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X-ray analysis of crystals of rat phosphatidylinositol-transfer protein with bound phosphatidylcholine

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Phosphatidylinositol-transfer protein (PITP) is a soluble, ubiquitously expressed, highly conserved protein encoded by two genes in humans, rodents and other mammals. A cDNA encoding the alpha isoform of the rat gene was expressed to high levels in *Escherichia coli*, the protein purified and the homogeneous protein used for crystallization studies. Crystals of rat PITP-α were obtained by vapor-diffusion techniques using the sitting-drop method. Crystals grow within two weeks by vapor-diffusion techniques in the presence of polyethylene glycol 4000. Both crystal forms pack in the monoclinic space group $P2_1$. Crystal form I has unit-cell parameters a = 44.75, b = 74.25, c = 48.32 Å and β = 114.14°. Unit-cell parameters for crystal form II are a = 47.86, b = 73.59, c = 80.49 Å and β = 98.54°. Crystal form I has a V_m of 2.295 Å^3 Da^{-1} and an estimated solvent content of 46.4% with one molecule per asymmetric unit, while crystal form II has a V_m of 2.196 Å^3 Da^{-1} and an estimated solvent content of 44.0%, assuming two molecules per asymmetric unit.

1. Introduction

Organisms from yeast to humans synthesize small monomeric cytosolic proteins which bind phospholipids such as phosphatidylinositol and phosphatidylcholine, and transfer them from one membrane to another (Helmkamp *et al.*, 1974; Helmkamp, 1990; Wirtz, 1997). They are referred to as phospholipid-exchange proteins or phospholipid-transfer proteins. In the yeast *Saccharomyces cerevisiae* the phospholipid-transfer protein is encoded by the *sec*14 gene, a gene essential for cell viability and cell secretion (Bankaitis *et al.*, 1989). In mammals, the phosphatidylinositol-transfer proteins (PITPs) are a functionally related family of proteins which show similar lipid-binding properties to the yeast proteins, but which have no amino-acid sequence similarity (Helmkamp *et al.*, 1974). Dickeson *et al.* (1989) have reported the sequence of a cDNA encoding rat brain PITP. Subsequently, the sequences of cDNAs encoding PITP have been determined for a number of other mammals. Other studies have shown that there are two related genes which encode PITPs of 270–271 amino acids (Tanaka & Hosaka, 1994). These proteins have more than 75% amino-acid sequence identity and have been termed PITP-α and PITP-β. The amino-acid sequences of PITPs are very highly conserved. For example, there are only two amino-acid differences and one deletion between rat and human PITP-α (Dickeson *et al.*, 1994). Interestingly, there is a membrane-bound 1054 amino-acid protein encoded by the retinal-degeneration gene B (*rdg*B) in *Drosophila* which contains an N-terminal segment showing strong sequence similarity to PITPs (Vihtelic *et al.*, 1993). Related *rdg*B genes have recently been identified in mice and humans.

It is now clear that PITPs interact with other cellular proteins and have important roles in such diverse functions as secretion and cell signalling (Cunningham *et al.*, 1995; Kauffmann-Zeh *et al.* 1995). One important function appears to be to replenish the phosphatidylinositol which is utilized in cell signalling. They may also be involved in presenting phosphatidylinositol to the lipid kinases which function during cell signalling.

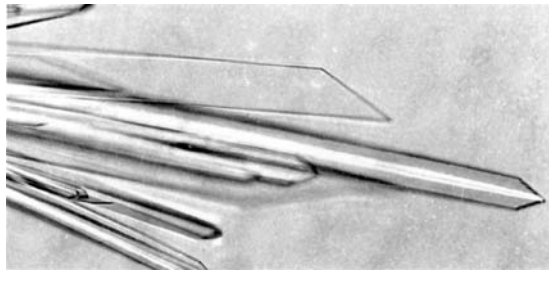

Figure 1
Crystals of PITP-α complexed with phosphatidylcholine. Two crystal forms are observed, occasionally in the same drop, as shown here. In rod-shaped crystals of form I, the four sides of the cross section of the elongated rod are of approximately the same dimension. An example is the longest crystal in this figure. The plate-shaped crystals of form II have one dimension of the cross section considerably shorter than the other. An example of this crystal form is directly above the longest (form I) crystal in the figure.

Table 1
Summary of crystals of PITP-α complexed to phosphatidylcholine.

Parameter	Crystal form I	Crystal form II
Space group	$P2_1$	$P2_1$
Crystal morphology	Rods	Plates
Unit-cell parameters (Å, °)	a = 44.75	a = 47.86
	b = 74.25	b = 73.59
	c = 48.32	c = 80.49
	β = 114.14	β = 98.54
Unit-cell volume (Å^3)	1.465×10^5	2.803×10^5
V_m (Å^3 Da^{-1})	2.295	2.196
Molecules per asymmetric unit	1	2
Estimated solvent content (%)	46.4	44.0
Diffraction limit (Å)	2.1	2.01

Recently, the three-dimensional structure of *Saccharomyces cerevisiae* phosphatidylinositol-transfer protein (sec14) was solved by X-ray diffraction (Sha *et al.*, 1998). In the crystal, the phospholipid normally bound had been replaced by two molecules of octylglucoside. Here, we report the crystallization of rat PITP-α complexed with phosphatidylcholine. Rat PITP-α has 271 amino acids, with a calculated polypeptide molecular weight of 31911. The protein binds the phospholipid in a 1:1 stoichiometric ratio.

2. Materials and methods

PITP-α was purified from recombinant *Escherichia coli* as described previously (Tremblay *et al.*, 1996) and the bound phosphatidylglycerol replaced with phosphatidylcholine. Protein concentration was determined by absorption spectroscopy, using a molar extinction coefficient at 280 nm of 79700 M^{-1} cm^{-1}. Crystals were grown from polyethylene glycol (PEG) using the sitting-drop vapor-phase equilibration method (McPherson, 1982). A solution containing 5 mg ml^{-1} of PITP-α, 15% PEG 4000, 0.1 *M* sodium acetate, 0.005 *M* 2-mercaptoethanol and 0.05 *M* Tris–HCl pH 8.5 was placed in the depression of a microbridge (Hampton Research) in a 24-well tissue-culture plate. The solution was equilibrated over 0.8 ml of a reservoir containing 30% PEG 4000, 0.2 *M* sodium acetate and 0.1 *M* Tris–HCl pH 8.5 and sealed with clear tape. Crystals grew at room temperature in about two weeks.

Preliminary X-ray diffraction data were collected at 149 K using Cu $K\alpha$ radiation from a rotating-anode X-ray source operated at 50 kV and 100 mA. Crystals were mounted in loops (Hampton Research) and flash cooled in the gas stream from the liquid-nitrogen cryostat (Area Detector Systems Corporation). The PEG present in the crystallization drops was sufficient for cryo-protection. Crystals were typically annealed by flash cooling, removing the crystal from the cryostat and placing it in 0.3 ml of a stabilization buffer for at least 3 min, then flash cooling a second time (Harp *et al.*, 1998). The stabilization buffer was composed of 31.5% PEG 4000, 0.2 *M* sodium acetate and 0.1 *M* Tris–HCl, pH 8.5. Space-group assignment and unit-cell parameters were based on the autoindexing routine of *DENZO* (Otwinowski, 1993).

Phosphatidylcholine analysis was made by extracting protein-bound phospholipid from dissolved crystals into chloroform–methanol (1:2 by volume). The crystals were dissolved in water. The extract was quantified by phosphorus analysis, as described previously (Voziyan *et al.*, 1996).

3. Results and discussion

It has been shown that the phospholipid is important in the folding and maintenance of the native conformation of PITP (Voziyan *et al.*, 1997). To confirm that PITP-α in the crystals contained bound phosphatidylcholine, crystals were removed from the crystallization solution, washed, dissolved and analyzed for phospholipids. The data showed that the protein contained 1.03 ± 0.05 (n = 3) mol of phosphatidylcholine per mole of protein. A control sample of PITP-α contained 1.05 ± 0.08 (n = 3) mol of phosphatidylcholine per mole of protein.

Two crystal morphologies were observed from similar conditions, occasionally within the same crystallization droplet. Both crystal forms belong to the monoclinic space group $P2_1$. A summary of the two crystal forms is provided in Table 1.

3.1. Crystal form I

Crystal form I has a long thin rod-like morphology, with unit-cell parameters of a = 44.75, b = 74.25, c = 48.32 Å and β = 114.14°. Typical crystal dimensions are 1.0 × 0.1 × 0.1 mm. A sample crystal is shown in Fig. 1. The number of molecules per asymmetric unit was estimated based on calculated values of V_m, the ratio of unit-cell volume to protein mass, and estimated values of V_s, the fractional volume occupied by solvent (Matthews, 1968). The fractional solvent content was estimated by $V_s = 1 - (\rho/V_m)$, where ρ is the crystal density. An average value of 1.23 g cm^{-3} was assumed for ρ. Values for V_m and V_s typical for soluble proteins are obtained only when assuming one molecule per asymmetric unit, and are reported in Table 1.

3.2. Crystal form II

Crystal form II has a thin plate-like morphology, with unit-cell parameters of a = 47.86, b = 73.59, c = 80.49 Å and β = 98.54°. Typical crystal dimensions are 0.8 × 0.15 × 0.05 mm. A sample crystal is shown in Fig. 1. The number of molecules per asymmetric unit was established in a similar manner as for crystal form I. Only when assuming two molecules per asymmetric unit were typical values for V_m and V_s obtained, and these are reported in Table 1. An X-ray data set was collected from this crystal, and was 98.9% complete to 2.25 Å with an $R_{\rm merge}$ of 5.3%.

A typical diffraction pattern, indicating resolution, is shown in Fig. 2. Both crystal forms are adequate for X-ray crystal-

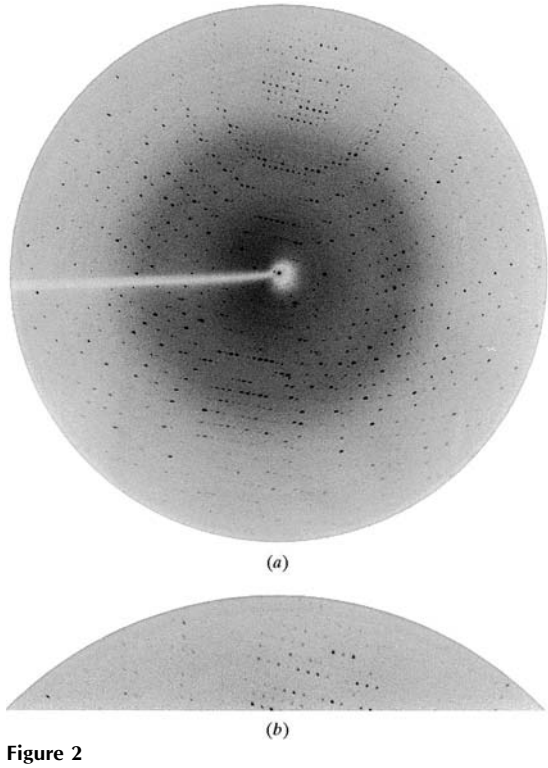

Figure 2
X-ray diffraction image of PITP-α crystal form II. The diffraction image was taken from a MAR Research detector, with a crystal-to-film distance of 150 mm. The oscillation range is 1° with an exposure time of 120 s. The crystal was flash cooled to 149 K. The detector edge corresponds to 2.01 Å resolution. (*a*) One diffraction image; (*b*) an enlargement of the top portion of the image in (*a*).

lographic structure analysis of the protein. A molecular-replacement solution for the structure, using the yeast PITP (sec14) structure (PDB code: 1AUA) as a model, has been unsuccessful. This is not surprising, due to the lack of detectable amino-acid sequence similarity. The PITP-α structure in complex with phosphatidylcholine will be solved with the use of multiple isomorphous replacement techniques.

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