

Expert Opinion

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Nicotinamide adenine dinucleotide metabolism as an attractive target for drug discovery

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Nicotinamide adenine dinucleotide (NAD⁺) has crucial roles in many cellular processes, both as a coenzyme for redox reactions and as a substrate to donate ADP-ribose units. Enzymes involved in NAD⁺ metabolism are attractive targets for drug discovery against a variety of human diseases, including cancer, multiple sclerosis, neurodegeneration and Huntington's disease. A small-molecule inhibitor of nicotinamide phosphoribosyltransferase, an enzyme in the salvage pathway of NAD⁺ biosynthesis, is presently in clinical trials against cancer. An analog of a kynurenine pathway intermediate is efficacious against multiple sclerosis in an animal model. Indoleamine 2,3-dioxygenase plays an important role in immune evasion by cancer cells and other disease processes. Inhibitors against kynurenine 3-hydroxylase can reduce the production of neurotoxic metabolites while increasing the production of neuroprotective compounds. This review summarizes the existing knowledge on NAD⁺ metabolic enzymes, with emphasis on their relevance for drug discovery.

Keywords: ADP-ribosylation, calcium signaling, FK866, indoleamine 2,3-dioxygenase, inosine mononucleotide dehydrogenase, kynurenines, multiple sclerosis, nicotinamide phosphoribosyltransferase, pre-B-cell colony-enhancing factor, protein deacetylation, sirtuins, tiazofurin

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1. Introduction

Nicotinamide adenine dinucleotide (NAD⁺) has crucial roles in many biochemical and biologic processes. Its function as a cofactor in oxidation/reduction (hydride transfer) reactions is well established. NAD⁺ can also be used as a substrate in several biochemical reactions, including mono- and poly-ADP-ribosylation, protein deacetylation, and ADP-ribose cyclization (**Figure 1**). A common feature of these reactions is that NAD⁺ donates its ADP-ribose group, such that the glycosidic bond between nicotinamide (NM) and ribose is broken, destroying the parent NAD⁺ molecule. The physiologic and pathologic importance of these reactions is becoming increasingly appreciated over the recent years [1-7]. As discussed in more detail in Section 2, poly ADP-ribosylation is crucial for DNA repair and genome stability (Section 2.1) [8-13]; protein/histone deacetylation is important for aging and longevity (Section 2.2) [14-19]; and ADP-ribose cyclization produces mediators of calcium signaling (Section 2.5) [1,20-22].

To replenish the NAD⁺ destroyed by these reactions, cells need to acquire new NAD⁺ molecules from the diet or from biosynthesis. Several different pathways are known for the biosynthesis of NAD⁺ [1,2,23]. In most eukaryotes, tryptophan is the precursor for the *de novo* pathway of NAD⁺ production (**Figure 2**). The

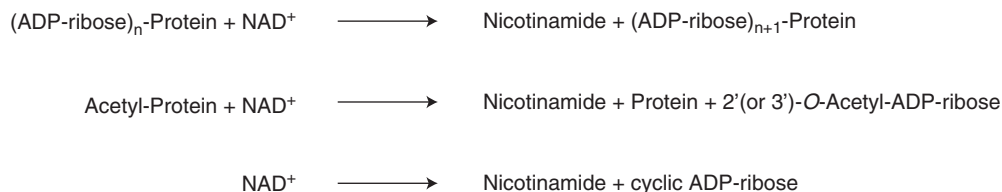


Figure 1. Chemical reactions in which NAD⁺ serves as a donor of ADP-ribose units.

kynurenine pathway converts tryptophan to quinolinic acid (QA) in five steps. The first step in this pathway is the oxidation of Trp to produce formylkynurenine, catalyzed by the enzymes tryptophan 2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase (IDO) (Figure 2) [1,2,24]. TDO is primarily expressed in the liver whereas IDO is expressed in most tissues. Kynurenine formamidase (KFase) then catalyzes the hydrolysis of formylkynurenine to produce kynurenine. The remaining enzymes of the pathway are kynurenine 3-hydroxylase (K3H, also known as kynurenine 3-monooxygenase, KMO), kynureninase and 3-hydroxyanthranilate 3,4-dioxygenase (3HAO). Crystal structure information is now available for most of the enzymes in this pathway, including TDO [25,26], IDO [27], kynureninase [28] and 3HAO [29]. The kynurenine pathway is the principal route for the metabolism of tryptophan into other chemical entities. However, not all the tryptophan flux through this pathway leads to NAD⁺ production, and some of the metabolites themselves (kynurenines) have important cellular functions [4,24,30,31]. In fact, the kynurenine pathway may be decoupled from NAD⁺ biosynthesis in *Drosophila* and *Caenorhabditis elegans* [23].

Quinolinic acid phosphoribosyltransferase (QAPRTase) catalyzes the formation of nicotinic acid mononucleotide (NAMN) from QA and phosphoribosylpyrophosphate (PRPP) (Figure 2) [32-34]. The addition of adenine mononucleotide to NAMN is facilitated by the enzyme nicotinic acid/nicotinamide mononucleotide adenylyltransferase (NMNAT) [31,35-37], producing nicotinic acid adenine dinucleotide (NAAD⁺). Finally, the carboxylic group in nicotinic acid is amidated by the enzyme NAD⁺ synthetase (NADS) to produce NAD⁺, using glutamine or free ammonia as the nitrogen donor [38-41].

Besides the *de novo* biosynthesis pathway, NAD⁺ can also be produced by salvage pathways [1,2,23]. The Preiss-Handler pathway starts with the conversion of nicotinic acid (NA) to NAMN [42], catalyzed by the enzyme nicotinate phosphoribosyltransferase (NAPRTase) (Figure 2) [43-45]. Another salvage pathway can convert NM, the breakdown product of NAD⁺, into nicotinamide mononucleotide (NMN) by the enzyme nicotinamide phosphoribosyltransferase (NMPRTase) [46-53]. NMN can then be directly turned into NAD⁺ by NMNAT, which is active towards both NAMN and NMN substrates. Alternatively, NM can be deamidated

Table 1. Potential drug discovery targets in NAD⁺ metabolism.

Target	Potential therapeutic areas
NMPRTase	Cancer
NMNAT (overexpression or agonists)	Neurodegenerative diseases
IDO	Cancer Autoimmune diseases Cataract (?)
K3H	CNS (neuroprotective agents) Huntington's disease Cataract
Kynurenine pathway inhibition (Kynurenine analogs)	Multiple sclerosis
IMPDH (NAD ⁺ analogs)	Cancer Autoimmune diseases Hepatitis C virus infection Other diseases (?)

IDO: Indoleamine 2,3-dioxygenase; IMPDH: Inosine mononucleotide dehydrogenase; K3H: Kynurenine 3-hydroxylase; NMNAT: Nicotinic acid/nicotinamide mononucleotide adenylyltransferase; NMPRTase: Nicotinamide phosphoribosyltransferase.

to NA [54,55], which can then enter the Preiss-Handler pathway. Analysis of genome sequences suggests that these two pathways are often mutually exclusive; many organisms contain either NM deamidase or NMPRTase [23].

Nicotinamide riboside (NR) can also be used as a precursor for NAD⁺ biosynthesis, and NR kinase (NRK) catalyzes the phosphorylation of NR to produce NMN (Figure 2) [56-59]. Another important function of NRK is the phosphorylation of the compounds tiazofurin, benzamide riboside and their analogs, which enables their further conversion into NAD⁺ analogs (TAD and BAD) by NMNAT [56,60]. These analogs are potent inhibitors of inosine mononucleotide dehydrogenase (IMPDH), the rate-limiting enzyme for guanine nucleotide biosynthesis [61]. Many cancer cells have elevated activity of this enzyme, and tiazofurin has been approved as an orphan drug for the treatment of chronic myelogenous leukemia [62-65]. IMPDH inhibitors are also being developed for the treatment of autoimmune diseases and hepatitis C virus infection [66,67]. As the active forms of these compounds are NAD⁺ analogs, some amount of toxicity is associated with their administration in the clinic [68].

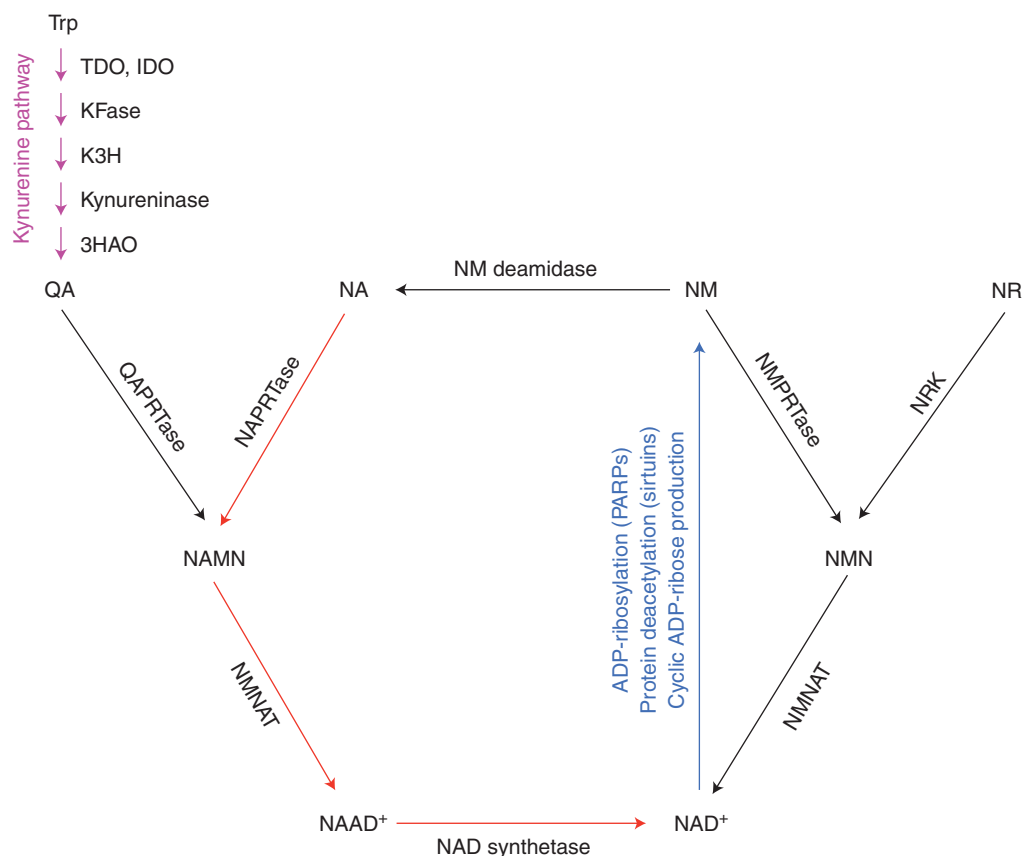


Figure 2. NAD⁺ metabolic pathways. Only those reactions that are directly linked to NAD⁺ metabolism are shown. The Preiss-Handler pathway is indicated in red and the kynurenine pathway in magenta. Reactions that break NAD⁺ down to ADP-ribose and NM are shown in blue.

3HAO: 3-Hydroxyanthranilate 3,4-dioxygenase; IDO: Indoleamine 2,3-dioxygenase; K3H: Kynurenine 3-hydroxylase; KFase: Kynurenine formamidase; NA: Nicotinic acid; NAAD⁺: Nicotinic acid adenine dinucleotide; NAMN: Nicotinic acid mononucleotide; NAPRTase: Nicotinate phosphoribosyltransferase; NM: Nicotinamide; NMN: Nicotinamide mononucleotide; NMPRTase: Nicotinamide phosphoribosyltransferase; NR: Nicotinamide riboside; NRK: Nicotinamide riboside kinase; PARP: Poly(ADP-ribose) polymerase; QA: Quinolinic acid; QAPRTase: Quinolinic acid phosphoribosyltransferase; TDO: Tryptophan 2,3-dioxygenase.

This review concentrates on enzymes directly involved in NAD⁺ metabolism, with special focus on those that could be potential targets for drug discovery (Table 1). Detailed discussions of the biochemical and biologic functions of these enzymes can be found in many excellent reviews [1-7], but are unfortunately beyond the scope of this review. Similarly, many of the enzymes that use NAD⁺ as a substrate, especially the PARPs and sirtuins, are important drug discovery targets as well, but space limitations preclude a detailed discussion on those topics here.

2. NAD⁺ metabolism and diseases

2.1 NAD⁺ metabolism and cancer

Tumor cells have a high rate of NAD⁺ turnover due to elevated ADP-ribosylation activity, predominantly mediated by the poly(ADP-ribose) polymerases (PARPs) [8-13]. Poly ADP-ribosylation of specific target proteins is crucial for genome stability, DNA repair, telomere maintenance, cell death and other biological functions. Proteins that bind mono

and poly(ADP-ribose) have been identified, suggesting that these molecules may have important cellular functions themselves [69,70].

DNA damage can stimulate NAD⁺ biosynthesis [71]. Expression levels of NMPRTase, which is the rate-limiting enzyme in the salvage pathway from the breakdown product NM [48], are upregulated in colorectal cancers [72,73], suggesting that NMPRTase may be crucial for maintaining cellular NAD⁺ levels in tumors. NMPRTase-deficient mice die during early embryogenesis [52].

FK866 is a potent small-molecule inhibitor of human NMPRTase, and the consequent reduction in NAD⁺ levels can cause apoptosis of tumor cells while having little (toxic) effects on normal cells [13,74,75]. This validates NMPRTase as a target for the development of novel anticancer therapeutic agents. FK866 also has antiangiogenic effects in a murine cancer model [76]. The compound (now renamed APO866) is in Phase II clinical trials against cancer. It could also be used in combination with other anticancer agents to potentiate their therapeutic effects [13,77].

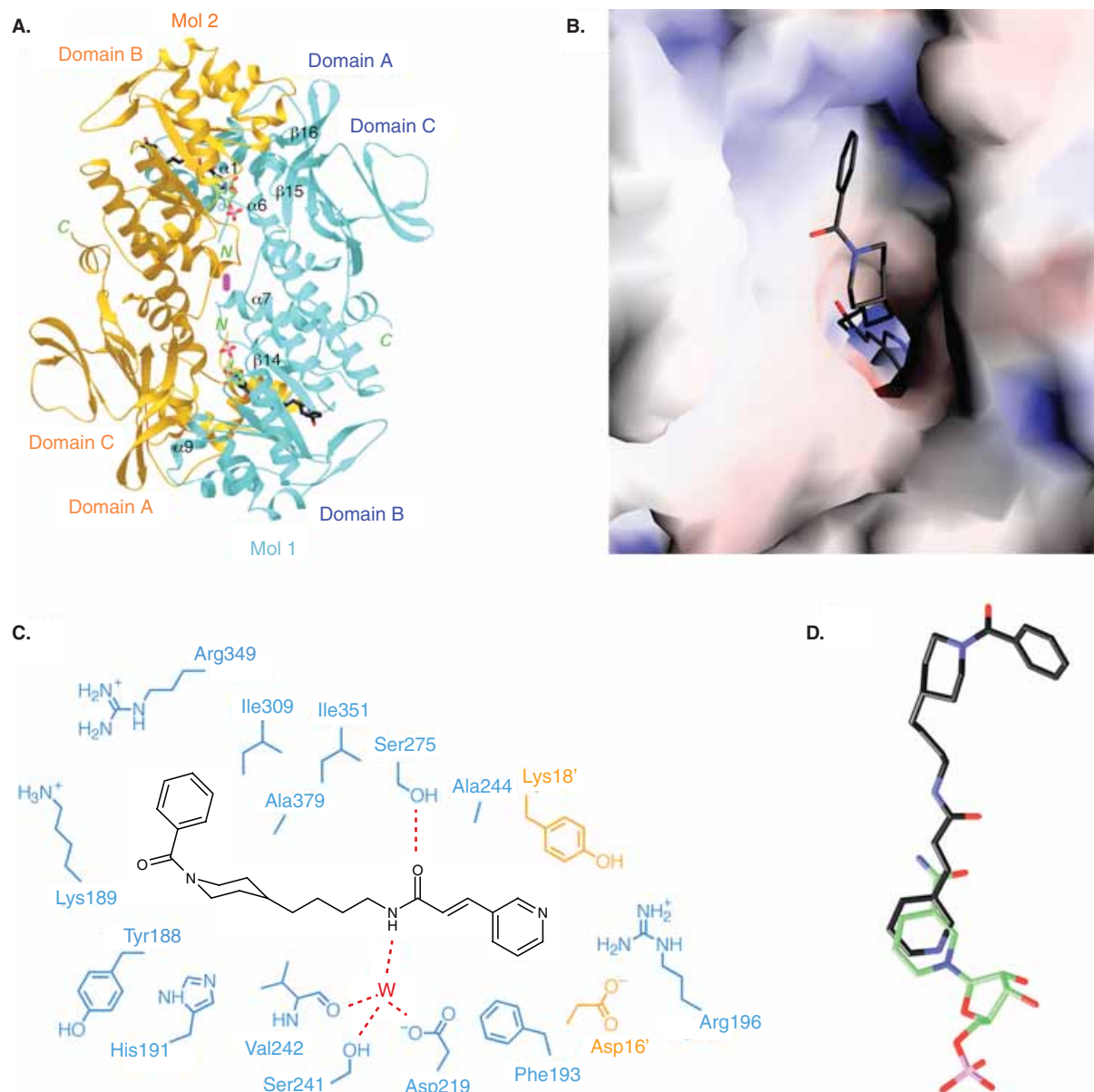


Figure 3. Structure and inhibition of NMPRTase. **A.** Schematic drawing of the dimer of human NMPRTase. The bound positions of NMN (in green) and FK866 (black) are shown for reference. **B.** FK866 is bound in a tunnel in the dimer interface of NMPRTase. **C.** Schematic drawing of the interactions between FK866 and NMPRTase. A water molecule is shown as W and dashed red lines indicate hydrogen bonds. **D.** Overlay of the binding modes of FK866 (in black) and NMN (in green). Panels **A** and **D** produced with Ribbons [128], and panel **B** with Grasp [129].

NMN: Nicotinamide mononucleotide; NMPRTase: Nicotinamide phosphoribosyltransferase.

The molecular basis for the inhibitory activity of FK866 has been revealed by the crystal structures of its complex with human or rat NMPRTase [46,49]. The active site of the enzyme is located at the interface of an intimately-associated dimer of the enzyme (Figure 3A) [46,49,50]. FK866 is bound in a tunnel in the center of this interface (Figure 3B), with hydrogen-bonding, π -stacking and van der Waals interactions (Figure 3C). The pyridyl ring of the inhibitor is sandwiched

between the side chains of Phe193 in one monomer and Tyr18 in the other. This is exactly the position where the nicotinamide ring of the substrate is located (Figure 3D). Therefore, the structure shows that FK866 is a competitive inhibitor versus the NM substrate.

Earlier kinetic studies demonstrated a decrease in the maximum initial velocity (V_{max}) of the enzyme in the presence of FK866, and a noncompetitive mechanism of inhibition was

proposed [13]. The structural information suggests a different interpretation of the kinetic data. FK866 is a tight-binding inhibitor of NMPRTase, with very slow off rate [46]. The apparent decrease in the V_{\max} is due instead to the sequestration of the active enzyme into the inactive enzyme-inhibitor complex in the kinetic experiments.

The structural information also defines the molecular mechanism for the specificity of FK866 towards NMPRTase [46,49]. Despite sharing little amino acid sequence homology with QAPRTase and NAPRTase, NMPRTase has a similar overall structure as these other two PRTases. However, the tunnel in the dimer interface exists only in NMPRTase, as there are also significant structural differences among the enzymes. These include a movement of a β -strand by ~ 2 Å and the replacement of several small residues in the tunnel in NMPRTase by larger residues in the other two PRTases. In fact, single-site mutations in the tunnel of NMPRTase can abolish the binding of FK866 [46].

Structural comparisons show that Asp219 in NMPRTase may be a determinant of the substrate specificity of this enzyme [46,50]. NMPRTase has high affinity towards the NM substrate, with K_m of ~ 2 μM [13,46-48], but it is inactive towards the NA substrate. The Asp219 side chain is hydrogen-bonded to the amide group of NM, suggesting an electrorepulsive interaction with NA in this active site. Mutation of Asp219 to Ser, which is present in NAPRTase, can enhance the activity of NMPRTase towards the NA substrate while maintaining the activity towards the NM substrate [46]. This suggests that additional changes in the enzyme are needed to discriminate against the NM substrate.

A histidine residue in the active site of NMPRTase may become phosphorylated during catalysis [46,50], and a phosphate group is bound near this residue in the structure of the NMN complex [46], possibly mimicking the phosphorylation. Phosphorylation of the equivalent His residue in NAPRTase can enhance the activity of that enzyme [43,78].

NMNAT may also be an attractive target for cancer therapy [31]. As discussed earlier, it catalyzes the conversion of the anticancer drug tiazofurin to its active form (TAD). In addition, it has been found that the expression level of NMNAT is low in cancer cells (except Burkitt's lymphoma and chronic myelogenous leukemia) [79]. However, the upstream NMPRTase is overexpressed in some cancers. A careful study correlating the expression levels of these two enzymes in different cancer cells could be very useful.

Three isoforms of NMNAT have been identified in humans, NMNAT-1, NMNAT-2 and NMNAT-3, with different tissue distribution and cellular localization [1]. All three enzymes can use either NMN or NAMN as the substrate, with similar K_m and k_{cat} values. Crystal structures of NMNAT-1 and NMNAT-3 are currently known, and the binding modes of NAD^+ , NAAD^+ and TAD have been determined [31,35-37]. The monomer of NMNAT contains a central parallel β -sheet that is surrounded by helices and the active site is located at the C-terminal end of this β -sheet. The

structural information elucidates the molecular mechanism for the dual specificity of this enzyme, as well as providing a foundation for structure-based drug design.

Enzymes in the kynurenine pathway may also be targets for anticancer agents. IDO is constitutively expressed in many human cancers and helps these cells escape the host immune system [80-82]. IDO inhibitors can potentiate chemotherapy in a breast cancer model [83]. As this is mostly linked to the effects of IDO on the immune system, it will be discussed in more detail in Section 2.4.

2.2 NAD^+ metabolism and aging

Sirtuins, a family of NAD^+ -dependent histone/protein deacetylases, are crucial for lifespan extension and the effects of caloric restriction in many organisms, including yeast, *C. elegans* and *Drosophila* [14-19]. Activation of sirtuins can deacetylate histones and promote the formation of silent heterochromatin, which is linked to the longevity effects.

Changes in NAD^+ level, $\text{NAD}^+:\text{NADH}$ ratio, or NM level have been proposed as possible mechanisms for the activation of sirtuins in yeast [16,18,84-87]. In mammals, Sirt1 activation is regulated by NMPRTase [7,48]. The reaction catalyzed by sirtuins leads to the breakdown of NAD^+ into NM, and the acetyl group is transferred to the 2'- and, subsequently, the 3'-hydroxyl of the ADP-ribose fragment (Figure 1). NM is a physiologic inhibitor of sirtuins, and removal of this inhibitor may be the master regulatory mechanism for the longevity effects mediated by the sirtuins [16]. The 2'/3'-*O*-acetyl ADP-ribose product may also be involved in gene silencing by binding to heterochromatin proteins [88-90].

In yeast, NM is converted to NA by NM deamidase (known as Pnc1 in yeast), which controls lifespan extension in that organism [85]. NAD^+ level in yeast nucleus is reduced under caloric restriction [86]. Pnc1 does not have a sequence homolog in animals [23]. Instead, NM may be directly salvaged back to NAD^+ through NMPRTase (Figure 2). It has been suggested that NMPRTase could be a crucial enzyme for longevity in mammals [16].

2.3 NAD^+ metabolism and neurodegenerative disorders

Enzymes involved in NAD^+ metabolism also have important roles in the nervous system. Increased activity of NMNAT-1 appears to protect against axonal degeneration, possibly through the activation of the sirtuin SIRT1 [91]. Stimulation of other enzymes in the NAD^+ biosynthetic pathways, including NMPRTase and NAPRTase, or the introduction of precursors of NAD^+ biosynthesis (NAMN, NMN), can also delay axonal degeneration [92]. Degenerating axons have reduced NAD^+ levels and the restoration of NAD^+ levels through activation of NAD^+ biosynthetic enzymes may be the mechanism for the protective effects [93]. Restoring cellular NAD^+ levels can also protect against axonal degeneration in experimental autoimmune encephalomyelitis (EAE), an

animal model for multiple sclerosis in humans [94,95]. Similarly, depletion of NAD⁺ levels, for example through excessive PARP activation, may be linked to ischemic brain injury [96,97].

However, the role of NAD⁺ in neuroprotection is still controversial. It has been suggested that the fusion with a ubiquitin assembly protein, as seen in Wallerian degeneration slow (Wlds) mice [98,99], is required for the protective effect [100,101]. The Wlds protein contains 70 residues from Ube4b fused to the N-terminus of NMNAT-1. Studies in *Drosophila* indicate that a catalytically inactive NMNAT still possesses neuroprotective effects [102].

In the kynurenine pathway, QA and some of the intermediates are known to be neurotoxins [1,24,103]. They may have important roles in neurodegenerative disorders such as Alzheimer's disease and schizophrenia [24,30,104,105]. On the other hand, kynurenic acid (KA), which can be produced from kynurenine by the enzyme kynurenine aminotransferase (KAT) [106,107], has neuroprotective effects [108]. Therefore, inhibitors of the kynurenine pathway can alter the ratio of QA versus KA, and achieve different effects in the CNS. It is not clear at present whether or not the kynurenine pathway is linked to NAD⁺ biosynthesis in the brain.

2.4 NAD⁺ metabolism and autoimmune diseases

IDO has an important role in controlling host immune system [24,81,82,109,110]. IDO expression is induced by IFN- γ , bacterial or viral infections, and other immunologic and inflammatory conditions. Immune suppression mediated by IDO expression helps prevent allogeneic rejection of the fetus [111], as well as the escape of cancer cells from host immunity. Both the reduction of local Trp levels and the production of various (toxic) metabolites from the kynurenine pathway are important for the immunoregulatory effects of IDO activation. An analog of 3-hydroxyanthranilic acid can reverse paralysis in EAE mice, suggesting that inhibition of this pathway could be beneficial for the treatment of autoimmune diseases [112].

Human NMPRTase was first identified as a secreted growth factor for early B cells, and was named pre-B-cell colony-enhancing factor (PBEF) [53]. PBEF does not have growth effects on its own, but instead it can synergize the pre-B-cell colony formation activity of stem cell factor and IL-7. PBEF can inhibit neutrophil apoptosis under inflammatory conditions and sepsis [113]. The expression level of PBEF is significantly elevated in acute lung injury [114].

2.5 Other biologic effects of NAD⁺ metabolism

Cyclic ADP-ribose (cADPR) is a potent activator of intracellular calcium signaling [1,20-22]. This is a crucially important cellular process; unfortunately, a detailed discussion of this topic is beyond the scope of this review. The ADP-ribose cyclases (CD38) that produce cADPR (Figure 1) are transmembrane proteins. Unexpectedly, the active sites of these enzymes are in the extracellular space (or vesicle matrix), and

the structure of the extracellular domain of CD38 has been determined [115,116]. CD38 also catalyzes an exchange reaction between NADP⁺ and NA to produce NAADP⁺, another potent activator of calcium signaling. Membrane transporters for both NAD⁺ and cADPR have been identified [20,21]. This suggests the exciting possibility of other roles for NAD⁺, and possibly NAD⁺ biosynthetic enzymes (such as NMPRTase), outside the cell. NMPRTase is ubiquitously expressed but lacks a secretion signal sequence. It is found intracellularly as well as extracellularly [47,51-53]. It is not clear how the protein is actively secreted from the cell.

Recently, NMPRTase was identified as an adipokine secreted by visceral fat tissues and named visfatin [52], and its expression levels may be elevated in diabetes and obesity [117,118]. Visfatin was also reported to have insulin-mimetic effects and can bind to the insulin receptor at a site distinct from that for insulin [52], although no further data have appeared that confirm these original observations [117].

Kynurenine and some of its derivatives function as UV filters in human lens and protect the retina from UV light [24,108]. The compounds are synthesized in the eye, with IDO catalyzing the first reaction from the Trp substrate. Kynurenine could also mediate the covalent modifications of lens proteins that contribute to age-related cataract [24,108].

NM is a weak product inhibitor of PARPs, sirtuins and CD38, and could be used as a therapeutic agent [1]. It is likely that NM exerts its beneficial effects by simultaneously regulating a collection of cellular processes [119].

3. Expert opinion and discussion

NAD⁺ is a central player in many cellular processes, both redox reactions where NAD⁺ participates in hydride transfer and nonredox reactions where NAD⁺ serves as a donor of ADP-ribose units. NAD⁺ analogs, such as tiazofurin have been found to be efficacious in the clinic in the treatment of some human cancers. At the same time, toxicity is a problem with such analogs, as they have the potential of disrupting other NAD⁺-dependent processes in the cell. This situation is somewhat reminiscent of the development of selective protein kinase inhibitors by using the ATP binding pocket. Despite original uncertainty, it is now clear that such compounds can be developed that target only a selected number of protein kinases, for example by establishing additional interactions with regions of the kinase that are outside the ATP binding envelope [120-122]. The same may hold true for NAD⁺ analogs. By designing compounds that can also interact with other regions of a targeted NAD⁺-dependent enzyme, highly selective inhibitors could be obtained that may have reduced toxicity in patients.

The pursuit of NAD⁺ metabolic enzymes themselves for drug discovery is still in its early stages. However, it is clear from the descriptions in the previous section that many of these enzymes are attractive targets (Table 1) [82,108,109,123]. An advantage of targeting these enzymes is that many of

them are induced primarily in the disease state, such that specific inhibitors will probably have less mechanism-based toxic side effects.

NMPRTase appears to be a highly attractive target, offering a novel mechanism for cancer treatment. Its inhibitor, FK866 (APO866), has entered Phase II clinical trials against several forms of human cancers. The molecular mechanism of the inhibition as well as the specificity of this compound towards NMPRTase has been defined by structural studies [46,49]. This should help the design and development of new chemical entities that target this enzyme. Such molecules should be long, mostly linear in shape, with π -stacking interactions with Phe193 and Tyr18 and hydrogen-bonding to Ser275 (Figure 3C). In addition, a compound that can replace the sole water molecule in the binding site (Figure 3C) could benefit from the resulting entropic effect. The compound could also establish interactions with residues in the binding site for the ribose and phosphate groups of NMN (Figure 3D). This part of the pocket is highly hydrophilic and introduction of such hydrophilic moieties could enhance the pharmacokinetic properties of the inhibitors. A potential concern with NMPRTase inhibitors is that PARPs are activated in stress conditions as well. It remains to be seen whether or not NMPRTase is also activated and what the effects are for its inhibition in these conditions.

Enzymes in the kynurenine pathway have significant potential as pharmaceutical targets, especially against cancer, Huntington's disease, multiple sclerosis, schizophrenia and other neurodegenerative and immunologic diseases (Table 1). Among these enzymes, IDO has been studied the most extensively [24,82,123]. Several inhibitors of IDO, including 1-methyltryptophan (1-MT), have been identified [82,110]. Natural product inhibitors of IDO have also been reported [124,125]. The best clinical outcome could be obtained if IDO inhibitors are used in combination with other therapeutic agents [82]. IDO and TDO share very low overall sequence homology, and recent structural studies show that there are significant differences in the active site region between the two enzymes [25]. Therefore, it should be feasible to develop compounds that can distinguish between the two enzymes, such as 1-MT [25].

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The 3-hydroxykynurenine product of K3H in the kynurenine pathway (Figure 2) has neurotoxicity in the CNS [1]. Therefore, K3H is an attractive target for the development of neuroprotective agents [109,123,126]. K3H inhibition should also lead to the accumulation of kynurenine and its transamination product KA, further enhancing the neuroprotective effects of K3H inhibitors. Crossing the blood-brain barrier is an important requirement for these compounds. K3H inhibition may also be beneficial for the treatment of Huntington's disease [127]. Kynureninase inhibitors have also been developed, although their cellular effects remain to be studied [109,123]. An analog of 3-hydroxyanthranilic acid showed promising effect in a mouse model of multiple sclerosis [112].

In addition to the inhibitors described above, compounds that can stimulate NAD⁺ biosynthesis may have potential as neuroprotective agents. As discussed in Section 2.3, if the effects of the Wlds protein are truly due to NMNAT-1 overexpression, it could be possible that agonists of this enzyme and possibly the upstream NMPRTase could also be neuroprotective. In fact, the intermediates of this pathway (or NAD⁺ itself) do have neuroprotective effects [91-93].

This is an exciting time in the studies of NAD⁺ metabolism and its relevance to diseases. The increasing recognition of the importance of NAD⁺ and its metabolites in pathologic conditions should lead to new efforts at discovering and developing inhibitors against enzymes involved in NAD⁺ metabolism. The availability of structural information on most of these enzymes should greatly aid this process. It may be anticipated that the coming years will see the identification of highly potent and selective inhibitors against these enzymes, which could prove efficacious in the treatment of a variety of human diseases. Such inhibitors should also be very useful in helping to further elucidate the biologic functions of the enzymes in NAD⁺ metabolism.

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