LYSOSOMAL α-MANNOSIDASE

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STRUCTURAL AND FUNCTIONAL ANALYSES OF β-DOMAINS OF UvrB AND TRCF

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In nucleotide excision repair system, UvrA-UvrB complex recognizes a wide range of DNA damages. Recently, we determined the crystal structure of UvrB from Thermus thermophilus HB8 (Nakagawa, et al. (1999) J. Biochem. 126, 986-990). Based on this structure and previous studies (Nakagawa, et al. (1997) J. Biol. Chem. 272, 22703-22713), we inferred that a discrete domain composed of β-sheets (β-domain) is the binding site of UvrA. Furthermore, sequence similar to the β-domain (UvrB-β) is found in transcription-repair coupling factor (TRCF). As TRCF also interacts with UvrA, this homologous region (TRCF-β) is considered as the UvrA-binding site. To prove this hypothesis and analyze the binding reaction in detail, we constructed the genes corresponding to UvrB-β and TRCF-β. The purified β-domains retained their secondary structures up to 75°C at neutral pH and were found to exist as two monomers in solution. The crystal structure of UvrB-β indicated that UvrB-β keeps the same structure as β-domain within the intact protein. Surface plasmon resonance analysis using BLAcore showed binding of UvrA both to UvrB-β and TRCF-β. Enzyme-coupled spectrophotometric assay indicated that β-domains have no effect on the ATPase activity of UvrA, although the activity was inhibited by the intact UvrB. Competition experiment between UvrB and β-domains with UvrA suggested that the binding region of UvrA to UvrB-β and TRCF-β were the same as the intact UvrB.

Keywords: DNA REPAIR THERMUS THERMOPHILUS DOMAIN

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THE CRYSTAL STRUCTURE OF THE CARBOXYTRANSFERASE SUBUNIT OF GLUTACONYL-CoA DECARBOXYLASE

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Glutaconyl-CoA decarboxylase from the Acidaminococcus fermentans is an ion pump, in which the free energy of decarboxylation drives the electrogenic transport of 1-2 Na+ from the cytoplasm to the outside of the membrane in exchange to one proton. This consists of four subunits of unknown stoichiometry. The carboxytransferase subunit (65 kDa) catalyzes the transfer of CO2 from glutacoyl-CoA to biotin, covalently attached to the subunit. The decarboxylation of the carboxybiotin by the subunit, the ion pump, drives the Na+ translocation. The d subunit is possibly a membrane-anchor for the a subunit. The carboxytransferase subunit could be crystallized in two different space groups: P41212 and P622. The phase problem was solved with an MAD-experiment at DESY/Hamburg with crystals of space group P41212 of the selenomethionine labeled protein, additionally soaked with platinum tetrachloride. Three platinum sites could be located and the program Shake & Bake identified 27 of the 34 possible selenium sites. With platinum tetrachloride the crystal a tetramer is formed by NCS and a crystallographic two-fold axis. SIRAS-phasing and molecular averaging using the NCS operator led to an electron density sufficient for model building. The overall structure of the monomer is formed by two mostly parallel β-sheets flanked by α-helices on both sides of the sheet and is comparable to the Clp_Crotonase-fold.

Keywords: GLUTACONYL-CoA DECARBOXYLASE, NA+-TRANSPORTING ION PUMP, COENZYME A

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STRUCTURE OF RAT BCKD Kinase: A PROTOTYPE FOR MITOCHONDRIAL PROTEIN Kinases

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Mitochondrial protein kinases (mPKs) are molecular switches that down regulate the oxidation of branched-chain α-ketocids and pyruvate. Elevated levels of these metabolites are implicated in disease states such as insulin-resistant Type II diabetes, branched-chain ketoaciduria, and primary lactic acidosis. We report the three-dimensional structure of a member of the mPK family, rat branched-chain α-ketocid dehydrogenase kinase (BCK). BCK features a characteristic nucleotide-binding domain and a four-helix bundle domain. These two domains are reminiscent of modules found in protein histidine kinases (PHKs), which are involved in two-component signal transduction systems. Unlike PHKs, BCK dimerizes through direct interaction of two opposing nucleotide-binding domains. Nucleotide binding to BCK is uniquely mediated by both potassium and magnesium. Binding of ATP induces disorder-order transitions in a loop region at the nucleotide-binding site. These structural changes lead to the formation of a quadruple aromatic stack in the interface between the nucleotide-binding domain and the four-helix bundle domain, where they induce a movement of the top portion of two helices. Phosphotransfer induces further ordering of the loop region, effectively trapping the reaction product ADP, which explains product inhibition in mPKs. The BCK structure is a prototype for all mPKs and will provide a framework for structure-assisted inhibitor design for this family of kinases.

Keywords: MITOCHONDRIAL PROTEIN Kinases BRANCHED CHAIN α-KETOACID DEHYDROGENASE Kinase MAPLE SYRUP URINE DISEASE