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Crystallization and preliminary X-ray analysis of human platelet profilin complexed with an oligo proline peptide

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Abstract

Profilin is an actin-monomer binding protein that regulates the distribution and dynamics of the actin cytoskeleton. Profilin binds poly-L-proline and proline-rich peptides *in vitro* and co-localizes with proline-rich proteins in focal adhesions and at the site of actin tail assembly on the surface of intracellular parasites such as *Listeria monocytogenes*. The crystallization of the complex between human platelet profilin (HPP) and an L-proline decamer [$(Pro)_{10}$] is reported here. Diffraction from these crystals is consistent with the space group $P2_12_12$ with unit-cell constants a = 68.25, b = 97.64, c = 39.10 Å. The crystals contain two HPP molecules per asymmetric unit and diffract to 2.2 Å.

1. Introduction

The actin cytoskeleton is involved in a wide range of cellular processes including motility, cytokinesis, the establishment and maintenance of cell morphology, and bacterial virulence. Profilin is a ubiquitous 12–15 kDa protein which binds actin monomers with an equilibrium dissociation constant (K_d) in the 0.1–10 μM range (Perelroizen, Marchand, Blanchoin, Didry & Carlier, 1994; Pollard & Cooper, 1986; Goldschmidt-Clermont, Machesky, Doberstein & Pollard, 1991), and regulates the normal distribution of filamentous actin structures *in vivo* (Haarer, Petzold & Brown, 1993; Verheyen & Cooley, 1994). Several mechanisms have been proposed to explain profilin regulation, including monomer sequestration (Pollard & Cooper, 1986) catalytic enhancement of actin-bound nucleotide exchange (Mockrin & Korn, 1980; Goldschmidt-Clermont *et al.*, 1992; Carlier, Jean, Roeger, Lenfant & Pantaloni, 1993) and the promotion of ATP-dependent monomer addition to the growing barbed end of actin filaments (Pantaloni & Carlier, 1993; Pring, Weber & Bubb, 1992). The interaction between profilin and actin can be disrupted by phosphoinositides such as phosphatidylinositol 4,5-bis-phosphate (PIP_2) (Goldschmidt-Clermont, Machesky, Baldassere & Pollard, 1990). Profilin's interaction with PIP_2 also inhibits the action of PLC_γ, an enzyme that hydrolyzes PIP_2 to the second messengers diacylglycerol (DAG) and IP_3, suggesting that profilin is a possible link between signal-transduction pathways and the cytoskeleton (Goldschmidt-Clermont, Machesky, Baldassere & Pollard, 1990). The structures of diverse profilins including plant (Fedorov, Ball, Mahoney, Valenta & Almo, 1997; Thorn *et al.*, 1997), amoeba (Fedorov *et al.*, 1993; Vinson, Archer, Lattman, Pollard & Torchia, 1993) and bovine profilins (Schutt, Myslik, Rozycki, Goonesekere & Lindberg, 1993) have been solved by NMR and crystallographic methods. These structures have identified the mode of binding between profilin and actin (Schutt *et al.*, 1993) and have suggested a mechanism for phosphoinositide regulation (Fedorov *et al.*, 1993).

All cellular profilins also bind poly-L-proline in the 5–50 μM range (Perelroizen *et al.*, 1994; Petrella, Machesky, Kaiser & Pollard, 1996). Fluorescence (Metzler, Bell, Ernst, Lavoie & Mueller, 1994), mutagenesis (Kaiser & Pollard, 1996; Björkegren, Rozycki, Schutt, Lindberg & Karlsson, 1993) and qualitative NMR studies (Metzler *et al.*, 1994; Archer, Vinson, Pollard & Torchia, 1994) have implicated a highly conserved patch of hydrophobic amino acids as the poly-L-proline binding site. The vasodilator-stimulated phosphoprotein (VASP), which localizes with actin filaments in focal adhesions (sites of substratum contact), has been identified as the first *in vivo* proline-rich ligand of profilin (Archer *et al.*, 1994; Reinhard *et al.*, 1992, 1995). Profilin binds to a $[(Pro)_5Gly]_3$ motif contained within VASP. Two recently identified homologues of VASP, Evl and Mena, also bind to profilin and contain highly proline-rich sequences [*e.g.* $(Pro)_{10}$, $(Pro)_{15}$] (Gertler, Niebuhr, Reinhard, Wehland & Soriano, 1996). Profilin can simultaneously bind actin and poly-L-proline (Perelroizen *et al.*, 1994) and the interaction with poly-L-proline does not effect profilin's actin-related activities. These observations suggest proline-rich proteins may serve to localize profilin to specific regions of the cell requiring actin filament assembly.

The interaction between VASP and profilin has recently been implicated as an important component of bacterial motility. Intracellular parasites such as *Listeria* and *Shigella* recruit components of the infected cell's cytoskeleton to form an actin-rich tail that propels the bacterium through the cytoplasm. This motility is necessary for intercellular spreading and virulence (Sanger, Sanger & Southwick, 1992). VASP has a unipolar distribution on the surface of *Listeria*, and is consequently thought to direct a similar distribution of profilin (Theriot, Rosenblatt, Portnoy, Goldschmidt-Clermont & Mitchison, 1994). The localization of profilin, *via* VASP, suggests a mechanism in which profilin enhances filament formation by delivering actin monomers to the site of assembly or by locally catalyzing nucleotide exchange (Chakraborty *et al.*, 1995; Southwick & Purich, 1996).

SH3 and WW domains also bind proline containing ligands, however, these ligands contain considerably fewer proline residues than those specific for profilin (Sudol, 1996). Comparison of profilin with SH3 and WW domains reveals no structural similarity, indicating that profilin possesses a novel proline-rich ligand binding motif. In order to identify the specific interactions involved in poly-L-proline binding, and the proximity of the actin binding site to this region, we have crystallized the complex between HPP and $(Pro)_{10}$.

2. Results and discussion

The 139-amino-acid human platelet profilin (M_r = 14 923 Da) was prepared as previously described with slight modifications

(Almo, Pollard, Way & Lattman, 1994). The *E. coli* strain BL21(DE3) was transformed with the T7 expression vector pMW172 containing the HPP coding region. Large scale cultures were grown overnight at 303–305 K in LB media without IPTG induction. HPP was purified by poly-L-proline affinity chromatography, concentrated to approximately 8 mg ml^{-1} by Amicon ultrafiltration, and stored at 273 K in 10 m*M* Tris pH 8.0, 40 m*M* KCl, 1 m*M* DTT (dithiothreitol). The $(Pro)_{10}$ peptide (M_r = 999.0 Da) was obtained from Peptidogenic Inc. (Livermore, CA, USA) and shown to be homogeneous by analytical high-pressure liquid chromatography (HPLC). The masses for the $(Pro)_{10}$ and HPP were confirmed by mass spectrometry.

Crystals were grown at room temperature using the hanging-drop vapor-diffusion method. 10 µl drops prepared from equal volumes of the HPP/$(Pro)_{10}$ complex (~8 mg ml^{-1} HPP mixed with peptide at a 1:1.5 molar ratio) and well solution were suspended over 1 ml reservoirs. Two related sets of conditions produced isomorphous crystals within one week. Reservoirs containing 2.0 *M* ammonium sulfate and 100 m*M* Tris pH 8.0 produced plate-like crystals of dimensions 0.6 × 0.04 × 0.4 mm. The substitution of lithium sulfate for ammonium sulfate resulted in bulkier crystals of dimensions 0.8 × 0.2 × 0.4 mm. The crystals from lithium sulfate diffracted to at least 2.2 Å resolution and were used for data collection. Two heavy-atom derivatives were obtained by soaking crystals in 10 m*M* $KAuCl_4$ for 3 d or 0.1 m*M* mercuric acetate (MA) overnight.

The composition of the crystals were analyzed by reverse-phase HPLC. Crystals were washed repeatedly in mother liquor to remove unbound peptide (~10^8 dilution), dissolved in distilled water, and applied to a reverse-phase C18 column which was developed with a gradient of 10–90% acetonitrile in 0.1% TFA (trifluoroacetic acid). Profiles of both HPP and the $(Pro)_{10}$ peptide alone were taken as controls. The presence of the complex was confirmed, as two species were identified with retention times of 24 and 46 min, corresponding to $(Pro)_{10}$ and HPP, respectively (Fig. 1).

Native and heavy-atom crystals were mounted in glass capillaries and data were collected at room temperature on a Rigaku RU-200 rotating-anode X-ray generator (fine focus, 50 kV × 80 mA) coupled to a Siemens X-1000 multiwire area detector. Data were reduced and scaled using the *XDS* and *XSCALE* programs (Kabsch, 1988). Preliminary phases were calculated using the *PHASES* software package (Furey & Swaminathan, 1990). Table 1 is a summary of the data-collection statistics. Diffraction from these crystals is consistent with the space group $P2_12_12$, with unit-cell constants a = 68.25, b = 97.64, c = 39.10 Å. Native data were collected to 2.2 Å with an R_{merge} of 5.3%. Data for the $KAuCl_4$ and MA derivatives were collected to 3 Å resolution, with R_{merge} values of 5.9 and 4.3% and R_{iso} values of 9.2 and 6.0%, respectively. Assuming two HPP molecules per asymmetric unit, the calculated Matthews coefficient is 2.2 Å^3 Da^{-1}, which corresponds to a solvent content of approximately 42%. Preliminary MIR maps confirm the presence of two HPP molecules in the asymmetric unit.

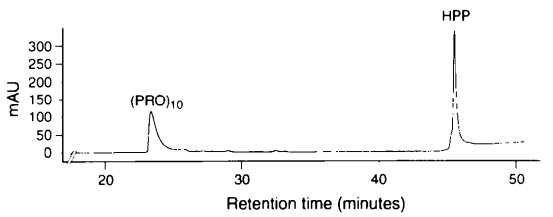

Fig. 1. HPLC elution profile of dissolved HPP/$(Pro)_{10}$ crystals. Peaks at 24 and 46 min correspond to the $(Pro)_{10}$ and HPP species, respectively.

Table 1. *Data analysis statistics*

	Native	MA	$KAuCl_4$
Resolution range (Å)	2.2	3.0	3.0
Reflections (observations/unique)	58289/13062	27658/5529	26119/5556
Completeness of data (%)	94.3	98.6	99.1
R_{merge} (%)†	5.3	4.3	5.9
R_{iso} (%)‡	N/A	6.2	9.2

† $R_{merge} = \sum |I_{obs} - \langle I \rangle| / \sum \langle I \rangle$ summed over all observations and reflections. ‡ $R_{iso} = \sum |F_{ph} - F_p| / \sum F_p$ summed over all unique reflections.

The refined structure of the HPP/$(Pro)_{10}$ complex will identify a novel mode for binding proline-rich ligands and allow for comparison with other modules which bind proline-rich ligands such as SH3 and WW domains. This structure will also show the proximity of the actin and proline-rich ligand binding sites and provide the structural basis for the independent binding of these macromolecules.

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