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Crystallization of human gelsolin

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Human gelsolin has been crystallized by microdialysis techniques to give single crystals that diffract to 3.5 Å resolution. The crystals belong to space group $P42_12$ and have cell dimensions a = 175.0 Å, c = 151.6 Å. They contain two gelsolin molecules in the asymmetric unit.

Gelsolin; Actin binding protein; Crystallization

1. INTRODUCTION

Gelsolin is a Ca^{2+}-dependent regulator of actin–filament structure [1]. In many types of eukaryotic cell, actin filaments are non-covalently cross-linked to form a gel-like cortex found beneath the cell membrane. As a gel has a certain resistance to deformation, the actin cortex plays an important part in defining cell shape [2] and is a barrier which limits cell organelles to the cytoplasm. Thus proteins that might locally promote a gel-to-sol transition in the actin cortex, such as gelsolin, have been implicated in cell locomotion [3] and in facilitating vesicle traffic between membrane and cytoplasm [5]. In vitro, the protein severs actin–filaments and caps their barbed ends; it will also form a ternary complex with two actin monomers that can nucleate polymerisation [1,4]. Although in the cell these activities seem contradictory, if severing and nucleation follow each other in time then gelsolin may be involved in a remodelling of actin-containing structures; the dispersal of local patches of the cortex could be followed by controlled nucleation of new actin structures, recruiting G-actin from the monomer pool [2].

The human form of the protein is an 82,000 M_r monomer that exists in cytoplasmic and plasma forms: the latter differs only in the presence of an additional 25 residues at its N-terminal end [6]. Analysis of amino acid sequences of gelsolins has shown that each has a marked internal homology [7], repeated six times. A single repeat can be described by a template of conserved residues that is itself also found repeated in a number of other actin binding proteins [8] that are widely separated in evolutionary history (amoebae to mammals). The repeat also delineates the pattern of limited proteolysis of gelsolin into groups of domains that retain distinct actin-binding properties [9]. An N-terminal fragment, delimited by the first repeat, binds (very tightly) to monomeric actin only; a fragment consisting of the second and third repeats only binds to filamentous actin; the minimal fragment capable of severing actin contains the first three repeats; nucleation of filament growth requires in addition the last three repeats; and Ca^{2+} control of these activities similarly requires the complete set of six repeats. Thus gelsolin is probably comprised of six similar, independently-folded domains. Yet, despite a probable common antecedent, these domains have evolved different and more elaborate modes of binding to the same molecule, actin. So far no three-dimensional structure is known for gelsolin or any protein in the family to which it belongs. Hence the structural basis of how the six domains of gelsolin act, individually or in concert, is unknown. As a step towards answering this problem we have crystallized human gelsolin.

Abbreviations: FXa, activated bovine plasma Factor X; V_m, volume of crystal unit cell/total protein relative molecular mass per unit cell (Å^3/dalton); $R_{merge} = \Sigma_i|I_i - \langle I\rangle|/\Sigma|\langle I\rangle|$ where I_i is the intensity measurement for a reflection, and $\langle I\rangle$ is the mean intensity of this reflection; EGTA, ethylene glycol-bis(β-amino ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid

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2. EXPERIMENTAL

Recombinant human plasma gelsolin, which had been ligated into the pLcIIFX plasmid in *E. coli* QY13 cells, was induced by heat shock and an extract made of the soluble proteins [10]. The protein was expressed as a fusion of a leader peptide (derived from the λ phage cII protein) linked by a proteolytic site specific for Factor Xa (FXa) [11] to the coding sequence of gelsolin. The fusion protein (cII-FX-gelsolin) was purified using two successive anion exchange chromatography steps on DEAE–cellulose (Whatman DE-52) at pH 8.0, first in the presence of Ca^{2+} (a condition in which the gelsolin is not retarded) then in its absence (in which condition gelsolin is bound and separated from *E. coli* proteins [12]). The cII leader peptide was cleaved by FXa treatment of the pooled gelsolin peak obtained after the first column. As the leader peptide is more basic than gelsolin it is efficiently separated by the second chromatograpy step. The FXa digestion was

carried out overnight at 4°C using a protease:protein ratio which was determined for each new batch of FXa. The concentration of protein digested was measured by the method of Bradford [13] in preference to spectrophotometric methods, because of the high A_{260}/A_{280} ratio at this stage of the purification. On occasions a further step was added, after the second DE-52 column, in which the gelsolin was bound to an Affi-Gel Blue (Sigma) column and was eluted with 1 mM ATP (based on the method of [14]).

This scheme was also used to purify recombinant plasma or cytoplasmic forms of gelsolin expressed in *E. coli* BL21 (DE3) cells under the control of the T7 promoter (based on [15]).

V8 (*Staphylococcus aureus*) protease digestion of expressed forms of gelsolin makes a single cut that removes 20 amino acids from the N-terminal extension sequence specific to the plasma form. Digestion was carried out in 25 mM Tris-HCl, pH 8.0, 0.1 mM Ca^{2+}, 50 mM NaCl, 1 mM NaN_3, overnight at 4°C at a protease:gelsolin ratio of 1:100 w/w. The gelsolin product is identical to the cytoplasmic form of the protein except for an additional five residues at its N-terminus.

Protein was dialysed into 25 mM Tris-HCl, pH 8.0, 1 mM dithiothreitol, 1 mM NaN_3 (buffer A) and concentrated using 30,000 M_r cut-off Centricons (Amicon) membranes to at least 50 mg/ml. The concentration of purified protein was calculated from the absorbance at 280 nm using an absorption coefficient of 1.24 cm^{-1} for a 1 $mg \cdot ml^{-1}$ solution [4]. Protein solutions were centrifuged for 20 min at top speed in an Airfuge (Beckman) and all other solutions were passed through 0.22 μm filters. Crystallization trials were set up in microdialysis buttons (Cambridge Repetitive Engineering, UK) using initial protein concentrations of 20 mg/ml and buttons were sealed with Spectraphor 10,000 M_r exclusion dialysis membrane. Crystal density was measured by flotation in Ficoll gradients as described by Bode and Schirmer [16].

Crystals were mounted in 1.5 mm diameter siliconised glass capillaries. A complete three-dimensional data set was measured at the EMBL outstation (HASYLAB line) at DESY, Hamburg, using the Hendrix–Lentfer image plate detector system. The patterns were indexed and reflection intensities obtained using the MOSFLM package [17]. Subsequent processing used the CCP4 program suite (SERC Lab., Daresbury, Warrington WA4 4AD, England).

Self-rotation functions were calculated using the POLARRFN program [18]. A control data set was constructed by dividing the experimental data set into 50 reflection resolution shells and cyclically permuting the order of the intensity measurements by 25 with respect to the order of the reflection indices. By this means we produce a randomised data set which still retains a sensible distribution of intensities with respect to resolution. We demand that any real peak is still present in self-rotation functions calculated using different, non-overlapping resolution shells, that such a peak does not disappear after leaving out the five strongest reflections in each of five resolution shells, and that candidate peaks are not sensitive to changes in the integration radius. Furthermore such a peak should not appear in a self-rotation function calculated using the randomised data.

3. RESULTS AND DISCUSSION

Large crystals of plasma gelsolin, derived by FXa cleavage of the cII-FX-gelsolin fusion protein, were obtained by microdialysis against buffer A containing 33% (v/v) saturated ammonium sulphate in the presence of either 1 mM $CaCl_2$ or 1 mM EGTA. The crystals diffract to 3.5 Å and are stable for at least 24 h in the X-ray beam from a rotating anode source. Examination of systematic absences and indexing of the X-ray data showed the space group to be $P42_12$ with cell dimensions a = 175.0 Å, c = 151.6 Å. The crystals grow as flat-plates, the {001} faces are most developed, and the dimensions are typically 0.5×0.5×0.2 mm^3 (see Fig. 1).

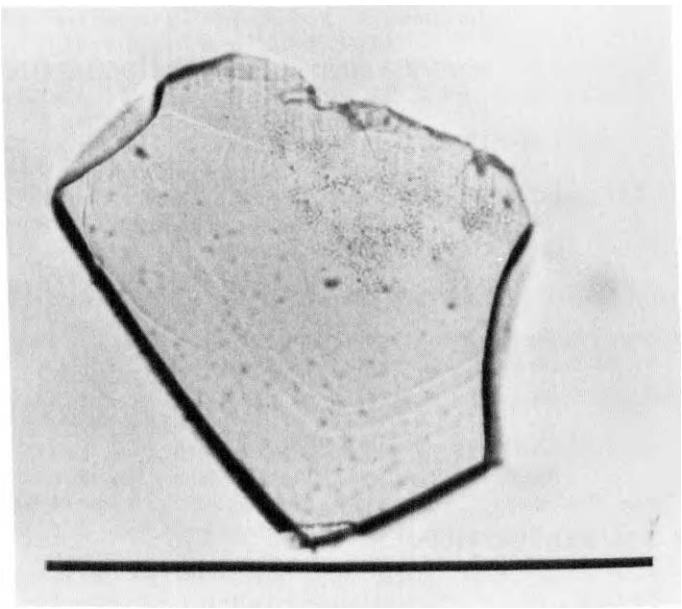

Fig. 1. A crystal of FXa cleaved cII-FX-gelsolin. The bar represents 0.5 mm.

They appear over the pH range 6.0–9.0, but the optimal size is obtained at pH 8.0. Temperature is not particularly critical: the quality of crystals is similar at 16°C and 20°C, but no crystals were obtained at 4°C, and at 37°C they were small and irregular.

Crystals were indistinguishable by their morphology, space group and unit cell parameters whether grown in the presence or absence of calcium. We conclude that the conformational change induced when gelsolin is activated by calcium is small or that Ca^{2+} cannot bind at the high ionic strength used for crystallization, or that only one conformation of the protein is stabilised under these conditions. However, until we collect three-dimensional data on the Ca^{2+} form, we cannot state definitely that the crystals are isomorphous.

Crystals were also obtained of the fusion protein containing the cII-derived peptide and the FXa-susceptible linker. These have similar morphology, identical space group and cell dimensions to those of the plasma protein. Although these crystals are harder to nucleate, they grow as thicker plates and are mechanically more robust. The pH optimum shifts from 8.0 to 6.5, which may be due to the presence of the more basic leader peptide.

The cytoplasmic form of the protein also gives crystals that are indistinguishable from the plasma form. In contrast, the protease V8-treates gelsolin, which is only five residues longer than the cytoplasmic form, has never crystallized. Although in the case of the plasma fusion protein the crystal lattice can accommodate a 38-residue longer N-terminal peptide (25 residues of plasma extension sequence plus 13 of the cII leader peptide), a gelsolin that is only five residues longer does not crystallize. It is probable that the whole plasma

extension sequence is solvent-accessible and disordered, and that it does not sterically interfere at crystal contacts. However, the nature of the residues in the N-terminal extension clearly affects crystallization properties, including final crystal size and pH optimum, possibly through long-range electrostatic effects on the residues at crystal contacts. These findings, and the observation that crystals of plasma gelsolin expressed with the T7 system were both harder to nucleate and on average gave thinner plates, show that subtle differences in the expressed protein have important consequences for growing the best quality crystals.

The density of plasma gelsolin crystals is 1.12 g/ml. One molecule per asymmetric unit would give a calculated density of 1.06 g/ml and three would give 1.18 g/ml. The measured value is consistent only with two molecules of gelsolin per asymmetric unit. On this basis, the volume occupied by one dalton of protein mass, V_m, as defined by Matthews [19], is 3.53 $Å^3$ dalton. Using this value, the fraction of the unit cell occupied by solvent is calculated to be 65%, which is within the range, 30–78%, commonly measured for protein crystals [20].

A native Patterson map calculated using X-ray data collected on a Ca^{2+}-free plasma gelsolin crystal (98% complete to 4.5 Å, R_{merge} = 0.12) did not show any large features. Several self-rotation functions were computed. There were no consistent peaks that indicated a non-crystallographic two-fold axis which, for example might relate segments within the gelsolin molecule or which might relate the two molecules in the asymmetric unit; and there was no evidence for three-fold symmetry which might reflect an arrangement of segments within either half of gelsolin. Similarly, there was no consistent peak close to the −166° rotation component that relates f-actin molecules in the filament [21]. Nevertheless, there are precedents where the final structure did show symmetry which was unclear in the self-rotation function [22].

Clearly the solution of this crystal structure will show the details of how the six domains in gelsolin are articulated. Knowledge of how this disposition may reflect the helical symmetry of f-actin subunits, or indeed how it may impose a different symmetry on the actin, should elucidate the mechanism of gelsolin action.

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