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Review

Enzymology of NAD^+ homeostasis in man

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Abstract. This review describes the enzymes involved in human pyridine nucleotide metabolism starting with a detailed consideration of their major kinetic, molecular and structural properties. The presentation encompasses all the reactions starting from the de novo pyridine ring formation and leading to nicotinamide adenine dinucleotide (NAD^+) synthesis and utilization. The regulation of NAD^+ homeostasis with respect to the physiological role played by the enzymes both utilizing NAD^+ through the nonredox NAD^+-dependent reactions and catalyzing the recycling of the common product, nicotinamide, is discussed. The salient features of other enzymes such as NAD^+ pyrophosphatase, nicotinamide mononucleotide 5′-nucleotidase, nicotinamide riboside kinase and nicotinamide riboside phosphorylase, described under 'miscellaneous', are likewise presented.

Key words. NAD^+ homeostasis; pyridine nucleotides; structure biology; recombinant enzymes; chromatin expression.

Introduction

Over the past decades, growing evidence accumulated on the paramount importance of the nonredox NAD^+ functions in the physiology of the cell. NAD^+ serves as the substrate for reactions involved in important protein covalent modifications, like adenosine 5′-diphosphate (ADP)-ribosylation reactions, catalyzed by two classes of enzymes: mono-ADP-ribosyltransferases (ARTs) and poly(ADP-ribose) polymerases (PARPs) [1]. It has been shown that PARP activation plays a decisive role in NAD^+ depletion, which is involved in the process of cellular death in many tissues and cells [2]. The maintenance of NAD^+ levels is also functional to the synthesis of signaling molecules such as cyclic ADP-ribose (cADPR) and nicotinic acid adenine dinucleotide phosphate ($NaADP^+$). Both metabolites are known to be Ca^{2+} mobilizing agents from intracellular stores acting via distinct mechanisms [3]. An important role of a new member of the PARPs family, tankyrase, has recently been described [4]. This enzyme, in fact, exerts an indispensable function in the maintenance of genome integrity through the regulation of telomere length homeostasis [5]. About three years ago it was discovered that NAD^+ is also an essential cofactor in histone/protein deacetylation reactions catalyzed by a new class of enzymes known as the SIR2 family, the products of silent information regulator genes [6]. This discovery suggests that silencing might be related to the metabolic cell rate and hence to NAD^+ concentrations, whose levels, therefore, seem to be a function directly proportional to a longer life span. The very recent observation that the SIR2 human homologue (SIRT1) acts as an NAD^+-dependent p53 deacetylase, thus inhibiting p53 cancer-preventing function, raises intriguing questions on the interrelationships governing aging with respect to cancer [7, 8]. The production of reactive oxygen species mediated by the action of free radicals causes irreversible cellular damage, which plays a major role in the pathogenesis of many diseases (e.g., atherosclerosis and neurodegenerative syndromes) generally associated with an altered cellular redox state [9]. The cellular redox state,

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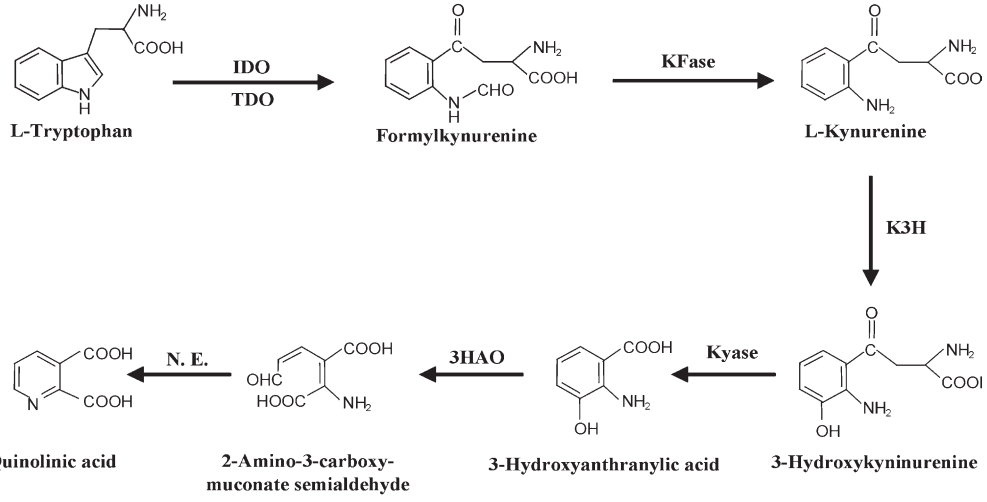
also indicated as the cellular redox potential, is related to the ratio of the pyridine dinucleotides in their respective oxidized and reduced forms (NAD^+/NADH and $NADP^+$/NADPH). It is conceivable that an altered regulation of the synthesis and degradation of these metabolites impairs the redox state of the cell and likely contributes to the mechanisms underlying the pathogenesis of the above-mentioned diseases. Even though little time has elapsed since the last comprehensive report on the enzymology of NAD^+ synthesis was published [10], the amount of data accumulated, ranging from archaea to eubacteria to yeasts to humans, is too large to be reviewed in a single chapter. Therefore, the present report will be devoted to the enzymology of NAD^+ homeostasis in human.

De novo pyridine ring formation

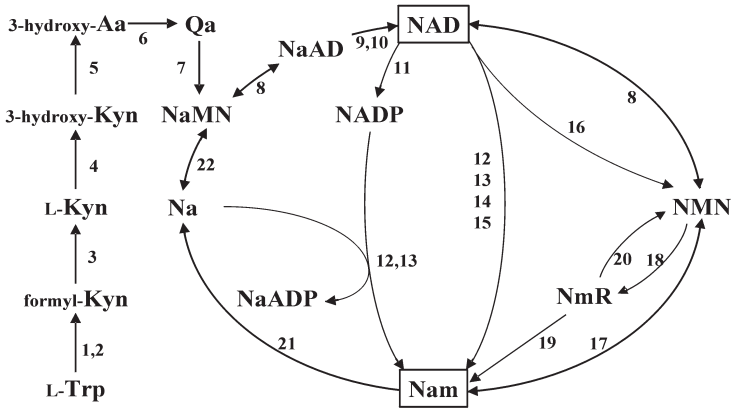
In case of restricted niacin availability, mammals are able to synthesize nicotinamide-containing nucleotides through the kynurenine pathway (scheme 1), whereby L-tryptophan is metabolized to quinolinate and subsequently to NAD^+ (scheme 2) [11]. Even though the liver is a major systemic site of the kynurenine pathway [12], the majority of the reports describes the central role played by many metabolites formed along the pathway in the human central nervous system (reviewed in [13–15]).

Indoleamine 2,3-dioxygenase and tryptophan 2,3-dioxygenase

In mammals, indoleamine 2,3-dioxygenase (IDO) (EC 1.13.11.17) and tryptophan 2,3-dioxygenase (TDO) (EC 1.13.11.11) catalyze the formation of N-formylkynurenine by cleavage of the pyrrole ring of L-tryptophan and the concomitant insertion of molecular oxygen (scheme 2, reactions 1 and 2). This reaction represents the first rate-limiting step in the tryptophan catabolic pathway. In humans, IDO is ubiquitous in extrahepatic tissues, where it is induced mainly by interferon-γ under a variety of physiological and pathological conditions, including antimicrobial and antitumor defense, immunoregulation and antioxidant activity [16–18]. Furthermore, IDO induction in several viral [19–21], bacterial [22–24] and intracellular protozoan [25] infections, as well as in tumor repression [26, 27], has been widely documented. Its positive role in the defense mechanisms may be related to the enzyme-mediated depletion of tryptophan, the least abundant among essential amino acids, and to the accumulation of its toxic catabolites [28–33]. Recently, many papers appeared on the important role of this enzyme as a modulator of immunological responses: cells expressing IDO are capable of inhibiting T-cell proliferation in vitro, suppressing maternal antifetal immunity during pregnancy and inhibiting T-cell-mediated responses to tumor-associated antigens [34–37]. The IDO-dependent immunoregulation seems to be related to the involvement of tryptophan degradation in T-cell apoptosis [38, 39]. In addition, IDO induction in the brain has been implicated in the pathogenesis of many neurological disorders ascribed to the accumulation of two neurotoxic tryptophan metabolites, quinolinic acid and 3-hydroxykynurenine [40]. Finally, IDO is a unique enzyme in that it can utilize as the substrate, in place of oxygen, the superoxide anion radical ($0_2^{\cdot-}$), thus acting as a radical scavenger. Therefore, its induction during inflammatory conditions has been proposed to prevent oxidative damage to host tissues



Scheme 1. Reaction pathway for quinolinate synthesis. Abbreviations used: IDO, indoleamine 2,3-dioxygenase; TDO, tryptophan 2,3-dioxygenase; KFase, kynurenine formamidase; K3H, kynurenine 3-hydroxylase; Kyase, kynureninase; 3HAO, 3-hydroxyanthranilate 3,4-dioxygenase; N.E., nonenzymatic.



Scheme 2. Panel *A*: Diagrammatic representation of human NAD^+ metabolism. Panel *B*: List of the enzymatic reactions involved in human NAD^+ metabolism. The reactions indicated refer to those included in the classification of the 'Enzyme-Catalyzed Reactions' recommended by the International Union of Biochemistry and Molecular Biology. Abbreviations used: Kyn, kynurenine; Aa, anthranilic acid; Qa, quinolinic acid; Na, nicotinic acid; Nam, nicotinamide; NmR, nicotinamide riboside; PRPP, 5-phospho-α-D-ribose 1-diphosphate; R-1-P, D-ribose 1-phosphate; NaMN, nicotinate mononucleotide; $NaAD^+$, nicotinate adenine dinucleotide; $NaADP^+$, nicotinate adenine dinucleotide phosphate.

Scheme 2, panel B

No.	Enzyme designation	EC	Reaction
1	Indoleamine 2,3-dioxygenase	1.13.11.17	Indole + O_2 ⇔ 2-formylaminobenzaldehyde
2	Tryptophan 2,3-dioxygenase	1.13.11.11	L-Trp + O_2 ⇔ L-formylkynurenine
3	Kynurenine formamidase	3.5.1.9	N-Formyl-L-kynurenine + H_2O ⇔ formate + L-kynurenine
4	Kynurenine 3-hydroxylase	1.14.13.9	L-Kynurenine + NADPH + O_2 ⇔ 3-hydroxy-L-kynurenine + $NADP^+$ + H_2O
5	Kynureninase	3.7.1.3	3-hydroxy-L-Kynurenine + H_2O ⇔ 3-hydroxyanthranilate + L-Ala
6	3-Hydroxyanthranilate 3,4-dioxygenase	1.13.11.6	3-Hydroxyanthranilate + O_2 ⇔ 2-amino-3-carboxymuconate semialdehyde
7	Quinolinate phosphoribosyltransferase	2.4.2.19	Quinolinate + PRPP ⇔ NMN + PPi + CO_2
8	NMN adenylyltransferase	2.7.7.1	NMN + ATP ⇔ NAD^+ + PPi NaMN + ATP ⇔ $NaAD^+$ + PPi
9	NAD^+ synthetase (ammonia-dependent)	6.3.1.5	$NaAD^+$ + ATP + NH_3 ⇔ NAD^+ + AMP + PPi
10	NAD^+ synthetase (glutamine-hydrolyzing)	6.3.5.1	$NaAD^+$ + ATP + L-Gln + H_2O ⇔ NAD^+ + AMP + PPi + L-Glu
11	NAD^+ kinase	2.7.1.23	NAD^+ + ATP ⇔ $NADP^+$ + ADP
12	NAD^+ glycohydrolase	3.2.2.5	NAD^+ + H_2O ⇔ nicotinamide + ADP-ribose
13	$NAD(P)^+$ nucleosidase	3.2.2.6	$NAD(P)^+$ + H_2O ⇔ nicotinamide + ADP-ribose(2′-phosphate)
14	Poly(ADP-ribose) polymerase	2.4.2.30	NAD^+ + $(ADP\text{-}ribose)_n$ ⇔ nicotinamide + $(ADP\text{-}ribose)_{n+1}$
15	Mono ADPribosyltransferase	2.4.2.31	NAD^+ + L-Arg ⇔ nicotinamide + N(2)-(ADP-ribosyl)-L-Arg
16	NAD^+ pyrophosphatase	3.6.1.22	NAD^+ + H_2O ⇔ AMP + NMN
17	Nicotinamide phosphoribosyltransferase	2.4.2.12	Nicotinamide + PRPP ⇔ NMN + PPi
18	5′-Nucleotidase	3.1.3.5	NMN + H_2O ⇔ nicotinamide riboside + Pi
19	Nicotinamide nucleoside phosphorylase	2.4.2.1	Nicotinamide riboside + Pi ⇔ nicotinamide + R-1-P
20	Ribosylnicotinamide kinase	2.7.1.22	ATP + nicotinamide riboside ⇔ ADP + NMN
21	Nicotinamide deamidase	3.5.1.19	Nicotinamide + H_2O ⇔ nicotinate + NH_3
22	Nicotinate phosphoribosyltransferase	2.4.2.11	Nicotinate + PRPP ⇔ NaMN + PPi

[18, 41]. IDO has been purified to homogeneity and characterized from human placenta [30]. The enzyme is a monomeric hemoprotein of about 40,000 M_r, exhibiting a pI value of 6.9. In addition to L-tryptophan, the purified enzyme oxidizes D-tryptophan, 5-hydroxy-L-tryptophan, 5-hydroxy-D-tryptophan and tryptamine, with the K_m value for L-tryptophan (21 μM) much lower than that found for the other substrates [30]. IDO cDNA has been cloned [42, 43], and the corresponding gene has been mapped to chromosome 8p12-p11 [44, 45]. The pure recombinant protein, expressed in *Escherichia coli* [40, 46], shows the same catalytic properties as the native enzyme [40]. A phylogenetic analysis revealed 25–30% identity of the mammalian IDO with myoglobins of archaeogastropod molluscs, suggesting the existence of a common ancestral gene [47, 48]. Indeed, resonance Raman spectroscopy measurements performed on human IDO heme environment show features common to globins and peroxidases. This analysis suggests a catalytic mechanism whereby a crucial role is played by the strong proximal Fe-His346 bond and the unique distal environment provided by L-tryptophan [49]. The human IDO gene has been fully sequenced and proven to be present in a single copy in the human genome [50]. Its promoter contains interferon-stimulated response element sequences, found in the regulatory region of most interferon-inducible genes [51]. In the mammalian liver, the tryptophan oxidation is catalyzed by the cytosolic enzyme TDO, clearly distinct from IDO. The human TDO gene is localized to chromosome 4q31 [52], and its regulatory region contains a glucocorticoid response-like element [53]. The amino acid sequence of the human enzyme is 88% homologous to that of the rat enzyme [53]. The rat TDO is a tetrameric protein and requires 2 mol of heme per mol of protein for its catalytic function [54]. Different polymorphisms of the human TDO gene have been identified, and the association of these functional variants with psychiatric disorders, characterized by abnormalities in tryptophan and serotonin levels, has been suggested [55, 56].

Kynurenine formamidase

N-Formyl-L-kynurenine is hydrolyzed to L-kynurenine by the action of kynurenine formamidase (KFase) (EC 3.5.1.9) (scheme 2, reaction 3). Surprisingly, to our knowledge no data exist in the literature on the characterization of this enzyme in humans. However, KFase has been isolated and partially characterized from several mammalian sources [10, 57].

Kynurenine 3-hydroxylase

L-Kynurenine is converted to L-3-hydroxykynurenine through a reaction catalyzed by kynurenine 3-hydroxylase (K3H) (EC 1.14.13.9) in the presence of NADPH and molecular oxygen (scheme 2, reaction 4). The enzyme is a flavin adenine dinucleotide (FAD^+)-containing monooxygenase localized on the outer membrane of mitochondria. In human tissues, K3H is mostly expressed in the liver and placenta and at lower levels in the kidney [58]. The enzyme is absent in astrocytes and neurons [12, 59], whereas it has been found to be present in microglia [60], blood macrophages [12] and monocyte-derived macrophages, where it can also be induced by bacterial lipopolysaccharides and proinflammatory cytokines [61]. The observation that 3-hydroxykynurenine induces an H_2O_2-mediated neurotoxicity in the central nervous system [62, 63] has stimulated extensive research on this enzyme. Indeed, K3H represents an attractive pharmacological target in that its inhibition both prevents the formation of a neurotoxic molecule and fosters the production of kynurenic acid, a known neuroprotective metabolite [64], through a divergent route catalyzed by kynurenine aminotransferase [65]. This strongly stimulates the search for novel inhibitors of the enzyme [66]. The human K3H cDNA has been isolated and expressed both in human embryonic kidney fibroblasts (HEK-293) and COS-1 cells [58, 65]. The corresponding gene is localized to chromosome 1q43 [67]. Homogeneous recombinant K3H shows a M_r of about 55,000, a neutral pI value, and it contains one molecule of non-covalently-bound FAD^+ per molecule of enzyme protein [65]. It shows an optimum pH at 7.5 and uses NADPH more efficiently than NADH [65]. Kinetic measurements reveal a random catalytic mechanism with dissociation constant values for NADPH and kynurenine of 153 μM and 100 μM, respectively. Furthermore, inhibition studies indicate that pyridoxal-5′-phosphate (PLP) is a noncompetitive inhibitor with respect to both NADPH and kynurenine substrates, whereas Cl^- ions competitively inhibit K3H with respect to NADPH and are noncompetitive inhibitors with respect to kynurenine [65].

Kynureninase

L-3-Hydroxykynurenine is converted to 3-hydroxyanthranilic acid and L-alanine by kynureninase (Kyase) (EC 3.7.1.3) (scheme 2, reaction 5). The enzyme is pyridoxal-5′-phosphate (PLP)-dependent, and its activity is depressed during vitamin B-6 deficiency [68, 69]. Similar to IDO and K3H, human kynureninase is significantly activated by bacterial lipopolysaccharides and proinflammatory cytokines in human monocyte-derived macrophages [61]. The human cDNA codes for a protein of 456 residues, with a M_r of 52,400 [70, 71]. Northern blot analyses performed on different human organs and tissues reveal that the enzyme is ubiquitous, being mostly expressed in the liver, placenta and lung [70]. Analysis of the protein primary structure shows a significant similarity with other eukaryotic enzymes and reveals a PLP-

binding motif typical of the class-V aminotransferases [70, 71]. The protein has been expressed in eukaryotic systems [70–72], and the pure recombinant enzyme possesses a homodimeric structure [72]. The enzyme activity is deeply influenced by the ionic strength and shows an alkaline optimum pH [71, 72]. The homogeneous recombinant enzyme is specific for L-3-hydroxykynurenine, exhibiting a sigmoidal kinetic behavior, with a K_s value of 3 μM, and it is inhibited by L-kynurenine and D-kynurenine [72]. Finally, it should be mentioned that novel kynurenine analogues potently inhibiting the enzyme have been recently synthesized [72–74].

3-Hydroxyanthranilate 3,4-dioxygenase

The last enzyme-catalyzed reaction in the kynurenine pathway is represented by the conversion of 3-hydroxyanthranilate to α-amino-β-carboxymuconate-ε-semialdehyde (ACMS) through the action of 3-hydroxyanthranilate 3,4-dioxygenase (3HAO) (EC 1.13.11.6) (scheme 2, reaction 6). ACMS is a relatively unstable compound undergoing spontaneous cyclization to quinolinic acid. 3HAO is a cytosolic enzyme belonging to the family of intramolecular dioxygenases containing nonheme ferrous iron (Fe^{2+}). The human cDNA, coding for a 286-amino-acid protein, has been cloned and expressed in *E. coli* and human embryonic kidney HEK-293 cells [75, 76]. Kinetic studies performed on purified recombinant 3HAO reveal a K_m value for 3-hydroxyanthranilic acid in the low micromolar range; the enzyme requires the presence of Fe^{2+} for the catalytic activity, whereas it is inhibited by metal cations like Cd^{2+} and Zn^{2+} [75]. Interestingly, the inhibition of 3HAO by Zn^{2+} has been linked to the neurotoxicity caused by an excess of Zn^{2+} ion accumulation [75]. A very recent paper reports on the identification and expression of a cDNA encoding human α-amino-β-carboxymuconate-ε-semialdehyde decarboxylase that can divert ACMS to α-amino-β-muconate-ε-semialdehyde (AMS), which unlike ACMS, is incapable of forming quinolinate [77]. The fluctuation of this enzyme activity could play a pivotal role in regulating quinolinate concentration and, indirectly, NAD^+ synthesis.

From quinolinate to NAD(P)$^+$

Quinolinate phosphoribosyltransferase

Quinolinate phosphoribosyltransferase (QaPRT) (EC 2.4.2.19) catalyzes the conversion of quinolinate and 5-phospho-α-D-ribose 1-diphosphate (PRPP) to nicotinate mononucleotide (NaMN), pyrophosphate (PPi) and CO_2 (scheme 2, reaction 7). The enzyme is specific for quinolinate and cannot use either nicotinic acid or nicotinamide as substrates. In addition to the reaction catalyzed by IDO, this reaction represents another rate-limiting step of the pathway from tryptophan to NAD^+ [78]. In humans, the enzyme has been hypothetically linked to neurological disorders such as epilepsy, Alzheimer disease and Huntington disease because its substrate, quinolinate, is a very well known endogenous excitotoxic agonist of N-methyl-D-aspartate (NMDA) receptors and thereby may modulate the effects of excitotoxins [79–84]. In particular, a substantial reduction in QaPRT activity has been reported in epileptic foci of the human brain [80], while its increase has been described in postmortem brains of Huntington disease victims [82]. Human QaPRT has been purified to homogeneity from both liver and brain tissues, showing identical molecular and kinetic properties [78]. The native protein (M_r 170,000) appears composed of five identical subunits of about 34,000. The K_m values for quinolinate and PRPP are 5.6 μM and 23 μM, respectively, and the activity is strictly dependent on the presence of Mg^{2+} ions. Human QaPRT is inhibited by PPi, non competitively with regard to quinolinate and uncompetitively with regard to PRPP [78]. Unfortunately, no data exist on the catalytic mechanism of the reaction catalyzed by the human enzyme, contrary to the detailed kinetic analyses performed on the enzymes from other sources [85–87]. Attempts to isolate quinolinate mononucleotide (QaMN) as the putative reaction intermediate failed because of its spontaneous decarboxylation through the formation of an intermediate nitrogen ylide [88, 89]. The cDNA of human QaPRT has been functionally expressed in *E. coli* [90]. A computer-aided search did not reveal any significant homology with the amino acid sequences of other phosphoribosyltransferases, including both nicotinate and nicotinamide phosphoribosyltransferases, suggesting that the three proteins might not have evolved from a common ancestor. The data obtained confirm the belonging of human QaPRT to the type II phosphoribosyltransferase class [86].

NMN adenylyltransferase

NaMN produced in the reaction catalyzed by QaPRT is linked to the adenosine 5′-monophosphate (AMP) moiety of adenosine 5′-triphosphate (ATP) and converted to nicotinate adenine dinucleotide ($NaAD^+$) in the reaction catalyzed by NMN adenylyltransferase (NMNAT) (EC 2.7.7.1) (scheme 2, reaction 8). The same enzyme also catalyzes the synthesis of NAD^+ and $NaAD^+$ utilizing both NMN and NaMN produced in the salvage routes (scheme 2, reactions 16, 17, 20, 22). Since its presence was first demonstrated in yeast [91], NMNAT has been studied for more than 50 years by assuming that it was a single product coded by a single gene [10]. However, in the past six months two new human variants of the enzyme have been identified and characterized [92, 93]. Therefore, the three enzyme forms will be indicated as NMNAT-1, NMNAT-2 and NMNAT-3.

NMNAT-1

The first enzyme purification to homogeneity from human source was achieved more than 10 years ago [94]. Recently, the cDNA encoding NMNAT-1 has been isolated and cloned, and the molecular and kinetic properties of the recombinant human enzyme have been elucidated [95, 96]. The human cDNA codes for a 279-amino-acid protein with a calculated molecular mass of 31,900 Da. Inspection of the primary structure of human NMNAT-1 revealed the presence of a single potential Asn-linked glycosylation site and a putative nuclear localization signal (NLS) [95, 96]. The nuclear localization of the enzyme [96–98] suggested a functional relationship between NMNAT-1 and the chromatin-bound enzyme PARP [99]. Indeed, an inhibitory effect exerted by NMNAT-1 on PARP activity, first observed in this laboratory [100], was later confirmed by others [96]. Therefore, it has been proposed to be an essential role for NMNAT-1 in preventing NAD^+ and ATP depletion occurring during the cellular rescue from DNA damage caused by PARP overactivation. The possibility that NMNAT-1 expression might also be regulated at the post-translational level has been suggested [96]. Despite its widespread distribution in all organisms, the enzyme is not highly conserved. Human NMNAT-1 lacks the N-terminal extension of about 150 amino acids present in both *Saccharomyces cerevisiae* isoenzymes, and a very low degree of similarity exists with respect to the prokaryotic counterparts [95]. However, human NMNAT-1 shares with all NMNATs so far characterized the conserved ATP-recognition motifs GXFXPXT/HXXH and SXTXXR [95, 101]. Like other NMNATs, the human enzyme absolutely requires divalent cations, with optimal activity occurring in the presence of 12 mM Mg^{2+}, even though various metal ions can replace magnesium in the reaction mixture [95]. Experimental data seem to indicate a similarity with respect to the cations requirement between human NMNAT-1 and its thermophilic counterpart, whereas yeast isoenzymes exhibit maximal activity with Ni^{2+} and Co^{2+} [102–104]. Initial-rate kinetic studies and product inhibition patterns, obtained analyzing the reaction on both directions [94], showed that the NMNAT-1 catalytic mechanism is ordered Bi-Bi in agreement with the stereochemical data [105]. Compared with the eubacterial enzyme, which appears to prefer NaMN over NMN, human NMNAT-1 seems to use both mononucleotides with the same efficiency, thus being capable of participating in both de novo and salvage pathways of NAD^+ synthesis [95]. Like other eukaryotic NMNATs [106, 107], NMNAT-1 is able to catalyze the synthesis of NADH from the reduced form of NMN (NMNH), while α-NMN is ineffective both as an inhibitor and as a substrate, suggesting that the nucleotidyl transfer is strongly stereospecific [94]. In addition, NMNAT-1 catalyzes the key step of the metabolic conversion of the antileukemic nucleoside drugs of the tiazofurin class into NAD^+ analogues, representing their active form, and loss of NMNAT-1 activity is responsible for drug resistance in resistant leukemic cell lines [108–110]. The human NMNAT-1 gene is localized to chromosome 1p36.2, and its entire structure has been determined [95, 111]. Northern blot analysis reveals a widespread expression in all the examined human tissues, including various parts of the brain, where it is present at a much lower level [95, 111]. Further analysis of NMNAT-1 mRNA expression among a panel of cancer cell lines indicated that, with the exception of Burkitt's lymphoma and chronic myelogenous leukemia, the enzyme is weakly expressed in tumor cells [95]. This result, in agreement with the previously reported data concerning a reduced NMNAT-1 activity after cancerous transformation, makes this enzyme a potential chemotherapeutic target [112–113]. The NMNAT-1 gene has recently been identified as the human homologue of D4Cole1e, the gene that contributes the great majority of amino acids to the chimeric protein that delays Wallerian degeneration in the Wlds mouse [111, 114]. As Wallerian degeneration has a prominent causative role in a spectrum of human neuropathologies, it should be mandatory to evaluate the involvement of the NMNAT-1 gene in neurodegeneration. In this regard, the availability of the entire NMNAT-1 gene structure will allow to screen exons for mutations in neurological disorders as well as to look for single nucleotide polymorphisms [111]. The three-dimensional structure of human recombinant enzyme protein was recently solved through X-ray crystallography, both as apoenzyme (fig. 1) and in different liganded states, thus paving the way to the understanding of its catalytic mechanism at the atomic level as a mandatory step for the rational design of selective and potent inhibitors [101, 115, 116]. The human NMNAT-1 monomer structure contains a central six-stranded parallel β-sheet, flanked by nine α-helices, and connecting loops. A stretch of 37 residues (from 109 to 146) is disordered in the crystal. This region contains the above reported NLS sequence and two serine residues as putative phosphorylation sites (Ser109 and Ser136) that represent a peculiar feature of the human enzyme. The crystal structure shows that human NMNAT-1 forms a barrel-like hexamer, with the NLS- sequence-containing region located at the outer surface of the barrel and therefore able to interact with the nuclear transporting proteins. A cluster of positively charged residues detected at the dimer-dimer interface could be involved in the interaction of human NMNAT-1 with PARP or protein kinases. A comparative analysis of the crystal structures of both complexes of human NMNAT-1 with NAD^+ and $NaAD^+$ [116] allowed clarification of the active site conformation and the structural mechanism underlying the unique dual specificity evolved by the human protein with respect to its prokaryotic counterpart. Further, the structure of human NMNAT-1 com-

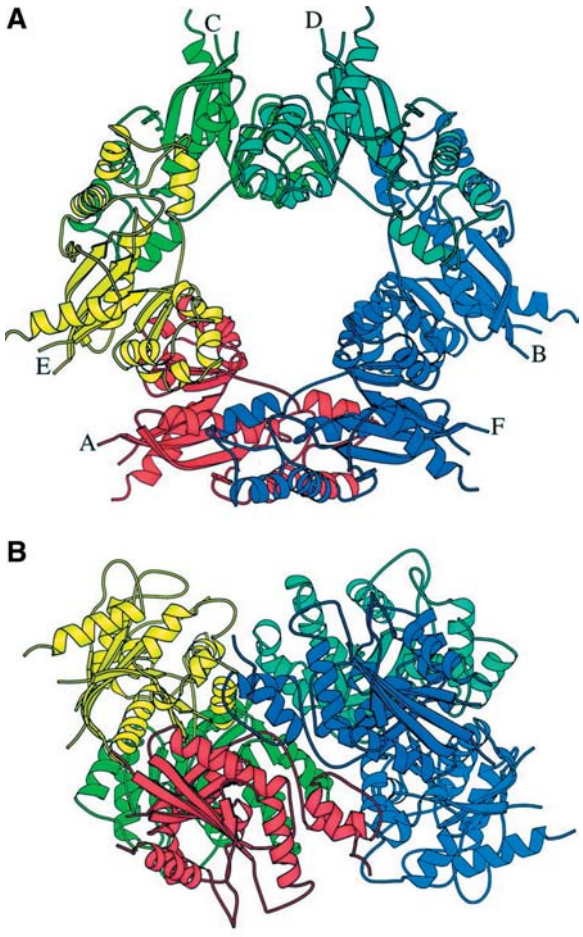


Figure 1. Ribbon representation of the hexamer of NMNAT-1 viewed along the local 3-fold axis (A) and the local dyad (B). From Garavaglia et al. [101].

plexed with a noncleavable tiazofurin adenine dinucleotide (TAD) analogue (β-CH_2-TAD) was solved, revealing that the TAD molecule adopts a similar conformation as the native ligand NAD^+, and that TAD and NAD^+ form essentially the same interactions with NMNAT-1, except for the extra interactions with the sulfur and nitrogen atoms on the tiazofurin molecule [116]. This finding represents a first step in understanding the molecular mechanisms of the metabolic conversion of nucleoside drugs of the tiazofurin class and producing strategies to prevent or overcome the development of resistance to this oncolytic drug.

NMNAT-2

The human NMNAT-2 isoform, distinct from the nuclear enzyme NMNAT-1, has been recently identified [92, 117]. Its gene has been mapped to chromosome 1q25 [118] and the corresponding cDNA codes for a 307-residue protein, which shares 34 % sequence identity with NMNAT-1. Northern blot analysis performed on RNA from several human tissues and organs revealed that NMNAT-2 is specifically expressed in the brain [92, 118]. Inspection of the protein primary structure with the PSORT II program suggests a cytoplasmic localization [92]. The presence of this isoform outside the nucleus has been confirmed by localization studies performed with NMNAT-2 tagged with green fluorescent protein (GFP) and overexpressed in cultured cells [93]. These studies demonstrated a highly compartmentalized localization outside the nucleus, which still remains to be exactly determined [93]. Surprisingly, gel filtration experiments on the pure recombinant enzyme expressed in *E. coli* indicated that NMNAT-2 exists as a monomeric protein, whereas the other human isoforms, NMNAT-1 and NMNAT-3, possess an oligomeric structure [92]. Like the nuclear isoform, recombinant NMNAT-2 has a broad pH optimum and performs the adenylyltransferase reaction equally efficiently with both the NMN and NaMN mononucleotides [92, 117]. However, a much lower specific activity, a significantly higher K_m for ATP (870 µM) and a lower optimal Mg^{2+} concentration (0.3 mM) are exhibited by NMNAT-2 [92]. In addition, the cytoplasmic enzyme contains nine cysteines versus the four cysteines present in NMNAT-1, and it is very prone to oxidation, which can be prevented by thiol protecting agents [92].

NMNAT-3

Very recently, a third isoform of human NMNAT (NMNAT-3) has been identified and structurally characterized [93]. Its cDNA codes for a 252-residue protein that shows 50 % and 34 % identity with NMNAT-1 and NMNAT-2, respectively. The overall mRNA levels in normal tissues are low compared with NMNAT-1. In particular, NMNAT-3 is present in the lung and spleen and, to a lesser extent, in the placenta and kidney [93]. Similarly to NMNAT-1, it is expressed in cancer cell lines at much lower levels [93]. Like NMNAT-2, NMNAT-3 is localized outside the nucleus, but besides being present in the cytosol, it is also present in mitochondria, as demonstrated by the localization experiments on the expressed GFP fusion protein [93]. The enzyme has been overexpressed in *E. coli,* and like the two other isoforms, it shows comparable activity with both NMN and NaMN [93]. The specific activity of NMNAT-3 is lower compared with both NMNAT-1 and NMNAT-2 [93]. The three-dimensional structure of the recombinant protein has been determined in its apo form and in complexes with its substrates or products. The overall structure of the NMNAT-3 monomer, including the active site residues arrangement, is very similar to NMNAT-1; however NMNAT-3 forms a tetramer versus the hexamer of the nuclear isoform [93]. Table 1 summarizes the properties of the human NMNAT isoforms.

NAD^+ synthetases

NAD^+ synthetases (EC 6.3.1.5, EC 6.3.5.1) catalyze the formation of NAD^+ from $NaAD^+$ through the amidation

Table 1. Properties of human NMNATs. [a]

Features	NMNAT-1			NMNAT-2			NMNAT-3		
Localization									
– chromosomal	1p32-35			1q25			ND		
– cellular	nuclear			cytosolic			cytosolic/mitochondrial		
Tissue distribution	ubiquitous			brain			ubiquitous		
Northern blot	4.1–3.1 kb			6.6–4.6 kb			1.1 kb		
Structure organization	hexameric			monomeric			tetrameric		
Isoelectric point	9.0			6.6			ND		
Optimal pH	6.0–9.0			6.0–9.0			ND		
Optimal [Mg^{2+}]	12 mM			0.3 mM			ND		
Kinetic parameters	K_m	V_{max}	V_{max}/K_m	K_m	V_{max}	V_{max}/K_m	K_m	V_{max}	V_{max}/K_m
(mM)	(mM)	(U/mg)		(mM)	(U/mg)		(mm)	(U/mg)	
ATP	0.036	51	1417	0.870	15	17.2	ND	ND	ND
NMN	0.023	51	2217	0.091	15	165	ND	4.7	ND
NaMN	0.116	76.5	659	0.125	10.5	84	ND	5.6	ND

ND, not determined.
[a] Adapted according to refs. 92, 93 and 95.

of its nicotinic moiety (scheme 2, reactions 9 and 10), the final step in the formation of NAD^+ both in the de novo synthesis and in the salvage pathways. The NAD^+-synthetases-catalyzed reaction can use either glutamine or ammonia as the amide group donor and requires stoichiometric hydrolysis of ATP. In a previous review we reported the literature on the existence of different enzymes from several sources utilizing both donors with distinct efficiencies [10]. In eukaryotes, the presence of glutamine-dependent NAD^+ synthetase activity has long been known in yeast and human erythrocytes [119, 120]. Recently, by using the deduced amino acid sequence of *Bacillus subtilis* NAD^+ synthetase as a probe in a homology search analysis [121, 122], two different forms of human NAD^+ synthetases, NADsyn1 and NADsyn2, encoding putative proteins of 706 and 275 amino acids, have been identified and expressed. As determined by SDS-polyacrylamide gel electrophoresis (PAGE), molecular masses of NADsyn1 and NADsyn2 were 80 and 34 kDa, respectively. In nondenaturating conditions, both enzyme forms appear to be oligomeric, consistent with the *B. subtilis* NAD^+ synthetase topological arrangement [123] and with the proposed two-step mechanism for the reaction [10, 119, 123]. The kinetic characterization showed that the K_m values of NADsyn1 and NADsyn2 for $NaAD^+$ and ATP are in the range previously reported for known synthetases [119, 120, 123], whereas the two enzymes differ in the affinities for glutamine and ammonia. The larger form, NADsyn1, shows K_m values of 1.44 mM for glutamine and higher than 10 mM for ammonium ion, while NADsyn2 shows K_m values of over 100 mM for glutamine and 34 μM for ammonium ion. Therefore, NADsyn1 appears to be a glutamine-dependent NAD^+ synthetase, while NADsyn2 is a strictly ammonia-dependent NAD^+ synthetase. This finding shows for the first time the presence of a strictly ammonia-dependent NAD^+ synthetase in eukaryotes. BLAST alignment of the NADsyn1 and NADsyn2 amino acid sequences evidenced highly conserved regions in the C-terminal portions comprising the NAD^+ synthase domain and the P-loop motif, characteristic of the N-type family of ATP-PPases [124]. In addition, the larger NADsyn1 sequence possesses within its N-terminal extension the carbon-nitrogen (CN) hydrolase domain shared by enzymes of the nitrilase family, whose presence is consistent with the ability of NADsyn1 to use glutamine. The CN hydrolase domain is also present in NAD^+ synthetase from *Mycobacterium tuberculosis* [125, 126], suggesting that glutamine-dependent NAD^+ synthetases from human and microbes may share a similar mechanism of catalysis and active site structure.

NAD^+ kinase

NAD^+ kinase (EC 2.7.1.23) catalyzes the transfer of a phosphate group from ATP to NAD^+, forming $NADP^+$ and ADP (scheme 2, reaction 11). This reaction represents the only known way to generate $NADP^+$ in all living organisms. The structural and functional characterization of the human enzyme has been presented only very recently [127]. Based on sequence similarity searches by using the eubacterial enzyme as the query, the human cDNA for NAD^+ kinase has been identified [127]. The gene is located on chromosome 1p36.21-36.33, and it is expressed in most tissues with the exception of skeletal muscle. The recombinant human enzyme has been expressed in *E. coli* [127]. The pure recombinant protein is composed of four identical subunits of about 49 kDa. Its catalytic activity is optimal at 55 °C and in the pH range of 7.0–8.0. The enzyme requires a divalent cation: Zn^{2+}, Mn^{2+} and Mg^{2+} are, in the order, the most effective. Human NAD^+ kinase is highly selective for the substrates NAD^+ and ATP, showing K_m values of 0.54 mM and 3.3 mM, respectively. It is not able to phosphorylate $NaAD^+$ to $NaADP^+$ [127], thus reinforcing the hypothesis that $NaADP^+$ synthesis in the cell

exclusively proceeds through the reaction catalyzed by CD38 [128] even though alternative pathways cannot be definitively discounted.

Non redox NAD^+-dependent reactions

With the name of nonredox NAD^+-dependent reactions, we indicate those reactions utilizing NAD^+ and leading to the formation, in addition to nicotinamide, of various metabolites such as ADP-ribose, cADPR, $NaADP^+$, O-acetyl-ADP-ribose and poly/mono-ADP-ribosylated proteins (scheme 2, reactions from 12 to 15). In the last few years, excellent reports appeared in the literature reviewing new, exciting data supporting the fundamental role played by these reactions and their products in the most relevant phases of cellular life [129–131]. Therefore, only a brief overview on this topic with specific emphasis on the last published data will be reported. About 30 years ago the demonstration that a large quantity of NAD^+ synthesis takes place in the nucleus suggested a possible involvement of the pyridine dinucleotide in the modulation of chromatin structure and function [132]. Moreover, NMNAT-1, a key enzyme in NAD^+ synthesis, was found to be a chromatin-associated protein [133] exhibiting a physical and functional interaction with PARP, another NAD^+-dependent enzyme [100]. PARP (EC 2.4.2.30) utilizes NAD^+ by transferring the ADP-ribose moiety to itself as well as to other proteins on specific amino acid residues. The enzyme has been studied and its major kinetic and structural properties characterized in detail for more than 15 years by assuming that it was a single product deriving from a single gene. In the late 1990s, however, it was demonstrated that PARP null mouse cells were able to synthesize ADP-ribose polymers, suggesting the existence of other PARPs [134]. Soon after, indeed, several papers appeared on the existence of multiple genes encoding different forms of the enzyme in animals and humans [135], including tankyrases 1 and 2, PARP-2, V-PARP and PARP-3. Tankyrase-1, a positive regulator of telomere length, possesses a catalytic domain very similar to PARP-1 but has a different relative mass (142,000) [135, 136]. Tankyrase-2, a second tankyrase with an overall amino acid identity of more than 80% to tankyrase-1, has been identified as a Golgi-associated protein [137]. PARP-2 is a 62-kDa protein very similar to PARP-1, and PARP-3 has not been extensively studied [138]. V-PARP is the 193-kDa protein species of the vault, a ubiquitous ribonucleic particle with unknown function [139]. PARPs belong to a larger family of enzymes that also include those catalyzing mono-ADP-ribosylation reaction, by transferring single ADP-ribose units from NAD^+ to specific amino acid residues, usually the guanidine group of an arginine or the sulphydryl group of a cysteine present in target proteins [140]. In humans, several ARTs (EC 2.4.2.31) have been isolated and functionally and structurally characterized [141]. The results provide good evidence on the involvement of this enzyme activity in the immune response [142]. Another class of enzymes utilizing NAD^+ is represented by the CD38 family (EC 3.2.2.5, EC 3.2.2.6). CD38 indeed possesses, besides its antigenic function [143], the capability to catalyze different reactions, such as the hydrolysis of NAD^+ and cADPR to ADP-ribose but also the cyclization of NAD^+ and nicotinamide guanine dinucleotide (NGD^+) to cADPR and cyclic GDP-ribose, respectively. Furthermore, it is able to catalyze a base exchange reaction between $NADP^+$ and nicotinic acid, thereby producing $NaADP^+$ and nicotinamide. Both $NaADP^+$ and cADPR act as second messengers, modulating the mobilization of intracellular Ca^{2+} in a large variety of cells including plant, protozoan and human [128]. CD38 is a transmembrane glycoprotein whose cDNA codes for a 30-kDa polypeptide chain. The structure of CD38 has not been determined. However, modeling of the structure of CD38 using the crystallographic data of a functional and structural homologue allowed the identification of a single amino acid residue, Glu146, as the determinant for the NAD^+ cyclizing and hydrolyzing activities of the protein [144]. It is noteworthy that CD38 has been described to be involved in a model proposed for necrotic beta-cell death. This model implies that the impairment of the CD38-cADPR signal system for insulin secretion is caused by a depletion of NAD^+ content. Such a depletion is generally secondary to PARP activation because of the occurrence of a cellular insult generating DNA strand breaks [145]. Many reports exist in the literature on the involvement of PARP activity and, consequently, of NAD^+ levels in multiple cellular processes such as DNA replication, repair and recombination, cell proliferation and death, and gene transcription. The role of NAD^+ in remodeling chromatin structure and thus in regulating its transcriptional activity through the post-translational modification of chromatin-associated proteins has been dramatically reinforced by the recent discovery that a class of histone/protein deacetylases are NAD^+-dependent. These enzymes, constituting the SIR2 family, are conserved from prokaryotes to humans [146]. SIR2 proteins, also known as 'sirtuins', catalyze the removal of one acetyl group from the epsilon-amino group of lysine with an accompanying cleavage of one NAD^+ molecule for each lysine being deacetylated [6, 147, 148]. The products of this reaction are the deacetylated histones/ proteins and a new compound, 2′-O-acetyl-ADP-ribose, which can be further converted to 3′-O-acetyl-ADP-ribose through a nonenzymatic isomerization [149]. The cellular role of these novel compounds is still un-clear even though their catalytic formation is evolutionarily conserved among sirtuins, including the human forms [150, 151]. In humans, seven sirtuin genes have been

identified whose cDNAs code for proteins of relative molecular masses ranging from 33,000 to 81,700 [146]. Although a silencing function of human sirtuins can be anticipated by analogy to their *S. cerevisiae* counterparts, little is known about their biological function. However, recent findings demonstrating that human hSIRT1 protein is capable of deacetylating/inactivating p53 [7] and that another human variant, hSIRT3, is localized in mitochondria [152] provide important clues to both their potential substrates and their biological function(s). In addition to poly(ADP-ribose), poly(ADP-ribosylated)-acceptors, deacetylated histones/proteins, cADPR, $NaADP^+$, ADPR and O-acetyl-ADP-ribose, all the above-described reactions produce nicotinamide. It is noteworthy that a common distinctive feature of these reactions is the inhibition exerted by nicotinamide toward all the enzymes involved. Therefore, it is conceivable to hypothesize that the fluctuations of nicotinamide concentration regulate the metabolic fluxes occurring in these pathways, which are known to be strictly associated to fundamental events of cellular life. The inhibitory role played by nicotinamide constitutes the basis of the rationale underlying its adoption in therapeutic trials to prevent cancer recurrence and insulin-dependent diabetes [153, 154]. The nicotinamide concentrations in turn fluctuate in response to the activities of the enzymes involved in NAD^+ recycling. We believe that these studies connecting intermediary metabolism and gene expression will develop into a new and exciting research area with a bright future.

Nicotinamide salvaging

The levels of nicotinamide produced by all the enzymes described in the previous section are intracellularly modulated by the action of three enzymes involved in nicotinamide salvaging, that is, nicotinamide deamidase, nicotinamide phosphoribosyltransferase (NamPRT) and nicotinate phosphoribosyltransferase (NaPRT) (scheme 2, reactions 21, 17 and 22, respectively). Surprisingly, no in-depth studies have been carried out on the characterization of these enzymes in humans despite their evident importance in niacinamide utilization.

Nicotinamide deamidase

Nicotinamide deamidase (EC 3.5.1.19) deamidates nicotinamide substrate to nicotinic acid. Since our previous review on NAD^+ synthesis [10], no further data are available in the literature on the enzyme from human sources. However, in the same review we did not mention a report showing that in *Plasmodium falciparum*-infected human erythrocytes, a marked nicotinamide deamidase activity can be detected, in contrast to the substantial lack of such activity observed in noninfected human erythrocytes. Nevertheless, the authors do not discriminate whether a preexisting, though undetectable, nicotinamidase activity is stimulated by the presence of the parasite or encoded by its genome [155].

Nicotinamide phosphoribosyltransferase

NamPRT (EC 2.4.2.12) catalyzes the conversion of nicotinamide and PRPP to NMN and PPi. The presence of this enzymatic activity was first demonstrated in the cytosolic fraction of human erythrocytes [156] and later in human fibroblasts, liver, kidney, lung, brain and spleen [157]; human lymphocytes and lung cancer cell lines [158]; and mitogen-stimulated human T-lymphocytes [159]. Possibly due to the repeatedly reported extreme instability of the enzyme from any source, its purification to homogeneity has never been obtained; thus, the molecular and kinetic characterization of the human enzyme has been sporadic and elusive. The partially purified enzyme from human erythrocytes showed an optimum pH in the range of 6.8–7.6 and an unphysiological K_m value of 100 mM for nicotinamide, suggesting that in humans, this enzyme might not play a significant role in NAD^+ synthesis [156]. On the other hand, NamPRT from human fibroblasts has been subsequently reported to possess a very low K_m for nicotinamide of 1.6 μM [157]. More recently, high-pressure liquid chromatography (HPLC)-based determinations also revealed low K_m values for the enzyme from human erythrocytes, corresponding to 12.7 μM for nicotinamide and 53.7 μM for PRPP [160, 161]. These low K_m values appear to provide a suitable explanation for the observed active synthesis of NAD^+ from nicotinamide in intact erythrocytes and NamPRT seems to catalyze the rate-limiting step of this pathway [161, 162]. In contrast to the enzyme from other sources, human NamPRT seems to be stimulated by low ATP concentration and even inhibited by ATP higher than 0.4 mM [162]. Very recently, the product of the murine homologue of the previously identified human pre-B-cell colony-enhancing factor (PBEF) gene [163] has been hypothesized to possess NamPRT enzymatic activity on the basis of its sequence homology to NamPRT from *Haemophilus druceyi* [164]. Indeed, the murine protein, partially purified by immunoprecipitation with anti-PBEF antibody-coupled beads, showed NamPRT activity with a K_m value for nicotinamide of 1.24 μM, consistent with the values reported for the human fibroblast enzyme [157, 164]. Since the PBEF gene has been reported to be involved in signaling events within the cell, the demonstration that it is endowed with NamPRT activity, though in mouse tissue, suggests that NAD^+ biosynthesis plays an important role in lymphocyte activation [163, 164]. This finding is consistent with the early reported enhancement of NamPRT activity in mitogen-stimulated human T-lymphocytes [159].

Nicotinate phosphoribosyltransferase

NaPRT (EC 2.4.2.11) catalyzes the conversion of nicotinate and PRPP to NaMN and PP_i. The enzyme-catalyzed formation of NaMN from nicotinate was first described in human erythrocytes [165], and NaPRT was subsequently purified to apparent homogeneity and characterized from the same source [166]. The protein shows a native molecular mass of 86,000 Da. The enzyme activity is optimal over a broad pH range, from pH 6.5 to pH 8.0. Like other mammalian NaPRT enzymes and in contrast with the enzyme from other sources, the activity of human NaPRT is stimulated by ATP, but its requirement is not obligatory. At saturating concentrations, ATP is hydrolyzed to ADP in a 1 : 1 molar ratio with respect to the formation of NaMN, whereas in the absence of ATP the enzyme is still active, though at a lower rate [166]. In the absence of ATP, K_m values for nicotinate and PRPP are 24 μM and 100 μM, respectively, whereas in the presence of saturating ATP, K_m values are 0.5 μM and 5 μM, respectively, and the V_{max} value increases by a factor of three. Thus, ATP appears to act both as an energy-yielding cosubstrate and as an enzyme allosteric modulator, and in this respect NaPRT represents a paradigm for a new energy-coupling mechanism of enzyme action [167]. More recently, NaPRT activity has been evaluated in crude extracts from human lymphocytes: the apparent K_m values for nicotinate and PRPP were 0.165 mM and 0.33 mM, respectively [168]. These values, obtained in the absence of added ATP, are much higher than those reported above for the purified erythrocyte enzyme.

Miscellaneous

In this section, the human enzymes involved in the degradation of NAD^+ by catalyzing reactions leading to the formation of the pyridine mononucleotide, nucleoside and base are described (scheme 2, reactions 16, 18, 19, 20). These routes have been poorly investigated in humans, and, indeed, the literature, refers mainly to sources other than humans [10]. The NAD^+ formed through the biosynthetic pathways described above can be subjected to the action of NAD^+ pyrophosphatase (EC 3.6.1.22) (scheme 2, reaction 16). This enzyme activity, catalyzing the cleavage of NAD^+ to NMN and AMP, has been mostly described in connection with bacterial DNA ligase. Recently, the presence of an activity yielding NMN from NAD^+ has been described in human blood serum, where its presence was found to be necessary in order to confer virulence to *H. influenzae* [169], thus confirming the early hypothesis on the presence of such an activity in humans [170]. The sera from the same *H. influenzae*-affected patients also contain an NMN phosphohydrolase activity able to cleave NMN to nicotinamide riboside (NMN 5′-nucleotidase, EC 3.1.3.5) (scheme 2, reaction 18), which is involved, together with NAD^+ pyrophosphatase, in the mechanism underlying the host–mediated virulence of *H. influenzae* [169]. The nicotinamide riboside formed in the reaction catalyzed by NMN 5′-nucleotidase can be reutilized by its conversion to NMN via a reaction catalyzed by human nicotinamide riboside kinase (EC 2.7.1.22) (scheme 2, reaction 20) [171]. The enzyme has been purified to near homogeneity from human placenta by means of a multiple-step purification procedure. The final preparation consists of a monomeric protein whose molecular weight was calculated to be 29,000 under denaturating conditions and 32,000 under native conditions. The pI value is 5.6, and the pH optimum is in the range of 6.5–6.9. The human enzyme exhibits broad substrate specificity, being able to phosphorylate, in addition to nicotinamide riboside, guanosine and, more importantly, tiazofurin and 3-deazaguanosine, known antineoplastic agents. The K_m values for these substrates are 9.6 μM, 115 μM, 90 μM and 16.5 μM, respectively. In addition to its salvaging to NMN, nicotinamide riboside can be cleaved to free nicotinamide by the action of the enzyme nicotinamide riboside phosphorylase (EC 2.4.2.1) (scheme 2, reaction 19). The only existing report on the conversion of nicotinamide riboside to nicotinamide in humans appeared 45 years ago [172]. This old, excellent paper describes several features of the enzyme, partially purified from the soluble fraction of human erythrocytes. The riboside cleavage occurs through a phosphorolytic mechanism, which in principle implies the reversibility of the reaction. Human nicotinamide riboside phosphorylase is subjected to uncompetitive inhibition by nicotinamide. The enzyme pH optimum is 7.5. The enzyme is strictly specific for nicotinamide riboside, being inactive toward other purine, pyrimidine and pyridine nucleosides. Guanosine is the only other riboside utilized as a substrate by the enzyme preparation; it also act as an inhibitor of nicotinamide riboside phosphorolysis.

Conclusions

Our work represents the first overview devoted to the description of human NAD^+ metabolism, and our aim is to illustrate the state-of-the-art knowledge on the enzymatic routes governing NAD^+ homeostasis. We hope to draw the attention of researchers who are active in different fields of science, such as biochemistry, pharmacology and experimental medicine, and who are involved in the elucidation of specific physiopathological phenomena where NAD^+ levels play important functions. In excellent old reviews and also in our comprehensive report, the enzymology of NAD^+ metabolism occurring in many organisms has been described [10, 173, 174]. Since the last review, many enzymes from human sources have been further molecularly and functionally characterized. How-

ever, detailed structural studies have been carried out only for NMNAT isoforms 1 and 3 and for the enzymes catalyzing the nonredox NAD^+-dependent reactions like PARPs and CD38. These studies single out these enzymes as druggable targets in a variety of different pathologies including diabetes, inflammatory syndromes, cancer and infectious disease. Furthermore, several enzymes of the pyridine nucleotide metabolism have been shown to perform specific functions within the nervous system. Recently, a therapeutic effect exerted by nicotinamide in brain disease has been suggested [175]. Its potential use as a drug has been expanded and is currently in trials for testing its effectiveness against various diseases [153, 154].

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