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Experimental Report – MX-742

"Damage recognition in formamidopyrimidine-DNA glycosylase from *Lactococcus lactis*"

Quality of measurement/data and status / progress of evaluation

Co-crystals from wild type FPG from *Lactococcus lactis cremoris* in complex with oligonucleotides containing carbacyclic-7,8-dihydro-8-oxoguanine (c8oxo-dG) diffracted X-rays to about 3 Å spacing at the microfocus beamline ID23-2. A complete data set to 3.2 Å was obtained by combining data collected at two different parts of the crystal. Data processing statistics are shown in Table 1.

Results

The structure was solved by molecular replacement using the coordinates of the inactive mutant missing the active-site proline (PDB code 1TDZ). The assymetric unit consists of two protein molecules bound to one double strand DNA. One protein molecule flips-out the c8oxo-dG lesion in syn-conformation almost 180dg from the DNA duplex into its active site with a comparable binding mode as observed in the structure of the cleavable 8oxo-dG lesion and the inactive FPG mutant (PDB code 1R2Y). The second protein molecule in the ASU binds to the end of the DNA. Figure 1 shows the overall structure of the wild type FPG in complex with the c8oxo-dG lesion and an example of the electron density of the c8oxo-dG lesion.

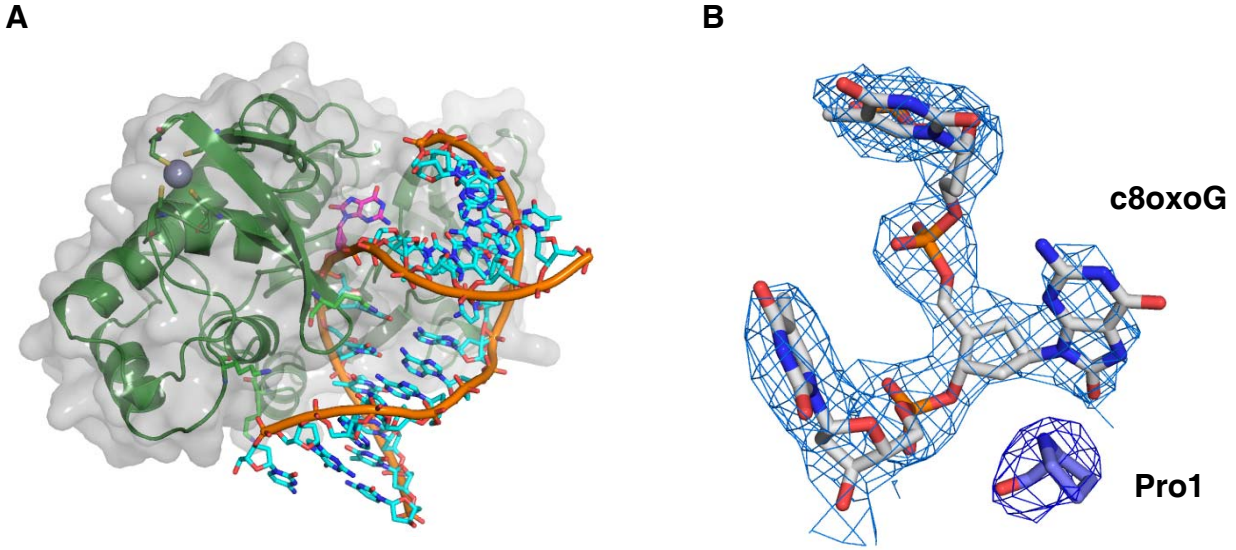

Figure 1 Overall structure of the FPG-DNA complex. A) The flipped-out c8oxo-dG lesion is highlighted in pink and the Zn^{2+} of the Zn-finger is shown as sphere. B) 2Fo-Fc electon density of the lesion and active-site prolinecontoured at 1 σ level.

The preliminary structure refinement statistics are summarised in Table 1. Model building, refinement as well as crystal optimisation are currently under way.

This data show that using non-cleavable carbocyclic ribose, where the sugar oxygen is substituted by carbon, are suitable lesion-analogues and omit the necessity of mutation of the repair enzyme.
But higher diffraction data will be necessary to provide better insight into the mechanism of screening of undamaged DNA, damage recognition and repair by DNA glycosylases.

Table 1: Data collection, processing and preliminary structure refinement statistics.

Data collection	
Wavelength (Å)	0.873
Spacegroup	$P2_12_12_1$
Cell dimensions	42.6 113.6 135.1
Rmerge	0.13 (0.47)
Number of observations	61159 (9064)
Number unique	11087 (1575)
Mean I/ σ I	11.9 (3.5)
Completeness	98.7 (99.3)
Multiplicity	5.5 (5.8)
Refinement	
Resolution	40.00 - 3.2 (3.37-3.2)
Rwork/Rfree	0.2321/ 0.2749
R.m.s Bond	0.0164
R.m.s. Angle	2.039