Session III

S10

CRYSTALLIZATION STUDIES OF PROTEIN TT81 FROM *THERMOCOCCUS THIOREDUCENS*

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Protein crystallography is a field of study of the three-dimensional structure of biological macromolecules. Based on the knowledge of the atomic structure of the studied biomacromolecules, it is possible to describe the processes in the cell or the catalytic reaction. An important biophysical method for determining the structure of biological macromolecules is X-ray diffraction. An essential requirement for using X-ray diffraction is to produce well-ordered crystals without defects that are large enough to provide diffraction data after passing the X-ray beam. The main goal of this research was to obtain suitable monocrystals of newly prepared protein Tt81 from *Thermococcus thioreducens*, which is considered to be haloacid dehalogenase. The crystallization experiment was performed using the microbatch and the sitting drop vapour diffusion techniques and crystallization screen Index HR2-144 (Hampton Research, USA). Diffraction data were collected to the resolution about 2.5 Å and will be used for further research, mainly for solving the structure of protein Tt81.

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S11

CRYSTALLIZATION STUDIES OF NEWLY PREPARED SUGAR-PHOSPHATASE DH TT80 FROM *THERMOCOCCUS KODAKARENSIS* KOD1

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Crystallization of macromolecules and structural studies have an important role in a number of biological branches, such as molecular biology, pharmacology, enzymology or biochemistry. The main goal of this research was to optimalize crystallization conditions and obtain suitable monocrystals of a newly prepared and yet uncharacterized protein DH Tt80 from *Thermococcus kodakarensis* KOD1, which is considered to be sugar-phosphatase. The crystallization experiment was performed using the sitting drop vapour diffusion technique and crystallization kit SaltRx (Hampton Research, USA). Diffraction data were collected to the resolution about 2.0 Å and will be used for further research, mainly for solving the structure of sugar-phosphatase DH Tt80.

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S12

CRYSTALLIZATION STUDIES OF RECENTLY PREPARED HALOALKAN DEHALOGENAS DGAA OF *GLACIEOCOLA AGARYLITICA* NO2

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The main goal is focused on crystalization study of newly prepared haloalkan dehalogenase DgaA from bacteria *Glaciecola agarylitica* NO2. The main target of this work is getting acquianted with methods of protein crystalization and usage of those methods for preparation of suitable DgaA protein crystals of that will be used for X-ray structural analysis. An essential requirement for using X-ray diffraction is to produce well ordered crystals without defects that are large enough to provide diffraction data after passing the X-ray beam. The crystallization experiment was performed using the sitting drop vapour diffusion techniques and crystallization screen Index HR2-144 (Hampton Research, USA). Results from difraction analysis of DgaA crystals will be starting point for further research focused on structure determination and and description of protein function.

The work was supported from GACR 17-24321S.

S13

Bilirubin OxidASE: Structural Analysis of Complexes with Ligands in Active Site and Study of Activities

BILIRUBIN OXIDÁZA: STRUKTURNÍ ANALÝZA KOMPLEXŮ S LIGANDY V AKTIVNÍM MÍSTĚ A STUDIE AKTIVIT

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Bilirubin oxidáza z rostlinného patogenu *Myrothecium verrucaria* (BOD; EC 1.3.3.5) katalyzuje oxidaci bilirubinu na biliverdin, čehož je využíváno v medicíně při stanovení hladiny bilirubinu v krvi při vyšetření jater. Kromě bilirubinu je tento enzym schopen katalýzy oxidace mnoha dalších aromatických a některých anorganických sloučenin. Fyziologická funkce bilirubin oxidázy v přírodě nebyla dosud odhalena.

Vlastnosti jako jsou katalýza oxidace širokého spektra látek, enzymatická aktivita v širokém rozsahu pH, využití kyslíku jako druhého substrátu či stabilita při vyšších teplotách činí BOD atraktivním pro mnohé biotechnologické a průmyslová aplikace [1, 2].

Přestože prostorová struktura BOD byla již popsána (PDB kód: 2XLL [3], 3ABG [4]), otázky týkající se místa vazby substrátu a aminokyselin účastnících se na přenosu elektronu ze substrátu k primárnímu akceptoru elektronu – iontu mědi (T1) nebyly dosud zodpovězeny. Hlavní motivací pro další studie BOD je právě hledání odpovědí na tyto otázky.

Byly vypěstovány krystaly komplexů BOD s navázanými ligandy v aktivním místě a vyřešena jejich struktura. Na základě těchto struktur komplexů byl navržen koncept existence dvou vazebných míst pro substrát (pro negativně nabité částice a pro aromatické sloučeniny). Inhibiční studie ukazují, že obě místa hrají důležitou roli. Na základě získaných výsledků byly navrženy cílené mutace BOD. Na základě naměřených aktivit BOD a jejích mutantů vůči různým typům substrátu byla diskutována nejpravděpodobnější cesta elektronu od substrátu k T1.

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S14

A SYNCHROTRON TOOL USED TO PROCESS HOME SOURCE DATA

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Laboratory instruments for X-ray diffraction measurements are becoming more powerful with new technologies such as the liquid anode sources or HPAD detectors. It allows efficient diffraction experiments, including data for native SAD phasing, on an increasing number of protein samples in house, without the need of a synchrotron source. The crucial part of the diffraction measurement is data processing. It is a very much standardized process for synchrotron data and users are used to processing data with tools such as XDS [1]. However, in house data collection has a few specific parameters compared to synchrotron: the measurement consists of several runs with various geometry settings and the geometries are non-orthogonal. Even though XDS can process such individual runs, settings are non-trivial and behind the scope of a general user. Therefore, *Xdskappa* was introduced to simplify processing of in house data using XDS. *Xdskappa* automatically generates input for XDS and runs XDS on multiple datasets simultaneously. Moreover, a possibility of manual fine tuning remains. *Xdskappa* is distributed under the GNU General Public License 3 [2].

1. W. Kabsch, *Acta Cryst. D*, **60**(2), (2010), 125-132.
2. GNU General Public License, Free Software Foundation, https://www.gnu.org/licenses/gpl-3.0.en.html.

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Session IV

S15

DIFFRACTION LIMIT IN MACROMOLECULAR CRYSTALLOGRAPHY

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Choice of the high resolution diffraction limit is an important step in macromolecular structure determination. Nowadays, there are several criteria for resolution cut-off estimation and this can lead to some confusion. To cite P. Evans: *"An appropriate choice of resolution limit is difficult and sometimes seems to be performed mainly to satisfy referees."* [1] Data from high resolution contain important information, which is needed to clarify structure details. It is also a significant part (tens of percent) of all dataset observations. Thus these data are not negligible and have a remarkable influence on the resulting structure model.

Diffraction data from a crystal of a FAD-dependent enzyme were collected on beam line P13, Petra III, Hamburg, using a PILATUS 6M detector. Diffraction spots were visible by eye up to resolution of 2.2 Å (Fig. 1). Various modern approaches of diffraction data processing were applied [2-3].

Attempts to estimate the diffraction limit using numerous criteria were performed. The main focus was on the R-factor analysis using the refinement statistics R_{work} and R_{free}. This criterion links crystallographic model and data quality [4], in contrast with conservative criteria, *e.g.*, $I/$, R_{merge}, or $CC_{1/2}$, that evaluate data quality without relation to model. Impact of various processing approaches on the overall structure quality has been analyzed. The resulting diffraction limit varied from 2.1 Å to 1.7 Å with respect to