FLUORESCENCE CHANGES ASSOCIATED WITH G–F TRANSFORMATION OF ACTIN*

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1. Introduction

Actin, the major protein component of the thin filaments of striated muscle, can exist either as a monomer, G-actin, of molecular weight 47 000 [1], or in a polymerized, filamentous form, F-actin. In the thin filaments, actin is associated with tropomyosin in a stoichiometric combination [2], and with a number of minor proteins which constitute the calcium sensitizing system, troponins and associated proteins [3]. As a prelude to an intensive study of conformational and microenvironmental changes in actin when it is polymerized and then associates with other thin filament proteins and with myosin using fluorescent probes, we undertook a study of the intrinsic fluorescence of actin and the changes which occur upon polymerization.

2. Materials and methods

G-actin was extracted from an acetone powder of rabbit white skeletal muscle, purified and separated from the minor proteins by gel filtration on Sephadex G-200 using the method of Rees and Young [1]. Electrophoresis of the G-actin on polyacrylamide gels in the presence of sodium dodecyl sulfate [4] yielded a single, homogeneous band. The reduced viscosity of the G-actin solutions was less than 0.1 dl/g. G–F transformation was brought about by the addition of KCl and $MgCl_2$ to final concentrations of 0.1 M and 0.001 M, respectively.

Fluorescence was studied using vertically polarized light of 280 nm for excitation and the spectrum of emitted light was measured in the horizontal and vertical planes in an Aminco–Bowman spectrophotofluorometer equipped with Glan–Thompson prisms and thermoregulated at 25°. Total fluorescence intensity (I) in arbitrary units was calculated from the vertically polarized emission ($I_{\parallel}$) and the horizontally polarized emission ($I_{\perp}$) according to Jablonski [5]:

$$I = I_{\parallel} + 2I_{\perp} \quad (1)$$

Anisotropy of emission (A) at 350 nm was also calculated after Jablonski [5]:

$$A = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} - 2I_{\perp}} \quad (2)$$

3. Results and discussion

The fluorescence emission spectra of G- and F-actin were similar; the apparent wavelength of maximum emission (uncorrected for fluorometer characteristics) was 342 nm for F-actin and 345 nm for G-actin. The polarization anisotropy of F-actin was appreciably greater than that of G-actin ($P < 0.001$) (table 1). The emission spectrum and polarization anisotropy were independent of actin concentration between 0.04

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Table 1
Effects of solvent perturbation on fluorescence polarization anisotropy.

Preparation	Solvent	*N**	Polarization anisotropy (±S.E.)**
F-actin	Buffer†	14	0.206 ± 0.004
G-actin	Buffer††	8	0.167 ± 0.005
L-tryptophan	Buffer†	6	0.120 ± 0.009
F-actin	8 M Urea	3	0.137 ± 0.005
G-actin	8 M Urea	2	0.138 ± 0.006
L-tryptophan	8 M Urea	2	0.126 ± 0.016

* *N* represents number of determinations.
** Samples were excited at 280 nm and fluorescence was measured at 350 nm.
† Tris-ATP – ascorbate with 0.1 M KCl and 1×10^{-3} M $MgCl_2$ [11].
†† Tris-ATP – acsorbate [11].

mg/ml and 2.5 mg/ml. As given in table 1, there was a 25% increase in emission anisotropy at 350 nm after transformation of G-actin to the F-form.

The emission maxima of G- and F-actin, 345 nm and 342 nm respectively, showed a shift to an emission maximum of 365 nm in both cases after exposure to 8 M urea, a perturbing solvent (fig. 1a). In contrast, no shift in the emission spectrum of L-tryptophan (maximum 370 nm) was observed after treatment with 8 M urea (fig. 1a). The actin and tryptophan solutions were approximately equimolar with respect to tryptophan and were at concentrations at which the emission spectra and polarization anisotropy were not concentration dependent. After urea perturbation, the emission spectra of both forms of actin resembled that of L-tryptophan alone.

The effects of urea on emission anisotropy of F-actin and L-tryptophan are given in table 1. Treatment with 8 M urea caused a decrease in the emission anisotropy of F-actin ($P < 0.001$), but no appreciable change was observed with free tryptophan ($P > 0.10$). The fluorescence anisotropy of urea-treated G- and F-actin was not significantly greater than that of free tryptophan, signifying that the effects of energy transfer, electronic transitions and rotational diffusion in urea-perturbed actin are similar to those of free tryptophan.

Proteins containing the amino acids phenylalanine, tyrosine and tryptophan exhibit primarily tryptophan fluorescence, as a result of energy transfer to the indole

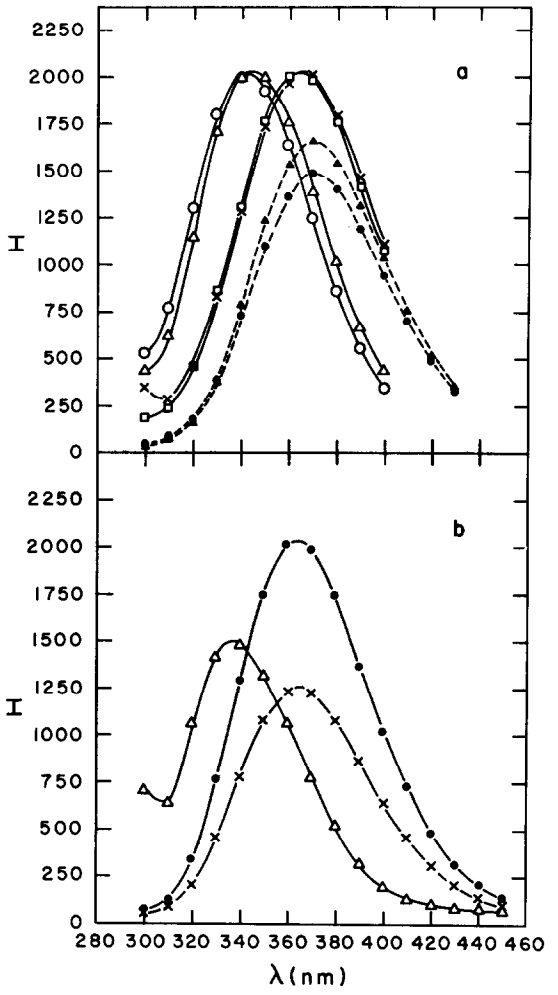

Fig. 1. Fluorescence emission spectra of actin, L-tryptophan and indole solutions. Panel a: (△—△—△): G-actin; (○—○—○): F-actin; (x—x—x): G-actin in 8 M urea; (□—□—□): F-actin in 8 M urea (●- -●- -●): L-tryptophan; (▲- -▲- -▲): L-tryptophan in 8 M urea. Panel b: (△—△—△): indole in dioxane; (x—x—x): indole in water; (●—●—●): indole in 8 M urea.

nucleus of tryptophan [6, 7]. Van Duuren [8] found the emission maxima of various indole compounds shifted to longer wavelengths with increased dielectric constant of the solvent. Fig. 1b shows that the fluorescence emission maximum of 0.001 M indole shifts from 336 nm in dioxane ($\epsilon = 2.2$) to 365 nm in water ($\epsilon = 78.5$) or 8 M urea ($\epsilon = 130$). A comparison between the two panels of fig. 1 shows that the red shift in the spectrum of G- and F-actin after exposure

to 8 M urea resembles the red shift when indole is transferred from a relatively apolar to a polar environment. Although the intensity of fluorescence of indole or tryptophan in aqueous solutions is greater than the fluorescence intensity in dioxane, a change in fluorescence intensity was not observed when G- or F-actin was put into 8 M urea. The red shift observed when actin solutions are treated with urea is thought to represent a movement of the indole nuclei of tryptophan residues from a relatively apolar microenvironment in the interior of the actin molecule to a more exposed, polar microenvironment. A similar red shift has been observed when G- or F-actin was treated with EDTA [9].

The increase in emission anisotropy of fluorescence which accompanied the G–F transformation (as given in table 1) is consistent with decreased rotational freedom of tryptophan or other fluorescent residues in F-actin. Such decreased rotational freedom would be expected if aromatic side chains were shifted from the outside to the interior of the molecule during G–F transformation as originally suggested by Higashi and Oosawa [10]. The emission spectra, however, indicate that the fluorescent groups in G- and F-actin are already in hydrophobic regions which tend to be in the interior of the molecule, making it unlikely that the change in anisotropy associated with G–F transformation reflects a change in rotational freedom exclusively. The change in emission anisotropy accompanying G–F transformation probably also reflects differences in Förster distances and orientation of tryptophan, tyrosine and phenylalanine residues in G- and F-actin.

Other possibilities may account for part of the fluorescence changes observed during the G–F transformation. Fluorescent indole nuclei lying on the surface of G-actin monomers may come to lie in a hydrophobic environment between two apposing molecules in the polymer. It also is possible that different electronic transitions are induced when G-actin and F-actin are excited at 280 nm. The latter possibility is suggested by the slight difference in emission spectra for G- and F-actin which may be due to a slightly greater contribution from tyrosine excitation in F-actin.

References

[1] M.K. Rees and M. Young, J. Biol. Chem. 242 (1967) 4449.
[2] D.L.D. Caspar, C. Cohen and W. Longley, J. Mol. Biol. 41 (1969) 87.
[3] S. Ebashi and M. Endo, Prog. Biophys. Mol. Biol. 18 (1968) 123.
[4] K. Weber and M. Osborn, J. Biol. Chem. 244 (1969) 4406.
[5] A. Jablonski, in: Luminescence of Organic and Inorganic Materials (John Wiley and Sons, New York, 1962) p. 110.
[6] G. Weber, Biochem. J. 75 (1960) 335.
[7] G. Weber, Biochem. J. 75 (1960) 345.
[8] B.L. van Duuren, J. Org. Chem. 26 (1961) 2954.
[9] S.S. Lehrer and J. Gergely, in press.
[10] S. Higashi and F. Oosawa, J. Mol. Biol. 12 (1965) 843.
[11] R.M. Dowben, W.M. Curry, K.M. Anderson and R. Zak, Biochemistry 4 (1965) 1264.