at different temperatures which gives information on the biological relevance of the protein structures solved at cryogenic conditions.

Keywords: serine protease, reaction mechanisms, high-resolution protein structures

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Electrochemically assisted protein crystallization. Applications to protein crystallography

Abel Moreno[1], Jean Jakoncic[2], Bernardo A. Frontana-Uribe[1], Vivian Stojanoff[2]
[1]Universidad Nacional Autonoma de Mexico (UNAM), Institute of Chemistry, Circuito Exterior, C.U., Mexico, D.F., 04510, Mexico, [2]Brookhaven National Laboratory, NSLS Upton New York 11973-5000 USA., E-mail：abel.moreno@mac.com

The electrochemically-assisted crystallization of non-purified (commercial purity) cytochrome c was successfully achieved inside of small cell of a dynamic light scattering (DLS) apparatus. The method combined batch crystallization conditions and an internal electrical field to favor the nucleation stage. This methodology crystallizes commercial cytochrome c without previous isoforms separation, decreasing costs and experimental time to obtain crystals. The effect of the electric field on the aggregation time and on the protein nucleation was observed in real time by means of dynamic light scattering methods. The results showed a marked decrease of the crystallization time (from 45 days to 5 days) highly improving the previous reported method of crystallization. The HPLC signal of re-dissolved crystals of these protein crystals showed that the protein corresponds to the same isoform previously crystallized by micro-seeding methods. The excellent crystal quality of the cytochrome c crystals obtained in the presence of electrical current was confirmed by protein X-ray crystallography reaching 1 angstrom of resolution.

Keywords: cytochrome c, protein electrocrystallization, high-resolution protein crystallography

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Invariom refinement of 5 K 0.66 Å data of the ethanol solvate of gramicidin A

Birger Dittrich[1], Alke Meents[2]
[1]Georg August Universitaet Goettingen, Institut fuer Anorgansiche Chemie, Tammannstr. 4, Goettingen, Niedersachsen, 37077, Germany, [2]HASYLAB at DESY, Notkestr. 85, D-22603 Hamburg, Germany, E-mail：bdittri@gwdg.de

It is now 20 years since the first structure of Gramicidin D has been reported [1]. Wild type Gramicidin D from bacillus brevis is a mixture of three peptides (Gramicidin A, B and C) each consisting of 15 residues that differ only at position 1 and 11. Gramicidin exhibits antibiotic activity against Gram-positive species by forming ion channels through cell membranes that preferably transport Na^+/K^+. Various solvate structures and ion-complexes of Gramicidin are known to date, as summarized recently [2]. We have re-examined the original ethanol solvate to illustrate the benefits of ultra-high resolution in protein crystallography. To minimize rotational disorder and to maximize resolution we collected data on purified Gramicidin A at a temperature of 5 K at the 3rd generation synchrotron SLS in Switzerland. The resulting Bragg data to 0.66 Angstrom resolution were evaluated with the non-spherical scattering factors of the invariom database [3,4] which is based on the Hansen-Coppens variation of the rigid pseudoatom formalism. Single crystal diffraction data evaluated this way provide a wealth of detailed and accurate information on structure, geometry and electron-density derived properties. For Gramicidin and its biological function an analysis of the electrostatic potential [5] is especially relevant in this respect.

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Keywords: charge density, X-ray structure of membrane proteins, synchrotron radiation

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Ultra-high resolution and very cold structure of lysozyme

Zbigniew Dauter[1], Elzbieta Nowak[1], Anna Brzuszkiewicz[1], Miroslawa Dauter[2], Stephen Ginell[3]
[1]National Cancer Institute, Argonne Natl. Lab., Bldg. 202, Rm. Q142, 9700 S. Cass Ave., Argonne, IL, 60439, USA, [2]SAIC-Frederick Inc., Argonne National Laboratory, Argonne, USA, [3]Structural Biology Center, Biosciences Division, Argonne National Laboratory, Argonne, USA, E-mail：dauter@anl.gov

Extension of data resolution from high to atomic level reveals a wealth of information about the intricate details of protein structures, important for discussing their chemical and biological behavior or their potential to interact with other molecules. The triclinic form of hen egg-white lysozyme is an example of protein crystals able to diffract beyond the ultra-high resolution limit of 0.8 Å. The ultra-high resolution data measured from these crystals at extremely low temperature of 16 K allowed us to obtain a very accurate model of the molecule. About half of the whole structure displays multiple conformations of the main and side chains. Electron densities for hydrogen atoms and bonding electrons are apparent in many fragments as well as strong indications about protonation states of potentially charged groups. Several discrepancies from the library of geometrical parameters are suggestive for reevaluation of some of such library targets. The structural model will be compared in detail with several available structures of lysozyme obtained with different data resolution limits and temperatures.

Keywords: lysozyme, ultra-high resolution, ultra-low temperature

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Crystal structure of fully oxidized human thioredoxin1 containing disulfide between Cys62 and Cys69

Jung Won Hwang[1,2], Jie-Oh Lee[2], Tae-Kwang Oh[1], Myung Hee Kim[1]
[1]Korea Research Institute of Bioscience and Biotechnology (KRIBB), Systems Microbiology Research Center, KRIBB, 52 Eoeun-Dong,

Yusung-gu, Daejeon, Daejeon, 305-806, Korea (S), [2]Department of Chemistry, KAIST, Daejeon, Korea 305-701, E-mail：jwhwang@kribb.re.kr

Human thioredoxin1 (hTRX1) is a small 12-kD oxidoreductase enzyme consisting of 105 amino acids and containing a dithiol/disulfide active site with multiple cellular functions. This enzyme has activity as a cellular reductase by a dithiol-disulfide exchange reaction using two cysteine residues (Cys32 and Cys35) in the conserved active site sequence. Apart from the two cysteines, there are three additional conserved cysteines, Cys62, Cys69, and Cys73 in the mammalian TRX, which have not been known to their biological functions. Although it has been identified that the Cys73 residue is involved in dimerization of hTRX via an intermolecular disulfide bond formation between Cys73 of each monomer in the oxidized state, biological function of the Cys62 and Cys69 residues in the non-active remain to be fully elucidated. In the previous paper, researchers proposed that the formation of a disulfide bond between Cys62 and Cys69 could give a way to transiently inhibit hTRX activity for redox signaling or oxidative stress. Furthermore, they proposed a model structure of the non-active site disulfide in the hTRX. Here, we present the high-resolution crystal structure of fully oxidized hTRX1, which shows an intramolecular disulfide bond between Cys62 and Cys69. The disulfide bond formation disengages a helix proximal to the active site and results in a conformational change of the hTRX enzyme, providing a structural basis for understanding the regulation mechanism of redox signaling or oxidative stress.

Keywords: human thioredoxin1, intramolecular disulfide, redox signaling

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Joint neutron and X-ray diffraction studies at 293 K of antifreeze protein

Andre Mitschler[1], Mathew Blakeley[2], Michael Haertlein[2], Christophe Mueller-Dickmann[3], Alexandre Popov[3], Eduardo Howard[4], Isabelle Haertlein[2], Alberto Podjarny[1]
[1]IGBMC, 1 rue Laurent Fries, Illkirch, CUS Strasbourg, 67404, France, [2]ILL, 6 rue Jules Horowitz, Grenoble, France, [3]ESRF, 6 rue Jules Horowitz, Grenoble, France, [4]IFLYSIB, 59 N 789, La Plata, Argentina, E-mail：mitschler@gmail.com

Type III Antifreeze Proteins (AFPs) are small globular monomeric proteins (66 aa, M.W.=7kDa), which are highly homologous. Their shared antifreeze property is linked to a network of hydrogen bonding between a specific lattice plane on ice and several conserved, polar and solvent accessible amino acids located along a flat Ice-Binding Surface (IBS). We shall present our developments: 1) - Neutron Laue data collection on the new LADI III (ILL) on an ab-initio fully deuterated tiny crystal (volume = 0.12 mm^3, resolution = 2 Å), including production of fully deuterated protein, crystallization by macro-seeding in D_2O. The ratio resolution/volume is similar to the Human Aldose Reductase [1]. 2) - X-ray diffraction at Synchrotron ESRF beamline ID29 on a fully deuterated crystal of the same crystallization batch at a resolution of 1.05 Å, necessary to carry out a joint Neutron - X-ray refinement like for Human Aldose Reductase h AR [2]. 3) Specific H labelling on Leucine and Isoleucine of the fully deuterated protein, in order to create a contrast useful for specific phasing methods for neutron diffraction data. (Human Frontier Science Program).

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Keywords: neutron diffraction, antifreeze protein, perdeuteraton

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X-ray induced perturbation in an ultra-high resolution protein structure

Kazuki Takeda, Kouji Kusumoto, Yu Hirano, Kunio Miki
Kyoto University, Graduate School of Science, Department of Chemistry, Kitashirakawa-oiwake-cho, Sakyo-ku, Kyoto, 606-8502, Japan, E-mail：ktakeda@kuchem.kyoto-u.ac.jp

Positions of hydrogen atoms and orientations of water molecules are important to functions of proteins. However, such information from protein crystals is easily disturbed by radiation damage. The damage can not be prevented completely even in the data collection at cryogenic temperatures. Therefore, influence of X-rays should be estimated exactly in order to bring out meaningful information from crystallographic results. Diffraction data from a single crystal of the high-potential iron-sulfur protein (HiPIP) from *Thermochromatium tepidum* were collected at SPring-8, and were merged into three data sets as exposure to X-rays. The maximum absorption doses were estimated to be 4.5×10^5, 9.0×10^5 and 1.4×10^6 Gy for the three data sets. Structures analyzed at 0.70 Å show detailed views of X-ray induced perturbation such as positional changes of hydrogen atoms of a water molecule. We will discuss about initial steps of radiation damage from the ultra-high resolution analysis.

Keywords: electron transfer, high-resolution protein structures, radiation damage

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High resolution crystals of human hematopoietic & lipocalin-type prostaglandin D synthases in space

Hiroaki Tanaka[1], Koji Inaka[2], Toshiharu Tsurumura[3], Kosuke Aritake[3], Masaru Sato[4], Sachiko Takahashi[1], Mari Yamanaka[1], Naoki Furubayashi[2], Erika Hirota[1], Satoshi Sano[4], Tomoyuki Kobayashi[4], Tetsuo Tanaka[4], Yoshihiro Urade[3]
[1]Confocal Science Inc., Wakamatsu Building 7F, 3-3-6 Nihonbashi Hon-cho, Chuo-ku, Tokyo, 103-0023, Japan, [2]Maruwa Foods and Biosciences, 170 Tsutsui-cho, Yamatokoriyama, Nara, 639-1123, Japan, [3]Osaka Bioscience Institute, 6-2-4, Furuedai, Suita-shi, Osaka, 565-0874, Japan, [4]Japan Aerospace Exploration Agency, 2-1-1 Sengen, Tsukuba, Ibaraki, 305-8505 Japan, E-mail：tanakah@confsci.co.jp

Hematopoietic and lipocalin-type prostaglandin (PG) D synthases (H-PGDS and L-PGDS) are responsible for production of PGD2, which acts as an allergic inflammatory mediator[1] and an endogenous sleep-promoting substance[2], respectively. The specific inhibitors of each enzyme are important for suppression of various diseases. To obtain high-quality crystals for structural analysis, we crystallized both H-PGDS and L-PGDS by using a counter-diffusion method under a microgravity environment on the International Space Station (ISS). The three-dimensional structure of human H-PGDS has already been determined in a complex with an H-PGDS inhibitor HQL-79 (PDB ID: 2CVD)[3]. Since novel H-PGDS inhibitors with affinities 100-fold higher than HQL-79 have recently been developed, we obtained high quality crystals of human H-PGDS in complexes with those novel inhibitors by using PEG 6000 as a precipitant in microgravity. The crystals showed diffraction up to 1.2