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Crystallization and preliminary X-ray crystallographic studies of a macromolecular antitumour antibiotic, C1027

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C1027 is a macromolecular antitumour antibiotic produced by *Streptomyces globispourus* C1027 and consists of an apoprotein and a non-protein labile chromophore. Little is known about how the thermally unstable chromophore is stabilized by the apoprotein. The purified C1027 was monodisperse according to dynamic light-scattering measurements and crystallized in two different crystal forms from two different starting conditions using the vapour-diffusion method. Condition I yielded hexagonal prism crystals having space group $P3_1/P3_2$ and unit-cell parameters $a = b = 66.8$, $c = 55.4$ Å. Diffraction data were collected to 2.1 Å resolution using an in-house Rigaku rotating Cu anode X-ray generator. Another condition produced rod-like crystals with space group $P3_121/P3_221$ and unit-cell parameters $a = b = 55.15$, $c = 55.87$ Å. A data set to 1.8 Å resolution was collected from a rod-like crystal using a MAR CCD detector at the SRS synchrotron source.

1. Introduction

C1027, a macromolecular antitumour antibiotic, is produced by *S. globispourus* C1027 (Hu *et al.*, 1988; Otani *et al.*, 1988). It has been found to show extreme potency towards cultured cancer cells *in vivo* (Sugimoto *et al.*, 1990; Zhen *et al.*, 1989) and marked growth inhibition of experimental tumours in mice (Zhen *et al.*, 1989, 1994; Xu *et al.*, 1994). It is a 1:1 complex of an enediyne chromophore and a carrier apoprotein. The non-protein chromophore is the active biological part of C1027 and possesses DNA-cleaving activity when activated. The sequence-specific DNA double-strand lesion (5′GTTA1T/5′ATA2A3C, damaged residues are numbered) was caused by a free-radical intermediate formed by the chromophore (Xu *et al.*, 1995, 1997; McHugh *et al.*, 1995).

The chemical structure of the chromophore determined by nuclear magnetic resonance (NMR) showed that it consists of three parts: a nine-membered 1,5-diyn-3-ene core structure in the 16-membered macrocyclin ring, together with benzoxazine and amino sugar moieties as its side chain (Minami *et al.*, 1993; Yoshida *et al.*, 1993). Since there is no covalent bond between the chromophore and apoprotein, the C1027 chromophore is unstable and is very readily separated from its apoprotein by extraction with organic solvents. The apoprotein-free C1027 chromophore is extremely labile to heat, light, alkaline pH (>6) and ultraviolet radiation (Hu *et al.*, 1988). Indeed, a similar situation has been also observed in the other chromophore antitumour antibiotics of this family such as actinoxanthin (AXN), neocarzinostatin (NCS), auromomycin (AUR) and kedarcidin (KDR). Of these, NCS is the only one for which the crystal structure of the protein–chromophore complex has been solved. The crystal structure revealed that the NCS chromophore is greatly stabilized by specific binding to the apoprotein and that the apoprotein undergoes a conformational change on binding of the chromophore. The apo C1027 is a 110 amino-acid acidic protein with two internal disulfide bridges (Otani *et al.*, 1991). Biological studies showed that the non-covalently bound apoprotein stabilizes and controls the release of the labile chromophore (Shao & Zhen, 1995). To date, little is known about the molecular mechanism of the thermally unstable chromophore stabilized and released by the apoprotein.

In order to understand the interaction between the chromophore and the apoprotein and design stable analogues of the chromophore, we have initiated the crystal structure determination of this potent macromolecular enediyne antitumour antibiotic. Here, we report the crystallization and preliminary X-ray diffraction analysis of C1027.

2. Materials and methods

2.1. Expression and purification

The holoantibiotic C1027 was expressed and purified from the fermentation broth of the producing *S. globisporus* C1027 strain as

Table 1
Data collection and processing statistics.

Values in parentheses are for the highest resolution shell: 1.85–1.80 Å for $P3_121/P3_221$ crystal data, 2.20–2.10 Å for $P3_1/P3_2$ crystal data.

	Crystal from condition I	Crystal from condition II
Space group	$P3_1/P3_2$	$P3_121/P3_221$
Unit-cell parameters (Å)	$a = b = 66.8$, $c = 55.4$	$a = b = 55.15$, $c = 55.87$
Resolution (Å)	30–2.1	50–1.8
R_{merge}† (%)	6.2 (25.5)	7.8 (38.6)
No. of unique reflections	15377 (1857)	9324 (757)
Completeness (%)	96.9 (95.0)	98.5 (98.2)
Redundancy	16.5	3.9
Mean $I/\sigma(I)$	20.8	12.38

† $R_{merge} = 100\sum|I_i - \langle I\rangle|/\sum I_i$, where I_i is the intensity of the ith observation, $\langle I\rangle$ is the mean value for that reflection and the summations are over all reflections.

reported by Hu *et al.* (1988). Briefly, the C1027 was separated from the broth filtrate of *S. globispourus* C1027, precipitated with ammonium sulfate and then purified by DEAE-cellulose anion-exchange chromatography and gel-filtration chromatography steps. In a final step before crystallization, the sample was run though a Superdex75 (HR, 10/30) gel-filtration column in 150 m*M* NaCl and 5 m*M* MES–NaOH pH 6.5. The purity of C1027 was >95% by SDS–PAGE.

2.2. Crystallization

The holoantibiotic C1027 was concentrated to a protein concentration of ~50 mg ml^{-1} in 150 m*M* NaCl and 5 m*M* MES/NaOH pH 6.5. Initial screening was performed at 291 K by the vapour-diffusion method using hanging drops. The drop contained 2 µl of protein and 2 µl of reservoir solution from a commercially available sparse-matrix screening kit (Hampton Research Crystal Screens I and II). C1027 was observed to display a strong propensity to form needle-like crystals in the presence of many precipitating agent such as ammonium sulfate, PEGs of various sizes and MPD. Two conditions were further optimized for crystal growth: condition I, 18% PEG 8000, 0.1 *M* sodium cacodylate pH 6.5, 0.2 *M* zinc sulfate (condition #45 in Crystal Screen I); condition II, 2.0 *M* ammonium sulfate (condition #32 in Crystal Screen I).

2.3. Data collection and processing

Crystals were picked up using a fibre loop and flash-frozen in a stream of cold nitrogen gas. Data from the the crystal from condition I were collected at ~115 K on a MAR345 image-plate detector using Cu $K\alpha$ radiation from an in-house Rigaku rotating-anode X-ray generator operating at 48 kV and 98 mA (λ = 1.5418 Å). The freezing solution contained 10% glycerol as cryoprotectant, but was otherwise identical to the precipitant solution. The crystal-to-detector distance was 170 mm. More than 300 frames were collected and each frame was exposed for 500 s and oscillated through 1.5°.

The data set of the crystals from condition II were collected at 100 K on a MAR CCD detector with a crystal-to-detector distance of 100 mm at a wavelength of 0.9798 Å at the SRS synchrotron-light source (Daresbury Laboratory, England). Paraffin oil (Hampton Research) was used as cryoprotectant. A total of 76 frames were collected and each frame was exposed for 120 s and oscillated through 1.0°. All the intensity data were integrated and scaled using the programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997), respectively.

3. Results and discussion

3.1. Crystals from condition I

This crystallization condition yielded hexagonal prismatic crystals from several conditions with PEGs of different sizes (PEG 4000, 6000 and 8000) at both 277 and 291 K in the initial search for crystallization conditions using Crystal Screen. Because crystals were observed to aggregate into large clusters which were not suitable for X-ray diffraction experiments, subsequent crystallization experiments were carried out to optimize the conditions, including addition of different divalent metal salts and variation of the protein concentration. Crystals of good morphology, which were optimized to yield maximum dimensions of 0.4 × 0.4 × 1.5 mm, were grown at a reduced concentration of protein (20 mg ml^{-1}) in 20% PEG 8000, 0.4 *M* zinc acetate, 0.1 *M* sodium cacodylate pH 6.5 (Figs. 1*a* and 1*b*). The crystals belonged to space group $P3_1/P3_2$, with unit-

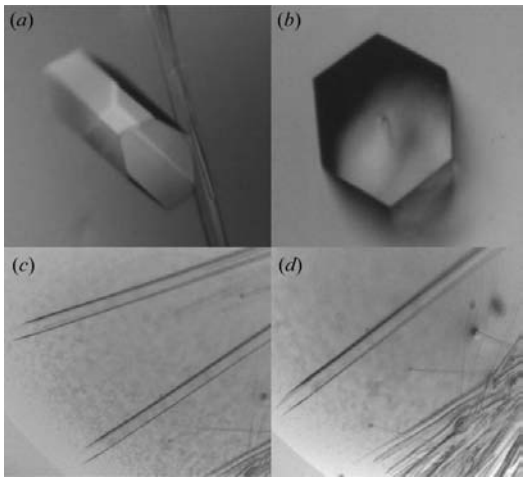

Figure 1
(*a*) and (*b*), hexagonal prism crystals of C1027 grown in 20% PEG 8000, 0.4 *M* zinc acetate, 0.1 *M* sodium cacodylate pH 6.5. (*c*) and (*d*), rod-like crystals of C1027 obtained from 1.8 *M* ammonium sulfate in 50 m*M* sodium cacodylate pH 6.5.

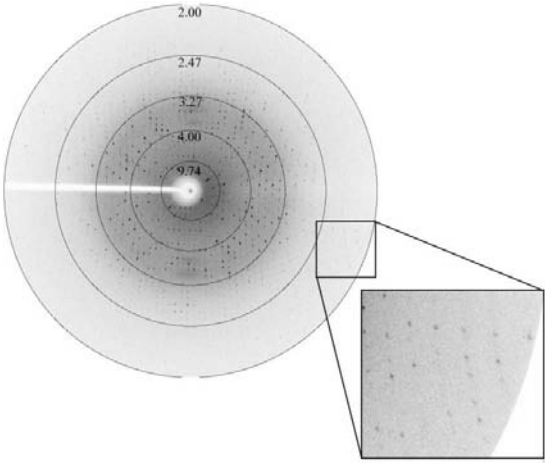

Figure 2
X-ray diffraction pattern of the hexagonal prism crystals of C1027 recorded on a MAR345 image plate. The space group is $P3_1/P3_2$.

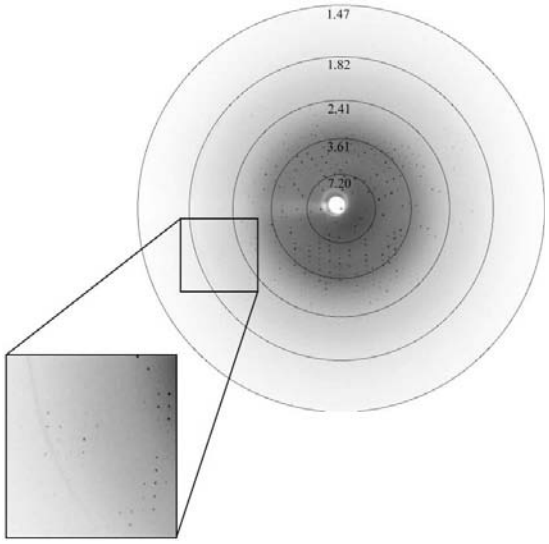

Figure 3
X-ray diffraction pattern of the rod-like crystal of C1027 recorded on a MAR CCD image-plate detector. The space group is $P3_121/P3_221$.

cell parameters $a = b = 66.8$, $c = 55.4$ Å. The hexagonal prismatic crystals diffracted to 2.1 Å in-house (Fig. 2). Assuming three molecules in the asymmetric unit, the solvent content is 45% (Matthews coefficient $V_M = 2.27$ Å^3 Da^{-1}; Matthews, 1968).

The self-rotation function of the data set was calculated using the program *POLARRFN* from the *CCP*4 program package for data in the range 10.0–5.0 Å and a Patterson function radius of 25.0 Å (Collaborative Computational Project, Number 4, 1994). Analysis of the $\kappa = 120^\circ$ section of the map showed the presence of a non-crystallographic threefold symmetry axis. The data statistics and the unit-cell parameters are shown in Table 1.

3.2. Crystals from condition II

Needle-like crystals were often observed from the starting condition II. This crystallization condition was optimized with different pH values and buffer systems to yield rod-like crystals in 1–2 weeks at 291 K (Figs. 1*c* and 1*d*).The crystals grew to maximum dimensions of 0.2 × 0.2 × 2.0 mm in solution of 1.8 *M* ammonium sulfate, 0.1 *M* sodium cacodylate pH 6.5 and diffracted to 1.8 Å at SRS station 9.4 (Fig. 3). The crystal belonged to space group $P3_121$/$P3_221$, with unit-cell parameters $a = b = 55.15$, $c = 55.87$ Å. Given that the molecular weight of C1027 is 10 500 Da, the calculated solvent content is 47% with a Matthews coefficient V_M of 2.34 Å^3 Da^{-1}, assuming one C1027 molecule in an asymmetric unit. The data statistics and unit-cell parameters are also shown in Table 1. The structure determination is currently in progress.

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