CRYSTALS OF SKELETAL MUSCLE ACTIN: PANCREATIC DNAase I COMPLEX

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1. Introduction

Skeletal muscle actin and the bovine pancreatic DNAase I inhibitor from calf-spleen are very similar by various criteria [1,2]. These include molecular weights, amino acid composition, tryptic fingerprints, and inhibition of DNAase I activity. DNAase I reacts with both G-actin, to form a stable ($K_a \simeq 10^8$ M^{-1}) 1:1 complex, and polymeric F-actin producing depolymerisation with concomitant formation of the 1:1 G-actin: DNAase I complex [3,4].

Crystals of the calf-spleen DNAase I inhibitor (now termed cytoplasmic actin) complexed with a 15 000 dalton protein (called profilin) suitable for an X-ray diffraction study have recently been described [2]. We describe below the crystallisation of the 1:1 complex formed by rabbit skeletal muscle actin and bovine pancreatic DNAase I from polyethylene glycol solutions.

Although the physiological significance of the interaction between skeletal muscle actin and DNAase I is not yet known, the elucidation of the structure of the complex formed by them is of particular importance. Firstly, actin plays a major role in muscular contraction and this complex provides a route to its three-dimensional structure. Secondly, DNAase I is one of the few proteins interacting with DNA that is well described [5] and only one other similar protein has been crystallised [6].

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2. Materials and methods

Bovine pancreatic DNAase I was purchased from Worthington (Catalogue number 2007) and further purified by gel filtration through Ultrogel AcA 54 (LKB) in a buffer containing 5 mM HEPES 0.1 mM $CaCl_2$, 30 mM KCl, 1 mM NaN_3, pH 7.5 (buffer A). Actin from rabbit psoas and back muscles was isolated according to Drabikowski and Gergely [7] including two cycles of depolymerisation–polymerisation followed by dialysis against buffer A without KCl. Actin: DNAase I complex was prepared by mixing G-actin with a slight molar excess of DNAase I in buffer A, followed by gel filtration on a column of Ultrogel AcA 44 equilibrated and eluted with a buffer containing 100 mM imidazole, 0.1 mM $CaCl_2$, 1 mM NaN_3, pH 6.6. All other chemicals were of analytical quality.

Protein concentrations were measured by ultraviolet absorption using $A^{1\%}_{280} = 12.3$ for DNAase I, $A^{1\%}_{290} = 6.6$ for G-actin, and the difference method of Ehresmann et al. [8] for the complex. Analytical SDS–polyacrylamide gel electrophoresis was as previously described [9].

DNAase I activity was determined with a pH-stat. by measuring the proton production at pH 8.0 and 30°C of a 5 ml solution of salmon sperm DNA (1 mg/ml) in 10 mM $MgCl_2$ and 0.1 mM $CaCl_2$. Under these conditions the rate of degradation of DNA is hyperbolic with DNAase I alone and sigmoidal with the actin: DNAase I complex.

Crystallisations were performed at 4°C and room temperature using polyethylene glycol (PEG 6000 – Serva) as a precipitant. Both the hanging-drop method and direct mixing of solutions to the desired final

concentrations of protein and PEG were used.

Photographs were obtained from crystals mounted in thin-walled quartz capillaries and exposed to CuK_α X-rays produced by a rotating anode tube (GX-6, Elliott Brothers Ltd.). Crystal density was measured in a gradient of xylol–carbon tetrachloride calibrated with CsCl solutions.

3. Results

Crystals of the actin: DNAase I complex were obtained at room temperature and at 4°C from solutions buffered with 50 mM imidazole pH 6.6 containing 0.1 mM $CaCl_2$, 1 mM NaN_3 and 2 mM Mg ATP. At room temperature, at protein concentrations between 2–8 mg/ml and PEG concentrations from 18–22% (w/w), crystals could be observed within 12 h and grew to about 0.5 mm long and 0.1 mm wide in 4–6 days. At 4°C crystals were obtained at lower PEG concentrations, 10–15%, but the crystal growth was much slower and only small crystals have been obtained. Crystals mostly grew as star-like clusters with a high incidence of twinning (fig.1).

In order to verify the composition of the crystals, some were carefully washed with artificial mother liquor containing no protein and analysed by SDS–polyacrylamide gel electrophoresis (fig.2). Furthermore, when similar washed crystals were dissolved and assayed for DNAase I activity the rate of DNA degradation was typical for the actin: DNAase I complex, i.e., a lag phase of about 5 min followed by a reduced rate compared to free DNAase I (Mannherz et al., unpublished results).

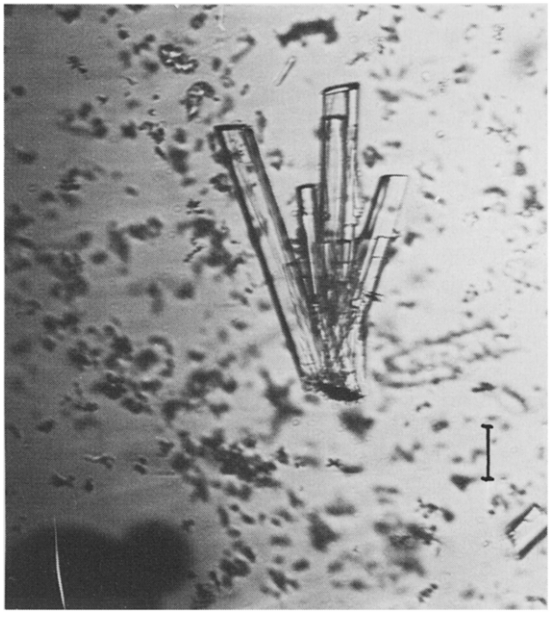

Fig.1. Crystals of the actin: DNAase I complex. Bar indicates 0.1 mm.

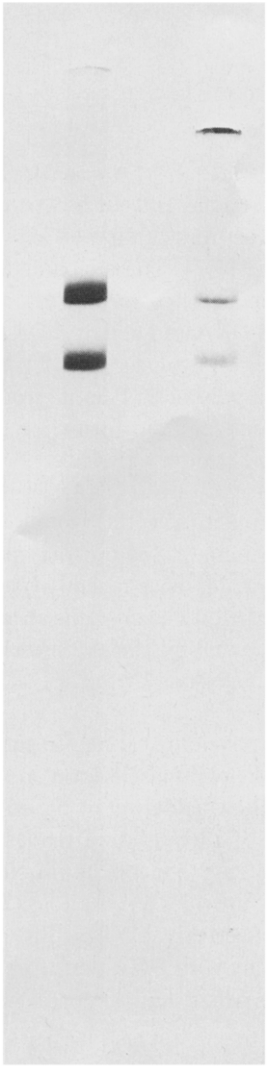

Fig.2. Polyacrylamide gels in the presence of SDS (10% acrylamide) of actin : DNAase I complex prior to crystallisation (a) and of carefully washed crystals (b) as described in text.

Table 1
Crystallographic parameters of the G-actin: DNAase I, 1:1 complex crystals

Parameters	Value/Description
Habit	Prismatic needles
Density	1.18 g/cm^3
Space group	$P2_12_12$
Cell dimensions	a = 42 Å
	b = 230 Å
	c = 77 Å
Cell volume	7.49 × 10^5 Å^3
V_m	2.55 Å/dalton
Molecules/asymmetric unit	1

X-ray precession photographs of the crystals were recorded and the crystallographic parameters derived from these photographs are presented in table 1. Figure 3 shows the hkO section of the reciprocal lattice at 5 Å resolution.

4. Discussion

Both skeletal muscle actin and cytoplasmic actin (bovine spleen DNAase I inhibitor) interact strongly with DNAase I and are so similar in many other respects, that unless evidence to the contrary is produced they can be considered identical proteins. The cytoplasmic actin: profilin complex [2] may represent a storage form of actin since it contains no nucleotide. In this complex the actin may have a different configuration to that in G-actin and the G-actin: DNAase I complex which both contain bound ATP. The role of profilin may therefore be to stabilise the cytoplasmic actin in its monomeric state devoid of nucleotide.

The results described above show that we have obtained crystals of the G-actin: DNAase I complex. The crystallographic data are consistent with one molecule of 74 000 daltons in the asymmetric unit, a value in agreement with the sum of the individual protein molecular weights. Although twinning is a problem in the present crystals, studies are underway to obviate this and also to find suitable heavy atom derivatives in order to proceed with the three-dimensional structure determination.

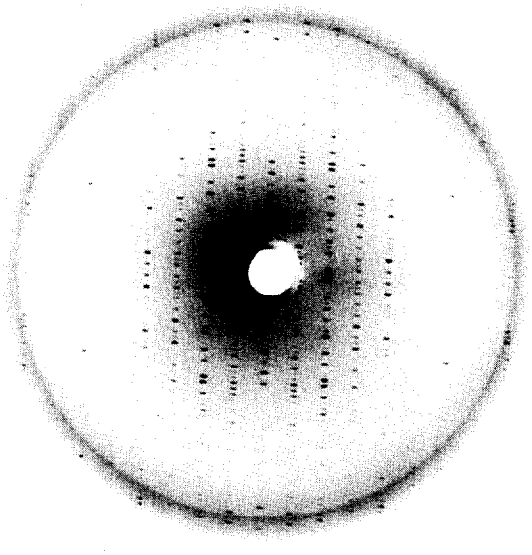

Fig.3. Precession photograph of the hkO plane of an actin: DNAase I crystal, $\mu = 9°$, CuK_α-radiation, exposure time 20 h at 10 kV, 40 mA.

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