ULTRA-VIOLET FLUORESCENCE OF ACTIN. DETERMINATION OF NATIVE ACTIN CONTENT IN ACTIN PREPARATIONS

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1. Introduction

Present day biochemical methods allow one to obtain actin adequately free of other proteins, however, it is much more difficult to produce actin free of inactivated actin. The high lability of g-actin and the time-consuming procedure for its extraction and purification are the reasons for actin preparations containing a considerable fraction of inactivated protein. The proportion of this inactive fraction may alter in the course of an investigation, during storage [1] or when material is being accumulated. Therefore a simple and reliable method for the rapid estimation of native actin is required. Existing methods [2,3] do not completely meet these requirements.

A very simple and rapid method for determining native actin is described here, based on the measurement of the spectral parameter

$$A = \left(\frac{I(320)}{I(365)}\right)_{296} \quad (1)$$

where $I(320)$ and $I(365)$ are the fluorescence intensities at 320 and 365 nm and the wavelength of the exciting light is 296 nm [4]. Changes in the fluorescence properties of actin preparations during purification have been studied and the fluorescence properties of native and inactivated actin have been determined.

2. Materials and methods

g-Actin was prepared from rabbit skeletal muscle acetone powder, prepared by Straub's method, by extraction at 4°C [5] with a solution containing 0.5 mM ATP, 0.2 mM $CaCl_2$, 0.5 mM β-mercapthoethanol, pH 7.5 [6]. The extract was purified by a slight modification of Mommaerts's method. The polymerization–depolymerization cycle was repeated 2, 3 or, sometimes, 4 times. Some preparations were additionally purified by gel-filtration on Sephadex G-200 [6] or by precipitation with 0.7 M KCl [7].

Protein concentrations of the solutions, 0.5–5.0 mg/ml, were determined either by a micro-biuret method [7] or spectrometrically using $E^{1}_{280}{}^{mg/ml} = 1.09$ [6].

Luminescence measurements were made with an apparatus previously described [8,9]. Luminescence was recorded at an angle of 90° to the direction of the exciting light, that spread from the front wall of cuvette. Fluorescence spectra and values of parameter A were corrected for the spectral sensitivity of the apparatus (for aqueous tryptophan $A = 0.38$).

Viscosity was measured at 20.5 ± 0.1°C with an Ubellode viscometer (outflow time for water, 88.5 sec).

3. Results and discussion

The spectral parameter A is a convenient quantitative characteristic of the fluorescence spectrum of protein tryptophan residues and it is easier to obtain than is the position of the spectral maximum, λ_{max}. Further, both λ_{max} and A equally characterize the fluorescence spectrum and, provided the shape of the spectrum is fixed, these parameters are unambiguously

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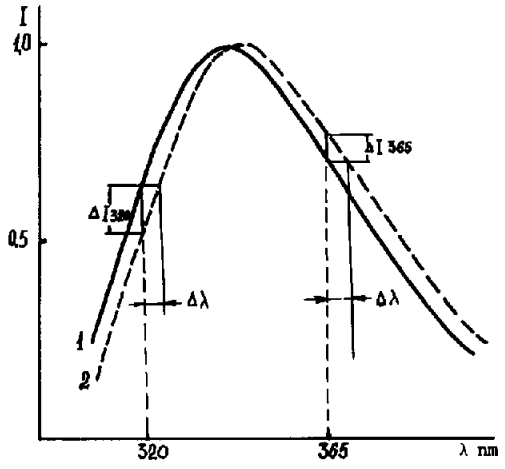

Fig.1. Scheme illustrating the meaning of spectral parameter A.

related. When the shape of the spectrum is complex due to superposition of the spectra of individual tryptophan residues of the protein [8], then λ_{max} and A no longer completely characterize the spectrum but measurement of both these parameters adds to our knowledge of the whole spectrum. Since, when the spectrum shifts the fluorescence intensity alters most in steep regions of the spectrum, at 320 and 365 nm in particular, and since changes of opposite sign occur in the numerator and denominator of the fraction $I(320)/I(365)$ (fig.1), the value of A is a sensitive indicator of spectral shifts, a shift of less than 1 nm being easily detectable.

It can easily be shown [10] that for a system consisting of two luminescent components (e.g. a mixture of native and inactivated actin)

$$A = A_1 \frac{[K_{21}(320) - 1]\alpha_1 - K_{21}(320)]}{[K_{21}(365) - 1]\alpha_1 - K_{21}(365)]} \qquad (2)$$

where $\alpha_1 = c_1/c$ is the proportion of component 1 in the mixture, $K_{21}(320) = I_2(320)/I_1(320)$, $K_{21}(365) = I_2(365)/I_1(365)$, and A_1 is the value of parameter A for component 1.

It follows from relation (2) that, to determine the native actin content from an experimentally measured value of A, calibration is required, i.e. values of A_1, $K_{21}(320)$ and $K_{21}(365)$ must be found. This poses a problem since it is almost impossible to guarantee that any one preparation of actin contains no inactivated material. To overcome this difficulty we

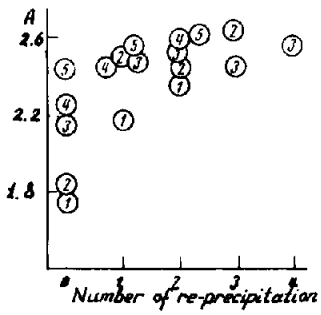

Fig.2. Change of parameter A during purification of g-actin preparations. The numbers of experiments are in circles.

studied changes in the properties of actin preparations during purification.

It has been found that, irrespective of the original properties of the preparation, successive polymerization precipitation and depolymerization cycles lead to: (1) Lowering of the protein content of the supernatant; (2) Increasing intrinsic viscosities of F-actin; (3) Increasing values of parameter A, tending to a limiting value (fig.2); and (4) The spectral shift to shorter wavelengths on actin polymerization [11,12] becoming more pronounced (fig.3).

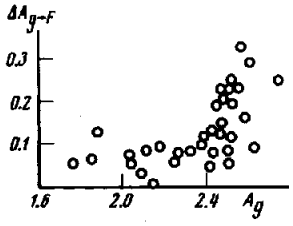

Fig.3. Change of parameter A during polymerization of g-actin preparations.

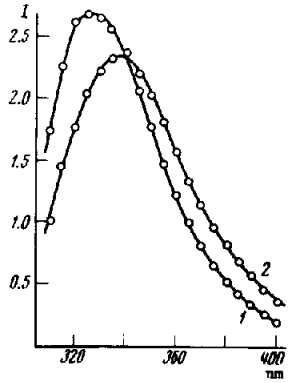

Fig.4. Fluorescence spectra of native (1) and inactivated (2) g-actin.

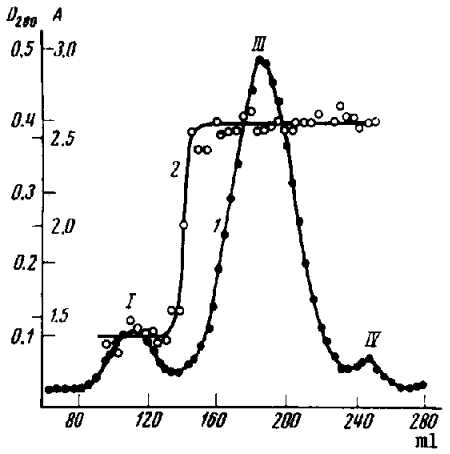

Fig.5. Elution profile of g-actin preparation on Sephadex G-200. (1) optical density; (2) parameter A. Peaks numbered according to Rees and Young [6].

The fluorescence spectrum of actin preparations with maximal values of A and $[\eta]$ (A = 2.60, $[\eta]$ = 8.8) was taken as the spectrum of native actin (fig.4, curve 1). For such preparations λ_{max} = 325 nm and $\Delta_{g\rightarrow F}$ = 0.25. The same fluorescence characteristics were found for preparations additionally purified by reprecipitation with 0.6 M KCl or by gel-filtration on Sephadex G-200.

An elution profile for gel-filtration of g-actin, with initial A = 2.45, is shown in fig.5. Both optical density and the A value were measured for each fraction. Proteins in peak I, that are not able to polymerize [6], had low A values. Native actin, eluting in the main peak III, had a higher A value than had the initial preparation, a value coinciding with the limiting value obtained after a series of precipitations. The constancy of the A value across the peak indicates that the protein in these fractions is homogeneous. The free nucleotides eluting in peak IV, that have no luminescence of their own, do not affect the A value.

Lower values of $[\eta]$ and longer wavelengths for λ_{max} of fluorescence have been reported for native actin (see, for example [11,12]). In our opinion these authors were dealing with preparations containing some inactive protein. The short wavelength position of the fluorescence spectrum of native actin demonstrates the compactness of the globular structure of its macromolecules. Heat denaturation of actin results in a considerable shift to longer wavelengths of the fluorescence spectrum (fig.4, curve 2), demonstrating destruction of the globular structure. For inactivated actin A = 1.30 and λ_{max} = 337 nm.

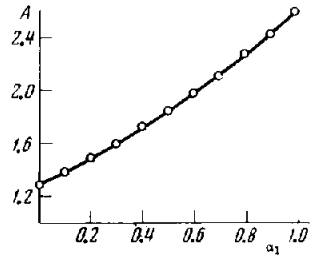

Fig.6. Dependence between parameter A and fraction of native actin (α_1) in preparations.

These results allow one to calculate the parameters in relation (2) for native actin, these are: $A_1 = A_{native}$ = 2.60; $K_{21}(320)$ = 0.67; and, $K_{21}(365)$ = 1.34. A relationship may then be determined between α_1 and A (fig.6) that allows the direct determination of the proportion of native actin in any preparation. The determination may be done directly on the solution being used without pre-treatment and the whole solution may then be used for further experiments.

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