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Biochemical and functional characterization of Nudix hydrolase enzymes with novel regulatory roles in Gram positive methylotrophic bacteria

Harm Kloosterman

The study described in this thesis was performed at the Department of Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute (GBB), University of Groningen, The Netherlands.

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RIJKSUNIVERSITEIT GRONINGEN

BIOCHEMICAL AND FUNCTIONAL CHARACTERIZATION OF NUDIX HYDROLASE ENZYMES WITH NOVEL REGULATORY ROLES IN GRAM POSITIVE METHYLOTROPHIC BACTERIA

Proefschrift

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Promotor:	Prof. Dr. L. Dijkhuizen
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	Prof. Dr. J. Kok

Contents

Aim and Outline of Thesis

At the start of this study no information was available about possible regulatory functions of Nudix (**Nu**cleotide **di**phosphate linked to some other moiety, **X**) hydrolase proteins. This protein family was regarded as a group of enzymes necessary to remove potentially toxic or mutagenic compounds from the cell. This is exemplified by the first member of this protein family, MutT of *E. coli,* which was shown to hydrolyze 8-oxo-dGTP, a potentially mutagenic form of dGTP when used for DNA synthesis. The entire Nudix hydrolase protein family subsequently was referred to as "housecleaning enzymes". The cloning and characterization of the methanol dehydrogenase (MDH) activator protein (ACT) of *Bacillus methanolicus* provided the first example of a Nudix hydrolase protein regulating an enzymatic activity involved in primary metabolism. Also a Nudix hydrolase encoding ORF on the indigenous plasmid pMEA300 of *Amycolatopsis methanolica* was shown to be an important regulator of various plasmid functions (pock formation, autonomous replication and stimulation of transformation frequency). The primary aim of this PhD study was to elucidate the physiological roles, regulatory functions and mechanisms of both Nudix hydrolases, which share significant overall amino acid sequence similarity.

Chapter 1 reviews the current knowledge of the Nudix hydrolase protein family, with emphasis on Nudix hydrolase proteins in microorganisms. Chapter 2 discusses the nicotinoprotein methanol dehydrogenase (MDH) enzymes in Gram-positive bacteria. The MDH enzymes in both *B. methanolicus* and *A. methanolica* are nicotinoproteins, with a tightly bound NAD(P)(H) cofactor. The *B. methanolicus* ACT protein hydrolyzes the NAD(H) cofactor of MDH (chapter 3). A detailed biochemical and mutational analysis of the catalytic function of this NAD(H) cofactor and its binding site in MDH are presented in chapter 4. Chapter 5 reports the identification of the minimal replicon of *A. methanolica* plasmid pMEA300, revealing that none of the regulatory genes -including the Nudix hydrolase encoding *orf192*- are essential for replication. The pMEA300 Rep protein shows no similarity with any other Rep protein in databases. The data suggest that pMEA300 belongs to a new family of Rolling Circle Replication plasmids. A functional analysis of the Nudix hydrolase Orf192 protein and other regulatory proteins of pMEA300 is described in chapter 6, revealing their effect on integration, pock formation and transformation frequency. Chapter 7 describes the regulation of key enzymes in the shikimate pathway of *A. methanolica.* Mutant enzymes affected in feedback inhibition/activation control were isolated by screening for mutant strains resistant to toxic phenylalanine analogs and subsequently characterized. Those mutants could only be isolated in the *A. methanolica* wild type strain, containing the indigenous plasmid pMEA300. No such mutants could be obtained in a strain devoid of pMEA300 sequences. Finally, chapters 8-9 summarize the results obtained, present concluding remarks and suggestions for further research.