FA1-MS14-O1

Elucidation of the Phosphate Binding Mode of Ding Proteins Revealed by Sub-Ångstrom X-Ray Crystallography. Dorothee Liebschner[a], Mikael Elias[b], Sebastien Moniot[a], Bertrand Fournier[a], Ken Scott[c], Benoit Guillot[a], Claude Lecomte[a], Eric Chabriere[b]. [a]*Nancy Universite, CRM2, Nancy, France.* [b]*CNRS-Université de la Mediterranee, AFMB, Marseille, France.* [c]*Auckland University, Auckland, New Zealand.*
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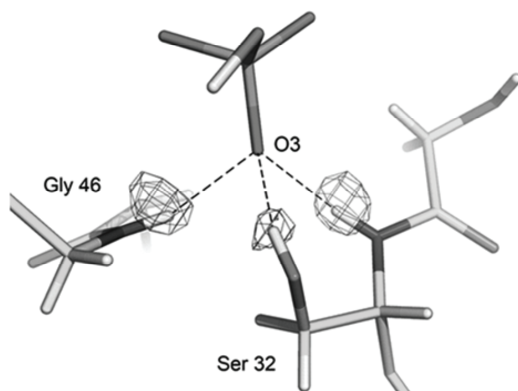
PfluDING is a bacterial protein isolated from *Pseudomonas fluorescens* that belongs to the DING protein family, which is ubiquitous in eukaryotes and extends to prokaryotes. DING proteins and PfluDING have very similar topologies to phosphate Solute Binding Proteins (SBP). The three-dimensional structure of PfluDING was obtained at sub-Ångstrom resolution (0.88Å and 0.98Å) at two different pH (4.5 and 8.5), allowing us to discuss the hydrogen bond network that sequesters the phosphate ion in the binding site. From this high resolution data, we experimentally elucidated the molecular basis of phosphate binding in phosphate SBPs. In particular, contrary to previous theories on phosphate SBPs, accurate electrostatic potential calculations shows that the binding cleft is positively charged. PfluDING structures reveal that only dibasic phosphate binds to the protein both at acidic and basic phosphate, suggesting that the protein binding site environment stabilizes the $HPO4^{2-}$ form of phosphate.



Phosphate binding mode in phosphate high affinity proteins

Keywords: sub-Ångstrom X-ray crystallography; phosphate binding mechanism; electrostatic potential calculation

FA1-MS14-O2

Ultra-High Resolution Protein Structures-Himalayas of Protein Crystallography. Zbigniew Dauter[a], Miroslawa Dauter[b]. [a]*Macromolecular Crystallography Laboratory, NCI, ANL, Argonne, IL, USA.* [b]*Basic Research Program SAIC Frederick, ANL, Argonne, IL, USA.*
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Whereas the average resolution among almost 50,000 macromolecular X-ray crystal structures in the PDB is about 2.0 Å, some protein crystals are able to diffract to a resolution higher than 0.8 Å, which is the usual limit of Cu-radiation applied to crystals of small organic compounds. Such ultra-high resolution analyses provide a wealth of information about details of stereochemical and electronic structures. The knowledge of such details helps to form the libraries of accurate geometrical targets but also may suggest which exceptions from the standard protein stereochemistry are possible. The intimate knowledge of structural and electronic details is important for understanding of (bio) chemical reactions and interactions among macromolecules and for faithful modeling of their complexes with various ligands. Similarly as the Himalayan mountains higher than 8,000 metres, the crystal structures at resolutions higher than 0.8 Å are there to be conquered – both provide very far-reaching perspectives.

Keywords: ultra-high resolution; stereochemistry; accuracy

FA1-MS14-O3

Protonation State Determination in Proteins Using High Resolution Protein X-Ray Crystallography: Effects of Resolution and Completeness. Stuart J. Fisher[a,b], Matthew Blakeley[a], Sean McSweeney[c], John R. Helliwell[b]. [a]*Institut Laue Langevin, 6 rue Jules Horowitz, Grenoble, 38042, France.* [b]*The University of Manchester, Department of Chemistry, Brunswick Street, Manchester, M13 9PL.* [c]*Macromolecular Crystallography Group, European Synchrotron Radiation Facility, 6 rue Jules Horowitz, 38043, Grenoble, France.*
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The advancement of synchrotron X-ray facilities, and the consequent increase in flux currently available to users at such facilities, in combination with advancements in protein crystallisation, means it is becoming increasingly possible to collect protein crystallographic data sets at, or within range of, atomic resolution. These data sets are often highly complete with large multiplicities making them excellent candidates for protonation state determination of amino acid groups using bond length analysis [1, 2]. By carefully considering the differences in bond lengths between, for example a C=O and C-OH bond, and the associated standard uncertainties, it is possible to determine whether these residues are in fact protonated to within a statistically significant level [1]. Here we consider the protonation state determinations in a muscle protein; myosin binding protein c [2] (MyBP-C) at 1.3Å (PDB Code 3CX2, 82% complete), trypsin [3] at 1.2Å (97% complete), and lysozyme [4] at 0.65Å (PDB code 2VB1, 98% complete). The estimated coordinate errors obtained using the Cruickshank DPI [5] (Diffraction component Precision Index), and those determined from the SHELX full matrix inversion have been determined and compared in order to gauge the effect of both resolution and completeness on those values. We find

that an increase in resolution by 0.1Å and in completeness by 16% yields a twofold improvement of the estimated coordinate errors (in the case of MyBP-C versus trypsin; 0.0625Å vs. 0.0325Å respectively), obviously making the assignment of protonation states much more significant. Although refinement of data at such resolution may seem somewhat detailed and complex; our results and procedures will be described.

[1] Ahmed, H.U., et al., *Acta Crystallographica Section D*, **2007**. 63: p. 906-922 [2] Fisher, S.J., et al., *Acta Crystallographica Section D*, **2008**. 64(6): p. 658-664. [3] McSweeney, S., Internal Data, Unpublished, **2009**. [4] Wang, J., et al., *Acta Crystallographic Section D*, **2007**. 63(12): p. 1254-1268.

Keywords: protein crystallography; enzymatic reaction mechanisms

FA1-MS14-O4

X-Ray and Neutron Structure Analyses of Proton Transfer Catalysis by EndoPG I. Mamoru Sato[a], Tetsuya Shimizu[b], Toru Nakatsu[b], Kazuo Miyairi[c], Toshikatsu Okuno[c], Hiroaki Kato[b]. [a]*Yokohama City University, Japan,* [b]*Kyoto University, Japan,* [c]*Hirosaki University, Japan.*
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Stereum purpureum is a pathogenic fungus that causes silver-leaf disease in apple trees, and it secretes large amounts of several endopolygalacturonases (endoPGs, EC 3.2.1.15) into the culture medium. Endopolygalacturonases catalyze the hydrolysis of the α-1,4-glycosidic linkages between adjacent α-D-galacturonic acid residues within the pectin main chain. Endopolygalacturonase I (endoPG I) is one of these endopolygalacturonases and consists of 335 amino acid residues. We have determined the respective X-ray crystal structures at resolutions of 0.96, 1.00 and 1.15 Å of endoPG I and its binary and ternary complexes with GalpA pyranose isomer and (GalpA + GalfA) at pH 5.0, and proposed three residues important for the catalysis and accounted for general acid-base catalysis of the enzyme. GalpA and GalfA are pyranose and furanose isomers as the reaction products, respectively. We also collected 0.96 Å resolution X-ray data of endoPG I in complex with GalpA at pH 2.5 and propose that Asp173 and Asp153 are the general acid and base, respectively. Furthermore, we observed a short and strong hydrogen bond forming between Asp153 and Asp156, where the proton between Asp153 and Asp156 is positioned at the center of the hydrogen bond at pH 5.0, but the proton is directly bound to Asp156 at pH 2.5. We therefore concluded that this is the low barrier hydrogen bond, which occurs when the pK values of the atoms sharing the proton are similar. It was, however, impossible to observe significant electron density corresponding to the carboxyl hydrogen (proton) of the catalytic acid residue (Asp173) at both pH 5.0 and 2.5. The Asp173 should be protonated for the catalysis at these two pHs. In order to solve this problem, we collected X-ray and neutron diffraction data of endoPG I up to resolutions of 0.68 Å and 1.5 Å on BL41-XU at SPring-8 and on BIX4 at JRR-3M reactor in Japan Atomic Energy Agency (JAEA), respectively. For the neutron diffraction experiment, we prepared large single crystals suitable for high resolution neutron crystallographic analysis with a hanging-drop vapor diffusion method, followed by macro-seeding in a sitting-drop (drop: 0.5 ml, reservoir: 4 ml) vapor diffusion. Typical size of the crystals grown was 3.0 x 1.9 x 0.8 mm, giving an approximate volume of 4.6 mm^3. The crystals were soaked for 40 days in a reservoir solution containing 25% PEG-4000, which is prepared with D_2O (pD 5.0), and then subjected to the neutron experiment on the BIX4. The ultra-high resolution structure of endoPG I was refined with SHELXL. On the other hand, the initial neutron structure obtained from the X-ray analysis at 1.0 Å resolution was refined with CNS, and manual modification made with O. After rigid body refinement, the neutron structure was further refined by simulated annealing and energy minimization. In this session we compare the X-ray and neutron structures and discuss on the catalytic mechanism of the enzyme in terms of the proton transfer at the general acid-base catalysis.

Keywords: crystal structure analysis; enzyme catalytic reaction mechanism; neutron X-ray diffraction

FA1-MS14-O5

Preliminary Deformation-Density Study of Cyanobacterial Cytochrome C_6. Maciej Kubicki[a], Szymon Krzywda[a], Mariusz Jaskólski[a], Wojciech Białek[b], Andrzej Szczepaniak[b], Zbyszek Dauter[c]. [a]*Faculty of Chemistry, Adam Mickiewicz University, Poznań, Poland.* [b]*Faculty of Biotechnology, University of Wrocław, Poland.* [c]*Macromolecular Crystallography Laboratory, NCI, Frederick, USA.*
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Cytochrome c_6 from mesophilic cyanobacterium *Synechococcus* sp. PCC 7002, containing an unusually high amount of alanines (25.8%) and glycines (9.7%), has well defined structure [1]. Crystals belonging to $P2_1$ space group diffract X-rays to subatomic resolution. All this make cytochrome c_6 an ideal metallo-protein object to study deformation density.
Protein was expressed and purified as described elsewhere [2]. We collected 0.75 Å data on a reduced cytochrome c_6 crystal with unit cell dimensions a = 31.81 Å, b = 27.87 Å, c = 43.85 Å, β = 101.03º and one molecule per asymmetric unit. The mean overall redundancy is 3.8 and is 3.5 in the highest resolution shell (0.78-0.75 Å). The data are 99.9% (99.9%) complete with an overall R_{merge} of 0.058 (0.537), the mean $I/\sigma(I)$ value is 20.3 (2.3). The picture below shows a haem molecule initially phased to 1.2 Å resolution.