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Crystallization and preliminary X-ray diffraction studies of perchloric acid soluble protein (PSP) from rat liver

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Perchloric acid soluble protein purified from the cytosol fraction of rat liver has been crystallized in a form suitable for high-resolution X-ray diffraction studies. Octahedral crystals reaching 0.5 mm in cross-sectional diameter were produced by the hanging-drop method using polyethylene glycol (M_r = 8 kDa) as precipitant. These crystals diffract to 2.44 Å on an in-house X-ray source and to 1.8 Å using a bending-magnet beamline at ESRF Grenoble. The crystals belong to the cubic space group $P2_13$ with a = 89.90 Å and two molecules per asymmetric unit, as indicated from a V_m value of 2.12 Å^3 Da^{-1} and self-rotation function computation. Screening for heavy-atom derivatives identified a platinum compound and xenon that bind to the protein.

1. Introduction

The novel perchloric acid soluble protein (PSP) is a homodimer of a subunit with 136 amino-acid residues and a molecular mass of 14149 Da (Oka *et al.*, 1995). It inhibits cell-free protein synthesis in the lysate of rabbit reticulocytes, but in a different manner to RNAase A. The incubation of the purified PSP with the lysate leads to the disaggregation of polysomes, suggesting that PSP exerts its inhibitory effects at the initiation stage of the cell-free protein synthesis. Among the several tissues examined by immunoblotting, Northern blotting and immunohistochemistry, PSP is prominently present only in liver and kidney. On the other hand, the expression of PSP is regulated in a differentiation-dependent manner in the kidney (Asagi *et al.*, 1998).

Recently, a 14 kDa translation-inhibitor protein has been characterized from human monocytes and mouse liver (Schmiedeknecht *et al.*, 1996; Samuel *et al.*, 1997). Furthermore, both proteins and their mRNAs are preferentially expressed in liver and kidney, like PSP. On the other hand, these cDNA sequences showed a high similarity to members of a new hypothetical family (YER057c/YJGF family) of small proteins with presently unknown function. Thus the sequences of PSP-like protein are highly conserved in prokaryotes, cyanobacteria, fungi and eukaryotes (Schmiedeknecht *et al.*, 1996). The high degree of evolutionary conservation of these proteins reflects an involvement in basic cellular regulation.

Despite the growing body of information on PSPs, no three-dimensional structures have been reported. In the following contribution we present the crystallization and preliminary crystallographic characterization of PSP.

2. Experimental

In this study, native PSP was purified from rat liver. The cytosolic fraction from rat liver was applied to a column (4.0 × 47 cm) of Sephadex G-75 equilibrated with 30 m*M* Tris–HCl (pH 9.0). The fractions containing PSP were identified by immunoblotting. The pooled fractions were subsequently applied to a column (2.5 × 20 cm) of DE-52 cellulose equilibrated with 30 m*M* Tris–HCl (pH 9.0). The flow-through fractions were collected,

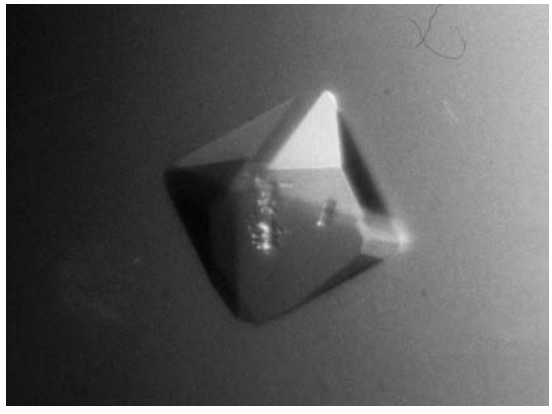

Figure 1
A single crystal of perchloric acid soluble protein grown from 25.5%(*w*/*v*) PEG 8000, 0.08 *M* ammonim sulfate and 0.1 *M* sodium acetate at pH 4.5.

Table 1
Data-collection statistics.

Values in brackets refer to the highest resolution shell.

Data set	Unit cell (Å)	Multiplicity	Unique	Resolution range (Å)	R_{merge}†	Completeness (%)
Native 1	89.90	7.8 (5.5)	9267	35.0–2.44	0.075 (0.272)	99.9 (99.6)
Native 2	89.74	3.3 (2.9)	22151	35.0–1.80	0.032 (0.277)	97.9 (96.6)
Pt derivative	89.92	7.0 (3.5)	5546	35.0–2.90	0.085 (0.237)	99.5 (91.6)
Xe derivative	89.99	5.9 (4.7)	6246	25.0–2.80	0.060 (0.224)	99.9 (99.7)

† $R_{merge} = (\sum |I_{hkl} - \langle I \rangle|)/(\sum I_{hkl})$, where I_{hkl} is the intensity of the individual reflection and $\langle I \rangle$ is the mean intensity of that reflection.

concentrated and then dialysed extensively against phosphate-buffered saline (PBS) at pH 7.4. After clarification centrifugation at 10000*g* for 10 min, the dialysate was applied to a column (1.0 × 3 cm) of IgGPSP–Sepharose 4B equilibrated with PBS (pH 7.4). The protein was eluted with glycine–HCl (pH 2.6), immediately neutralized and dialyzed against 50 m*M* Tris–HCl (pH 8.0). The final preparation appeared homogeneous by SDS–PAGE analysis and migrated as a 14 kDa protein in reducing conditions. Samples were routinely concentrated to 10 mg ml^{-1} and could be stored for several months at 277 K.

The hanging-drop vapour-diffusion method (McPherson, 1982) was used to screen for crystallization conditions. Small octahedral-like crystals appeared at room temperature after several weeks with a well solution containing 30%(*w*/*v*) PEG 8000 and 0.2 *M* ammonium sulfate. Showers of small crystals were subsequently obtained from well solutions containing 30–33%(*w*/*v*) PEG 8000, 0.2 *M* ammonium sulfate and 0.1 *M* sodium acetate at pH 4.5. These crystals (0.05 × 0.05 × 0.05 mm) were subsequently used for macroseeding of pre-equilibrated crystallization drops, equilibrated against reservoir solutions containing 25.5%(*w*/*v*) PEG 8000, 0.08 *M* ammonim sulfate and 0.1 *M* sodium acetate at pH 4.5. They grew to a final size of 0.4 × 0.4 × 0.4 mm within one week (Fig. 1) at room temperature.

For the X-ray diffraction and heavy-atom soaking experiments the crystals were transferred to a solution containig 33%(*w*/*v*) PEG 8000 and 0.05 *M* sodium cacodylate pH 6.5 in order to avoid possible problems with heavy-atom soaks owing to low pH and complexation. The native crystals were flash-frozen to a temperature of 100 K in a cold nitrogen-gas stream and diffraction data were collected to 2.44 Å resolution on a rotating-anode (Cu target) X-ray source equipped with a MAR image-plate detector. All data processing was carried out using the *HKL* program suite (Otwinowski, 1997). The crystals were found to belong to the cubic space group $P2_13$ with cell dimension a = 89.90 Å. Assuming a partial specific volume for protein of 0.746 cm^3 g^{-1}, the number of molecules per asymmetric unit is predicted to be two, corresponding to a V_m of 2.12 Å^3 Da^{-1} (Matthews, 1968) and a solvent content of 41.7%. A native data set of 72696 observations, yielding 9267 unique reflections, was collected to 2.44 Å resolution and scaled to give an overall R_{merge} of 0.075 using *HKL*. Subsequently, a native data set to 1.8 Å resolution was collected on the BM14 beamline at ESRF in Grenoble (λ = 0.9183 Å). From the self-rotation analysis we found that the two molecules in the asymmetric unit are related by a pseudo-twofold axis of 147°.

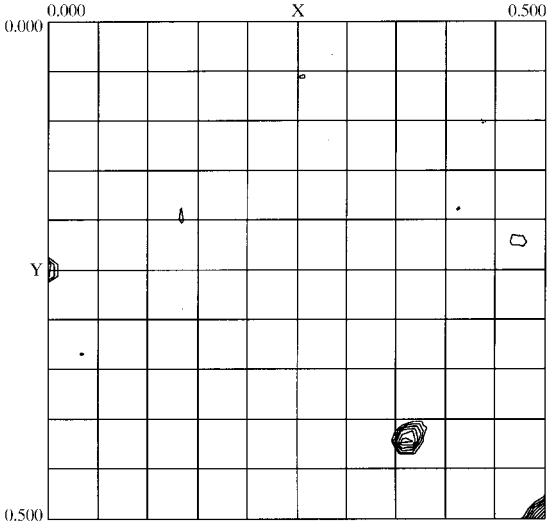

Figure 2
Harker section (x, y, 0.5) of the difference Patterson map for the platinum derivative, computed with diffraction data in the resolution range 9.0–3.5 Å. The map is contoured at 3σ cutoff with steps of 0.5σ.

Searches for heavy-metal derivatives were performed on the in-house X-ray source using an Oxford Cryosystems Cryostream. Promising derivative data sets (to 2.9 and 2.8 Å, respectively) were obtained from a crystal soaked for 48 h in 2 m*M* potassium tetrachloroplatinate (IV) and from a crystal pressurized in a xenon cell at 0.4 MPa for 15 min (Djinović Carugo *et al.*, 1998). These crystals were isomorphous to the native crystal and the data set was scaled to the native set with $R_{iso}(F)$ of 0.173 and 0.142, respectively. A summary of data-collection statistics from the native and derivative data sets is reported in Table 1. The inspection of the Harker sections (x = 0.5, y = 0.5, z = 0.5) of an isomorphous difference Patterson synthesis map (Fig. 2) computed with the *PHASES*95 package (Furey & Swaminathan, 1997) revealed the position of one Pt atom. The parameters of the heavy atom were refined with the program *PHASES*95, and the position of the second Pt atom was located from difference Fourier maps. The positions of the bound Xe atoms were determined from isomorphous and anomalous cross-difference Fourier synthesis maps. Two xenon sites were readily identified, while the remaining two were located from difference Fourier maps after phase refinement. Phase refinement in combination with solvent flattening and averaging yielded an interpretable experimental density map which reveals the prominent secondary-structure elements.

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