nucleotide is dephosphorylated on polymerization of the actin to the filamentous form, giving F-actin with bound ADP. It is still not known what biological function the bound nucleotide has (1).

ε-ATP and ε-ADP[1], fluorescent derivatives of ATP and ADP (2), have been used in a number of recent studies to investigate the interaction between the nucleotides and actin (3-5). The fluorescence lifetime of the ethenoadenosine nucleotides is 27 nsec in water at 4°C. On binding to actin, the lifetime increases dramatically: the lifetime of ε-ATP on G-actin is 36 nsec (3). In this paper, we show that the longer lifetime is due to the binding

1. Abbreviations: ε-ATP and ε-ADP, 1,N^6-ethenoadenosine 5'-triphosphate and -diphosphate, respectively.

of the nucleotide base in a region of the protein where it is inaccessible to water.

MATERIALS AND METHODS

ε-ATP was synthesized using the method of Secrist, et al (2). Actin was prepared from acetone powder following the procedure of Spudich and Watt (6). Actin concentrations were determined spectrophotometrically, using a molecular weight of 42,300 (7) and an extinction coefficient of 11.1 cm^{-1} at 280 nm, for a 1% solution (8). In the experiments where the nucleotide was bound to actin, the G-actin was dialyzed overnight against a solution containing 0.2 mM ε-ATP, 10mM Tris, pH 7.0. Immediately before measuring the lifetime, the free nucleotide was removed by treating the sample three times, for three minutes each time, with Dowex AG1-X2 resin, adding the resin to the sample in a v:v ratio of 1:10 (9). The quenching agent, if any, was added after resin treatment.

Fluorescence lifetimes were measured with a single photon fluorometer (10) which was built in our laboratory. An excitation wavelength of 336 nm was used throughout. Emission was observed through a Corning 3-144 filter followed by a Corning 3-72 filter, giving a cutoff wavelength near 450 nm.

Deconvolution of the lifetime data was accomplished by either of two computer programs, one using the method of moments (11), the other employing the Marquardt algorithm (12) to fit the data by the method of least squares (13).

RESULTS AND DISCUSSION

We have shown (S. C. Harvey and H. C. Cheung, manuscript submitted to Arch. Biochem. Biophys.) that the fluorescence of ε-ATP is quenched by water, and that this quenching has both a static component (occurring in the ground state, prior to excitation) and a dynamic component (occurring in the excited state, competing with emission). The dynamic component is due to collisions of the fluorophore with water molecules. The 33% increase in lifetime on binding of ε-ATP to G-actin may be due to the binding of the nucleotide in such a way that water molecules do not have access to the fluorophore, so they cannot quench the fluorescence. If this is true, then other collisional quenching agents should not have access, either, and they should not be able to affect the lifetime of the bound fluorophore.

To investigate this possibility, we selected four reagents which are known to be collisional quenchers of the fluorescence of ε-ATP in solution. Each of these was added to a sample of ε-ATP-G-actin, to see if any of them quench the bound nucleotide. Table I gives the results of these experiments.

These measurements show clearly that the four reagents do collisionally

TABLE I

EFFECTS OF COLLISIONAL QUENCHING AGENTS ON THE FLUORESCENCE LIFETIME OF ε-ATP IN SOLUTION AND BOUND TO G-ACTIN

SAMPLE[a]	QUENCHING AGENT	LIFETIME (nsec)[b]
ε-ATP	- - -	27.0 ± 0.2 (S.D.)
ε-ATP	20 mM KI	13.3
ε-ATP	80 mM Methionine	8.7
ε-ATP	5 mM Tryptophan	16[c]
ε-ATP	100 mM Cysteine	15.0
ε-ATP-G-actin	- - -	36.0 ± 0.7 (S.D.)
ε-ATP-G-actin	20 mM KI	35.4
ε-ATP-G-actin	67 mM Methionine	35.4
ε-ATP-G-actin	5 mM Tryptophan	36.4
ε-ATP-G-actin	100 mM Cysteine	35.6

a. All experiments performed at 4°C, pH 7.0 ± 0.2 (range). The concentrations of ε-ATP and ε-ATP-G-actin ranged between 20 μM and 60 μM.
b. All of the decay curves are single exponentials except the one indicated.
c. The decay curve is not a single exponential. The given value is the weighted harmonic mean of the best fit values for a two exponential fit.

quench the fluorescence of ε-ATP in solution, but they do not appreciably affect the lifetime of the bound nucleotide. We conclude that the etheno-adenine base of ε-ATP is bound to G-actin in such a way that these collisional quenching agents do not have free access to the base.

As mentioned above, we have previously shown that water also quenches the fluorescence of ε-ATP. Since the free nucleotide fluorescence is quenched (τ = 27 nsec) when compared to the fluorescence of the bound nucleotide (τ = 36 nsec), and since these other collisional quenchers cannot reach the bound nucleotide, the bound fluorophore must be inaccessible to water. Apparently, the ethenoadenine base is not bound in an exposed position on the surface of the protein.

What do these results say about the binding position of ATP, the nucleotide normally associated with G-actin? Although there is some risk in extrapolating from ε-ATP to ATP, the two nucleotides do have similar binding constants to G-actin, and actin can be polymerized with either nucleotide (3,4), so the interaction of ε-ATP with G-actin is similar to that of ATP. We believe it is likely that the adenine base of ATP is also inaccessible to water when the nucleotide is bound to G-actin.

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