L18

PROTEIN-CARBOHYDRATE INTERACTION: STRUCTURAL AND THERMODYNAMIC CHARACTERISATION OF HIGH AFFNITY BINDING BETWEEN LECTINS FROM PATHOGENS AND HOST CARBOHYDRATES

M. Wimmerová[1,2], M. Pokorná[1], C. Sabin[3], S. Perret[3], J. Adam[1], E.P. Mitchell[4], A. Imberty[3]

[1]National Centre for Biomolecular Research and [2]Department of Biochemistry, Faculty of Science, Masaryk University, Kotlarska 2, 611 37 Brno, Czech Republic
[3]CERMAV-CNRS, 601 rue de la Chimie, BP 53, 38041 Grenoble, France
[4]ESRF, Experiments Division, BP 220, 38043 Grenoble, France
e-mail:michaw@chemi.muni.cz

Carbohydrate-mediated recognition plays an important role in the ability of pathogenic bacteria to adhere to the surface of the host cell in the first step of their invasion and infectivity. Lectin-carbohydrate interactions are usually characterised by a low affinity for monovalent ligands that is balanced by multivalency resulting in high avidity for complex glycans or cell surfaces. Usually, a millimolar affinity is observed for plant lectin binding to monosacharides. In contrast, bacterial lectins involved in pathogenesis display much higher affinity than that observed for plant or animal lectins [1].

Contribution is focused on bacterial lectins from Pseudomonas aeruginosa and their homologues from other pathogens displaying sub-micromolar range affinity towards their carbohydrate ligands. The combination of X-ray crystallography and isothermal titration microcalorimetry approaches is used to decipher the structural and thermodynamical basis for high affinity binding of these lectins to host carbohydrates. Discovery of a three amino acid motif of the „ligand binding loop" that is responsible for lectin sugar preferences toward different monosaccharides will be discussed.

1. A. Imberty, E.P. Mitchell & M. Wimmerová, *Curr. Opin. Struct. Biol.*, **15**, (2005), 523.

Lectures - Saturday, June 24, morning

L19

STRUCTURE OF -GALACTOSIDASE COMPLEXES

J. Dohnálek[1], T. Skálová[1], J. Dušková[1], H. Petroková[1], J. Hašek[1], P. Lipovová[2], V. Spiwok[2], H. Strnad[3], B. Králová[2]

[1]Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic, Heyrovského nám. 2, 16206 Praha 6, Czech Republic
[2]Institute of Chemical Technology, Technická 5, 16628 Praha 6, Czech Republic
[3]Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Flemingovo nám. 2, 16637, Praha 6, Czech Republic
dohnalek@imc.cas.cz

The unliganded structure of -galactosidase from the soil bacterium *Arthrobacter* sp. C2-2 isolated in Antarctica (GH family 2) revealed compact hexameric organization of the enzyme [1-2]. It is the first structure of a cold-active -galactosidase. The structure was determined by single crystal X-ray crystallography with use of synchrotron radiation at the ESRF beamline ID29. The diffraction data were recorded in 1,800 oscillation images at 0.1 slicing and the scaled set of intensities contained 577,572 reflections in space group $P2_1$ (a = 140.1 Å, b = 205.7 Å, c = 140.5 Å, = 102.3). Six monomers of the enzyme are arranged with approximate 32 point symmetry into a sphere-like object and the individual active sites face the internal cavity. The cavity is connected with outer environment mainly by three different types of channels. The hexameric form is present in solution and is assumed to be the relevant biological oligomerization state. Therefore, ligands, substrates, ions and products interacting with the enzyme in the vicinity of the active site must enter and leave through the major openings. *E.coli* -galactosidase from the same glycoside hydrolase family (a mesophilic counterpart) was extensively studied as for its oligomerization state (tetramer), activity, enzymatic mechanism and the -complementation

phenomenon [3]. Conclusions based on the new structure bring the two enzymes into contrast and raise questions regarding the hexamer's function.

Diffraction data for several different complexes of the cold-active enzyme with various types of ligands were collected and processed, some of them with use of synchrotron radiation. It was confirmed that ligands must enter the internal cavity of the complex and thereby access the active sites of the hexamer.

1. H. Petroková, E. Vondráčková, T. Skálová, J. Dohnálek, P. Lipovová, V. Spiwok, H. Strnad, B. Králová, J. Hašek, *Collect. Czech. Chem. Commun.*, **70**, (2005), 124.
2. T. Skálová, J. Dohnálek, V. Spiwok, P. Lipovová, E. Vondráčková, H. Petroková, J. Dušková, H. Strnad, B. Králová, J. Hašek, *J. Mol. Biol.*, **353**, (2005), 282.
3. D.H. Juers, T.D. Heightman, A. Vasella, J.D. McCarter, L. Mackenzie, *Biochemistry*, **40**, 14781.

Acknowledgements. *This project was supported by the Grant Agency of the Academy of Sciences of the Czech Republic (KJB500500512).*

L20

HIPHOP REFINEMENT OF PROTEIN STRUCTURES

Jan Ondráček

Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Flemingovo nám. 2, 16637 Prague 6

A novel multisolution refinement method for protein diffraction data is described. HipHop refinement explores the conformational landscape by modification of the phases by introduction of water molecules into the model, followed by many cycles of refinement and removal of water molecules that do not comply with a minimal electron density, ball shape and a distance from protein. The resulting ensemble of generated models represents the graphical expression of structural variances of the crystal structure. The shape of the distribution function (histogram) of clusters (arising by overlapping of models) of water molecules expresses the probability of the correctness of the refinement. The method was successfully used for refinements of many protein crystal structures, e.g. lysozyme soaked with periodate [1] or bromate [2]. Noteworthy, the integrity of this approach was recently corroborated by Furnham *et al.* [3]. The general paper is in preparation [4]. Program versions for ShelX and Refmac are available free on http://www.img.cas.cz/hiphop.

1. Ondráček, J., Weiss, M.S., Brynda, J., Fiala, J., Jursík, F., Řezáčová, P., Jenner, L.B. & Sedláček, J. (2005). *Acta Crystallogr.* D61, 1181-1189.
2. Ondráček, J. & Mesters J.R. (submitted to print). *Acta Crystallogr*. **D**.
3. Furnham, N., Blundell, T.L., DePristo, M.A., Terwilliger, T.C. (2006). *Nature Struct. Mol. Biol.* 13, 184-185.
4. Ondráček, J., Weiss, M.S., Hilgenfeld, R., Sedláček. J. & Mesters,J. (manuscript in preparation). *Structure.*

L21

A COMPLEX OF HIV-1 PROTEASE AND PHENYLNORSTATINE INHIBITOR ANALYZED AT ATOMIC RESOLUTION

J. Brynda[1], P. Řezáčová[1], M. Fábry[1], M. Hořejší[1], R. Štouračově[1], M. Souček[2], M. Hradílek[2], M. Lepšík[2], J. Konvalinka[2] and J. Sedláček[1]

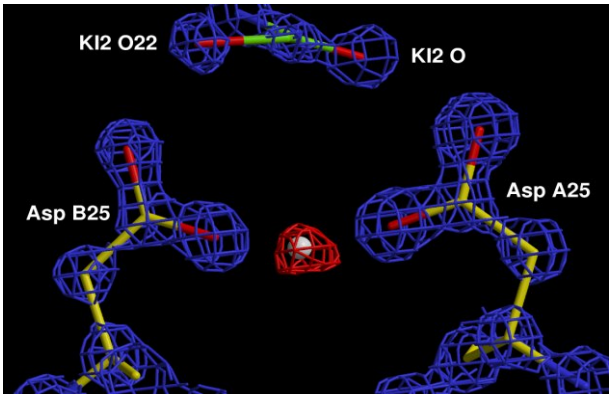
[1]*Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Flemingovo nám. 2, 16637 Prague 6*

[2]*Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo nám. 2, 16610 Prague 6*

The X-ray structure of a complex of HIV-1 protease (PR) with a phenylnorstatine inhibitor Z-Pns-Phe-Glu-Glu-NH_2 has been determined at 1.03 Å, the highest resolution so far reported for any HIV PR complex. The inhibitor shows subnanomolar Ki values for both the wild-type PR and the variant representing one of the most common mutations linked to resistance development. The structure comprising the phenylnorstatine moiety of (2*R*,3*S*) chirality displays a unique pattern of hydrogen bonding to the two catalytic aspartate residues. This high resolution allows to assess the donor/acceptor relations of this hydrogen bonding, and to indicate a proton shared by the two catalytic residues.

A structure-aided design of HIV PR inhibitors has led to a class of drugs useful in clinical anti-HIV intervention [see ref. 1 for review]. Nevertheless, mutational development of HIV PR drug resistance presents a major medical complication.

A combinatorial chemistry approach [2] yielded a series of novel pluripotent HIV PR inhibitors having a picomolar range of their Ki values for the wild-type HIV PR as well as various degrees of insensitivity of their inhibitory potency to HIV PR variants with mutations in positions 48, 82, 84 and 90, often found in drug-resistant PR strains [3]. In this work, the structure of wild-type HIV PR, complexed with one of these inhibitors, Z-Pns-Phe-Glu-Glu-NH_2 (Z, benzyloxycarbonyl; Pns, phenylnorstatine, (2*R*,3*S*)-3-amino-2-hydroxy-4-phenylbutanoic acid, termed hereafter KI2), is described. The phenylnorstatine group, an untypical inhibitor moiety, served the purpose to investigate the potential of replacement of amino acid residues with larger groups (the "main chain" between the aromatic groups occupying S1' and S1 pockets is longer by one carbonyl group, as compared to common, *e.g.,* hydroxyethylene or hydroxyethylamine isosteres). This compound inhibits the Val82Ala mutant of HIV PR with a Ki value 0.11 nM, while the wild-type HIV PR inhibition has Ki = 0.18 nM[3].



1. Wlodawer, A.; Vondrasek, J. Inhibitors of HIV-1 Protease: A major success of structure-assisted drug design. *Anu. Rev. Biophys. Biomol. Struct.* **27** (1998) 249-284.

2. Houghten, R. A.; Pinilla, C.; Blondelle, S. E.; Appel, J. R.; Dooley, C.T.; Cuervo, J. H. Generation and use of synthetic peptide combinatorial libraries for basic research and drug discovery. *Nature* **354** (1991) 84-86.

3. Rinnova, M.; Hradilek, M.; Barinka, C.; Weber, J.; Soucek, M.; Vondrasek, J.; Klimkait, T.; Konvalinka, . A picomolar inhibitor of resistant strains of human immunodeficiency virus protease identified by a combinatorial approach. J. *Arch. Biochem. Biophys.* **382** (2000) 22-30.

Acknowledgement

The authors wish to thank the staff of ESRF in Grenoble, beamline ID14.

L22

COMPLEX INTERMETALLIC COMPOUNDS AND METAL HYDRIDES FROM POWDER DIFFRACTION

Radovan Černý

Laboratory of Crystallography, University of Geneva, Radovan.Cerny@cryst.unige.ch

Intermetallic compounds are playing important role in many technological applications (high performance construction materials, magnetic materials, hydrogen storage). The characterization of their crystal structure is not an easy task due to often presence of lattice defects, chemical disorder, pseudosymmetry and weak superstructures. The fact that the intermetallic structures do not contain easily recognizable building units like molecules, and that they are often build up from atoms with large scattering contract, does not simplify their structural characterization by diffraction methods either. The single crystals are often not available, and structure size can reach few hundreds of independent atoms.

In the talk we will review the pitfalls of the structural characterization of intermetallic compounds by X-ray and neutron powder diffraction. Different tools of structure characterization of intermetallic compounds and their hydrides like high reciprocal space resolution [1], resonant diffraction [2], *in-situ* studies [3], methods and programs for structure solution [4] will be discussed with examples from our work.

1. Černý R., Renaudin G., Favre-Nicolin V., Hlukhyy V. and Pöttgen R.: $Mg_{1+x}Ir_{1-x}$ (x = 0, 0.037 and 0.054), a Binary Intermetallic Compound with a New Orthorhombic Structure Type from Powder and Single Crystal X-ray Diffraction. *Acta Cryst. B***60** (2004) 272-281.

2. Joubert J.-M., Černý R., Latroche, M., Percheron-Guégan, A. and Yvon K: Site Occupancies in the Battery Electrode Material $LaNi_{3.55}Mn_{0.4}Al_{0.3}Co_{0.75}$ as Determined by Multiwavelength Synchrotron Powder Diffraction. *J. Appl. Cryst.* **31** (1998) 327-332.

3. Joubert J.-M., Černý R., Latroche M., Percheron-Guégan A. and Schmitt B.: Hydrogenation of $LaNi_5$ studied by in situ synchrotron powder diffraction. *Acta Materialia* **54** (2006) 713–719.

4. Favre-Nicolin V. and Černý R.: A better FOX: using flexible modeling and maximum likelihood to improve direct-space ab initio structure determination from powder diffraction. *Z. Krist.* **219** (2004) 847-856.

L23

STRUCTURAL ANALYSIS OF ORGANIC POWDERS AND MICROCRYSTALS WITH SYNCHROTRON RADIATION

J. Maixner, M. Hušák

Institute of Chemical Technology Prague, Technická 5, 166 28 Prague, Czech Republic
maixner@vscht.cz, husakm@vscht.cz

Molecular and crystal structure determination used to be connected with 3D analysis of reciprocal space of suitably perfect and large enough single crystals (average size of 0.500 mm and volume of 0.1mm^3). The difficulties to grow such single crystals mainly due to the time consuming partly trial crystallization procedures are cause that directs the structure analysis of organic compounds to use of either very small single crystals (microcrystals with avarage size of 0.050 mm and volume of 0.0001 mm^3 up to submicrocrystals with size of a powder like crystallite with size of 1 m and volume of 1 m^3) or to use polycrystalline materials - powders when suitable single micro- or submicrocrystals are not available. Both approaches need to use high flux of primary beam and ab initio structure solution from powders furthermore high resolution to separate the number of severely overlapping reflections due to a compression of a reciprocal space into one dimension. These requirements can be achieved by exploitation of synchrotron radiation.

Ab initio structure determination from microcrystals and powder diffraction data is currently of great interest in crystallography and materials science. A number of crystal structures have been solved using either solution in reciprocal space (single crystal direct methods modified to use powder diffraction data for phasing - Altomare et al., 2004) or so-called direct-space methods as new approaches for structure solution using the Monte Carlo method, the simulated annealing method or the genetic algorithm. The later methods directly fit a calculated diffraction pattern to the observed pattern, and thus they do not require the pattern decomposition process for extracting integrated intensities. To solve structures having a large number of unknown parameters is, however, practically impossible without utilizing data other than those of diffraction intensities and a rigid-body or flexible rigid-body approximation is introduced to reduce the number of variables.

The lecture will give some information about handling micro crystals, filling capillaries to avoid texture formation, indexing powder data as the bottleneck of ab initio structure solution (CRYSFIRE-Shirley, 1999), application of the simulated annealing (FOX – Favre-Nicolin,V. and Černý R., 2002) and Rietveld refinement (GSAS - Von Dreele 2000, EXPGUI – B.H. Toby).

We will present the structure solution of metergoline form II (solution form powder data with preferred orientation and from micro crystal) and the results of the temperature behavior of simvastatin in the range from 150 K up to 295 K giving 2 new polymorphic forms solved from synchrotron powder data.

1. Altomare,A., Caliandro,R., Camalli,M., Cuocci,C., Giacovazzo, C., Moliterni, A.G. G., and Rizzi, R. "Automatic structure determination from powder data with EXPO2004,"*J. Appl. Crystallogr.* **37** (2004) 1025–1028.
2. Shirley, R. (1999). http://www.ccp14.ac.uk/tutorial/crys/program/crysfire.htm
3. Favre-Nicolin, V. and Černý, R. (2002). "FOX, 'Free Objects for Crystallography:' A mo-dular approach to ab initio structure determination from powder diffraction," *J. Appl. Crystallogr.* **35**, (2002) 734–743.
4. A.C. Larson, R.B. Van Dreele, "General Structure Analysis System (GSAS)", Los Alamos National Laboratoy Report LAUR 86-748 (2000).
5. B.H. Toby, "EXPGUI, a graphical user interface for GSAS", *J. Appl. Crys.***34**, 210-21 (2001).

L24

CONTRIBUTION TO THE CRYSTALLOGRAPHY OF OWYHEEITE

F. Laufek[1,2], R. Pažout[3]

[1]*Czech Geological Survey, Geologická 6, Praha 5, 152 00, Czech Republic*
[2]*Charles University, Faculty of Science, Albertov 6, Praha 2, 128 43, Czech Republic*
[3]*Institute of Chemical Technology, Technická 5, Praha 6,166 28, Czech Republic*
laufek@cgu.cz

The mineral owyheeite belongs to complex sulphosalts of Ag, Pb and Sb whose structures present one of the most complicated problems in crystallography of inorganic compounds. Uncertainty existed about the crystallographic symmetry which was for a long time considered to be orthorombic [1] although a monoclinic symmetry with *â* near 90 was suggested. The crystal structure of owyheeite, structure formula $Ag_2Pb_5Sb_5S_{14}$, was refined from powder X-ray synchrotron data of a sample from hydrothermal veins at the Turkaňk Lode of the Kutná Hora Ag-Pb-Zn polymetallic deposit, Central Bohemia, Czech Republic. The data were collected on the Swiss-Norwegian diffractometer BM1B at the European Synchrotron Radiation Facility (ESRF, Grenoble, France, = 0.49953 Å, sample in a 0.3 mm glass capillary). The powder X-ray synchrotron diffraction pattern was indexed using the first 20 lines. The monoclinic symmetry was indicated by DICVOL04 [2] program with figures of merit $M_{20} = 37$, $F_{20} = 267$ (0.0014, 53). The synchrotron powder diffraction pattern was refined with the cell found by DICVOL04 using the "profile matching" option [3] of the program FullProf [4] in order to determine the space group. The systematic absences were consistent with the space group $P2_1/c$. The initial structural model was obtained by preliminary single crystal X-ray diffraction experiment (Makovicky, *pers. com*). The final structure was obtained through the Rietveld refinement in the program FullProf and refined to R factors $R_{wp} = 0.041$, $R_B = 0.034$ and $^2 = 4.52$.

Owyheeite, $Ag_2Pb_5Sb_5S_{14}$, space group $P2_1/c$, a = 4.1034(1) Å, b = 27.3130(5) Å, c = 22.9352(4) Å, = 90.359(2) , Z = 4. The asymetric unit contains 26 atoms. The average structure exhibits fourteen S, one Ag, four Pb, five Sb sites and also two mixed sites, (Pb, Sb) and (Ag, Sb). The structure is presented in Fig. 1.

1. J. W. Anthony, *Handbook of Mineralogy, Elements, Sulfides, Sulfosalts*. Mineral Data Publishing, USA, 1990.
2. A. Boultif, D. Louër, *J. Appl. Cryst*. **37**, (2004), 724-731.
3. A. LeBail, H. Duroy, J. L. Fourquet, *Mater. Res. Buletin*., **23**, (1988), 447-452.
4. J. Rodríguez-Carvajal, *FullProf.2k*, Laboratoire Léon Brillouin, France, 2006.

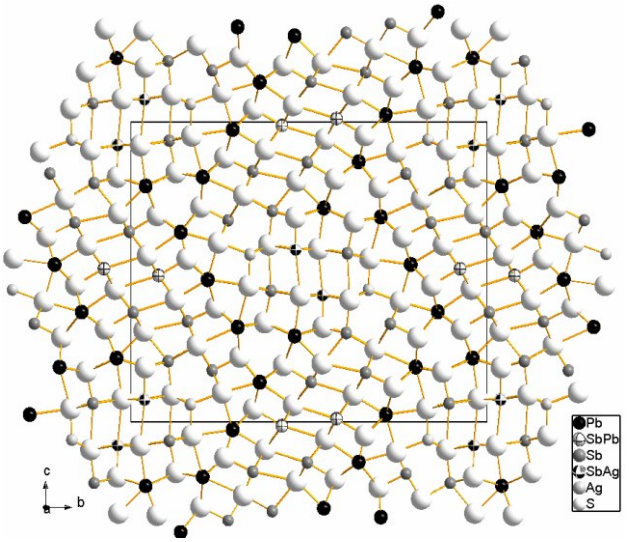


Fig. 1. Crystal structure of owyheeite