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Crystallization and preliminary X-ray diffraction studies of DNA decamer d(CCAGGCCTGG) complexed with cobalt(III)-pepleomycin

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Crystals of the self-complementary oligonucleotide d(CCAGG-CCTGG) [Heinemann & Alings (1989), *J. Mol. Biol.* **210**, 369–381] complexed with the hydroperoxide of cobalt-pepleomycin (CoPEP) were obtained by the hanging-drop vapour-diffusion method at 298 K. An X-ray diffraction data set was collected to 2.8 Å at 100 K. The crystal belonged to space group $P2_12_12_1$, with unit-cell parameters a = 34.52, b = 59.88, c = 72.93 Å.

1. Introduction

Bleomycins (BLMs) are natural products extracted from *Streptomyces verticillus* and are used clinically to treat a number of specific tumour types (Boger & Cai, 1999; Claussen & Long, 1999; Burger, 1998). BLMs bind to and cleave duplex DNA in the presence of required cofactors, iron and oxygen. There are cleavage hot spots on double-stranded DNA with regard to BLM and one BLM molecule may effect two cleavage events, resulting in a double-strand break (Steighner & Povirk, 1990; Povirk *et al.*, 1989; Absalon *et al.*, 1995). DNA-repair enzymes are induced in the presence of BLM, supporting the hypothesis that DNA cleavage is the cause of BLM-induced cytotoxicity (Robertson *et al.*, 2001; Ramana *et al.*, 1998).

Although a number of models by which metallo-BLMs bind to DNA, based on biochemical and physicochemical methods such as footprinting and two-dimensional NMR experiments, have been presented (Kuwahara & Sugiura, 1988; Wu *et al.*, 1994, 1996; Vanderwall *et al.*, 1997; Caceres-Cortes *et al.*, 1997*a*,*b*), possible mechanisms for the sequence specificity of their binding and the chemical specificity of their cleavage have not been established.

Pepleomycin is an anti-tumour antibiotic that is related structurally to bleomycin and derived from it: the two antibiotics differ in their C-terminal substituent. Pepleomycin is reported to exhibit greater anti-cancer activity and lower pulmonary toxicity than bleomycin (Oka, 1980; Takahashi *et al.*, 1979; Matsuda *et al.*, 1978). We have initiated a structural analysis of the hydroperoxide of cobalt(III)-pepleomycin (CoPEP) complexed with DNA in order to elucidate the mechanism of DNA binding and cleavage.

Here, we report the crystallization and preliminary crystallographic analysis of the CoPEP–DNA complex.

2. Sample preparation

A self-complementary 10-mer DNA, CCAGGCCTGG, which has a CoPEP-binding site (GC sequence) in the middle, was purchased from Amersham Pharmacia Biotech. A lyophilized sample of CoPEP was prepared as described previously (Sugiyama *et al.*, 1990).

3. Crystallization

Crystallization trials were conducted using the hanging-drop vapour-diffusion method (Berger *et al.*, 1996). Preliminary crystallization conditions were established using the Hampton Research Nucleic Acid Mini Screen kit at 277 K, followed by refinement of the conditions by variation of concentration, precipitant, temperature, drop volume and additives. Optimal conditions for crystal growth were obtained using 2.0 µl drops containing 2.0 m*M* purified DNA and CoPEP and reservoir solution containing 10% MPD, 40 m*M* sodium cacodylate, 12 m*M* spermine.4HCl, 80 m*M* KCl, 20 m*M* $BaCl_2$ pH 6.0. Under the optimized conditions, green rhombic crystals (Fig. 1) with dimensions of approximately 0.4 × 0.2 × 0.04 mm were obtained in three to four weeks.

The green colouration of the crystals indicated the presence of CoPEP and high-performance liquid chromatography (HPLC) analysis revealed two peaks corresponding to DNA and CoPEP (data not shown).

4. Data collection

For preliminary characterization, crystals were mounted in glass capillaries with a small amount of mother liquor and intensity data for unit-cell parameter and space-group determination were collected on an R-AXIS IV image-plate detector with Cu $K\alpha$ radiation from a

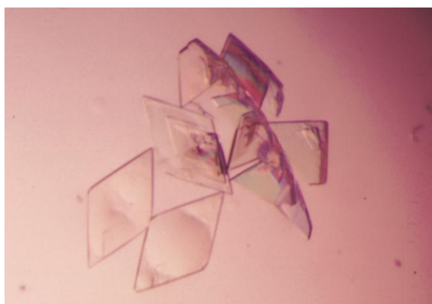

Figure 1
Photograph of the CoPEP–DNA complex crystals obtained by the hanging-drop vapour-diffusion method. Crystals showed the green colour characteristic of the presence of Co^{III}.

Rigaku rotating-anode generator operated at 40 kV and 150 mA.

Because CoPEP–DNA crystals contained cobalt, we decided to use this atom as an anomalous scatterer for structure determination using the multiwavelength anomalous dispersion (MAD) method. For this purpose, a MAD experiment at the cobalt edge was performed using the synchrotron-radiation source at SPring-8 BL44B2 and a MAR CCD 165 detector system (Hyogo, Japan).

CoPEP–DNA complex crystals were flash-frozen for data collection. Prior to flash-freezing, crystals were soaked for about 20 s in a cryoprotectant solution containing 8 µl reservoir solution and 2 µl MPD. The crystals were then picked up using a 1.0 mm fibre loop (Hampton Research) and flash-frozen in a nitrogen-gas stream at 100 K. The data were processed using *MOSFLM* (Leslie, 1992) and *SCALA* from the *CCP*4 suite (Collaborative Computational Project, Number 4, 1994). Data-collection statistics are given in Table 1.

Table 1
Data-collection statistics.

Space group	$P2_12_12_1$
Unit-cell parameters (Å)	a = 34.52, b = 59.88, c = 72.93
Resolution (Å)	2.8
Completeness (%)	93.8
No. of measurements	43235
No. of unique reflections	5822
$I/\sigma(I)$	7.4
R_{sym} (%)	9.5

† $R_{sym} = \sum|I - \langle I \rangle|/\sum I$, where I is the observed intensity and $\langle I \rangle$ is the average intensity from multiple observations of symmetry-related reflections.

5. Preliminary X-ray diffraction analysis

The systematic absences and symmetry were consistent with the space group $P2_12_12_1$, with unit-cell parameters a = 34.52, b = 59.88, c = 72.93 Å. The real-space self-rotation function with origin removal showed a single strong peak at κ = 180°, indicating twofold non-crystallographic symmetry. Attempts to solve the structure by MAD methods have so far failed because of the high internal symmetry and high mosaicity of the data set. We are presently searching for suitable heavy-atom derivatives in order to apply the multiple isomorphous replacement method to the CoPEP–DNA crystal structure determination.

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