PURIFICATION AND CHARACTERISATION OF AN UNUSUAL DNA GLYCOSYLASE IN DIATOMS

Martin Vejle Andersen[1], Berit Doseth[2], Cecilie Dahl Hem[1], Tore Brembu[1], Geir Slupphaug[2] & Atle M. Bones[1]

[1]Department of Biology, Norwegian University of Science and Technology
[2]Department of Cancer Research and Molecular Medicine, Norwegian University of Science and Technology

A large number of DNA repair enzymes ensure that damage to the DNA by spontaneous mutations, radiation and chemical mutagens is rapidly and efficiently repaired. DNA glycosylases recognise damaged bases in the DNA and removes these as part of the base excision repair (BER) pathway. Two classes of DNA glycosylases are Uracil N-glycosylase (UNG), which recognises uracil, and Nei-like glycosylase (NEIL), which recognises oxidised bases.

A database search for UNG genes in the sequenced genomes of two diatoms, *Thalassiosira pseudonana* and *Phaeodactylum tricornutum,* identified two *UNG* orthologues. One of these genes encodes a DNA glycosylase with a novel domain organisation, where a C-terminal UNG domain has been fused to an N-terminal NEIL-like domain. This glycosylase, termed Dual DNA Glycosylase (DDG), appears to be conserved in, and is unique for diatoms.

DDG from *P. tricornutum* was cloned as full-length DDG and the single NEIL and UNG domains into the expression vector pBADM-30, which contains both a histidine tag and a glutathion S-transferase (GST) tag. Recombinant proteins expressed in *E. coli* were purified by the use of both histidine tag and GST tag affinity columns, and verified by MALDI-TOF analysis. Enzyme activity assays using the purified fusion proteins showed that PtDDG carries both UNG and NEIL activity. The activity optima of PtDDG was also analysed with regard to pH, temperature and NaCl concentration.