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# Crystallization and preliminary X-ray diffraction analysis of a cold-adapted uracil-DNA glycosylase from Atlantic cod (*Gadus morhua*)

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Uracil-DNA glycosylase (UDG) is a DNA-repair enzyme involved in the removal of uracil from DNA. The Atlantic cod UDG (cUDG) possesses typical cold-adaptation features, with higher catalytic efficiency and lower thermal stability than the mammalian counterparts. cUDG has been crystallized by the vapour-diffusion method using sodium citrate as the precipitant at pH 7.5. The crystals are monoclinic and belong to space group $P2_1$, with unit-cell parameters $a = 68.58$, $b = 67.19$, $c = 68.64$ Å, $\beta = 119.85^\circ$. There are two molecules in the asymmetric unit, with a corresponding $V_M$ value of 2.71 Å$^3$ Da$^{-1}$ and a solvent content of 54.7%. Synchrotron diffraction data have been collected to 1.9 Å resolution using cryogenic conditions (120 K).

## 1. Introduction

Uracil-DNA glycosylase (UDG; E.C. 3.2.2.3) is the first enzyme in the base-excision repair (BER) pathway which removes uracil from DNA. A uracil base in DNA results either from incorporation of deoxyuridine triphosphate instead of thymidine triphosphate during replication or from deamination of cytosine in DNA. Spontaneous cytosine deamination occurs at a rate of about 100–500 events per human cell per day (Lindahl, 1993). Uracil-DNA glycosylase is highly conserved in organisms as diverse as bacteria and vertebrates (Krokan *et al.*, 1997), and catalyzes the first step of the BER pathway by hydrolyzing the N-glycosylic bond between uracil and the deoxyribose sugar; it is able to remove uracil from both single- and double-stranded DNA. The archetypical vertebrate UDG is a monomeric protein with a MW in vertebrates ranging from about 25 to 35 kDa, depending on the degree of processing and phosphorylation after subcellular localization into mitochondria or the nucleus (Bharati *et al.*, 1998; Muller-Weeks *et al.*, 1998). Targeting of UDG to the nucleus and mitochondria is determined by two different N-terminal domains containing a nuclear and mitochondrial signal sequence, respectively, produced by alternative transcription and splicing of the same gene (Nilsen *et al.*, 1997). The catalytic domain of cod UDG is highly conserved between the vertebrates and consists of 220 amino acids with a sequence identity of 77 and 75% to the mouse and human sequences, respectively (Lanes *et al.*, 2001). The crystal structures of the catalytic domain of UDG has been determined for the human (Mol *et al.*, 1995), herpes simplex virus 1 (HSV-1) (Savva *et al.*, 1995) and *Escherichia coli* (Ravishankar *et al.*, 1998) UDGs and contains the classic $\alpha/\beta$-fold with a central four-stranded parallel $\beta$-sheet surrounded by eight $\alpha$-helices. The active-site groove is located at the C-terminal end of the parallel $\beta$-sheet. UDG compresses the DNA–phosphate backbone and extrudes a conserved leucine residue into the minor groove of DNA, facilitating base flipping of the nucleotide into the specificity pocket. Owing to the tightly formed specificity pocket and complementary hydrogen bonding, only uracil fits into this pocket and formation of the productive complex causes conformational changes in the human UDG structure (Parikh *et al.*, 1998).

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Atlantic cod UDG has been shown to have both a higher catalytic efficiency at low temperatures and a lower stability at high temperatures compared with the human

enzyme (Lanes *et al.*, 2000, 2001). These are the main characteristics of the so-called cold-adapted enzymes. Structural data at atomic resolution is of crucial importance to gain insight into the mechanisms responsible for cold adaptation. To facilitate comparative studies between the mesophilic warm-adapted mammalian UDGs and the cold-adapted UDG from Atlantic cod, we have crystallized UDG with the aim of solving its three-dimensional structure using X-ray crystallography.

## 2. Materials and methods

### 2.1. Protein purification and crystallization

Recombinant Atlantic cod uracil-DNA glycosylase, expressed in *Escherichia coli*, was purified as described elsewhere (Lanes *et al.*, 2001). The purified enzyme was concentrated to 10 mg ml$^{-1}$ in 60 m*M* NaCl, 0.5 m*M* EDTA and 0.5% glycerol buffered with 15 m*M* Tris–HCl at pH 8.0. Initial screening for crystallization conditions was initiated using the vapour-diffusion technique (McPherson, 1976) at room temperature (291 K). Conditions were surveyed using Hampton Research crystallization kits (Crystal Screens I and II; Cudney *et al.*, 1994; Jancarik & Kim, 1991). In each well, hanging drops were made by mixing equal amounts of protein and precipitant solution. The drops had an initial volume of 4 µl and were equilibrated against a reservoir solution containing 1 ml of the precipitant solution. Rosettes formed by thin plates of crystals appeared in condition number 38 (0.1 *M* HEPES pH 7.5 and 1.4 *M* sodium citrate) of the Crystal Screen I kit after one week (Fig. 1). From the rosettes, single crystals suitable for X-ray data collection could be isolated. Further optimization of the crystallization conditions did not improve the crystal quality.

### 2.2. X-ray data collection

From a single plate-shaped crystal having approximate dimensions 0.1 × 0.3 × 0.05 mm, a native data set from cUDG was collected at the Swiss–Norwegian beamline (SNBL) at the European Synchrotron Radiation Facilities (ESRF), Grenoble, France. Prior to data collection, the crystal was immersed for a few seconds in a cryoprotecting solution containing 0.1 *M* HEPES pH 7.5, 1.4 *M* sodium citrate and 20% glycerol followed by flash-cooling in liquid nitrogen. Intensity data were then collected at 120 K using a MAR345 image-plate detector in the 180 mm mode at a wavelength of 0.873 Å and a crystal-to-detector distance of 150 mm. A total of 180 images were collected with 1° oscillations. The data were subsequently indexed and scaled with the programs *DENZO* (Otwinowski & Minor, 1997) and programs from the *CCP*4 package (Collaborative Computational Project, Number 4, 1994). Data-collection statistics are shown in Table 1.

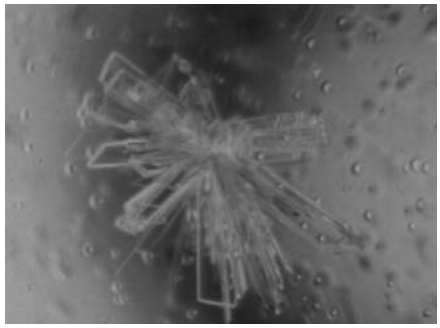

**Figure 1**
Crystals of cUDG.

## 3. Results and discussion

Many crystals that were cut from the rosettes and tested in the X-ray beam did not diffract at all. In most cases, this might be explained by the limited scattering volume of the plate-shaped crystals, which were less than 0.1 mm thick. Still, regardless of size, the quality of the crystals varied significantly, even for crystals of comparable size from one droplet.

From the unit-cell parameters, it is evident that in addition to the monoclinic $P2_1$ unit cell, both trigonal/hexagonal *C*-centered orthorhombic and *C*-centered monoclinic lattices could be indexed well below the distortion criteria recommended in the indexing program *DENZO* (Otwinowski & Minor, 1997). However, the true space group is determined by the intensity distribution, not the unit-cell parameters, and in this case the data could only be scaled and merged in $P2_1$. All other crystal systems tried gave $R_{\rm merge}$ values in the 50–60% range.

Molecular replacement trials in *CNS* (Brunger *et al.*, 1998) using the crystal structure of the catalytic domain of the human UDG (PDB code 1akz; Mol *et al.*, 1995) as a search model gave two clear individual solutions about 8$\sigma$ above the mean value for the rotation search and 6$\sigma$ above the highest non-related peak. The occurrence of two molecules in the asymmetric unit was expected from a previously run self-rotation function. The translation search indicated two solutions, 5.2$\sigma$ and 4.5$\sigma$ above the following peak; the subsequent translation search for the dimer, fixing the best solution, resulted in one solution only. A rigid-body refinement of the two molecules using all data in the resolution range 12–2.45 Å lowered the $R_{\rm cryst}$ and $R_{\rm free}$ values to 38.0 and 38.3%, respectively. Further refinement and model building of the crystal structure of cod uracil-DNA glycosylase is in progress.

**Table 1**
Data-collection and processing statistics.

| | |
|---|---|
| Wavelength (Å) | 0.873 |
| Space group | $P2_1$ |
| Unit-cell parameters | |
| *a* (Å) | 68.58 |
| *b* (Å) | 67.19 |
| *c* (Å) | 68.64 |
| $\beta$ (°) | 119.85 |
| Unit-cell volume (Å$^3$) | 274164.4 |
| Estimated water content (%) | 54.7 |
| Resolution range (Å) | 12.0–1.9 (2.0–1.9) |
| No. of measurements | 137511 |
| No. of unique reflections | 42162 |
| Completeness (%) | 98.7 (97.5) |
| Multiplicity (%) | 3.3 (2.9) |
| $R_{\rm merge}$† (%) | 9.6 (52.2) |
| Average $I/\sigma(I)$‡ | 10.2 (2.0) |

† $R_{\rm merge} = \sum_h \sum_i |I_i(h) - \langle I(h)\rangle| / \sum_h \sum_i I(h)$, where $I_i(h)$ is the *i*th measurement of reflection *h* and $\langle I(h)\rangle$ is the weighted mean of all measurements of *h*. ‡ Intensity signal-to-noise ratio.

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