

# Lack of nucleotide cleavage on the binding of G-actin-ATP to plasma gelsolin

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G-Actin-ATP bound to plasma gelsolin to form a 2:1 complex. The complex contained approximately equivalent amounts of nucleotide and actin. More than 84% of this nucleotide was ATP. Half of the bound nucleotide was displaced by cold chase and the remainder did not exchange, implying that the two actins in the complex are not equivalent.

*Actin      Gelsolin      ATP      Actin polymerisation*

## 1. INTRODUCTION

Plasma gelsolin binds two actin monomers with high affinity in the presence of  $\text{Ca}^{2+}$  [1–3]. G-Actin binds 1 mol adenine nucleotide per mol protein; when F-actin assembles from G-actin-ATP, the nucleotide is cleaved to ADP, with  $\text{P}_i$  release [4]. Since actin:gelsolin complex is an efficient nucleator of actin polymerisation [5] and therefore seems likely to mimic the structure of an end of an actin filament, it was of interest to determine whether cleavage of ATP to ADP accompanied complex formation.

## 2. MATERIALS AND METHODS

### 2.1. Materials

Pig plasma gelsolin [5,6] and rabbit skeletal muscle F-actin [7] were prepared by published procedures. To prepare G-actin, pelleted F-actin was resuspended in 0.2 mM ATP, 0.2 mM Tris-Cl, pH 8.0, 0.2 mM DTT, 0.1 mM  $\text{CaCl}_2$  and 1 mM sodium azide to 2–5 mg/ml, and dialysed 3 days against this buffer. The dialysed protein was centrifuged for 30 min in a Beckman Airfuge and the supernatant G-actin used within about 10 days. Protein concentrations were determined spec-

troscopically, using  $A_{280} = 12.4$  for  $1 \text{ mg} \cdot \text{ml}^{-1}$  gelsolin (A. Weeds, personal communication) and  $A_{290} = 0.63$  for  $1 \text{ mg} \cdot \text{ml}^{-1}$  actin [8]. [ $^3\text{H}$ ] Adenosine 5'-triphosphate (ammonium salt) ( $^3\text{H}$ ]ATP) was obtained from Amersham and stored at  $-20^\circ\text{C}$ . [ $\gamma\text{-}^{32}\text{P}$ ]ATP (Amersham) was a gift from Dr A.G. Weeds and was stored at  $-20^\circ\text{C}$  after addition of 0.2 mM cold carrier.

### 2.2. Preparation and analysis of actin:gelsolin complexes

G-Actin was incubated for 14–36 h at  $0^\circ\text{C}$  with radiolabelled nucleotide. The extent of nucleotide exchange under these conditions was tested [9]. In experiments with [ $^3\text{H}$ ]ATP, creatine phosphate (1 mM) and creatine phosphokinase (0.1 mg/ml) were added. Actin was mixed with gelsolin (in 10 mM succinate, pH 6.0, 0.1 mM  $\text{CaCl}_2$ , 1 mM azide, 50% glycerol) at actin:gelsolin mole ratios between 1:1 and 2:1. The protein was loaded onto a Sephadex G-150 column,  $90 \times 1.4 \text{ cm}$ , and eluted with 5 mM imidazole, pH 7, 50 mM NaCl, 0.1 mM  $\text{CaCl}_2$ , 1 mM azide. 1.3 ml fractions were collected. Eluant was analysed for absorbance and radioactivity. Protein peaks were concentrated by Amicon ultrafiltration with a PM10 membrane (Amicon) and Centricon-30 centrifugation.

### 2.3. Nucleotide analysis

Protein-containing samples (G-actin or actin:gelsolin complexes) were made 10% in trichloroacetic acid and denatured protein was removed by centrifugation. Trichloroacetic acid was removed from the supernatants by 2 extractions with 5 vols diethyl ether and samples were analysed by thin layer chromatography on polyethyleneimine cellulose (Camlab, Cambridge) [10]. 50% methanol/water was the first solvent, and 1 M LiCl, 0.5% formic acid the second. Unlabelled ATP and ADP were included as markers, and the spots located under UV light. The chromatograms were cut into strips, placed in vials with scintillation fluid and counted in a Packard Tricarb liquid scintillation spectrometer.

## 3. RESULTS

G-Actin incubated overnight with [ $^3\text{H}$ ]ATP contained  $0.95 \pm 0.14$  (SD) mol ( $n=5$ ) of nucleotide per mol protein. Gel filtration of mixtures of actin and gelsolin containing radiolabelled nucleotide yielded a peak of actin:gelsolin complex coincident with a peak of radioactivity, and a shoulder of unlabelled gelsolin (fig.1). Control experiments showed that gelsolin did not bind nucleotide. Similar column profiles were obtained with both [ $^3\text{H}$ ]ATP and [ $\gamma\text{-}^{32}\text{P}$ ]ATP. The actin:gelsolin ratio in the complex was  $1.9 \pm 0.2$  ( $n=3$ ) by densitometry of SDS polyacrylamide gels. Moles of nucleotide bound per mole of a 2:1 complex were  $1.5 \pm 0.2$

( $n=5$ ). The difference between these numbers was not significant.

The relative amounts of ATP and ADP present were determined for the column-purified complex, for control samples of G-actin and for unbound nucleotide eluted from the column ( $V_\infty$ ) (table 1). In all samples more than 84% of the nucleotide was recovered as ATP, although complex contained a slightly lower proportion of ATP than  $V_\infty$ . When [ $\gamma\text{-}^{32}\text{P}$ ]ATP was used, 14% of the counts in the complex were recovered as  $\text{P}_i$ .

To test whether complex-bound nucleotide was exchangeable, complex isolated by gel filtration was concentrated, incubated with unlabelled ATP for 3–7 days and rechromatographed. Complex was recovered as a single peak, with an actin:gelsolin ratio not significantly different from 2. The relative amounts of bound and free ( $V_\infty$ ) radioactive nucleotide were evaluated (table 2). In the absence of added ATP, 85% of the counts remained bound. After treatment with cold nucleotide at

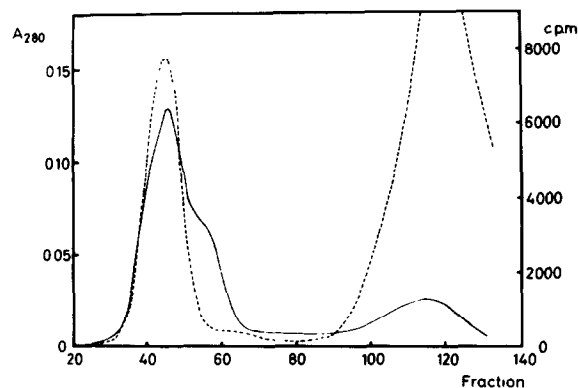


Fig.1. Gel filtration of a 1:1 molar ratio mixture of actin:gelsolin containing [ $^3\text{H}$ ]ATP. (—)  $A_{280}$ ; (---) cpm per 0.1 ml.

Table 1

Identity of nucleotide in actin:gelsolin complexes

Experiment	Content of ATP(%) in			
	Complex	$V_\infty$	Actin (A)	Actin (B)
1. [ $^3\text{H}$ ]ATP	88.8	97.8	96.0	94.0
2. [ $^3\text{H}$ ]ATP	92.2	97.8	98.3	97.9
3. [ $^3\text{H}$ ]ATP	84.2	94.0	98.4	84.0
4. [ $\gamma\text{-}^{32}\text{P}$ ]ATP	86.3	—	—	—

ATP content was evaluated as % of (ATP + ADP) for [ $^3\text{H}$ ]ATP or (ATP +  $\text{P}_i$ ) in the case of [ $\gamma\text{-}^{32}\text{P}$ ]ATP. Samples were complex and free nucleotide,  $V_\infty$ , separated by gel filtration. Controls of G-actin in the presence of free nucleotide were taken before (A) and after (B) running each column.

Table 2

Effect of rechromatography of actin:gelsolin complex on nucleotide content

Labelled nucleotide	Cold ATP (mM)	% total nucleotide	
		Complex	$V_\infty$
[ $^3\text{H}$ ]ATP	0	85.0	15.0
	0.5	53.6	46.4
	5.0	48.2	51.8
[ $\gamma\text{-}^{32}\text{P}$ ]ATP	0.5	55.4	44.6

0.5 or 5 mM, more nucleotide was displaced, but almost exactly half of the label always remained in the complex. [ $^3\text{H}$ ]ATP and [ $\gamma\text{-}^{32}\text{P}$ ]ATP gave similar results.

#### 4. DISCUSSION

The 2:1 complex formed from G-actin-ATP and gelsolin in  $\text{Ca}^{2+}$  contains nucleotide which is predominantly ATP. Although a small proportion ( $\leq 16\%$ ) of the bound nucleotide is ADP, most molecules of complex must contain only ATP, therefore ATP splitting is not obligatorily coupled to complex formation.

The stoichiometry of nucleotide binding to complex was variable and consistently lower than the expected value of 2.0, although the mean of 1.5 mol per complex was not significantly different from the measured actin:gelsolin ratio of 1.9. This suggests that both actins bind nucleotide.

Almost exactly half this nucleotide was displaced from the complex in cold chase experiments. The simplest model consistent with these data is that the two actins bound to gelsolin are not equivalent; one contains exchangeable nucleotide, but in the other, the nucleotide is tightly sequestered.

The nucleotide-binding properties of actin:gelsolin complex resemble those of chemically cross-linked actin dimers [11]. These were shown to contain 2 mol of nucleotide, 85–90% as ATP and 10–15% as ADP  $\cdot$  P $_i$ . Furthermore the two nucleotide sites could be distinguished by their exchange rates; the  $t_{1/2}$  for ATP exchange at one site was 55 min, the other site exchanged very much more slowly. Both crosslinked dimer [11,12] and gelsolin-bound dimers resemble the end of an actin filament in their ability to nucleate monomer assembly [1,2,5]. During rapid filament elongation, monomer addition is faster than ATP hydrolysis [13–15] so that growing actin filaments are capped with ATP-containing monomers [16], probably at both ends [17]. The dimers therefore also resemble the ends of growing filaments in that they contain ATP rather than ADP. Crosslinked and gelsolin-bound dimers differ in that the cross-linked dimers nucleate filaments which elongate at both ends

[12], whereas the actin:gelsolin complexes permit growth only at the pointed ends of filaments, because plasma gelsolin caps the barbed ends [6]. Monomer addition to actin:gelsolin complex is therefore probably an analogous interaction to that at the pointed end of growing filaments.

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