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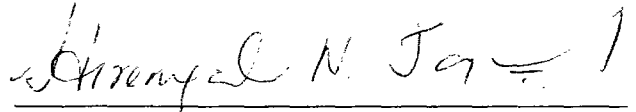
IDENTIFICATION AND CHARACTERIZATION OF A
FAMILY OF NICOTINAMIDE 5'-MONONUCLEOTIDE
ADENYLYLTRANSFERASES FROM YEAST AND HUMANS

Joel A. Yalowitz

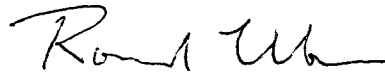
Submitted to the faculty of the University Graduate School
in partial fulfillment of the requirements
for the degree
Doctor of Philosophy
in the Department of Biochemistry and Molecular Biology
Indiana University

July 2004

Accepted by the Faculty of Indiana University, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

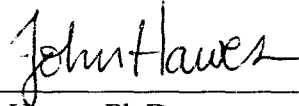


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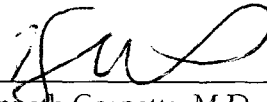
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ABSTRACT

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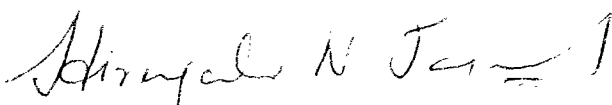
NICOTINAMIDE 5'-MONONUCLEOTIDE ADENYLYLTRANSFERASES: IDENTIFICATION AND CHARACTERIZATION OF A FAMILY OF HUMAN ENZYMES

The pathways of NAD metabolism are becoming better understood through the recent cloning of nicotinamide 5'-mononucleotide adenylyltransferase (NMNAT). At the start of this research project, the only cloned NMNAT enzymes were the yeast gene *NMA1*, now known as *NMA1*, and 3 bacterial genes. We cloned a new second NMNAT gene from yeast, designated *YGR010W* or *NMA2*. *NMA2* was expressed as a recombinant 6×His fusion protein and purified by nickel chromatography. The protein was shown to possess NMNAT activity and synthesized tiazofurin adenine dinucleotide (TAD) from tiazofurin monophosphate (TRMP). We propose that the purpose of the two separate NMNAT enzymes in yeast is to confer stress responses via the Sir2 pathway, and perhaps to regulate metabolic mode.

Our laboratory has cloned a new cDNA encoding NMNAT from human brain, designated hNMNAT-2. Using human genomic databases, hNMNAT-2 was localized to chromosome 1q25 within a 171 kb gene. Northern blot analysis revealed highly restricted expression of hNMNAT-2 to brain, heart, and muscle tissues. Different regions of the brain exhibited slightly differential expression of hNMNAT-2, and interestingly hNMNAT-2 expression is lower in the spinal cord. Substitution mutations of either of two conserved residues, histidine-24 or tryptophan-92, abolished enzyme activity. Anti-peptide antibody to a unique epitope within hNMNAT-2 was produced, and immunohistochemical analysis of sections of adult human pancreas revealed that hNMNAT-2 protein was markedly expressed

in the islets of Langerhans. Pancreatic exocrine cells exhibited weak expression of hNMNAT-2 protein. Subsequently, human NMNAT has been shown to be expressed as at least 3 different isoforms with differing properties, including intracellular and tissue expression. We propose that the different NMNAT enzymes have arisen to fulfill different biochemical functions within the NAD synthetic pathway.

Mycophenolic acid (MPA) is a fungally-derived inhibitor of inosine 5'-monophosphate dehydrogenase (IMPDH). MPA analogs were synthesized containing an adenosine moiety. C2-MAD, C4-MAD, and C6-MAD were obtained by linking adenosine 5'-methylenebis(phosphonate) with mycophenolic alcohols containing 2-, 4-, and 6-carbon atoms in their aliphatic side chain, respectively. In K562 cells, C2- and C4-MAD analogs were significantly more potent than native MPA. This indicates that rationally-synthesized MPA analogs could potentially be important clinical agents.



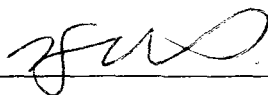
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