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Ligand binding phenomena that pertain to the metabolic function of renalase

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ABSTRACT

Renalase catalyzes the oxidation of isomers of β-NAD(P)H that carry the hydride in the 2 or 6 positions of the nicotinamide base to form β-NAD(P)$^+$. This activity is thought to alleviate inhibition of multiple β-NAD(P)-dependent enzymes of primary and secondary metabolism by these isomers. Here we present evidence for a variety of ligand binding phenomena relevant to the function of renalase. We offer evidence of the potential for primary metabolism inhibition with structures of malate dehydrogenase and lactate dehydrogenase bound to the 6-dihydroNAD isomer. The previously observed preference of renalase from *Pseudomonas* for NAD-derived substrates over those derived from NADP is accounted for by the structure of the enzyme in complex with NADPH. We also show that nicotinamide nucleosides and mononucleotides reduced in the 2- and 6-positions are renalase substrates, but bind weakly. A seven-fold enhancement of acquisition (k_{red}/K_d) for 6-dihydronicotinamide riboside was observed for human renalase in the presence of ADP. However, generally the addition of complement ligands, AMP for mononucleotide or ADP for nucleoside substrates, did not enhance the reductive half-reaction. Non-substrate nicotinamide nucleosides or nucleotides bind weakly suggesting that only β-NADH and β-NADPH compete with dinucleotide substrates for access to the active site.

1. Introduction

Renalase is an FAD-dependent oxidase that catalytically oxidizes two isomeric forms of β-NAD(P)H to form β-NAD(P)$^+$ and H_2O_2 (Scheme 1). These isomers are presumed to occur when β-NAD(P)$^+$ is reduced non-specifically forming, in addition to the native β-NAD(P)H (4-dihydroNAD(P)), 2-dihydroNAD(P) (2DHNAD(P)) and 6-dihydroNAD(P) (6DHNAD(P)) [1,2]. Both 2DHNAD and 6DHNAD have been shown to be highly inhibitory to specific primary metabolism dehydrogenases [2]. This suggests that renalase has an intracellular metabolic housekeeping function that alleviates metabolic suppression by these isomers. This proposal differs starkly from the consensus view that renalase is a mammalian serum borne protein that is associated with an array of aberrant physiological conditions. The enzyme was originally claimed to be a kidney-derived hormone that down-modulates vascular tone in animals by the oxidation of catecholamines [3–7] and then later to be a cytokine that ameliorates myocardial damage resulting from an ischemic event [8]. More recently it has been said to be a suppressor of pancreatic cancer and/or an exacerbatory factor for melanoma [4,9–12]. Here we present the first structures of 6-DHNAD in complex with malate dehydrogenase and lactate dehydrogenase, confirming our assertion that β-NAD(P)H [dihydronicotinamide] isomers are detrimental to normal metabolic activity and reasserting the verified catalytic function of renalase. These structures show that 6DHNAD occupies the β-NADH-binding site occluding the association of the native nicotinamide substrate.

With a modest two-electron reduction potential of −320 mV, the potential for non-specific redox reactions of β-NAD(P)$^+$ that form toxic β-NAD(P)H isomers exists in all living systems. It is therefore reasonable to expect that an intracellular detoxification activity such as renalase would be found in multiple kingdoms of life. However, homology searches based on the human renalase amino acid sequence return almost exclusively homologs from *animalia*. The structure of human renalase (isoform 1; HsRen) was solved in 2011 by the Aliverti group who noted that the renalase structural topology was common to numerous redox active

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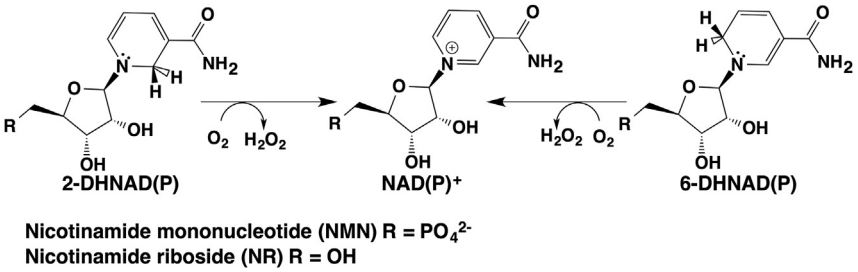
Abbreviations

ADP	adenosine diphosphate
ADP-ribose	adenosine diphosphate ribose
AMP	adenosine monophosphate
β-NAD$^+$	oxidized nicotinamide adenine dinucleotide
β-NADP$^+$	oxidized nicotinamide adenine dinucleotide phosphate
β-NADPH	reduced nicotinamide adenine dinucleotide phosphate
FAD	flavin adenine dinucleotide
HPLC	high performance liquid chromatography
HsRen	renalase from *H. sapiens*
MDH	malate dehydrogenase
LDH	lactate dehydrogenase
NMN	nicotinamide mononucleotide
NR	nicotinamide riboside
PpRen	renalase from *P. phaseolicola*
6DHNAD	6-dihydronicotinamide adenine dinucleotide
2DHNAD	2-dihydronicotinamide adenine dinucleotide
6DHNADP	6-dihydronicotinamide adenine dinucleotide phosphate
2DHNADP	2-dihydronicotinamide adenine dinucleotide phosphate
4DHNMN	4-dihydronicotinamide mononucleotide
6DHNMN	6-dihydronicotinamide mononucleotide
2DHNMN	2-dihydronicotinamide mononucleotide
4DHNR	4-dihydronicotinamide riboside
6DHNR	6-dihydronicotinamide riboside



Scheme 1. The chemistry catalyzed by renalase.

flavoproteins [13]. In this structure the open active site was observed to have only a small number of conserved amino acids indicating that sequence alignments based on overall alignment scores (E-values) may not detect this motif and will not necessarily identify distant forms of renalase. We have recently characterized a renalase from a Pseudomonad (19% identity to Human) [14]. As part of this study we solved the crystal structures of this form of renalase (PpRen) in complex with β-NADH (PDB ID 4ZCC, 2.1 Å; a fascimile of the ES complex) and β-NAD$^+$ (PDB ID 4ZCD, 1.7 Å; the EP complex). These structures revealed that the *si* face of the FAD isoalloxazine is closely associated with the inner surface of the active site offering the *re* face for interaction with the substrate nicotinamide base. The few conserved residues that line the dihydronicotinamide-binding cavity are: PpRen H232 (HsRen H245), W267 (HsRen W288) and R280, which is apparently equivalent to HsRen R193, as both residues offer their guanidino group to a similar location in the active site. In both proteins, the active site cavity forms one end of an extended cleft to which β-NAD(P)-derived substrate(s) associate (Fig. 1). Within this cleft are multiple hydrogen bonds and charge pairing interactions that in the *Pseudomonas* enzyme form a rather symmetrical set of interactions with respect to each nucleotide half of the substrate. However, the majority (8 of 11) form hydrogen bonding and/or charge pairing interactions with the pyrophosphate moiety of the substrate/product. Only one direct contact is observed for the nicotinamide amide and two for the 6 amino group of the adenine base with no hydrogen bonds from the protein engaging any of the hydroxyl groups of either ribose. This arrangement raises interesting questions about what part of the molecule contributes to the binding energy given that renalase must function in an environment in which non-substrate mono- and dinucleotides predominate. Using truncated forms of the substrate we offer evidence that, at least for human renalase, binding of the pyrophosphate moiety of the substrate has a primary contribution to stabilizing the pre-reduction E·S complex.

The substrate/product binding pose exposes one face of the ligand to direct interactions with solvent (Fig. 1). Both the human (HsRen) and bacterial (PpRen) forms of renalase exhibit a preference for substrates derived from β-NAD$^+$ over those derived from β-NADP$^+$ and this bias is notably more pronounced in the bacterial enzyme [14]. While this specificity bias is consistent with renalase serving to preferentially detoxify the inhibitory effect that 2DHNAD and 6DHNAD would exert on primary metabolism enzymes, the structural basis for this selectivity was hitherto unknown. In this study we present the structure of renalase from *Pseudomonas phaseolicola* in complex with β-NADPH and evaluate the influence of the ribose 2-phospho group in regard to the observed substrate specificity.

2. Materials and methods

2.1. Materials

Dibasic potassium phosphate and sodium phosphate, monobasic potassium phosphate and sodium chloride were obtained from ACROS. β-NADH (disodium salt, trihydrate) was obtained from Amresco. β-NAD$^+$, β-NADP$^+$, nicotinamide mononucleotide (NMN), ADP-ribose, methyl nicotinamide and nicotinic acid were purchased from Sigma. Nicotinamide riboside (NR) was purchased from High Performance Nutrition. Renalases from *H. sapiens* (HsRen) and *P. phaseolicola* (PpRen) were expressed and purified according to previously published methods [14,15]. Old yellow enzyme was prepared as previously described [2]. 6DHNAD and 6DHNADP were prepared and purified as described [2].