Abstract

Cyanophycin is a natural biopolymer consisting of a poly-L-Asp backbone with L-Arg residues attached to their β-carboxylate side chains by isopeptide bonds. First discovered in cyanobacteria in 1886, cyanophycin is produced by a wide range of bacteria and is important for cellular nitrogen storage. In addition to being a ubiquitous natural product, it is also studied due to its potential biotechnological applications. Cyanophycin can be synthetized by two different enzymes: cyanophycin synthetase 1 (CphA1) makes it from Asp and Arg, and cyanophycin synthetase 2 (CphA2) polymerizes β-Asp-Arg dipeptides. The polymer is degraded in two steps: in the first, cyanophycinase breaks it down into β-Asp-Arg dipeptides; in the second, enzymes with isoaspartyl dipeptidase activity degrade these dipeptides into Asp and Arg. Although cyanophycin has been known for over 100 years, many questions about its biosynthesis and biodegradation remained unanswered. This Ph.D. thesis first gives a current summary of the relevant literature about cyanophycin metabolism and its biotechnological applications. This is followed by chapters that present structural, biochemical and bioinformatic studies that show how the enzymes involved in its metabolism function.

The 2nd, 3rd and 4th chapters discuss the biosynthesis of cyanophycin. They present the X-ray crystallography and cryo-EM structures of CphA1 and CphA2, and propose models for these enzymes' activity. Chapter two presents co-complex structures of CphA1 with substrates and substrate analogs. Together with accompanying biochemical experiments, they show how this 3-domain enzyme binds its substrates and catalyzes two ATP-dependent reactions for the polymerization of cyanophycin. Chapter 3 describes the discovery that CphA1 has a third, hydrolytic active site that can cleave long cyanophycin chains into small segments that serve as primers for polymerization. Chapter 4 describes the characterization of nine different CphA2s, and illustrates the range of activity levels and oligomerization displayed by these enzymes. It also presents the crystal structure of a CphA2, highlighting differences and similarities between it and those of CphA1. The structural data, coupled with mutagenesis experiments and activity assays, show the roles of CphA2's domains and their importance for activity and stability.

The 5th, 6th and 7th chapters describe the biodegradation of cyanophycin. Chapter 5 presents the structure of a covalent enzyme-substrate intermediate of cyanophycinase with its substrate cyanophycin. The structure shows how this enzyme is able to bind and cleave cyanophycin which, despite its peptide-like nature, is resistant to proteolytic degradation. Biochemical experiments and comparison to a structure of an inactive cyanophycinase-like protein help identify regions around the active site which are important for enzymatic activity. Chapter 6 presents a bioinformatic analysis describing the co-occurrence and clustering of genes with isoaspartyl dipeptidase activity with other cyanophycin metabolizing genes. It then describes the structural and biochemical characterization of two such enzymes. The results show that despite being clustered with *cphA1* and cyanophycinase, these enzymes retain broad substrate specificity, similarly to other isoaspartyl dipeptidases. Chapter 7 describes the identification and biochemical and structural characterization of a novel isoaspartyl dipeptidase with specific activity towards dipeptides derived from cyanophycin degradation. *In vivo* data show that this common proteobacterial enzyme allows *Pseudomonas aeruginosa* to utilize β-Asp-Arg as a nitrogen source.

Together, the results presented in this thesis expand our knowledge on important aspects of cyanophycin metabolism. The insights gained from these studies will hopefully promote various areas of cyanophycin research and allow us to better understand the biological and biotechnological processes in which this polymer is involved.

McGill University

Graduate and Postdoctoral Studies

Final Oral Examination
for the Degree of
Doctor of Philosophy
of **ITAI SHARON**

of the Department of Biochemistry**, on Friday, November 11, 2022 @ 2:30 PM In-person**: **Goodman Cancer Research Centre, Karp Conference Room (501).**

COMMITTEE:

Dr. Massimo Avoli, Neurology & Neurosurgery	(Pro Dean)
Professor Albert Berghuis	(Deputy Chair)
Professor Martin Schmeing	(Thesis Supervisor)
Professor Natalie Zeytuni	(Internal Examiner)
Professor Jean-Francois Trempe	(Internal Member)
Professor Lyle Whyte	(External Member)

Agricultural & Environmental Sciences, McGill

Dr. Josephine Nalbantoglu
Dean

Members of Faculty and Graduate
Students are invited to be present

CURRICULUM VITAE

NAME: **Itai Sharon**
CITIZENSHIP: **Israel**

ACADEMIC BACKGROUND:

Ph.D. McGill University
Date Department of Biochemistry
Thesis Supervisor: Martin Schmeing

Thesis title: Structural insights into the biosynthesis and biodegradation of cyanophycin

B.Sc. Biology and Chemistry, Tel Aviv University
Date August 2013

PUBLICATIONS:

12. **Sharon I**, Hilvert D, Strauss M, Schmeing TM. Cyanophycin and its biosynthesis. *Imminent submission to* ***Natural Product Reports***
11. Markus LMD*, **Sharon I***, Munro K, Grogg M, Hilvert D, Strauss M, Schmeing TM. Structure and function of a hexameric cyanophycin synthetase 2. *Imminent submission to* ***Protein Science***
10. **Sharon I***, McKay G*, Nguyen D, Schmeing TM. Specific cyanophycin dipeptide hydrolase enzymes suggest widespread utility of this natural polymer. *Minor revisions requested,* ***Proceedings of the National Academy of Sciences USA***
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