# ABSENCE OF GROSS CHANGES IN THE SECONDARY STRUCTURE OF ACTIN AT G–F TRANSITION

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## 1. Introduction

Upon the addition of neutral salts, actin undergoes polymerization from monomeric globular (G) form to doublestranded helical fibrous (F) form. The G–F transition may involve some intramolecular structural changes that allow the monomer to associate, or it can be the consequence of a rearrangement of the specific quaternary structure of F actin.

Some changes accompanying the G–F transition have been shown to occur by ultraviolet difference spectrophotometry [1], electron paramagnetic resonance [2], circular dichroism (CD) in the near ultraviolet region [3], fluorescence [4] and proteolytic digestion [5]. These changes have usually been attributed to 'conformational' changes in the actin molecule.

These results, obtained by using methods that are sensitive enough to changes in protein secondary structure (CD in the far ultraviolet region and infrared spectroscopy in the region of the amide I band), suggest that there are no fundamental changes in the secondary structure of actin on polymerization.

## 2. Materials and methods

Actin was isolated from acetone-dried rabbit skeletal muscle powder prepared according to [6]. The powder was extracted with 0.2 mM ATP, 5 mM Tris–HCl (pH 7.6) at 0°C, and G actin was polymerized by addition of 50 mM KCl. For further purification the method in [7] was used. For infrared measurement an additional polymerization–depolymerization cycle was carried out in $D_2O$, 0.2 mM ATP, 5 mM Tris–DCl (pD 7.6).

Chemical modification of actin at Tyr-53 was made according to [8]. The modified actin samples for infrared measurements were concentrated by ultrafiltration on Amicon UM 10 membrane and $H_2O$ was replaced with $D_2O$ by subsequent dialysis.

Protein concentration was measured by ultraviolet absorption [9] after turbidity correction according to [10]. Ultraviolet spectral measurements were made in a Hitachi EPS-3T recording spectrophotometer.

CD spectra were measured in a Jasco ORD-UV-CD-5 spectropolarimeter in 1, 2, 10 and 20 mm jacketed cells. The instrument was calibrated with *d*-10-camphorsulfonic acid according to [11].

Infrared spectra were measured in a Perkin-Elmer M 180 spectrophotometer. Spectra of heavy water solutions were registered at cell widths of 80 and 120 $\mu$m, at 4–8 mg actin/ml. All CD and infrared measurements were made at 10°C.

Calculating the values of ellipticity per residue and infrared molar absorption coefficients, the value of the mean residue weight was taken as 112.

## 3. Results and discussion

### 3.1. *Circular dichroism*

CD spectra of G actin, F actin, chemically modified unpolymerizable actin in the presence and absence of 0.1 M KCl, and actin at subcritical concentrations (0.02–0.03 mg/ml) at which a rise in ionic strength does not lead to polymerization (e.g., see [5]), were measured.

All these CD spectra (data not shown) completely coincided with those for G actin in [13,14] and did not differ within the accuracy of measurement (±3%).

The above results indicate that neither addition of salt without polymerization (chemically modified actin and actin at subcritical concentration) nor polymerization itself leads to a change in the α-helix content of the actin molecule.

### 3.2. *Infrared spectra*

CD in the far ultraviolet region is more sensitive to changes in α-helix content than to changes in the amount of β-form. The opposite is true of infrared spectra. Methods for the quantitative estimation of β-form content in polypeptides and proteins have been developed by measuring infrared absorption in heavy water in the range of the amide I band [14].

Infrared absorption spectra of F actin, G actin, difference spectra of F actin against G actin and difference spectra of chemically modified actin in the presence of 0.1 M KCl against the same preparation in the absence of KCl are shown in fig.1.

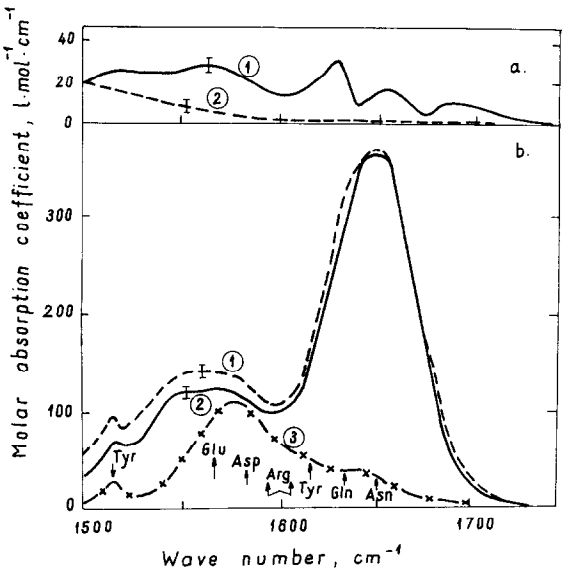


Fig.1. Infrared spectra of actin (4–8 mg/ml) in $D_2O$ containing 0.2 mM ATP, 5 mM Tris–DCl (pD 7.6): l = 80–120 μm; $t$ = 10°C. (a) Difference spectra of F actin against G actin (1) and difference spectra of chemically modified actin with 0.1 KCl against the same actin without KCl (2) (b) Absorption spectra of F actin (1), G actin (2) and the summarized absorption (3) of the relevant side groups (Tyr, Glu, Gln, Asp, Asn, Arg) based on the spectral data of [16] and the amino acid composition of actin as given in [15]. All curves were obtained by averaging 3–4 measurements from independent actin preparations. Vertical bars show the deviation from the mean. Arrows indicate the frequency of maximal absorption of the amino acid side groups.

Difference infrared spectra reveal that in F actin there is only a small, but reproducible ~3% (accuracy of measurement ±0.5%) increase in β-form content relative to G actin. Besides this, a difference spectrum in the absorbance region of side groups of glutamic acid and asparagine appears on polymerization.

However, this slight increase in the absorption band of the β-form in F actin and particularly the changes in the absorption of side groups can be interpreted as being a result of contact surface interaction. An intermolecular extension of β-structure upon association of two inherent β-structures with each other can cause such increase in intensity of the band of the β-form.

In addition, perturbation of the absorption of glutamic acid and asparagine side groups takes place as a result of changes in the environment of the contact surfaces. These changes are most probably induced by polymerization, they do not seem to result from a simple salt effect because no changes were obtained in the absorption band of the β-form (1625 and 1685 $cm^{-1}$) and of glutamic acid and asparagine (1560–1580 $cm^{-1}$) in the difference spectra of chemically modified actin upon addition of 0.1 M KCl.

Thus the results obtained support the assumption that are no fundamental changes in the secondary structure of actin upon polymerization.

The changes of 'conformation' of actin on G–F transition [1–4] or addition of neutral salts [5] as observed by other methods, are the consequence of local structural changes, mainly resulting from a change in the environment of the chromophores and/or the rigidity of the molecule. These changes cannot essentially alter the shape or size of the actin monomer itself.

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