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.

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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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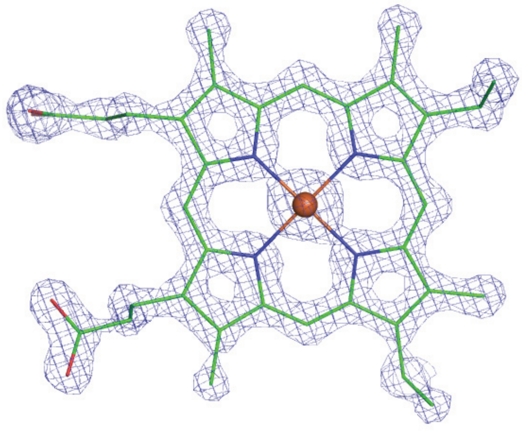
Cytochrome c_6 from mesophilic cyanobacterium *Synechococcus* sp. PCC 7002, containing an unusually high amount of alanines (25.8%) and glycines (9.7%), has well defined structure [1]. Crystals belonging to $P2_1$ space group diffract X-rays to subatomic resolution. All this make cytochrome c_6 an ideal 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 = 101.03° 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.



In the communication we will present the experimental details, anisotropically refined structure and preliminary deformation density maps.

[1] Białek W., Krzywda., Jaskólski M., Szczepaniak A., **2009**, to be published. [2] Białek W., Nelson M., Tamiola K., Kallas T., Szczepaniak A., Biochemistry, **2008**, 47, 5515.

Keywords: deformation-density;cytochrome c_6; metallo-proteins