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PROGRESS IN THE STRUCTURAL STUDIES OF THE SUCCINATE-UBIQUINONE DEHYDROGRENASE AND THE bc1 COMPLEX

D. Cobessi L.S. Huang E. A. Berry
Lawrence Berkeley National Laboratory, University of California 250, Melvin Calvin Lab BERKELEY CALIFORNIA 94720 USA

The bc1 complex (bc1) is a redox-driven proton pump of the mitocondrial respiratory chain. Available structures of vertebrate bc1 have insufficient resolution to elucidate the detailed binding of ligand and solvent molecules important for understanding its function. Here we report the 2.5 Å structure of beef bc1 from a new crystal form (unit cell: 144×180×226 $Å^3$, $P2_12_12_1$). It is now possible to assign water molecules and lipids, and to complete and correct the low resolution models. Details observed at this resolution will be presented and compared with the yeast bc1 structure solved in complex with a Fv fragment. Succinate-ubiquinone oxidoreductase is another membrane protein complex of the respiratory chain, oxidizing succinate into fumarate in the matrix and reducing quinone to quinol in the membrane. The enzyme from chicken heart mitochondria was crystallized in $P2_12_12_1$ space group (69×83×291$Å^3$). The data were phased by molecular replacement with a polyalanine model of only the extrinsic part of *E. coli* fumarate reductase. Positive peaks corresponding to the three iron sulfur clusters, to the FAD, and to the heme of the membrane part were observed in a Fo-Fc map validating the solution. The model is currently being rebuilt in a 3Fo-2Fc map calculated after solvent flattening. It is possible to locate the heme iron and assign some α-helices of the membrane part. An anomalous map at Cu-Kα-wavelength using the current phases shows peaks for the Fe-S clusters and heme.

Keywords: MEMBRANE PROTEINS ELECTRON TRANSFER OXIDOREDUCTASE

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CRYSTAL STRUCTURES OF OXYGENASE AND REDUCTASE MODULES OF NITRIC OXIDE SYNTHASE: ENZYME MECHANISM AND REGULATION OF ELECTRON TRANSFER REVEALED

E.D. Garcin[1] S.J. Lloyd[1] A.S. Arvai[1] H. Tsuruta[3] A. Meade[2] D.J. Stuehr[2] J.A. Tainer[1] E.D. Getzoff[1]
[1]The Scripps Research Institute, The Department of Molecular Biology and The Skaggs Institute for Chemical Biology, MB4 10550 North Torrey Pines Road LA JOLLA CA 92037 USA [2]The Cleveland Clinic, The Department of Immunology, Cleveland, Ohio, 4410 [3]Stanford Synchrotron Radiation Laboratory, Stanford Linear Accelerator Center, Stanford, CA 9430

Two high resolution X-ray crystallographic structures of the oxygenase and reductase modules of nitric oxide synthase (NOSox and NOSred) provide a structural basis for understanding the molecular mechanisms underlying electron transfer and regulation that is crucial to NO production. The structure of NOSox, containing heme, zinc, tetrahydrobiopterin and L-arginine, confirms the highly conserved overall folds and active site structures among the different NOS isozymes[1-4]. The 2.2Å structure of the reductase module including the FMN-, FAD- and NADPH-binding domains, the connecting domain, part of the autoinhibitory element and the C-terminal tail provides the first image of NOSred with all its cofactors and regulatory elements.

These two structures, in combination with mutagenesis, biochemical characterization and small-angle X-ray scattering experiments on both the independent modules and the full-length enzymes, provide the foundation for formulating hypotheses that address key points relevant to the structural biochemistry of all NOS enzymes.

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Keywords: NITRIC OXIDE SYNTHASE, ELECTRON TRANSFER, REGULATION

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INSIGHTS INTO THE STRUCTURAL MECHANISM BEHIND COMPLEX FORMATION BETWEEN A SNAKE VENOM METALLOPROTEINASE AND ITS NATURAL INHIBITOR BY SYNCHROTRON SMALL-ANGLE X-RAY SOLUTION SCATTERING

H. Fischer[1] A. G. C. Neves-Ferreira[3] J. Perales[3] A. M. Moura-da-Silva[5] G. B. Domont[4] D. H. F. Souza[2] R. C. Garratt[2] A. F. Craievich[1]
[1]Institute of Physics - University of Sao Paulo Department of Applied Physics Rua Do Matao, Travessa R, 187 SAO PAULO SAO PAULO 05508900 BRAZIL [2]Institue of Physics of Sao Carlos, Department of Physics and Informatics, USP [3]Departament of Phisiology and Pharmacodynamics, Oswaldo Cruz Institute, Fiocruz [4]Departament of Biochemistry, Institute of Chemistry, UFRJ [5]Imonopathology Laboratory, Butanta Institute, Sao Paulo

DM43 is an opossum serum glycoprotein inhibitor of snake venom metalloproteinases. It is homologous to human α1B-glycoprotein, a plasma protein of unknown function and a member of the immunoglobulin supergene family. Size exclusion, dynamic laser light scattering and small angle X-ray scattering (SAXS) data indicate that two monomers of DM43, each composed of three immunoglobulin-like domains, associate to form a homodimer in solution. DM43 inhibits the fibrinogenolytic activities of jararhagin, a PIII snake-venom toxin consisting of metalloproteinase, desintegrin and cysteine-rich domains. Evidence suggests that DM43 forms a 1:1 stoichiometric stable complex with jararhagin and that the metalloproteinase domain is essential for such interacion. Homology modeling, based on the crystal structure of a killer cell inhibitory receptor, suggests the existence of an I-type Ig fold for each DM43 domain, a hydrophobic dimerization surface and six exposed loops potentially forming the metalloproteinase binding interface. Jararhagin is shown to have a more compact structure than DM43 with a similar maximum dimension [110(5) Å] but a slightly larger radius of gyration; 34.5(2) Å and 33.7(3) Å respectively. *Ab-initio* models showed that DM43 is a flattened compact structure whilst Jararhagin is more globular, presenting an internal cavity revealing a structure composed of one large domain and one or two smaller ones. The former probably corresponds to the metalloproteinase domain. These results will be important in the refinement of crystal structures currently in progress.

Keywords: SNAKE VENOM METALLOPROTEINASE PROTEIN COMPLEXES SAXS

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CRYSTAL STRUCTURES OF THREE LECTINS FROM THE ROOTS OF POKEWEED

Y. Hata[1] T. Fujii[1] M. Hayashida[1] Y. Aso[2] M. Ishiguro[2]
[1]Institute for Chemical Research, Kyoto University, Uji, Kyoto 611-0011, Japan [2]Laboratory of Protein Chemistry and Engineering, Faculty of Agriculture, Kyushu University, Fukuoka 812-8581, Japan

Five kinds of lectins have been isolated and characterized from the roots of pokeweed. PL-B is composed of seven sequentially similar cystine-rich chitin-binding domains like hevein with highest mitogenic activity. PL-A is a part of PL-B. PL-C is a dimer of three-domain subunits with medium mitogenic activity. PL-D1 and PL-D2 each consist of two domains, and PL-D1 is completely same as PL-D2 in amino-acid sequence except for addition of two C-terminal residues. However, PL-D2 exhibits low mitogenic activity but PL-D1 does not. In order to understand relationships between the domain-repeating structures and physiological properties, we have determined three crystal structures of PL-C, PL-D1 and PL-D2 at 1.8, 1.6 and 1.5 Å resolutions, respectively. Each hevein-like domain of PL-Ds lacks a distinct secondary structure but has the tertiary structures maintained by four disulfide bonds. The corresponding domain structures are same between the PL-Ds, although both overall structures are different from each other because of the flexibility of the short linker. Two additional residues in PL-D1 were missing because this region protrudes from the molecular surface. The flexibility of this region is expected to disturb the interaction with a target molecule, which is the reason that PL-D1 has no mitogenic activity. Each subunit of PL-C consists of three hevein-like domains and is related to the other by the non-crystallographic 2-fold axis. The backbone structures of domains forming these three lectins are quite similar, indicating the gene-duplication during their evolution.

Keywords: LECTIN POKEWEED CRYSTAL STRUCTURE