CRYSTALLOGRAPHIC ANALYSIS OF THE HOLLIDAY JUNCTION-CUTTING ENZYME (Hjc) FROM *ARCHAEOGLOBUS FULGIDUS*

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Holliday junction-resolving enzymes are ubiquitous proteins that play a key role in DNA repair and reorganization by homologous recombination. They catalyze the critical step of junction resolution in a highly precise manner. The Hjc family (for Holliday junction cutting enzyme) was recently found to be a conserved type of junction-resolvases in archaea with similarity to type II restriction endonucleases (Komori et al., 1999; Kvaratskhelia & White, 2000). Two structures of Hjc from *Sulfolobus solfataricus* and *Pyrococcus furious* have been solved so far (Bond et al., 2001; Nishino et al., 2001). We recently obtained crystals of another member of this family originating from *Archaeoglobus fulgidus*. The crystals grown in a sparse matrix screen diffracted up to 2.5 Å, they showed an elongated trigonal unit cell with parameters of a = b =3 7.2 Å and c = 270.9 Å. A first data set was collected on beamline ID14-EH1 at ESRF to a resolution of 2.7 Å. A homology model was established based on sequence analysis using the available structures. The crystallization conditions are being refined to reach higher resolution and completeness and the phase determination by molecular replacement is in progress. By solving this new structure, we will be able to compare Hjc from three different archaeons and draw more general conclusions about this resolvase family, its evolution and consequences for DNA recognition.

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Keywords: HJC, HOLLIDAY JUNCTION, JUNCTION-RESOLVING ENZYME

STRUCTURAL IMPACT OF 8-OXOGUANINE ON HUMAN TOPOISOMERASE I

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8-OxoG (7,8-dihydro-8-oxoguanine) is the most common form of oxidative DNA damage humans. No structural information exists on the impact of 8-oxoG on key DNA metabolic proteins. We present the 3.1 Å crystal structure of human topoisomerase I in non-covalent complex with a DNA oligomer containing 8-oxoG adjacent to the enzyme's single-strand cleavage site. Topoisomerase I relaxes DNA superhelical tension and is vital for replication, transcription and recombination. Biochemical studies have revealed that the presence of 8-oxoG increases topoisomerase I's affinity for DNA, but decreases the enzyme's DNA cleavage activity. In our structure, we find that the 8-oxoG lesion causes the active site of human topoisomerase I to reorganize into an inactive conformation relative to the numerous structures of topoisomerase I-DNA complexes elucidated previously. The catalytic Tyr-723-Phe is rotated away from the DNA cleavage site and packed into the body of the molecule. A second active site residue, Arg590, becomes disordered and is not observed in the structure. These observations lead to a model in which human topoisomerase I binds to DNA first in an inactive conformation, and then the enzyme rearranges its active site to allow for catalysis. 8-oxoG impedes topoisomerase I activity by trapping it in its inactive state bound to DNA.

Keywords: **TOPOISOMERASE, PROTEIN-DNA COMPLEX, DNA DAMAGE**

CRYSTAL STRUCTURE ANALYSIS OF HUMAN CCG1/TAFII250-INTERACTING FACTOR B (cib)

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Transcription factor TFIID is a multi-protein complex composed of the TATA box-binding protein (TBP) and its associated factors (TAFs), and is required for accurate and regulated initiation of transcription by RNA polymerase II. The largest subunit of TFIID, CCG1, which serves a central architectural role in the assembly of TFIID has the histone acetyltransferase (HAT) activity. To investigate the functional regulation of CCG1 HAT domain, we screened some interacting factors with this domain using a yeast two-hybrid system. In this search, we have obtained the cDNA of a novel factor CIB (CCG1-interacting factor B). As the role CIB protein in the cell is not clearly known, we have initiated crystallographic, biochemical and genetic studies on this protein. The CIB protein crystals were grown in 1.4-1.5M ammonium sulfate, 15mm urea, 70mM Tris-Cl, pH7.5, using hanging drop vapor diffusion method. Thin plate-like crystals in the orthorhombic space group $P2_12_12$ with one molecule in the asymmetric unit, with cell dimensions of a= 110.70, b=44.45, c=43.60 Å, appeared in one week. A complete data set was collected to a maximum resolution of 1.7 Å, using the beam-line BL18B at the synchrotron facility, Photon Factory, Tsukuba. The structure was solved by MIR method using SOLVE package and the refinement was carried out using CNS. The present R-factor and R-free is 17.5% & 22.0%, respectively at 1.7 Å resolution. Surprisingly, the overall tertiary structure of CIB protein is similar to that of α/β-hydrolases. Details of the structure and function of the protein will be discussed.

Keywords: **TRANSCRIPTIONAL REGULATION HYDROASE STRUCTURE**

THE MOLECULAR BASIS OF THE COLORATION MECHANISM IN LOBSTER SHELL: β-CRUSTACYANIN AT 3.2 Å RESOLUTION

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The binding of the carotenoid astaxanthin in the protein multi-macromolecular complex crustacyanin is responsible for the blue coloration of lobster shell. The structural basis of the bathochromic shift mechanism has long been elusive. A change in the colour occurs from the orange red of the unbound astaxanthin (λ_{max} = 472 nm in hexane), the well-known colour of cooked lobster, to slate-blue in the protein bound live lobster state ((λ_{max} = 632 nm in crustacyanin). Intriguingly, extracted crustacyanin goes red upon dehydration and on rehydration goes back to blue. Recently the innovative use of softer X-rays and xenon derivatisation yielded the 3-D structure of the A1 apoprotein subunit of crustacyanin. This has now provided the molecular replacement search model for a completely new crystal form of the (β-crustacyanin holo complex that is an A1 with A3 subunit assembly including two bound astaxanthin molecules. We have thereby determined the structure of the A3 molecule *de novo*, and also the structural chemistry of the biological coloration mechanism at the detailed molecular level in this β-complex. Lobster has clearly evolved an intricate structural mechanism for the coloration of its shell utilizing astaxanthin and a bathochromic shift. Blue/purple caroteno-proteins are ubiquitous amongst invertebrate marine animals, particularly the Crustacea. For the first time, 3-D structural results on such a coloration mechanism are now available with our study on the lobster shell.

Keywords: **β CRUSTACYANIN BATHOCRHOMIC SHIFT ASTAXANTHIN**