m01.p01

Refinement of Taka-amylase crystallized in microgravity environment at 1.0Å resolution

Akifumi Higashiura[a], Hiroaki Tanaka[b], Koji Inaka[d], Masaru Sato[c], Shigeru Sugiyama[d], Sachiko Takahashi[b], Mari Yamanaka[b], Moritoshi Motohara[c], Satoshi Sano[c], Tomoyuki Kobayashi[c], Mamoru Suzuki[a], Tetsuo Tanaka[c], Atsushi Nakagawa[a]

[a]Institute for Protein Research, Osaka University, Osaka, 565-0871 JAPAN, [b]Japan Space Forum, Tokyo, 100-0004 JAPAN, [c]Japan Aerospace Exploration Agency, Ibaraki, 305-8505 JAPAN, [d]Maruwa Food Industries, Inc., Nara, 639-1123 JAPAN.

Keywords: microgravity crystallization, high-resolution crystal structures, Taka-amylase

Recently, high brilliance and highly focused synchrotron beam lines, X-ray data collection at low temperature and technical advances in crystallographic analysis have significantly improved the resolution of X-ray crystallography. Crystallization in microgravity environment is one of the potential techniques for improving quality of protein crystals. In this study Taka-amylase, a member of alpha-amylase family derived from *Aspergillus oryzae*, was used as a model protein for high resolution X-ray crystal structure analysis. Space-grown crystals were obtained in the JAXA-GCF (Japan Aerospace Exploration Agency - Granada Crystallization Facility) project [1]. Data collections was performed using synchrotron radiation from SPring-8 beamline BL44XU, equipped with a DIP6040 image plate detector (MAC Science / Bruker-AXS) at 90K under nitrogen vapour stream. Two data sets were collected for high- and low-resolution data to obtain wide dynamic range diffraction data. The diffraction data were observed up to 0.94Å resolution. The data were integrated, scaled and merged using the *DENZO* and *SCALEPACK* programs. The crystals belong to space group $P2_12_12_1$, with the following cell dimensions: a=50.4Å, b=67.4Å, c=130.5Å. The overall R_{merge} based on intensities for all data was 7.7% with its completeness of 97.6%. The starting model for the refinement was the coordinate set 6TAA from the Protein Data Bank [2]. Refinement was carried out by *SHELX-93.* The last stage of refinement against data to 1.0Å converged to an R factor of 13.1% and a free R of 16.1%. The final model of taka-amylase consists of 4210 non-hydrogen atoms, 3148 hydrogen atoms and 671 water molecules. 13 side chains were modelled in two conformations. B factors were 11.7, 13.7 and 23.6Å^2 for main-chain, side-chain and water molecules. This high-resolution structure was providing us more reliable geometric and conformational properties of the protein.

[1] H. Tanaka, K. Inaka, S. Sugiyama, S. Takahashi, S. Sano, M. Sato and S. Yoshitomi, J. Synchrotron Rad, 2004, 11, 45-48.
[2] HJ. Swift, L. Brady, ZS. Derewenda, E.J. Dodson, GG. Dodson, JP. Turkenburg, AJ. Wilkinson, Acta Cryst. 1991, B47, 535-44.

m01.p02

Trapping and mobility of soluble and insoluble impurities in ice monitored via Cryo-Synchrotron-Tomography

Markus M. Miedaner[1], T. Huthwelker[2], F. Enzmann[1], M. Kersten[1], M. Amman[2], A. Groso[3], M. Stampanoni[3]

*[1]Environmental Geochemistry, Johannes Gutenberg University, Mainz, Germany. [2]Radio and Environmental Chemistry, Paul Scherrer Institute, Villgen Switzerland. [3]Swiss Light Source, Paul Scherrer Institute, Villigen, Switzerland. * miedaner@uni-mainz.de*

Keywords: synchrotron micro-tomography, ice, impurities

Synchrotron micro tomography is an ideally suited tool to study non-destructively micrometer-sized impurity inclusions in ice. To perform cryo-tomography the standard setup of the SLS-XS04 Beam line was modified by a cryojet and a double walled Kapton cage, which provided cooling for the ice samples embedded in cyclohpetane and mounted in a small polyamide cup. A spatial resolution of 1.4 μm was obtained with this setup. Water soluble as well as insoluble impurities were studied due to their importance in atmospheric chemistry and physics [1,2]. Thereby a strong dependence of the size of the trapped inclusions on the temperature to which the samples were exposed to for freezing, was found. For samples frozen between 200 and 260 K, the pore volume occupied by either brine or air increased with decreasing exposure temperature. Contradicting behaviour was found for temperatures between 270 and 260 K. Here the voids decrease with temperature. These results can be modelled based on a 1-dimensional 'Freezing-Temperature-Approximation' approach [3]
to describe the accumulation of impurities at the front of the growing ice. The nucleation of inclusions (brine or air) at the growing ice front is modelled by a material dependent nucleation rate.
For the first time we also visualized the transport of impurities along grain boundaries by performing several scans of one sample and varying the experimental temperature. Therefore, scans were performed below the eutectic as well as above the eutectic point of the system. Using a sequence of these points a sincere growth of the large trapped inclusions by merging with smaller ones became evident.
Water insoluble impurities and mixtures of soluble and insoluble impurities were scanned. Thereby strong morphological changes were found depending on the chemical composition of the samples.

[1] Seinfeld J, Pandis S. (1997) Atmospheric Chemistry and Physics, Wiley Interscience.
[2] McElroy C, McLinden C, McConnell J. (1999) Evidence for bromine monoxide in the free troposphere during the arctic polar sunrise, Nature 397:338-341.
[3] Davis S H, Freund L B, Leibovich S, Tvergaard V. (2001) Theory of solidification, Cambridge University Press.