methylated proteins tend to diffract to higher resolution and show lower isotropic temperature factors. A number of well-ordered methylated lysines have been identified. Some lysine residues remain unmethylated or monomethylated although excess of reagents was used. These methylated residues make both inter- and intra-molecular contacts. We describe a detailed protocol, results, success rates and specific interactions in protein crystals that contribute to improved crystallization properties of some proteins. This work was supported by National Institutes of Health Grant GM074942 and by the U.S. Department of Energy, Office of Biological and Environmental Research, under contract DE-AC02-06CH11357.

Keywords: reductive methylation, crystal packing, isotropic temperature factor

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Many are called but few are chosen: 20 years of crystallizing HIV-1 reverse transcriptase

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Our research efforts focus on crystallographic studies of HIV-1 reverse transcriptase (RT), a multifunctional enzyme of the AIDS virus and the target of many of the most widely used anti-AIDS drugs. Over the past 20 years, we have crystallized and solved a diverse array of three-dimensional structures representing distinct conformational states of HIV-1 RT and clinically relevant drug-resistant mutants; these include HIV-1 RT in complexes with inhibitors, nucleic acid substrates, and a monoclonal antibody Fab fragment [1]. Our desire to improve both crystal quality and diffraction resolution led to improvements in purification methods and successful crystal engineering studies by site-directed mutagenesis, leading to 1.8 angstrom resolution diffraction for several crystal forms [2]. Recently, a longstanding collaboration between our lab and Janssen Pharmaceutica/Tibotec, Belgium, culminated in FDA approval of the potent non-nucleoside RT inhibitor etravirine/Intelence/TMC125 for treatment of HIV-1 infections resistant to other antiretroviral agents. Another related non-nucleoside RT inhibitor, TMC278, is more effective against drug-resistant HIV-1 variants than any other compound reported to date, and is currently in Phase III clinical trials [3]. Various methodologies used by our laboratory to produce diffraction-quality crystals of a number of RT complexes are described.
[1] Clark A.D., et al, Methods Enzymol, 1995, 262, 171-85, [2] Bauman J.D., et al, 2008, submitted, [3] Das K., et al, PNAS, 2008, 105, 1466-71.

Keywords: HIV-1 reverse transcriptase, protein purification crystallization, HIV drug design

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Studies on enzymes belonging to the crotonase superfamily

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The crystal structure of delta3-delta2-enoyl-CoA isomerase from human mitochondria (hmEci), complexed with the substrate analogue octanoyl-CoA, has been refined at 1.3 Å resolution. This enzyme takes part in the β-oxidation of unsaturated fatty acids by converting both cis-3 and trans-3-enoyl-CoA esters (with variable length of the acyl group) to trans-2-enoyl-CoA. hmEci belongs to the hydratase/isomerase (crotonase) superfamily. Most of the enzymes belonging to this superfamily are hexamers, but hmEci is shown to be a trimer. The mode of binding of the ligand, octanoyl-CoA, shows that the ω-end of the acyl group binds in a hydrophobic tunnel formed by residues of the loop preceding helix H4 as well as by side chains of the kinked helix H9. From the structure of the complex it can be seen that Glu136 is the only catalytic residue. A cavity analysis shows the presence of two large, adjacent empty hydrophobic cavities near the active site, which are shaped by side chains of helices H1, H2, H3 and H4. The structure comparison of hmEci with structures of other superfamily members, in particular of rat mitochondrial hydratase (crotonase) and yeast peroxisomal enoyl-CoA isomerase highlights the variable mode of binding of the fatty acid moiety in this superfamily.

Keywords: oxyanion hole, coenzyme A, isomerase

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Structure analysis of ligand-independent activation of Fushi tarazu factor-1 ligand binding domain

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Drosophila melanogaster Fushi tarazu factor 1 (Ftz-F1) is an orphan nuclear receptor of which ligand has not been identified until now. The Ftz-F1 regulate gene expression for development, reproduction and cholesterol homeostasis. Also, It is known that the Ftz-F1 interacts with segmentation gene 'Fushi tarazu' (Ftz) for activation of the Ftz-F1. The Ftz-F1 is divided two parts, DNA-binding domain (DBD) and ligand-binding domain(LBD). It is known which ligand binding domain of Ftz-F1 is crucial part to regulate gene expression. Here we report the crystal structure analysis of the Ftz-F1 LBD bound to the peptide containing LXXLL co-activator motif of Ftz. The Ftz-F1 LBD structure consists of eleven helix and two beta strand which form a fourth-layer alpha-helical sandwich. Compared to the structures of Liver receptor holmologue-1 and Steroidogenic factor-1 of the same subfamily of nuclear receptor, the Ftz-F1 LBD does not have enough space for ligand-binding which explains in structural points why the ligand for Ftz-F1 have not been found even though extensive efforts searching for it. Interestingly Ftz-F1 has the AF-2 in the active conformation without ligand binding. These suggest that Ftz-F1 is a constitutively active nuclear receptor which does not need ligand implying the another regulation mechanism of the Ftz-F1

different from that of other nuclear receptors.

Keywords: nuclear receptor, Fushi tarazu factor 1, FTZ-F1

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X-ray diffuse scattering from protein crystals caused by the lattice defects

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High resolution X-ray protein crystallography needs a single crystal of high quality. The quality has been often described with a mosaicity. However, the intrinsic nature of the quality of protein crystal has not yet been understood well. Phenomenologically speaking, a crystal of poor quality causes the decrease of the Bragg reflection intensity and does not give higher order Bragg reflections. It has been developed to estimate the quality of proteins by measuring the B-factor.1) The B-factor consists of static and dynamic components and the quality of protein crystals may correspond to the orientation disorder of molecules in the crystal. Therefore the disorder structure will be determined by analyzing X-ray diffuse scattering on the foot of the Bragg reflections. We have carried out the measurement of the X-ray diffuse scattering from a cubic insulin crystal which has given medium resolution data (2.2Å). The size of the sample is about $0.3\text{mm} \times 0.3\text{mm} \times 0.3\text{mm}$. We have used 4-circle diffractometer installed at BL10A in Photon Factory in KEK, Japan. The beam divergence is 1.23×10^{-6}[rad]. We have measured several rocking curves of Bragg reflections of [100], [110] and [111] series at the ambient temperature and succeeded in observing the diffuse scattering on the foot of these Bragg reflections. In order to make the origin of the diffuse scattering clear, we are planning to measure several crystals which have grown under different crystallization condition and have different qualities.

1) S.Arai, T.Chatake, N.Suzuki, H.Mizuno and N.Niimura: Acta Cryst. D60, 1032-1039 (2004)

Keywords: static and dynamic disorder, quality of protein crystals, diffuse scattering

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Structure of membrane-bound quiohemoprotein alcohol dehydrogenase

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Many Gram-negative aerobic bacteria can grow on alcohols and sugars as the sole carbon and energy sources. In the periplasm of acetic acid bacteria, quinoprotein alcohol dehydrogenases (ADH) containing pyrroloquinoline quinine (PQQ) instead of nicotinamide or compounds as the prosthetic group catalyze the first step of acetic acid production, oxidation of ethanol to acetaldehyde. There are three types of ADHs. Type I ADH is a soluble, dimeric protein of identical subunits having a PQQ and a calcium ion in each active center, but no other redox cofactors. Type II ADH is a soluble, monomeric, having a PQQ-containing catalytic domain and an additional *c* domain with a covalently bound heme *c*. Type III ADH is a quinohemoprotein complex with three nonidentical subunits that catalyzes the oxidation of ethanol and the subsequent reduction of ubiquinone, and attached on the cytoplasmic membrane of acetic acid bacteria. We report here 3.0 Å crystal structure of the type III membrane-bound quinohemoprotein ADH from *Gluconobacter suboxydans* refined to *R*-factor 29 %. Our structure reveals that the enzyme contains a large subunit A similar to the type II quinoprotein ADHs which have a eight-stranded propeller domain and a cytochrome *c* domain, a membrane-bound subunit B which has a novel three-heme cytochrome *c* structure, and a small subunit C which has unknown function. The PQQ is located near the axis of the propeller domain about 14 Å from the in subunit A. The shortest distances between four hemes are about 9 Å, 4 Å, and 8 Å, respectively.

Keywords: crystal structure analysis, membrane protein structures, heme proteins

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Rational crystallization of β-lactoglobulin and vitamin D_3 complex reveal a secondary binding site

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β-lactoglobulin (β-LG) is a major bovine milk protein with a predominantly β structure. The function of the only α-helix with three turns at the C-terminus is unknown. Vitamin D binds to the central calyx formed by the β-strands. Despite being one of the most investigated proteins whether there are two vitamin D binding-sites in each β-LG molecule has been a subject of controversy during the past forty years. In this study, we chose vitamin D3, instead of vitamin D2, and use rational approach to successfully form a β-LG-vitamin D3 complex for crystallization. The only difference of vitamin D3 from D2 is the latter being a double bond between the carbon positions 22 and 23. Vitamin D3 is well-fitted into the bulk of electron density at 2.4 Å-resolution around the calyx and the exosite. In the central calyx binding mode, the aliphatic tail of vitamin D3 clearly inserts into the binding cavity, where the 3-OH group of vitamin D3 binds externally. The electron density map suggests that the 3-OH group interacts with the carbonyl of Lys-60 forming a hydrogen bond. The second binding site, however, is near the surface at the C-terminus containing part of an α-helix and a β-strand I with 17.91 Å in length, while the span of vitamin D3 is about 12.51 Å. A remarkable feature of the second exosite is that it combines an amphipathic α-helix providing non-polar residues and a β-strand providing a non-polar and a buried polar residue. They are linked by a hydrophobic loop. Thus, the binding pocket furnishes strong hydrophobic force to stabilize vitamin D3 binding. This finding provides a new insight into the interaction between vitamin D3 and β-LG, in which the exosite may provide