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Crystallization and preliminary X-ray studies of metallothionein II from rabbit liver

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Metallothionein II (M_w = 6.8 kDa), induced by cadmium and purified from rabbit liver, has been crystallized in space group $P6_222$ or $P6_422$. The unit-cell parameters were $a = b = 113.4$, $c = 219.1$ Å, $\alpha = \beta = 90.0$, $\gamma = 120.0^\circ$ when crystallized from sodium citrate buffer and $a = b = 113.4$, $c = 219.6$ Å, $\alpha = \beta = 90.0$, $\gamma = 120.0^\circ$ when crystallized from Tris–HCl buffer. There are 12 molecules per asymmetric unit and the solvent content is about 57%.

1. Introduction

Metallothioneins (MTs) are a special class of proteins which bind unusually large amounts of metal ions (covering almost all heavy metals). These proteins are ubiquitously distributed in the cytoplasm of animals, plants and microbes. They are unusually rich in cysteine but contain few aromatic or histidine residues. Their amino-acid sequences are highly conserved. MTs possess two domains of metal–mercapto clusters and have a very distinctive absorption spectrum (Kojima, 1991). The physiological function of MTs is concerned with the delivery and storage of rare elements, resistance to metal and chemical poisons and protection against alkylating agents, radicals and radiation. They have important applications in medicine, ecology and molecular biology (Vallee, 1991).

The crystal structure of a tetragonal form (Melis *et al.*, 1983) of metallothionein II (MT-II) from rat liver crystallized using a repetitive seeding technique has been reported previously (Robbins *et al.*, 1991). It appears to be the only X-ray crystal structure of a metallothionein deposited in the Protein Data Bank, although other metallothionein structures have been solved in solution by NMR. We now report the crystallization of a second MT-II, obtained from rabbit liver, in a hexagonal crystal form. This second MT structure may give further insight into structure–function relationships in the MT family of proteins.

2. Protein induction and purification

A rabbit was subcutaneously injected six times with $CdCl_2$: 1 mg Cd per kilogram body weight on the first and third days, 2 mg Cd per kilogram body weight on the fifth and seventh days, and 4 mg per kilogram body weight on the tenth and twelfth days. On the 14th day, the rabbit was killed and its liver immediately removed.

The liver was cut into small pieces and homogenized in a pre-cooled solution containing 0.01 *M* Tris–HCl buffer (pH 8.6), ethanol and chloroform (1.00:1.03:0.08 by volume). Three volumes of the above solution were added, followed by centrifugation at 15 000*g* at 277 K for 20 min. The supernatant was maintained at 353 K for 5 min, cooled on ice and centrifuged at 10000*g* at 277 K for 30 min. Three volumes of pre-cooled ethanol (253 K) were subsequently added to the supernatant. The solution was allowed to stand at 253 K overnight. The precipitate was collected by centrifugation at 277 K for 20 min, dissolved in 0.25 ml 0.01 *M* Tris–HCl buffer (pH 8.6) per gram of wet tissue and centrifuged at 8000*g* at 277 K for 10 min. The supernatant was loaded onto a Sephadex G-50 column and eluted with 0.01 *M* Tris–HCl buffer (pH 8.6). The fractions containing MT were collected and applied to a DEAE Sepharose Fast Flow column pre-equilibrated with 0.01 *M* Tris–HCl buffer (pH 8.6). Elution was carried out using a linear gradient from 0.01 *M* Tris–HCl (pH 8.6) to 0.25 *M* Tris–HCl buffer (pH 8.6). MT-I and MT-II were collected and concentrated to suitable volumes by lyophilization and applied to a Sephacryl S-100 column equilibrated with 0.01 *M* ammonium carbonate for desalination. MT-I and MT-II, each having M_w = 6.8 kDa, were finally lyophilized and stored separately at 253 K.

3. Crystallization and X-ray diffraction analysis

A precipitate only appeared when the crystallization conditions were based on the parameters reported for MT-II from rat liver (Melis *et al.*, 1983). Crystallization trials were carried out using the hanging-drop vapour-diffusion

Table 1
Crystallization and preliminary X-ray studies of rabbit liver MT-II.

Protein buffer (*P*)	Sodium citrate (0.2 *M*, pH 5.6)	Tris–HCl (0.2 *M*, pH 8.5)
Protein concentration (mg ml^{-1})	5	10
Reservoir solution (*R*)	Ammonium sulfate (2.4 *M*, 1.0 ml)	Ammonium sulfate (2.4 *M*, 1.0 ml)
Hanging drop	10 µl *P* + 5 µl *R*	10 µl *P* + 10 µl *R*
Crystals appeared after	1 week	1 week
Crystallization time (weeks)	3	4
Crystal shape	Hexagonal prism	Hexagonal prism
Crystal dimensions (mm)	0.25 × 0.25 × 0.10	0.30 × 0.30 × 0.20
Resistance to X-rays (h)	>18	>22
Space group	$P6_222$ or $P6_422$	$P6_222$ or $P6_422$
Unit-cell parameters (Å, °)	$a = b = 113.4$, $c = 219.1$ $\alpha = \beta = 90.0$, $\gamma = 120.0$	$a = b = 113.4$, $c = 219.6$, $\alpha = \beta = 90.0$, $\gamma = 120.0$
Molecules per asymmetric unit	12	12
Matthews coefficient† (Å^3 Da^{-1})	2.49	2.50
Solvent content‡ (%)	57	57

† M_r = 6.8 kDa. ‡ v = 0.64 ml g^{-1}.

Table 2
Data collection for rabbit liver MT-II.

Resolution (Å)	2.8	2.6
Number of data	169369	166833
Completeness (%)	93.6	95.7
$R_{\rm merge}$ (%)	6.6	5.1
Redundancy	8.8	6.8
$I > 3\sigma(I)$ (%)	81.2	83.5

method at 298 K. Crystallization droplets of 15 µl initial volume, prepared on siliconized glass cover slips and suspended over a reservoir solution containing 1.0 ml of 2.4 *M* ammonium sulfate, were composed of 10 µl of 5 mg ml^{-1} protein in 0.2 *M* sodium citrate buffer (pH 5.6) and 5 µl reservoir solution. Crystals appeared within one week, grew to maximum dimensions ∼0.25 × 0.25 × 0.10 mm in 20 d and had a hexagonal prism shape. A single crystal was mounted in a thin-walled glass capillary, and X-ray diffraction data were collected using a Rigaku R-AXIS II imaging-plate detector mounted on a Riguku RU-200 rotating-anode X-ray generator operated at 50 kV and 100 mA with Cu $K\alpha$ radiation filtered by a graphite monochromator.

These crystals were resistant to X-rays for more than 18 h without significant decay during data collection. The data were indexed, integrated and reduced on a Silicon Graphics INDIGO workstation using the UNIX version of the R-AXIS software provided with the instrument. The space group is $P6_222$ or $P6_422$, with unit-cell parameters $a = b = 113.4$, $c = 219.1$ Å and $\alpha = \beta = 90.0$, $\gamma = 120.0^\circ$. The crystal density was measured (Westbrook, 1985) to be 1.24 g cm^{-3} with 12 molecules in the asymmetric unit; the Matthews coefficient (Matthews, 1968) was determined to be 2.49 Å^3 Da^{-1} and the solvent content to be 57% (Table 1). A data set to 2.8 Å has been collected (Table 2).

A crystal was also grown from Tris–HCl buffer. A crystallization droplet consisting of 10 µl of 10 mg ml^{-1} protein in 0.2 *M* Tris–HCl buffer (pH 8.5) and 10 µl reservoir solution was equilibrated against a reservoir containing 2.4 *M* ammonium sulfate. A single crystal appeared after one week and continued to grow slowly over four weeks. The crystal had a hexagonal prism shape. The maximum dimensions achieved by this crystal were ∼0.30 × 0.30 × 0.20 mm. As well as diffracting X-rays to a slightly higher resolution, the larger crystal was more stable in the X-ray beam. This crystal is also in space group $P6_222$ or $P6_422$, with essentially the same unit-cell dimensions, Matthews coefficient and solvent content as the previous crystals. A data set to 2.6 Å has been collected.

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