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IMP Dehydrogenase: the Dynamics of Reaction Specificity

Lizbeth Hedstrom

Departments of Biology and Chemistry, Brandeis University, 415 South St., Waltham MA 02454, U.S.A.

E-Mail: hedstrom@brandeis.edu

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Abstract

Subtle changes in enzyme structure can have enormous impact on catalysis, as vividly illustrated in IMP dehydrogenase (IMPDH) and GMP reductase (GMPR). These proteins share a common structure and set of catalytic residues and bind the same ligands with similar affinities. IMPDH catalyses a hydride transfer reaction involving a nicotinamide cofactor, with formation of the covalent intermediate E-XMP*. Hydrolysis of this intermediate produces XMP, which is converted to GMP by the action of another enzyme. In the GMPR reaction, E-XMP* is formed by the deamination of GMP, and is subsequently reduced via a hydride transfer reaction with a nicotinamide cofactor. In both cases, a conformational change separates the two chemical transformations. The protein moves in the case of IMPDH, while the cofactor moves in GMPR. Thus conformational dynamics control reaction specificity in the IMPDH/GMPR family, with intriguing implications for the evolution of these enzymes.

Introduction

The remarkable versatility of the $(\beta/\alpha)_8$ barrel fold, also known as the TIM barrel, is well recognized [1 – 6]. The current SCOP and CATH databases list approximately thirty $(\beta/\alpha)_8$ barrel protein superfamilies [7, 8], which catalyse over twenty-five different reactions [9]. One $(\beta/\alpha)_8$ barrel protein superfamily contains just two proteins, inosine monophosphate dehydrogenase (IMPDH) and guanosine monophosphate reductase (GMPR), which catalyse

similar reactions with opposing metabolic consequences (Figure 1; [10]). IMPDH catalyses the oxidation of IMP to XMP with concomitant reduction of NAD^+. This reaction is the first committed and rate-limiting step in the biosynthesis of guanine nucleotides, and therefore controls the size of the guanine nucleotide pool. IMPDH inhibitors block cell proliferation, which makes IMPDH an attractive drug target. IMPDH inhibitors are currently used as immunosuppressive [11], anticancer [12, 13] and antiviral therapy [14], and also show promise as antimicrobial agents [15]. GMPR catalyses the reduction of GMP to IMP and ammonia with the concomitant oxidation of NADPH. This reaction allows guanine nucleotides to be recycled into the adenine nucleotide pool. These two enzymes bind the same ligands and have the same catalytic residues. The same covalent intermediate E-XMP* is formed during both reactions. Recent work provides some insights into why this intermediate partitions in different directions on the two enzymes.

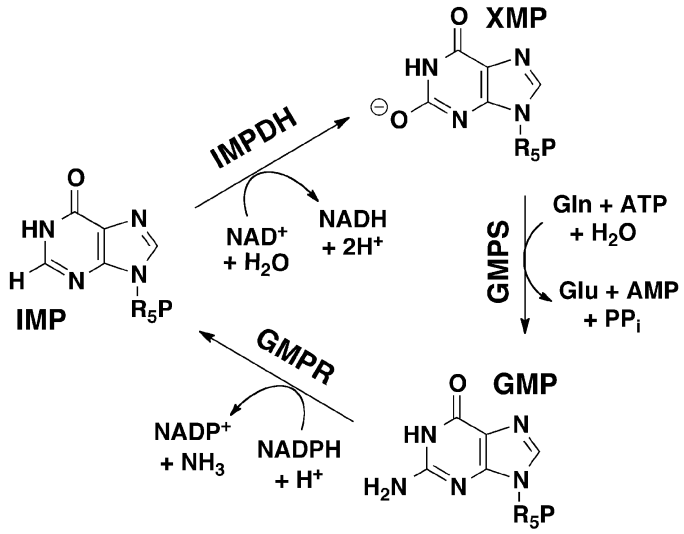

Figure 1. The interconversions of IMP and GMP.

The IMPDH Reaction

IMPDH catalyses two distinct chemical transformations. In the first step, Cys319 attacks C2 of IMP and hydride is transferred to NAD^+ to produce the covalent intermediate E-XMP* (Figure 2; *Tritrichomonas foetus* IMPDH numbering will be used throughout). This step is likely to proceed via a tetrahedral intermediate, although experiments have yet to address this point. The hydrolysis of E-XMP* produces XMP; this step is rate-limiting in most IMPDHs [10, 16].

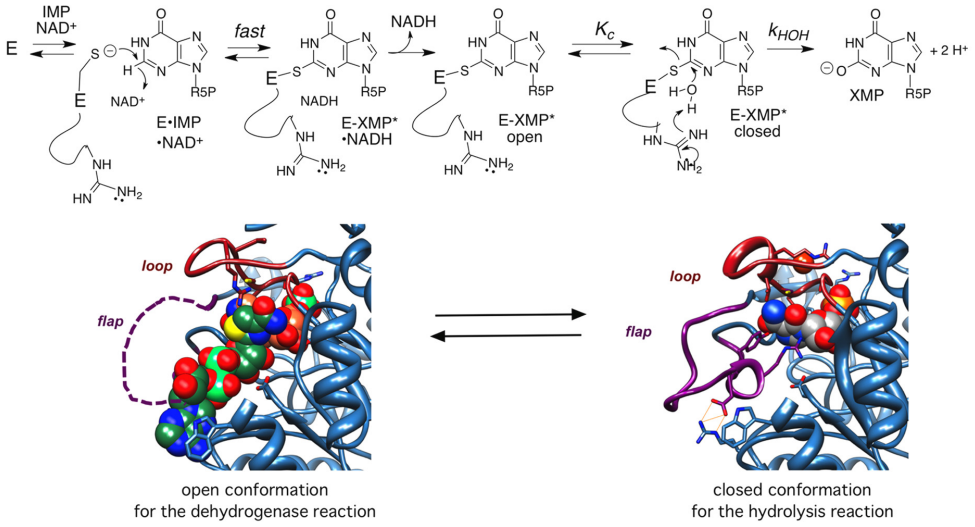

Figure 2. The mechanism of IMPDH. The model for the open conformation is the E●IMP●tiazofurin adenine dinucleotide complex (1lrt; [36]) and the model for the closed conformation is the E●MZP complex (1pvn; [17]).

The central challenge of the IMPDH reaction is "how can a single active site catalyse two different chemical transformations?" IMPDH solves this problem with a conformational change that remodels part of the active site, as revealed by the structure of the IMPDH●mizoribine monophosphate (MZP) complex [17]. Unlike other IMP analogues, the affinity of MZP negatively correlates with the activity of mutant enzymes [18], which is behaviour characteristic of transition state analogues. The structure of the E●MZP complex does indeed resemble a transition state [17]. Cys319 and a putative catalytic water molecule are positioned in a tetrahedral arrangement as expected for the hydrolysis of E-XMP*. If E-XMP* is modelled in place of MZP, the water is poised for attack at the C2 position (Figure 3). A mobile flap (residues 412 – 432), disordered in previous IMPDH structures, is found in a "closed conformation", and folded into the cofactor site. The catalytic water molecule forms hydrogen bonds to Thr321 from the same loop that contains the catalytic Cys319, as well as two residues of the flap, Arg418 and Tyr419 (Figure 3). Thr, Arg and Tyr residues are not usually candidates for general base catalysts, though precedence exists for each (see examples in [19]). The substitution of Thr321 decreases both the hydride transfer and hydrolysis steps by a factor of ~20 [20]. In contrast, mutations of both Arg418 and Tyr419 selectively perturb the hydrolysis step (Table 1). However, only the substitution of Arg418 decreases the rate of the hydrolysis step by the magnitude expected for loss of a general base [21]. As expected, the mutation of Arg418 also perturbs the equilibrium between the open and closed conformations of the flap, but the magnitude of this effect is not sufficient to account for the decrease in k_{cat} (Table 1). Guanidinium analogues rescue the

activity of Arg419 mutants and Bronsted analysis of the rescue reaction suggests that the proton is almost completely transferred in the transition state, providing further support for the role of Arg418 as the base that activates water [22].

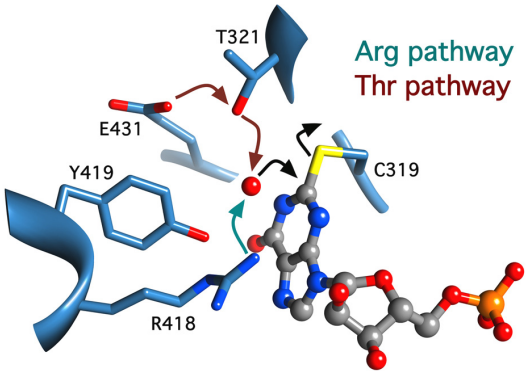

Figure 3. The activation of water in IMPDH. Model of the E-XMP*$_{closed}$ complex based on the structures 1pvn and 1jr1. The Arg pathway for water activation is depicted in teal and the Thr pathway for water activation is shown in maroon.

Table 1. Effects of mutations of the residues that interact with the catalytic water
a. [21]; b. [20]

Variant	k_{cat} (s^{-1})	Hydride transfer (s^{-1})	NADH release (s^{-1})	K_c	k_{HOH} (s^{-1})
Wild-type [a]	1.9	93	8.5	140	4
Thr321Ala [b]	0.18	1.7	≥8	≥20	0.18
Arg418Ala [a]	0.004	42	11	1	0.008
Arg418Gln [b]	0.0069	≥400	≥4	10 – 50	0.007
Arg418Lys [b]	0.15	83	6.5	≤0.1	≥1
Tyr419Phe [a]	0.22	70	10	20	0.22

While all of the biochemical experiments are consistent with Arg418 playing the role of general base in the activation of water in the IMPDH reaction, none are definitive. Therefore, we performed combined molecular mechanics/quantum mechanics simulations to gain further insight into this transformation [23]. In the lowest energy pathway for the hydrolysis of E-XMP*, neutral Arg418 abstracts a proton from the catalytic water as it attacks the C2 position of IMP (Figure 3). Assuming that the pK_a of Arg418 is ~12.5, the calculated energy barrier is in good agreement with the barrier observed experimentally. Proton transfer is rate-limiting and almost complete in the transition state, also consistent with experimental findings. More importantly, the simulations also revealed a second pathway for the activation of water that operates when Arg418 is protonated: Thr321 activates water via a proton relay with Glu431 (Figure 3C). This pathway is also consistent with experimental findings. The

energy barrier is similar to that observed when Arg418 is mutated. Proton transfer is rate limiting in the simulations, and a larger solvent isotope effect is observed when Arg418 is mutated, consistent with the transfer of two protons. These simulations suggest that the Thr321 pathway activates water at low pH, while the Arg418 pathway dominates at high pH, which further predicts that the substitution of Glu341 with Gln will disable the Thr321 pathway and shift the pH rate profile to the right. This behaviour is in fact observed experimentally. Thus water appears to be activated by both the Arg418 pathway and Thr321 pathway.

Why would IMPDH possess two sets of catalytic machinery to activate water? We hypothesize that the Thr321 pathway is a remnant of evolution. Phylogenetic analysis indicates that the closest relative to IMPDH is GMPR, and that the ancestral IMPDH/GMPR contained the catalytic Cys, Thr and Glu residues [23]. IMPDH obtained the more efficient Arg418 pathway with the attendant conformational change later, at which point the Thr321 pathway became redundant. Indeed, the mutation of Glu341 to Gln has little effect on the IMPDH reaction other than shifting the pH rate profile, and many modern IMPDHs contain this substitution. Thus the simulations provided unexpected insight into the evolution of IMPDH.

The GMPR Reaction

The GMPR reaction is effectively the reverse of the IMPDH reaction, reducing GMP to IMP with the release of ammonia. Thus GMPR also must perform two chemical transformations, a deamination reaction and a hydride transfer. Comparatively little was known about the mechanism of GMPR beyond steady-state kinetic experiments that indicated that the E●GMP●NADPH ternary complex must form before the reaction proceeds in the human enzyme [24, 25]. We and others recently confirmed that a similar pattern is observed in the reaction of *Escherichia coli* GMPR [26, 27]. Surprisingly, an isotope effect is observed on hydride transfer, indicating that hydride transfer is rate-limiting [26, 27]. These observations indicate that the cofactor is present throughout the entire reaction cycle, so the GMPR reaction cannot simply follow a reaction sequence that is the reverse of IMPDH.

If the above phylogenetic analysis is correct, then the analogous Cys186, Thr188 and Glu289 residues should be necessary for GMPR catalysis (*E. coli* GMPR numbering). Moreover, these residues should perform similar roles in the two reactions, that is, Cys186 attacks C2 to form E-XMP*, with Thr188 and Glu289 activating the leaving ammonia. The formation of E-XMP* from GMP was demonstrated by radiolabeling and mass spectroscopy, but this intermediate only forms in the presence of cofactor [26]. E-XMP* also forms from 2-Cl-IMP, but again only in the presence of cofactor. Curiously, E-XMP* forms in the presence of $NADP^+$ in addition to NADPH, which indicates that the cofactor induces a protein conformational change required for the deamination reaction. The structure of E●GMP (PDB accession number 1ble) reveals that the Cys186 and Glu289 point away from the active site and Arg286 folds across the face of the guanine ring, further protecting GMP

from reaction (Figure 4; [28]). As found in the structure of the E●IMP●NADPH (PDB accession number 2c6q), when the cofactor binds, Arg286 forms part of the 2'-phosphate binding site. Movement of this segment allows Cys186, Thr188 and Glu289 to rearrange into catalytically competent alignment (Figure 4; [26]). Surprisingly, this crystal structure finds the cofactor in two conformations: (1) the 'in' conformation, where the nicotinamide is stacked against the hypoxanthine ring, optimally aligned for hydride transfer (Figure 4) and (2) the 'out' conformation, where the nicotinamide is too far from the hypoxanthine ring for hydride transfer to occur (Figure 4). An electron density, modelled as water, is observed within hydrogen bonding distance of Thr188 and the carboxamide of the cofactor, in a similar position to the catalytic water of the closed conformation of IMPDH. Therefore we hypothesize that the cofactor moves to the 'out' conformation for the deamination reaction. Further, these observations suggest that cofactor amide may be part of the machinery that activates the amine leaving group.

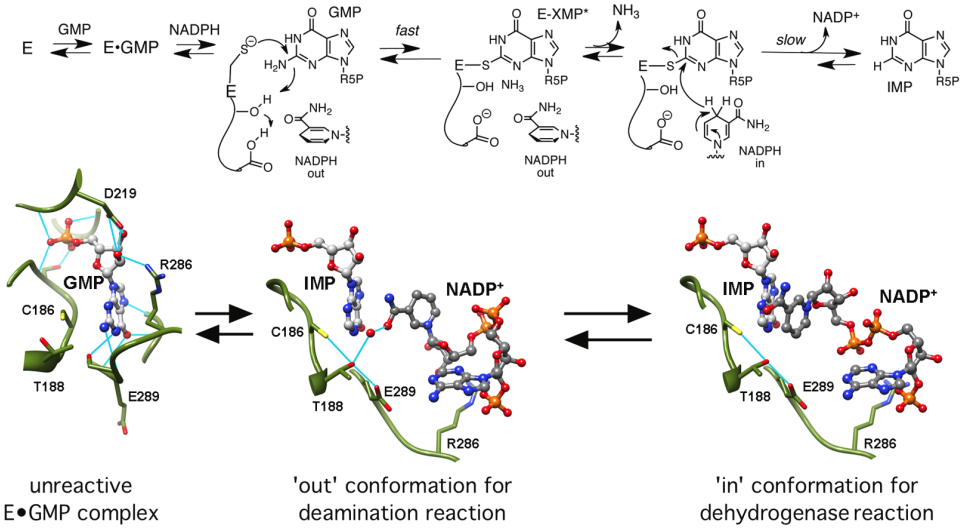

Figure 4. The mechanism of the GMR reaction. Structure of the inactive E●GMP complex is 2a7r [28]). Structure of E●IMP●NADPH in the "out" position is subunit E from 1c6q; [26]. Structure of E●IMP●NADPH in the "in" position for is subunit C from 1c6q[26]).

We examined the reactions of GMPR with 2-Cl-IMP and reduced acetyl pyridine adenosine dinucleotide phosphate (APADPH) to further probe the mechanism of the deamination reaction (Table 2). The value of k_{cat} for the reaction of 2-Cl-IMP and NADPH is equivalent to that of the physiological reaction with GMP. Importantly, chloride is a good leaving group, so the 2-Cl-IMP reaction should proceed even if the residues that activate the leaving group of GMP have been removed. As expected, substitution of Cys186 with Ala completely blocks both the reactions of GMP and 2-Cl-IMP [26]. The mutation of Thr188 decreases the value of k_{cat} for the GMP reaction by a factor of 500, but reduces that for the 2-Cl-IMP

reaction by only a factor of 14. This observation indicates that Thr186 is part of the machinery that activates the amine leaving group of GMP. Likewise, mutation of Glu289 also decreases the value of k_{cat} for the GMP reaction by a factor of 103, but reduces that for the 2-Cl-IMP reaction by only a factor of 20, again consistent with the involvement of this residue in leaving group activation. In APADPH, the cofactor amide is replaced with a methyl ketone. This analogue cannot catalyse the reduction of GMP ($V_{max} \leq 0.6\%$ of the reaction with NADPH), but can catalyse the reaction with 2-Cl-IMP ($V_{max} = 20\%$ of the reaction with NADPH). Thus the cofactor amide is part of the catalytic machinery activating the amine leaving group of GMP. We believe that this may be an unprecedented role for a nicotinamide cofactor. These experiments suggest that GMPR uses a complementary reaction strategy to that of IMPDH, with the cofactor moving to accommodate both chemical transformations.

Table 2. Reactions of GMPR variants. Data from [26].

Reaction	k_{cat} (s^{-1})			
	wild-type	C186A	T188A	E289Q
GMP + NADPH	0.35 ± 0.01	≤ 0.0001	$(8.8 \pm 0.6) \times 10^{-4}$	$(3.8 \pm 0.3) \times 10^{-4}$
2-Cl-IMP + NADPH	0.40 ± 0.01	≤ 0.0001	0.021 ± 0.001	0.027 ± 0.001
GMP + APADPH	< 0.0008	n.d.	n.d.	n.d.
2-Cl-IMP + APADPH	0.08 ± 0.01	n.d.	n.d.	n.d.

Can a similar cofactor conformational change occur on IMPDH? The cofactor conformational change requires a rotation about the 5' carbon of the adenosine ribose of NADPH (Figure 4). The NAD binding site of IMPDH appears to be incompatible with this motion. The cofactor occupies a different portion of the barrel domain than in GMPR in the structure of IMPDH●cofactor complexes that are currently available. This observation suggests that the adenosine binding portion of the cofactor binding site is a critical determinant of reaction specificity. Curiously, some prokaryotic IMPDHs may bind NAD^+ in the same manner that GMPR binds NADPH. A recent structure of *Cryptosporidium parvum* IMPDH finds an inhibitor binding in a site that resembles the adenosine subsite of GMPR [29]. The inhibitor interacts with a Tyr residue in the neighbouring subunit, much as the adenosine of $NADP^+$ does in GMPR (Figure 5). Many bacterial IMPDHs share this motif [30]. Perhaps these bacterial IMPDHs can catalyse both the IMPDH and GMPR reactions.

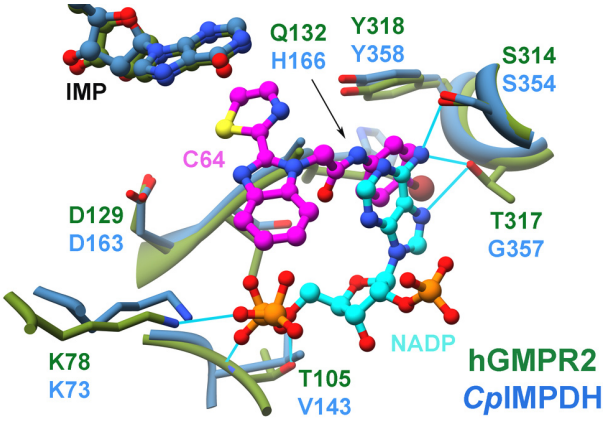

Figure 5. Cofactor binding sites in human GMPR2 and *C. parvum* IMPDH. The nicotinamide riboside portion of NADPH is omitted for clarity, as are the residues that interact with the 2'-phosphate (2c6q). *C. parvum* IMPDH inhibitor C 64 is shown (3khj). IMP (sticks) and inhibitor C 64 (ball and stick) are shown.

How does GMPR Distinguish Water and Ammonia?

The GMPR reaction involves the same E-XMP* intermediate as the IMPDH reaction, yet this intermediate does not react with water. In the presence of $NADP^+$, E-XMP* forms readily from both IMP and 2-Cl-IMP [26]. XMP is more stable than GMP, so XMP would form if GMPR catalysed the reaction with water, but E-XMP* is stable for days in the absence of ammonia. Thus GMPR prefers ammonia over water by a factor of at least 10^6. What are the kinetic barriers that prevent GMPR from reacting with water?

The preference of GMPR for ammonia must derive from a combination of intrinsic reactivity and selective binding. Ammonia is a stronger nucleophile than water, by ~10^4–fold [31], but an additional factor of 100 is still required to account for the selectivity of GMPR. Ammonia has one more hydrogen bond donor than water, which could provide ~3 kcal/mol of specific binding energy, which could translate into a factor of 500 in selectivity. The ammonia channels AmtB and RhAG display ammonia/water selectivity of this order [32].

Was the Ancestral Enzyme a GMPR?

The primordial environment is believed to have been ammonia-rich and reducing [33], conditions that would favour the emergence of a GMPR operating to produce GMP. This hypothesis is supported by the observation that the over-expression of *E. coli* GMPR can complement bacteria lacking IMPDH and attenuated in GMPS [26]. Curiously, *Buchnera*, the aphid symbiont, is missing genes for both IMPDH and GMPS but contains a GMPR [34]. Insect guts are having high concentrations of ammonia/ammonium, reaching as high as

130 mM [35], so it is quite possible that GMPR is responsible for the synthesis of guanine nucleotides. Approximately 20 bacteria/archaea appear to be missing genes encoding GMPS, suggesting that these organisms also synthesize GMP directly from IMP and ammonia (The SEED, http://theseed.uchicago.edu/FIG/index.cgi accessed June 11, 2011).

If the ancestral enzyme was indeed a GMPR, then IMPDH would have evolved as the environment became oxidative and ammonia became limiting. Water activation would have initially depended on the Thr pathway, and therefore have involved a cofactor conformational change. However, with the installation of the Arg pathway for water activation, cofactor conformational changes would no longer be required. The hydride transfer step could be optimized and the cofactor binding site could migrate. Since Thr321 pathway is no longer required to activate water, the catalytic Glu431 could be substituted with Gln, which may represent a further specialization to the IMPDH reaction. We are now devising experiments to test the feasibility of this pathway.

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