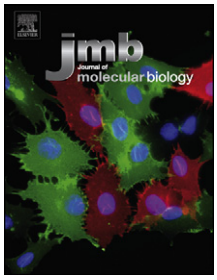

Structural and Functional Characterization of *Plasmodium falciparum* Nicotinic Acid Mononucleotide Adenylyltransferase

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Abstract

Nicotinic acid mononucleotide adenylyltransferase (NaMNAT) is an indispensable enzyme for the synthesis of NAD and NAD phosphate. It catalyzes the adenylylation of nicotinic acid mononucleotide (NaMN) to yield nicotinic acid adenine dinucleotide (NaAD). Since NAD(H) and NAD phosphate(H) are essentially involved in metabolic and redox regulatory reactions, NaMNAT is an attractive drug target in the fight against bacterial and parasitic infections. Notably, NaMNAT of the malaria parasite *Plasmodium falciparum* possesses only 20% sequence identity with the homologous human enzyme. Here, we present for the first time the two X-ray structures of *P. falciparum* NaMNAT (PfNaMNAT)—in the product-bound state with NaAD and complexed with an α,β-non-hydrolizable ATP analog—the structures were determined to a resolution of 2.2 Å and 2.5 Å, respectively. The overall architecture of PfNaMNAT was found to be more similar to its bacterial homologs than its human counterparts although the PPHK motif conserved in bacteria is missing. Furthermore, PfNaMNAT possesses two cysteine residues within the active site that have not been described for any other NaMNATase so far and are likely to be involved in redox regulation of PfNaMNAT activity. Enzymatic studies and surface plasmon resonance data reveal that PfNaMNAT is capable of utilizing NaMN and nicotinamide mononucleotide with a slight preference for NaMN. Surprisingly, a comparison with the active site of *Escherichia coli* NaMNAT showed very similar architectures, despite different substrate preferences.

Introduction

Nicotinamide adenine dinucleotide (NAD) is indispensable for living organisms. Together with its phosphorylated derivative NAD phosphate, NAD acts as an electron carrier in metabolic redox reactions by serving as a cofactor for a variety of enzymes, especially dehydrogenases. NAD(P) holds a central position in energy metabolism and also in (redox) regulation and cellular signal transduction. NAD deficiency has been linked to neurodegenerative diseases, the metabolic syndrome, diabetes, and cancer [1,2].

In 1958, Preiss and Handler discovered the classic metabolic pathway for NAD synthesis, which is today known as the Preiss–Handler pathway or niacin salvage pathway [3–5]. This pathway comprises three reactions: (i) conversion of nicotinic acid and phosphoribosyl pyrophosphate to nicotinic acid mononucleotide (NaMN) and pyrophosphate (PP_i), (ii) adenylation of NaMN to yield nicotinic acid adenine dinucleotide (NaAD) and PP_i, and (iii) conversion of NaAD, glutamine, and ATP to NAD, glutamate, adenine mononucleotide (AMP), and PP_i [4]. However, the Preiss–Handler pathway is only one among several—some authors report up to six—NAD biosynthetic routes [5]. Figure 1 gives an overview of NAD biosynthesis pathways. In *de novo* synthesis, Tyr and Asp are converted to quinolinic acid, which is utilized to yield NaMN [2,5]. Furthermore, nicotinamide riboside and nicotinic acid riboside give a Preiss–Handler-independent route to NAD [2,6].

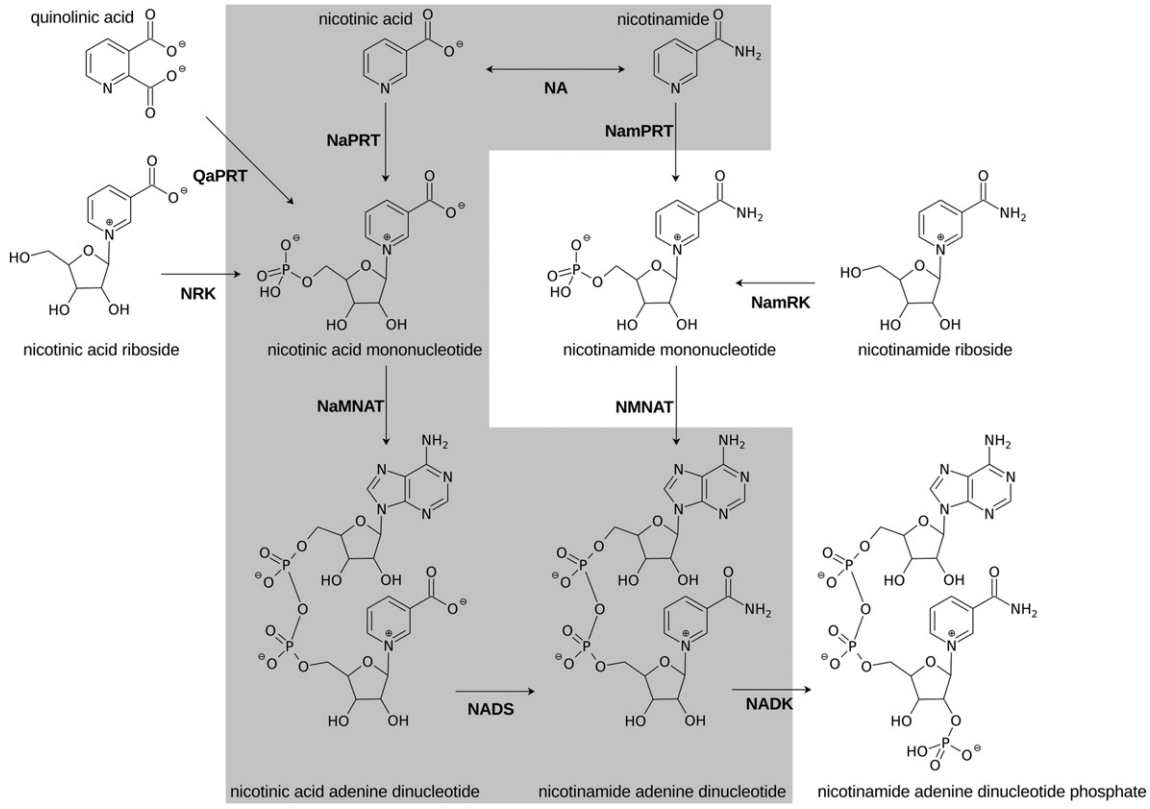

Fig. 1. NAD biosynthesis pathways. A variety of molecules can serve as a starting point in NAD biosynthesis. The metabolic pathway actually used depends on the respective organism but is highlighted in gray for intraerythrocytic stages of *Plasmodium falciparum*. *P. falciparum* relies on the Preiss–Handler pathway, starting with nicotinic acid, and results in the production of NaAD. Enzyme names are written in boldface and abbreviated as follows: NA, nicotinamidase; QaPRT, quinolinic acid phosphoribosyltransferase; NaPRT, nicotinic acid phosphoribosyltransferase; NamPRT, nicotinamide phosphoribosyltransferase; NRK, nicotinate riboside kinase; NamRK, nicotinamide riboside kinase; NaMNAT, nicotinic acid mononucleotide adenylyltransferase; NMNAT, nicotinamide mononucleotide adenylyltransferase; NADS, NAD synthetase; NADK NAD kinase.

Which pathway is used to generate NAD depends on the respective organism, but there is a common step for every pathway: dinucleotide formation is always achieved by NaMN adenylyltransferase (NaMNAT, EC 2.7.7.18) or nicotinamide mononucleotide (NMN) adenylyltransferase (NMNAT, EC 2.7.7.1) [2], emphasizing the importance of these enzymes for NAD synthesis.

Due to the essential role of NAD as a cofactor, its biosynthesis is considered a potential drug target [5,7], with NaMNAT being the focus of current research activities. These include studies on bacterial pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Mycobacterium tuberculosis*, and *Bacillus anthracis* [5,7–13]. Furthermore, NAD synthesis in *Plasmodium* has been described as a promising target for the development of new antimalarial drugs [14]. In 2015, the WHO estimated 214 million malaria cases with 438,000 deaths [15]. Considering that the spread of resistance to antimalarial drugs has significantly increased, the development of new drugs is mandatory [15]. In this context, *P. falciparum* NaMNAT (PfNaMNAT) is a very attractive drug target. O'Hara *et al.* confirmed the lack of *de novo* NAD synthesis in *P. falciparum* and concluded from their experiments that the parasite produces NAD through the canonical Preiss–Handler pathway—with an essential role of NaMNAT [14]. Furthermore, PfNaMNAT is highly divergent from its human homologs but very similar to bacterial NMNATs. This high level of conservation was confirmed by complementing the essential *E. coli* NadD *in vivo* [14]. Furthermore, derivatives of bacterial NaMNAT inhibitors were able to inhibit *P. falciparum* growth *in vivo* [14]. The most promising among those inhibitors showed MIC_{50} values in the lower micromolar range. However, due to the lack of a crystal structure, it has so far been difficult to correlate MIC_{50} values with specific features of novel inhibitors.

Here, we present the first three-dimensional structure of *P. falciparum* NaMNAT. In fact, two complex structures of the enzyme were obtained: one with the product NaAD and the other with an ATP analog bound. Additionally, kinetic analysis of