BETA-GALACTOSIDASE FROM PSYCHROTROPHIC MICROORGANISM (STRAIN ARTHROBACTER)

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Abstract
The cold active ß-galactosidase isolated from *Arthrobacter* sp. C2-2 was expressed and fully characterized. The diffraction measurements were completed and the 3D structure determination is in progress. Comparison of preliminary structure shows that the enzymes from psychrophilic and thermophilic organisms have high conformation similarity in spite of homology 32 % only. A role of mutations important for activity and the conformational stability of the enzyme are analyzed by energy calculations.

Introduction

The ß-galactosidase from Arthrobacter sp. C2-2, main subject of this study, was derived from bacteria living in Antarctica under low temperatures (permanently below 5 °C). Its low temperature activity deserves special attention because of many practical and theoretical impacts of enzymatic processes under ambient temperatures [1, 2]. The elucidation of low temperature adaptation of enzymes requires knowledge of detailed molecular geometry and an analysis of conformational changes in the active site. The only known method being able to give complete determination of structure of large proteins is the X-ray crystallography. Therefore, the first part of our project is to determine the structure the above mentioned cold-active enzyme using X-ray diffraction.

Structure determination

Cold active ß-galactosidase from Arthrobacter sp. C2-2 is a large protein - homotetramer with molecular weight 500 kDa, having 4092 residues. It is homological to the ß-galactosidase from Escherichia coli (sequence identity 32%). The details concerning the expression, purification and determination of basic characteristics of the protein performed in the Institute of Chemical Technology in Praha were published in [1, 2].

The protein was crystallized using hanging drop method. Crystallization drops contained 1 µl protein solution and 1 µl reservoir solution. Protein solution: 4 mg/ml in 10 mM potassium phosphate buffer pH 7.5, 1 mM $MgSO_4$, 1 mM NaN_3 . Reservoir solution: 20% PEG 4000, 200 mM NaCl, 200 mM ammonium sulphate in 100 mM Na citrate buffer, pH 5.6. The final crystals were obtained by microseeding, i.e. doping the crystallization drop by several microcrystals obtained in preliminary crystallization attempts. The crystal was soaked in the mother liquor with 20 % ethylene glycol shortly before measu-rement.

In spite of large and optically perfect crystals, the successful X-ray diffraction measurement required high intensity of primary X-ray beam. The final measurement was performed at the source of synchrotron radiation ESRF in Grenoble at the beam line ID 29. Because of large unit cell (a = 140.1, b = 205.7, c = 140.5 Å, ß = 102.3°, space group $P2_1$), the diffraction data was collected with small oscillation angle 0.1°. Total 1800 frames gave 28 millions of measurements up to resolution 1.9 Å. They were processed by special version of the HKL package of programs [3] for viruses. All data processed up to 1.9 Å gave 579289 unique reflections, R_{lin} = 8.5 % and completeness 95 %. The asymmetric unit contains one tetramer, i.e. 32 800 symmetrically independent non-hydrogen atoms.

The phase problem was solved by the program EPMR (molecular replacement by evolutionary search [4]) using the structure of ß-galactosidase from *Escherichia coli* (PDB code 1DP0, resolution 1.7 Å) as a model [5]. In spite of low homology, the global structure appears similar. The structure building of the protease tetramer into the maps of electron density and the refinement is under progress.

The Fig. 1 shows a cartoon-view of a monomer of the ß-galactosidase from Arthrobacter sp. C2-2 obtained by homology modeling using program Modeler [6] starting from the ß-galactosidase from *Escherichia coli* (homology ~30 %). Conformations of side chains of the Glu442 and Glu521 in the active site of ß-galactosidase are shown in stick and ball model.

Conclusion

The x-ray structure determination using synchrotron radiation provides a reliable and sufficiently exact experimental view of the molecular structure even in the case of large protein macromolecule like this structure. This information gives a complete geometrical view on binding and mobility of the products, intermediate products and substrates (measured after slight mutation of the active site) which is necessary for a reliable description of the enzymatic process. The mutation experiments and research of ß-galactosidase complexes necessary for a reliable interpretation of low temperature activity of ß-galactosidase from psychrotrophic microorganism Arthrobacter sp. C2-2 are under way.

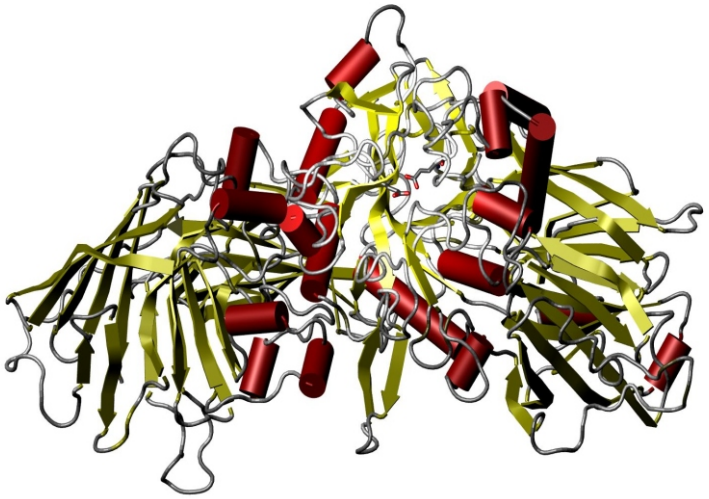

Figure 1. Monomer of ß-galactosidase from psychrotrophic microorganism Arthrobacter sp. C2-2 living in Antarctica under low temperature.

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SOME RECENT NON-TRADITIONAL APPLICATIONS OF X-RAY SCATTERING TECHNIQUES IN BIOLOGY

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In the last decade, X-ray diffraction of protein single crystals has become quite a routine and well-known technique of their structure analysis. Of course, synchrotron radiation is usually required and preparation of suitable single crystals may be not so easy.

However, this is not the only use of X-ray diffraction and X-ray scattering in biology. In this short review, several other examples will be mentioned - use of powder diffraction, X-ray reflectivity, grazing incidence, standing waves.

Powder diffraction

In last years, *structure refinement* and even *structure determination* from powder diffraction is more and more popular. This has been routinely used in chemistry, physics and also materials science. For large molecules it is rather complicated because the information in powder pattern is much lower than in full single-crystal pattern and moreover, huge number of diffraction lines leads to their strong overlap. However, recently successful attempts of protein structure refinement have been realized using fast development in instrumental techniques, namely high-resolution diffraction with synchrotron radiation. Pioneer work was presented by von Dreele [1, 2] and it started series of other experiments, especially at ESRF high-resolution beamline [e.g. 3a, 3b]. Other experiments were performed in Japa-