EXTENSION OF HOME LABORATORY PHASING CAPABILITIES USING CHROMIUM RADIATION

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A home laboratory high-intensity chromium X-ray source appears to be ideally suited for use in enhancing the weak anomalous signals from sulfur, selenium, calcium and other atoms found in protein crystals. Specifically, the Δf for sulfur is 1.14 at CrKα which is similar to the Δf of calcium collected at CuKα. Since calcium anomalous scattering has been used to phase trypsin [1] from CuKα diffraction data, we expect a high quality CrKα data set to provide even more phasing power and allow for routine phasing of macromolecular diffraction data without the need for synchrotron data or isomorphous replacement. In order to test this hypothesis we have commissioned Osmic, Inc. to design and manufacture a Confocal Max-Flux™ optic optimized specifically for Cr radiation. We are now performing experiments with this optic to determine how to maximize the anomalous signal from sulfur and other light elements within different proteins with the goal of solving the phase problem more readily. Special attention will be given to the details required to offset the strong absorption of Cr radiation by the experimental setup typically used for diffraction studies.

Keywords: PHASING

CRYSTAL STRUCTURE OF ACYL-CoA DEHYDROGENASE OF THERMUS THERMOPHILUS HB8

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A gene product from Thermus thermophilus HB8 consists of 410 amino acid residues and displays 48% sequence identity to mammalian medium-chain acyl-CoA dehydrogenase. Acyl-CoA dehydrogenases catalyze the first step in the cycle of fatty acid β-oxidation. Acyl-CoA thioesters are oxidized to the corresponding trans-2,3-enoyl-CoA products with concomitant reduction of enzyme-bound FAD. In this study, we characterized the enzymatic activity and determined the crystal structure of Acyl-CoA dehydrogenase of T. thermophilus HB8 at 2.4 Å resolution using the molecular replacement program CNS with human medium-chain acyl-CoA dehydrogenase structure (1EGE). The crystal contains two molecules forming a dimer in an asymmetric unit. The monomer structure includes 379 residues (32-410 residues) folded into three parts (N-terminal and C-terminal α-helix parts and the middle β-sheet part) and a FAD molecule. The FAD is in the crevice between the two helical parts and β-sheet part of the enzyme. The catalytically essential glutamate residue (Glu393) that initiates catalysis by abstracting substrate α-hydrogen is located near the flavin ring. Structural comparison with the structure of human shows that the overall structures are substantially the similar except for several loop regions, and that the relative positions of FAD and the several residues interacting with FAD are well conserved. In the enzyme structure, the active site is filled with a string of ordered water molecules. It suggests that these water molecules should be replaced by the fatty acyl thioester moiety of the substrate. The plasmid for the protein expression was provided by the RIKEN Structural Genomics Project (Project Code: 162).

Keywords: CRYSTAL STRUCTURE, ENZYME, ACYL-COA DEHYDROGENASE

RIKEN STRUCTURAL GENOMICS BEAMLINES AT SPring-8 / DEVELOPMENT OF SAMPLE AUTO-CHANGING SYSTEM

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RIKEN Structural Genomics Beamline I & II have been developed for high throughput protein crystallography (HTPX). The final goal of the beamlines is their automatic operation. Sample auto-changing system is a key component of the automated beamlines. The sample auto-changer will enhance the data-collection efficiency, as well as remove human-error. We are developing a new sample auto-changing system based on a turntable pin design. The turntable sample pin has rigid and left handed screw at both ends and then can be mounted or unmounted on the goniometer by rotating the pin to one direction. The pin has a cryoloop to hold a crystal sample at the end. During the sample operation, the crystal is kept inside the screw hole of the changer arm to avoid exposure the air. The system is controlled by a PC and is easy to operate other computer via TCP/IP socket connection. We have tested successive 500 time sample exchanges without error. It takes 5 sec to make one sample exchange.

Keywords: HIGH THROUGHPUT SPRING-8 STRUCTURAL GENOMICS

Crystal Structure of Escherichia coli YodA, A Protein of Unknown Function

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While attempting to overexpress a heterologous protein in E. coli as a host, we induced the expression of the E. coli protein YodA, a protein whose function is not known. To date, the YodA gene had been described in two studies, both concerned with the overall response of E. coli to different external stimuli. In the first case, Laurent-Winter et al., 1997 observed a decrease of YodA expression in E. coli mutants defective in the histone-like nucleoid-structuring protein (H-NS). In the second case, Ferianc et al., 1998 found an increase of YodA expression when submitting E. coli to increased levels of cadmium in the medium. YodA, as well as YrPE from B. subtilis and pX01-130 from B. Anthracis show a strong sequence similarity with the C-terminal part of adhesion AdA from Streptococcus pneumoniae. Interestingly, AdA was suggested to be a lipoprotein containing a metal-binding site, belonging to a new family of metal-binding bacterial receptors (Dintilhac & Claverys, 1997). We have expressed and crystallized YodA alone and in the presence of the divalent cations cadmium, zinc and nickel. The structure determination is currently in progress, using MAD phasing with zinc. In parallel, we are trying to understand its physiological function, in particular when expressing heterologous proteins in fermentors. Given the manner in which this protein was obtained, we think our study may be of special interest in the context of structural genomics.

References


Keywords: PROTEIN STRUCTURE; MAD; UNKNOWN FUNCTION