

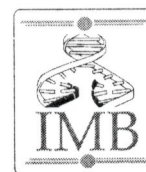


INSTITUTE OF MOLECULAR BIOLOGY

Slovak Academy of Sciences

Dúbravská cesta 21, 845 51 Bratislava 45, Slovakia

Tel.: +421 2 5930 7411, E-mail: umbidir@savba.sk



Opponent's review on PhD. thesis

Name of the student: **Andrii Mazur**

Thesis title: ***Crystallographic studies of multimeric and ancestral haloalkane dehalogenases***

Supervisor: prof. Mgr. Ivana Kutá Smatanová, PhD.

Referee: RNDr. Ľubica Urbániková, CSc.

Referee's affiliation: Institute of Molecular Biology Slovak Academy of Sciences,
Bratislava, SR

Human activity very often produces toxic industrial by-products, including halogenated compounds. Haloalkane dehalogenases, as enzymes capable of cleaving the halogen-carbon bond and degrading various types of halogenated compounds, may be useful in their biodegradation. Thus, haloalkane dehalogenases have great potential not only in biotechnology (such as the production of alcohols) but also in bioremediation.

The current thesis is aimed in structural functional studies of several selected haloalkane dehalogenases. To the main results belong the crystallization and structural studies of several haloalkane dehalogenases: DpaA from *Paraglaciecola agarilytica*, newly synthesized AncLinB-DmbA which is the most common ancestor of LinB from *Sphingobium japonicum* and DmbA from *Mycobacterium bovis* and cooperation on the crystallization of the mutant haloalkane dehalogenase from *Bradyrhizobium elkanii*. The results have contributed to better knowledge of structure, oligomerization state and influence of individual amino acid residues to the properties of these biotechnologically important enzymes.

I can conclude that the scientific objectives were well defined and were achieved. This is evidenced by three interesting publications in peer-reviewed scientific journals Crystals (in 2019), Acta Crystallographica D and International Journal of Molecular Sciences (both in 2021).

Some comments to the thesis:

- It would help the reader if the abbreviated names of the individual haloalkane dehalogenases mentioned in the Introduction were also given in the list of abbreviations. Some of them, e.g. LinB, DmbA, and others are specified only in the introductory parts of the articles included in the thesis.
- In the Introduction chapter, the author cites 55 articles, but only 7 of them were published in the last 5 years, with the most recent one in 2020. I think that such an interesting topic would deserve an introduction supplemented by the most up-to-date knowledge.

- There is only one figure in the chapter Introduction. It illustrates the tertiary structure of LinB haloalkane dehalogenase. However, both the PDB code and the citation are missing. It would certainly be possible and useful to prepare more interesting pictures to illustrate the text.
- In the chapter Materials and Methods, the principles of protein crystallization and X-ray structure determination are very briefly explained. However, some steps are omitted; for example, refinement is only mentioned in one sentence and data processing and structure validation are not mentioned at all.
- URL address should be given when citing PDB database.
- The incorrect PDB code is given on page 7, 5ERS is not a haloalkane dehalogenase.
- The list of references is not uniformly formatted. In addition, I think that all authors should be listed, as well as the doi numbers of the works cited.

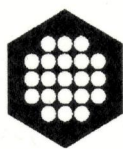
Questions

- Could you please explain the principle of protein crystallization by the vapor diffusion method in a hanging/sitting drop?
- Some crystals do not diffract or diffract very weakly after flash cooling and mounting on a goniometer. What can you do to improve diffraction?
- In the thesis you state that haloalkane dehalogenases are mostly monomeric, but some of them can form different types of dimers. Are these dimers only crystallographic or also functional? How are the activity and possibly other properties affected by the oligomerization state of the enzyme?

In conclusion, I would like to mention that the above comments do not detract from the scientific value of the work, of which there is not the slightest doubt. The PhD student had to master a number of techniques to reach the scientific goals and he succeeded. I propose to accept the thesis and after its successful defence to award Andrii Mazur the degree of PhD.


RNDr. Lubica Urbaniková, CSc.

Bratislava, 6th June, 2023



ÚOCHB AV
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IOCB PRAGUE

Institute of Organic Chemistry and Biochemistry of the
Czech Academy of Sciences
Flemingovo náměstí 542/2
160 00 Praha 6
Czech Republic

Prague, June 4th, 2023

Opponent's review of the doctoral thesis by

Andrii Mazur MSc. Entitled

„Crystallographic studies of multimeric and ancestral haloalkane dehalogenases “

The doctoral thesis presented by colleague Andrii Mazur is focused on a structural study of haloalkane dehalogenases (HLDs; EC 3.8.5.1). These enzymes catalyze hydrolytic cleavage of the carbon-halogen bond in various halogenated compounds into corresponding alcohols, halides, and protons. Halogenated compounds, such as 1,2-dichloroethane, 1,2,3-trichloropropane, 1,2-dibromoethane, are important and widely used in industry. These pollutants represent the greatest threats to nature, and thus their biodegradation by bacterial enzymes, such as haloalkane dehalogenases, is a promising way to the protection of environment. Haloalkane dehalogenases have wide substrate specificity, which pushes forward the research to improve existing dehalogenases. Using several biochemical techniques would reveal features of novel types of these enzymes with additional unique properties.

The thesis is written in a shortened form and has a standard structure. In the literature review (chapter Introduction) the author briefly describes facts about haloalkane dehalogenases, their structure, and the multimeric nature of some of these species. In this chapter, the reaction mechanism of HLDs is well described. The last part is dedicated to the HLDs originating from marine organisms.

The final part of the thesis consists of three publications, which were published in good-quality international journals with IF. Andrii Mazur is the first author in two of these publications and co-author in the last one. The author's contribution is reported for all publications in percentage.

The first publication describes a structural feature of novel haloalkane dehalogenase DpaA which was isolated from the psychrophilic and halophilic bacterium *Paraglaciecola agarilytica* NO2, which was found in marine sediment collected from the East Sea near Korea. The second publication analyses the ancestral enzyme AncLinB-DmbA, which was crystallized using the sitting-drop vapor-diffusion method, giving crystals that diffracted X-rays to 1.5 Å resolution. Structural comparison of AncLinB-DmbA with their closely related descendants LinB and DmbA revealed some interesting differences in overall structure and tunnel architecture. The last publication presents crystallization and crystallographic Analysis of a *Bradyrhizobium Elkanii* USDA94 haloalkane Dehalogenase mutation variant with an eliminated halide-binding site.

I found several interesting methodical and technical details in all publications. These led me to the following questions related to the presented thesis:

1. In the first publication, the author mentions the twin refinement because of “almost ideal merohedral twinning” in apparently tetragonal data. Could you please comment on this problem in a more detailed manner?
2. The second question relates to the first article as well. There was mentioned that protein was migrating in gel filtration as a dimer. However, the title of the publication is “The tetrameric structure of the novel haloalkane dehalogenase DpaA....”. Therefore, I would need you to clarify the multimerization of HLD DpaA.
3. Third question is inspired by the method of the reconstruction of an ancestral sequence, mentioned in the second publication. I would like to know the author’s general view about, or estimation of the common ancestor of the haloalkane dehalogenase protein family.
4. The last question points to the issue of crystallization seeding, conferring to the third publication. I would like to hear more about the different seeding methods used, and how they influenced the crystals diffraction quality.

To summarize my review, I would like to mention that the formal and graphic quality of the submitted dissertation is good. The quality of the English, at least as far as I can judge, is very good. In conclusion, the doctoral thesis presented by colleague Andrii Mazur represents a significant contribution to structural studies of the haloalkane dehalogenases protein family. The thesis is written in an intelligible language and obtained results were published in international journals with IF. The presented thesis demonstrates that MSc. Andrii Mazur is able of independent scientific work. I hope that the presented thesis satisfies all requirements for the doctoral thesis, and **I fully recommend its acceptance.**

Doc. RNDr. Jiří Brynda, CSc.

tel.: +420 220183210
e-mail: brynda@uochb.cas.cz