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Preliminary X-ray diffraction study of a new crystal form of C-1027-AG, the apoprotein of the macromolecular antitumor antibiotic C-1027 from *Streptomyces globisporus*.

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Abstract

A new crystal form of C-1027-AG, the apoprotein of the macromolecular antitumor antibiotic C-1027 isolated from *Streptomyces globisporus* was obtained by the vapor-diffusion procedure using lithium sulfate as a precipitant. In the present crystallization, it is noteworthy that large-sized single crystals successfully grew from very small droplets (less than 1.0 µl). The present crystals belong to the trigonal system, space group $P3_121$ or $P3_221$ with cell dimensions of $a = b = 62.6$ and $c = 54.2$ Å. Assuming that the asymmetric unit contains one molecule, the V_m value is calculated as 2.9 Å^3 Da^{-1}. A total of 3654 independent reflections from two native crystals was obtained up to 2.5 Å resolution with synchrotron radiation, the merging R factor being 0.097.

The macromolecular antitumor antibiotic C-1027 is an acidic protein containing its specific chromophore isolated from the culture filtrate of *Streptomyces globisporus* C-1027 (Hu *et al.*, 1988; Otani, Minami, Marunaka, Zhang & Xie, 1988*a*). C-1027 belongs to the antitumor antibiotic family of neocarzinostatin (NCS), in which actinoxanthin (AXN) and auromomycin (AUR) are also included. The apoproteins of these antitumor antibiotics have a similar size and show a high degree of sequence homology (Otani *et al.*, 1991; Sakata, Ikeno, Hori, Hamada & Otani, 1992). It is remarkable that the cytotoxicity of C-1027 towards human cancer cell lines *in vitro* is much stronger than those of others in the NCS family (Zhen *et al.*, 1989). This high activity is thought to be solely as a result of the chromophore, C-1027-Chr, which has the ability to cleave DNA. The structures of unstable C-1027-Chr and its cyclo-aromatized product have been determined by the two-dimensional NMR studies (Yoshida, Minami, Azuma, Saeki & Otani, 1993).

C-1027-AG, a selective antagonist, is the apoprotein of C-1027 (Otani, Minami, Marunaka, Zhang & Xie, 1988*b*) and consists of a single chain of 110 amino-acid residues with the calculated molecular weight of 10 500 Da (Otani *et al.*, 1991). Although C-1027-AG does not show any antimicrobial or antitumor activity by itself, it serves as a carrier for its chromophore. It is suggested that C-1027-AG is a targeting vehicle for C-1027-Chr through possible recognition of surface proteins on the target cells (Sakata, Tsuchiya *et al.*, 1992). Therefore, it is important to determine the three-dimensional structure of C-1027-AG to know how the apoprotein stabilizes the chromophore and promotes its interaction with DNA. The crystal structures of the apoproteins of AXN (Pletnev, Kuzin, Trakhanov & Kostetsky, 1982) and AUR (Van Roey & Beerman, 1989) and of the holo-NCS (Kim, Kwon, Myers & Rees, 1993) are now available. The comparison between these structures and that of C-1027-AG might give an implication about the mechanism of the high cytotoxic activity of C-1027.

We have already obtained the crystals of C-1027-AG in the orthorhombic form (the space group $P2_12_12_1$ with unit-cell dimensions $a = 55.1$, $b = 61.3$ and $c = 79.1$ Å), in which two or three molecules are included in the asymmetric unit (Briozzo, Inaka, Minami, Otani & Miki, 1993). The structure determination of this crystal is currently underway but the structure has not yet been solved. In this paper, we report a new crystal form of C-1027-AG, the preliminary X-ray characterization of which indicated that the asymmetric unit contains only one molecule and that the crystals are suitable for high-resolution crystallography.

C-1027-AG was purified as described (Otani *et al.*, 1991). Crystallization conditions were re-screened at 293 K by the sitting-drop vapor-diffusion procedure using various precipitants. The new crystals were obtained by the use of lithium sulfate as a precipitant, whereas the orthorhombic crystals grew from 2-methyl-2,4-pentanediol (MPD) solutions (Briozzo *et al.*, 1993). The best crystals grew in the form of hexagonal prisms, when 0.5 µl droplet containing 27.5 mg ml^{-1} C-1027-AG, 0.50 *M* lithium sulfate and 50 m*M* citrate was vapor equilibrated against 1.15 *M* lithium sulfate at 293 K for one month (Fig. 1). In this crystallization, it is noteworthy that large-sized single crystals as shown in Fig. 1 successfully grew up from a little amount of droplets (less than 1.0 µl). In larger amounts of droplets which are usually employed in protein crystallization, the crystals tended to form several clusters consisting of many thin crystals and did not grow up into a large size.

For crystallographic characterization, precession photographs were taken using a Huber procession/rotation camera with Cu $K\alpha$ radiation generated by an M18X X-ray generator (MAC Science Co. Ltd, Tokyo) (Fig. 2). The crystals were sealed in a glass capillary with a minimum amount of the solution that consists of 1.15 *M* lithium sulfate and 115 m*M* citrate. Two precession photographs (*hk*0 and *h*0*l*) revealed that the crystals belong to the trigonal system with the space group of $P3_121$ or $P3_221$. The unit-cell dimensions are determined as $a = b = 62.6$ and $c = 54.2$ Å by a least-squares fit of diffraction spots on DIP100S imaging-plate rotation-camera system (MAC Science Co. Ltd, Tokyo). Assuming that the

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asymmetric unit contains one molecule, the V_M value is calculated as 2.9 Å^3 Da^{-1}, resulting in a solvent content of 59% (Matthews, 1968).

Intensity data were collected with synchrotron radiation at the BL-6A$_2$ beamline of the Photon Factory, the National Laboratory of High Energy Physics, Tsukuba, Japan. The X-ray beam was monochromatized to 1.00 Å by a Si(111) monochromator system. On Sakabe's macromolecular screenless Weissenberg camera (Sakebe, 1991), a full set of diffraction data was collected using two native crystals rotated along the c^* and $2a^* + b^*$ axes, respectively. Diffraction intensities, which were digitized on a Fujix BA100 read-out system (Miyahara, Takahashi, Amemiya, Kamiya & Satow, 1986), were recorded beyond 2.5 Å resolution which is the resolution limit for the previous orthorhombic crystals (Briozzo *et al.*, 1993). The intensities were evaluated and processed by *WEIS* (Higashi, 1989) and *PROTEIN* (Steigemann, 1992) programs, respectively. A total of 3654 independent reflections (2.0σ cutoff) was obtained up to 2.5 Å resolution after merging of two native crystals with an merging R value of 0.097 ($R_{\text{merge}} = \sum_h \sum_j |I_{hj} - \langle I \rangle_h| / \sum_h \sum_{hj} I_{hj}$, where $\langle I \rangle_h$ is the mean intensity of a reflection h and I_{hj} is the jth measurement of reflection h). The overall completeness of reflections is 82%.

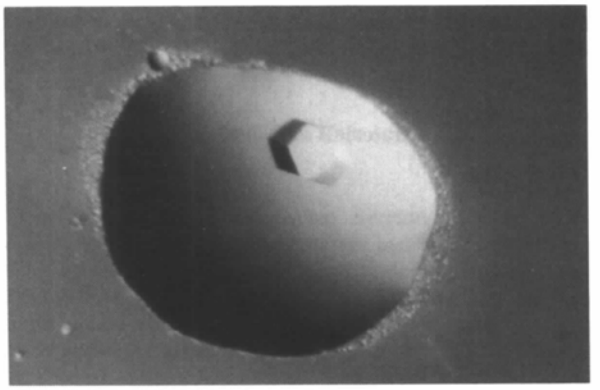

Fig. 1. A trigonal form crystal of C-1027-AG. The crystal was obtained by sitting-drop vapor diffusion as described in the text. The dimensions of this crystal are 0.15 × 0.15 × 0.10 mm.

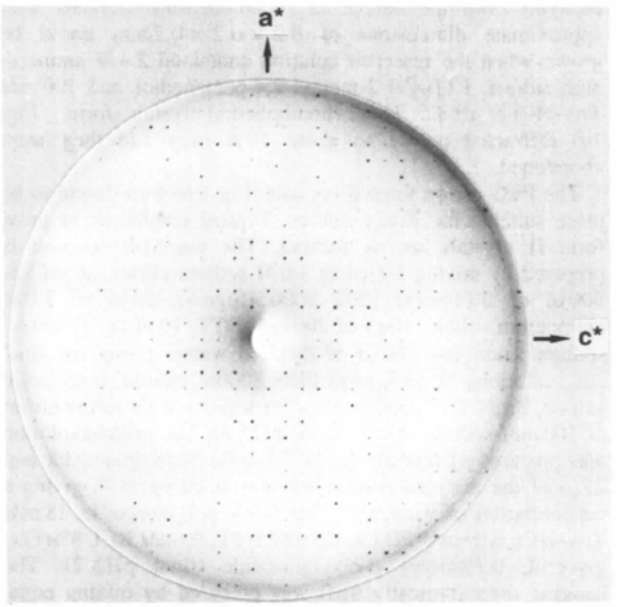

Fig. 2. A precession photograph of the *h0l* zone from the trigonal crystal of C-1027-AG. The crystal-to-film distance was 100 mm. The precession angle was 12.7°. Exposure time was 32 h at 45 kV and 90 mA. The outer edge of the photograph corresponds to a 3.5 Å resolution.

We have been trying to solve the structure by the molecular-replacement method using the structure of AXN as a model which shares more than 90% sequence similarity with C-1027-AG (Knokhlov *et al.*, 1976; Otani *et al.*, 1991). The present crystal form might be more suitable for the molecular-replacement technique than the orthorhombic form judging from the number of molecules in the asymmetric unit.

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