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Crystal structure of human nicotinic acid phosphoribosyltransferase

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Nicotinic acid phosphoribosyltransferase (EC 2.4.2.11) (NaPRTase) is the rate-limiting enzyme in the three-step Preiss–Handler pathway for the biosynthesis of NAD. The enzyme catalyzes the conversion of nicotinic acid (Na) and 5-phosphoribosyl-1-pyrophosphate (PRPP) to nicotinic acid mononucleotide (NaMN) and pyrophosphate (PPi). Several studies have underlined the importance of NaPRTase for NAD homeostasis in mammals, but no crystallographic data are available for this enzyme from higher eukaryotes. Here, we report the crystal structure of human NaPRTase that was solved by molecular replacement at a resolution of 2.9 Å in its ligand-free form. Our structural data allow the assignment of human NaPRTase to the type II phosphoribosyltransferase subfamily and reveal that the enzyme consists of two domains and functions as a dimer with the active site located at the interface of the monomers. The substrate-binding mode was analyzed by molecular docking simulation and provides hints into the catalytic mechanism. Moreover, structural comparison of human NaPRTase with the other two human type II phosphoribosyltransferases involved in NAD biosynthesis, quinolinate phosphoribosyltransferase and nicotinamide phosphoribosyltransferase, reveals that while the three enzymes share a conserved overall structure, a few distinctive structural traits can be identified. In particular, we show that NaPRTase lacks a tunnel that, in nicotinamide phosphoribosiltransferase, represents the binding site of its potent and selective inhibitor FK866, currently used in clinical trials as an antitumoral agent.

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1. Introduction

Nicotinamide adenine dinucleotide (NAD) and its phosphorylated form (NADP) are essential ubiquitous coenzymes playing fundamental roles in living cells. Beyond renown redox roles in energy metabolism, NAD(P) is also intimately involved in signaling pathways through a number of consuming reactions that underscore a wealth of physiopathological conditions [1–4]. Indeed, the NAD(P) derivatives nicotinic acid adenine dinucleotide phosphate (NaADP) and cyclic ADP-ribose (cADPR) are among the most potent intracellular calcium-mobilizing agents [4,5]. Moreover, NAD is the substrate for poly(ADP-ribosyl)ation reactions that in higher eukaryotes regulate chromatin function and gene expression, as well as for mono(ADP-ribosyl)ation modifications of target proteins in both mammalian and prokaryotic cells [4]. In all organisms, NAD can also be consumed by important regulatory enzymes, named sirtuins, that catalyze NAD-dependent deacetylation reactions of target proteins [6]. Clearly, physiological NAD depletion caused by overall NAD-consuming reactions necessitates permanent regeneration of this cofactor. Therefore NAD biosynthesis appears of therapeutically value for the treatment of pathological conditions arising from severe altering of NAD(P) homeostasis like in the case of neurological, neoplastic, and infectious disorders, as well as in the process of ageing [1,3,7–10]. Based on current knowledge, four different substrates can be used as a source of the pyridine ring in the NAD biosynthesis: quinolinic acid (QA) in the *de novo* pathway; nicotinic acid (Na), nicotinamide (Nam) and nicotinamide riboside (NamR) in the salvage pathways. While this latter compound is phosphorylated by action of an

Abbreviations: Na, nicotinic acid; NaAD, nicotinic acid dinucleotide; NAD, nicotinamide adenine dinucleotide; NaMN, nicotinic acid mononucleotide; NamR, nicotinamide riboside; NaPRTase, nicotinic acid phosphoribosyltransferase; NMN, nicotinamide mononucleotide; NMNAT, nicotinamide mononucleotide adenylyltransferase; PRPP, 5-phosphoribosyl-1-pyrophosphate; QA, quinolinic acid

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ATP-dependent kinase activity [11], the three other precursors, QA, Na and Nam, can be individually transferred to a phosphoribosyl pyrophosphate moiety (PRPP) by respective phosphoribosyltransferase activities [12]. The resulting mononucleotide products, nicotinic acid mononucleotide (NaMN) and nicotinamide mononucleotide (NMN), are then converted to dinucleotide forms, i.e. nicotinic acid dinucleotide (NaAD) and NAD, by action of a single enzymatic activity represented by nicotinamide mononucleotide adenylyltransferase (NMNAT) [13,14]. NaAD is finally amidated to NAD by a NAD synthetase activity (Fig. 1) [7,12,15].

Although the Nam salvaging biosynthetic route appears physiologically the main contributor to keep adequate NAD homeostasis [16–18], the supplementation of Nam does not seem so effective in elevating cellular NAD beyond the basal concentration. Indeed, some mammalian tissues like heart, kidney [19], and red blood cells [20] use preferentially Na for NAD synthesis even in the presence of higher Nam levels physiologically available [21]. Thus, both precursors appear relevant for NAD biosynthesis but with distinct and possibly complementary roles in different tissues [22]. This view is also supported by the observation that human embryonic kidney (HEK293) cell line, when supplemented with Na, but not with Nam, markedly elevated intracellular NAD levels, and showed beneficial effects versus the cytotoxic stress symptoms caused by H_2O_2 [22]. This effect of exogenously administered Na likely accounts for at least some effects of this vitamin precursor and suggests novel applications for the treatment of conditions associated with cellular NAD depletion, such as in photodamaged skin [23]. Of note in this context is also the finding that Na is one of the oldest drugs known for its unique anti-lipolytic effects [24], that has been attributed in more recent years to its specific binding to selected receptors present on the plasma membrane of adipocytes [25,26]. In mammals, Na can derive either directly from the diet, or indirectly from the enzymatic deamidation of dietary Nam operated by the gut flora. As shown in Fig. 1, Nicotinate Phosphoribosyltransferase (EC 2.4.2.11) is the first enzyme that catalyzes the synthesis of NaMN and PPi from Na and PRPP. The enzyme, originally named NaMN pyrophosphorylase, was first described by Handler in human erythrocytes, where it plays a key role in elevating NAD levels [20]. In both mice and humans, NaPRTase appears differentially expressed in tissues, being more abundant where Na is the preferential source for NAD biosynthesis [22,27]. Like the bacterial counterpart enzymes, human NaPRTase is strictly specific for Na as a substrate and ADP, NaAD and NAD do not affect its activity [27,28]. The central compounds of cellular metabolism, ATP and Pi, were found to affect human NaPRTase (hNaPRTase) activity. In particular ATP utilization seems to promote allosteric interactions between subunits [27]. Such ATP-driven conformational change can also be inferred by the observation that, in microbial NaPRTs, ATP binding protects from proteolysis [29] and heat inactivation [30].

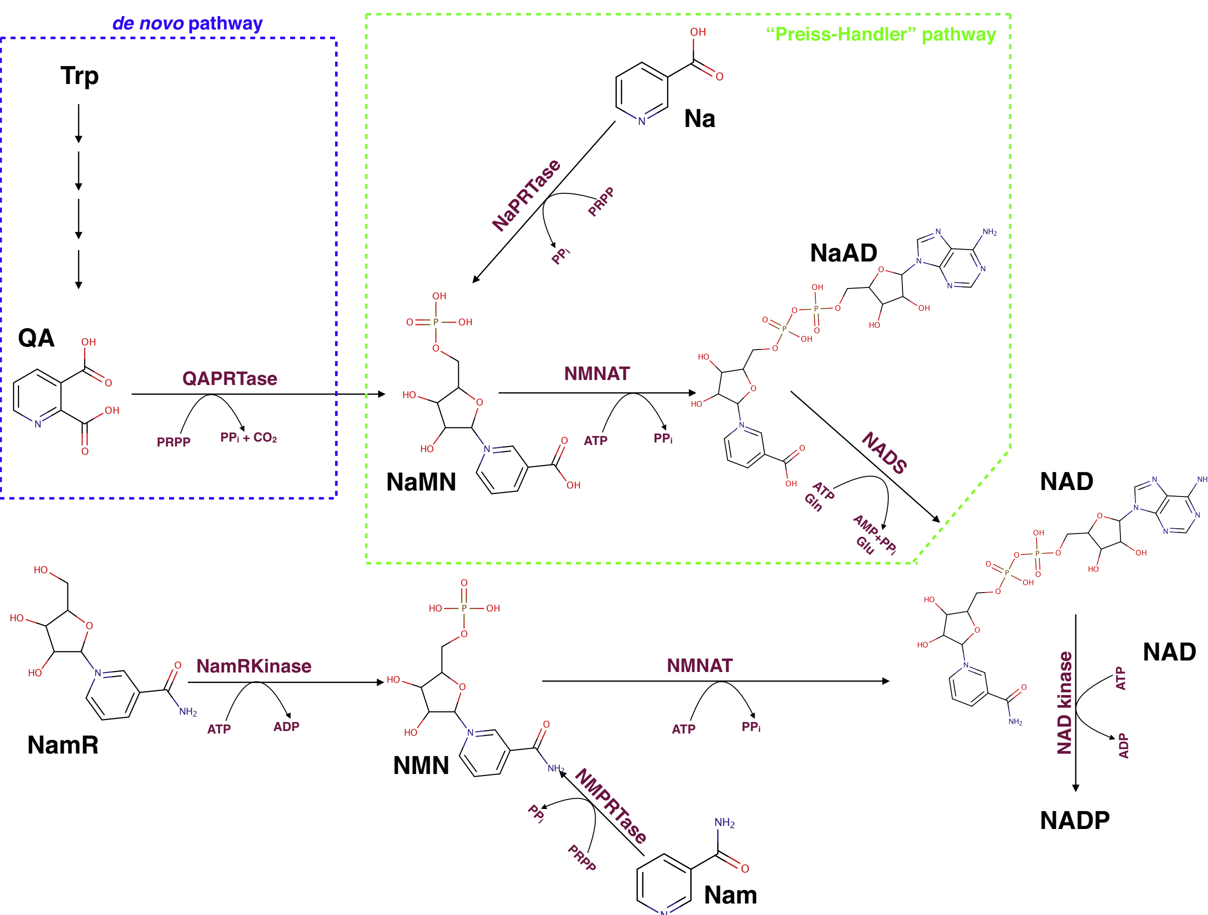

Fig. 1. Metabolic pathway for NAD(P) biosynthesis in humans. NAD biosynthesis starting from four different sources of the pyridine ring, namely QA, Na, Nam and NamR, through four distinct ways. The *de novo* pathway (blue dotted line) allows NAD biosynthesis starting from QA derived from Trp and Na is processed to NAD through the Preiss–Handler pathway enzymes (green dotted line). Through a salvage pathway Nam and NamR can be transformed in NMN by NMPRTase and NamRKinase respectively. Finally, some of the cellular NAD can be converted into NADP by NAD kinase (EC 2.7.1.23). QAPRTase, quinolinic acid phosphoribosyltransferase (EC 2.4.2.19); NaPRTase, nicotinic acid phosphoribosyltransferase (EC 2.4.2.11); NMPRTase, nicotinamide phosphoribosyltransferase (EC 2.4.2.12); NamRKinase, nicotinamide riboside kinase (EC 2.7.1.22); NMNAT nicotinamide mononucleotide adenylyltransferase (EC 2.7.7.1); NADS, NAD synthetase (EC 6.3.5.1).

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