ULTRAHIGH RESOLUTION CRYSTAL STRUCTURE OF SQUID GANGLION DFPase

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Diisopropylfluorophosphatases (DFPases) are capable of detoxifying chemical warefare agents like diisopropylfluorophosphate (DFP), soman, sarin, tabun, and cyclosarin by hydrolysis. The protein reported here belongs to the subclass of squid-type DFPases and was originally isolated from squid head ganglion of Loligo vulgaris, but in this work we used the protein recombinantely expressed in E. coli. The X-ray crystal structure of this enzyme has been refined to a resolution of 0.85 Å and a crystallographic R-value of 9.42%. Reversible flash-cooling improved both, mosaicity and resolution of the crystals considerably. The overall structure of this protein represents a six-bladed ß-propeller with two calcium ions bound in a central water filled tunnel. 496 water, 2 glycerol, 2 MES-buffer molecules, and 18 PEG fragments of different length could be refined in the solvent region. 45 of the 314 residues have been refined with alternative orientations. Hydrogen atoms have been omitted from these residues. In the residues of the inner ß strands, hydrogen atoms are visible in a normal Fo-Fc difference map of a hydrogen deficient structural model. The 208 most reliable residues, without disorder or reduced occupancy in their side-chains, were finally refined without restraints. The following full matrix refinement cycle for the positional parameters yielded estimated standard deviations (e.s.d.s.) by matrix inversion. The herewith-calculated bond-lengths and bond-esds were used to obtain averaged bond-length, which had been compared to the restraints used in preceding refinement rounds. Also very accurate dimensions for the Ca^{2+} coordination polyhedrons could be obtained.

Keywords: PHOSPHOTRIESTERASE ß-PROPELLER REVERSIBLE FLASHCOOLING

DESKTOP SYSTEM FOR DIFFRACTION STUDIES OF PROTEIN CRYSTALS AND MACROMOLECULES BASED ON KUMAKHOVS OPTICS

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Herewith a desktop system for X-ray crystallographic analysis of protein crystals and macromolecules is described. It includes a system developed at IRO with a point-focus source of 40 W power and poly-capillary half-lens transforming divergent beam into quasi-parallel beam. Diffraction studies are performed using MAR 345 detector. Preliminary investigations of protein crystals using an 8 W X-ray source complete with a half-lens featuring 0.04 radian angle of capture have shown that quasi-parallel beam of Cu Kα radiation with 3 mrad divergence and 300 micron diameter corresponds to the flux characterized by the same parameters from a source with rotating anode that has a power of several kilowatts. Using pointed source of X-ray of 40 W power and latest generation poly-capillary systems, it is possible to produce quasi-parallel monochromatic beams with a flux density of 10^{10} photon/sec/mm^2 at 2-3 mrad divergence, which is a significant achievement for laboratory sources.

Keywords: PROTEIN DIFFRACTION ANALYSIS, POLY-CAPILLARY OPTICS

THE CRYSTAL STRUCTURE OF DISSIMILATORY SULFITE REDUCTASE D (DsrD) PROTEIN THAT HAS A DNA-BINDING MOTIF

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DsrD (dissimilatory sulfite reductase D) is a compact protein located on dsr operon, which encodes dissimilatory sulfite reductase (DsrAB). The function of this molecule was expected as a sulfite-binding protein. However, biochemical experiments have not indicated the sulfite-binding of DsrD with high affinity. Therefore, the real function of this protein has not been clearly identified. However, DsrD should have an important role for DsrAB (dissimilatory sulfite reductase AB) because a fused DsrB-DsrD subunit was found in Bilophila wadsworthia recently. DsrD in the anaerobic sulfate-reducing eubacterium, Desulfovibrio vulgaris Hildenborough was crystallized by using ammonium sulfate as a precipitant. High resolution native data was collected at 100 K up to 1.2Å on beamline BL41XU of SPring-8 using a MAR CCD detector. This crystal belongs to the space group $P2_12_12_1$ with unit cell dimensions of a = 60.03Å, b = 64.56Å, and c = 45.22Å. The structure was determined by a combination of single isomorphous replacement and anomalous scattering (SIRAS) using an Au derivative. The structure was refined by the program of SHELX with a R-factor of 15.21% at 1.2Å. The DsrD protein showed a DNA binding (winged-helix) motif, and had five sulfate anions per two molecules in an asymmetric unit of a crystal cell. It is still possible that DsrD plays a role of the binding of sulfite anions. In addition, it is also expected that DsrD is a DNA binding protein and have a function such as a transcriptional factor.

Keywords: SULFATE-REDUCING BACTERIA HIGH RESOLUTION STRUCTURE DNA-BINDING MOTIF

N-GLYCOSYLATION AT ATOMIC RESOLUTION IN THE 1.12Å STRUCTURE OF RHAMNOGALACTURONAN ACETYLESTERASE

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Protein glycosylation is one of the most prevalent post-translational modifications of eukariotic proteins. Many biological functions have been attributed to oligosaccharides, and the role of carbohydrates as hardware in biological information transfer and storage has become a subject of increasing interest. In spite of this only a few glycoprotein structures have been determined to high resolution. The crystal structure of the glycoprotein rhamnogalacturonan acetylesterase from Aspergillus aculeatus has been refined to a resolution of 1.12Å using synchrotron data collected at 263 K. There are two putative N-glycosylation sites at Asn104 and Asn182, and both are glycosylated. Due to crystal contacts, the glycan structure at Asn182 is unusually well defined in the electron density maps, and a total of seven carbohydrate residues could be modelled into the density. Equivalent carbohydrate parameters were restrained to be similar, but were refined without target values. The refined bond lengths and angles were compared to the values from the carbohydrate dictionaries that are used for glycoprotein refinement, and which are obtained from small-molecule studies.

Keywords: RHAMNOGALACTURONAN ACETYLESTERASE CARBOHYDRATE GLYCOSYLATION