Acta Cryst. (1993). D**49**, 372–374

Crystallization and Preliminary X-ray Diffraction Studies of C-1027-AG, the Apoprotein of the Macromolecular Antitumor Antibiotic C-1027 from *Streptomyces globisporus*

BY PIERRE BRIOZZO AND KOJI INAKA

Research Laboratory of Resources Utilization, Tokyo Institute of Technology, Nagatsuta, Midori-ku, Yokohama 227, *Japan*

YOSHINORI MINAMI AND TOSHIO OTANI

Tokushima Research Center, Taiho Pharmaceutical Co. Ltd, Kawauchi-cho, Tokushima 771-01, *Japan*

AND KUNIO MIKI*

Research Laboratory of Resources Utilization, Tokyo Institute of Technology, Nagatsuta, Midori-ku, Yokohama 227, *Japan*

(*Received* 14 *January* 1993; *accepted* 15 *March* 1993)

Abstract

C-1027-AG, the apoprotein of the macromolecular antitumor antibiotic C-1027, isolated from *Streptomyces globisporus*, was crystallized by the vapor-diffusion procedure using 2-methyl-2,4-pentanediol as a precipitant. The crystals belong to the orthorhombic system, space group $P2_12_12_1$, with unit-cell dimensions $a = 55.1$, $b = 61.3$ and $c = 79.1$ Å. Assuming that the asymmetric unit contains two or three molecules, the V_m value is calculated as 3.2 or 2.1 Å^3 Da^{-1}, respectively. A total of 7630 independent reflections was obtained up to 2.5 Å resolution with synchrotron radiation, the merging R factor being 0.077 for 24713 measurements.

Introduction

The macromolecular antitumor antibiotic C-1027 is an acidic protein isolated from the culture filtrate of *Streptomyces globisporus* C-1027 (Hu *et al.*, 1988; Otani, Minami, Marunaka, Zhang & Xie, 1988*a*). C-1027-AG, a selective antagonist, is the apoprotein of C-1027 (Otani, Minami, Marunaka, Zhang & Xie, 1988*b*) and consists of a single polypeptide chain of 110 amino-acid residues; the minimum molecular weight calculated from the sequence is 10500 Da (Otani, Yasuhara *et al.*, 1991).

C-1027 belongs to the antitumor antibiotic family of neocarzinostatin (NCS), also including actinoxanthin (AXN), auromomycin (AUR), and some others. The apoproteins of this family have similar sizes and show a high degree of sequence homology (Otani, Yasuhara *et al.*, 1991). These apoproteins serve as carriers for their respective non-protein chromophores and as regulators for their release. The unstable chromophore itself is the active component. The cytotoxicity of C-1027 towards human cancer cell lines *in vitro* is much stronger than that of NCS. C-1027 is also effective at tolerable doses against a panel of transplantable tumors in mice (Zhen *et al.*, 1989). C-1027-AG does not show any antimicrobial or antitumor activity by itself. These activities are thought to be solely as a result of the chromophore, C-1027-Chr, capable of cleaving DNA. However, 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 *et al.*, 1992). Although the detailed structure of the unstable C-1027-Chr is still unknown, it seems to be different from other chromophores of the NCS family. For instance, unlike NCS, C-1027 induces DNA strand scission without any supplement of reducing agent (Sugimoto, Otani, Oie, Wierzba & Yamada, 1990) and the HPLC profile of C-1027-Chr differs from that of AUR (Otani, Minami, Sakawa & Yoshida, 1991).

The crystal structures of the AXN apoprotein (Pletnev, Kuzin, Trakhanov & Kostetsky, 1982), which shares more than 90% primary sequence similarity with C-1027-AG (Khokhlov *et al.*, 1976; Otani, Yasuhara *et al.*, 1991), and of the AUR apoprotein (Van Roey & Beerman, 1989) are known. The three-dimensional solution structures of NCS apoprotein (Adjadj, Quiniou, Mispelter, Favaudon & Lhoste, 1992; Gao, 1992) and of holo-state NCS containing its chromophore (Tanaka *et al.*, 1991) have been elucidated by means of nuclear magnetic resonance

* Author to whom all correspondence should be addressed.

ISSN 0907-4449

spectroscopy. These three apoproteins have an overall similar folding defining a binding site for their respective chromophores. Despite their shape similarity, the cleft-like binding sites differ by the locations of their functional side chains, explaining the chromophore-binding selectivity (Van Roey & Beerman, 1989).

Here we report the crystallization and the preliminary X-ray characterization of C-1027-AG.

Experimental

C-1027-AG was purified as described (Otani, Yasuhara *et al.*, 1991). Crystallization conditions were screened at 293 K by the hanging-drop vapor-diffusion technique using various precipitants. Protein solutions were adjusted in 10 m*M* sodium phosphate buffer. Once crystals had grown, crystallization conditions were refined systematically.

For crystallographic characterization, precession photographs were taken using a Huber precession/rotation camera with Cu $K\alpha$ radiation generated by an M18X X-ray generator (MAC Science Co. Ltd, Tokyo).

Intensity data were collected with synchrotron radiation at the BL-6A$_2$ beamline of the Photon Factory, the National Laboratory for High Energy Physics, Tsukuba, Japan. The X-ray beam was monochromatized to 1.00 Å by an Si(111) monochromator system. A screenless Weissenberg camera for macromolecular crystals was used with a 0.1 mm aperture collimator and a cylindrical cassette of radius 286.5 mm (Sakabe, 1991). The diffraction intensities were recorded on 200 × 400 mm imaging plates (Fuji Photo Film Co. Ltd), which were digitized at 100 μm intervals on a Fujix BA100 read-out system (Miyahara, Takahashi, Amemiya, Kamiya & Satow, 1986). Intensity data were evaluated by the *WEIS* program (Higashi, 1989) and reduced by the *PROTEIN* program (Steigemann, 1974).

Results and discussion

The crystals appeared when 2-methyl-2,4-pentanediol (MPD) was used as a precipitant. The best crystals grew when a droplet of protein solution (pH 7.0) with 40 mg ml^{-1} C-1027-AG and 60% (*v*/*v*) MPD was equilibrated against a 70% (*v*/*v*) MPD solution. Needle-shaped colorless crystals as shown in Fig. 1 grew for two weeks. Their size was up to 1.5 mm in length but only 0.08 mm in diameter.

Crystals were sealed in a glass capillary with a minimum amount of 80% MPD solution for crystallographic characterization. The X-ray diffraction pattern symmetry in precession photographs indicates that the crystal system is orthorhombic. Systematic absences of reflections are consistent with those from space group $P2_12_12_1$. The unit-cell parameters are $a = 55.1$, $b = 61.3$ and $c = 79.1$ Å ($V = 2.67 \times 10^5$ Å^3). Assuming that the unit cell contains eight or twelve molecules (two or three molecules per asymmetric unit, respectively), the V_m value is calculated as 3.2 or 2.1 Å^3 Da^{-1}, resulting in a solvent content of 61 or 42%, respectively (Matthews, 1968).

Using the macromolecular Weissenberg camera with synchrotron radiation, a full set of intensity data was collected using one native crystal rotated along the a^* axis. The total oscillation range of 100.5° was covered by ten serial Weissenberg photographs (10.5° oscillation each), one image pattern of which is shown in Fig. 2. The crystals were stable towards X-ray exposure and the diffraction was recorded beyond 2.5 Å resolution. Intensity data evaluated from the imaging plates were scaled and merged up to 2.5 Å resolution. Consequently, a total of 7630 independent reflections was obtained, which corresponds to 78% of the number of theoretically possible reflections. The merging R factor is 0.077 for 24713 measurements ($R_{\rm merge} = \sum_h \sum_j |I_{hj} - \langle I \rangle_h| / \sum_h \sum_{hj} I_{hj}$, where $\langle I \rangle_h$ is the mean intensity of a reflection h and I_{hj} is the jth measurement of reflection h), in which reflections with $|I - \langle I \rangle|/I$ greater than 0.4 were rejected, where I and $\langle I \rangle$ are individual and mean intensities of symmetry-equivalent reflections.

The determination of the three-dimensional structure of C-1027-AG should contribute to a better understanding of the mechanism by which the apoprotein stabilizes the chromophore and promotes its interaction with the target DNA. It could also allow structural comparison with the respective

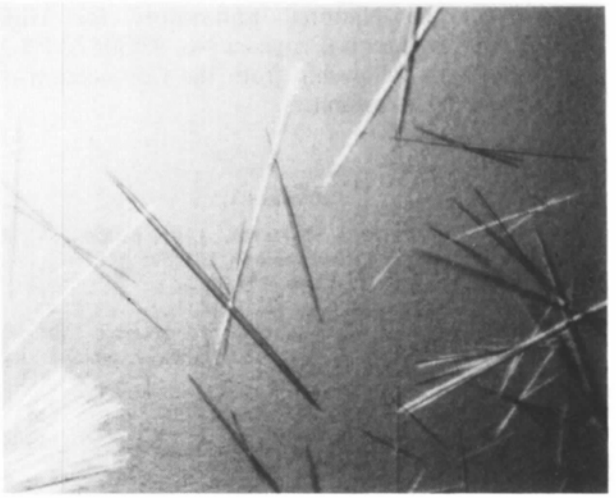

Fig. 1. Crystals of C-1027-AG, the apoprotein of the macromolecular antitumor antibiotic C-1027 from *Streptomyces globisporus*.

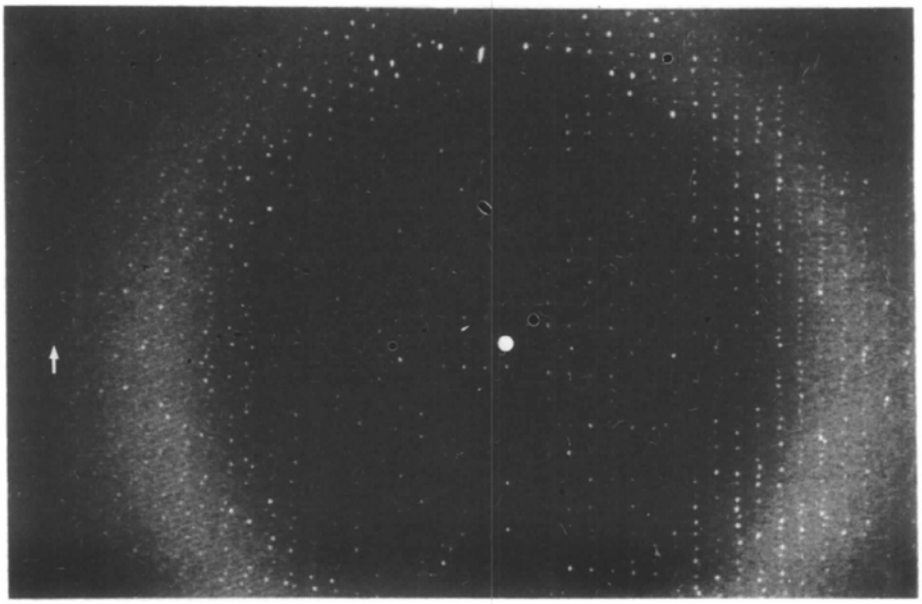

Fig. 2. An image pattern of a screenless Weissenberg photograph from a C-1027-AG crystal taken with synchrotron radiation, recorded on imaging plates and digitized on a Fujix BA100 read-out system. The oscillation range was 10.5° and the exposure time was 42 s. The white circle indicates the direct beam position, whereas the arrow shows where diffraction spots at 3.0 Å resolution are recorded.

apoproteins of NCS, AUR and AXN, and help the understanding of why C-1027 exhibits such a high cytotoxicity.

We thank Professor N. Sakabe and Dr A. Nakagawa of the Photon Factory, National Laboratory for High Energy Physics (BL-6A$_2$) for their kind help in data collection. This work was supported in part by Grant-in-aids for Scientific Research to KM from the Ministry of Education, Science and Culture, Japan, and performed under the approval of the Photon Factory Advisory Committee, the National Laboratory for High Energy Physics, Japan (Proposal No. 93G067). PB is supported by a fellowship from the Commission of the European Communities.

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