IMP dehydrogenase: the dynamics of drug selectivity

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ABSTRACT
Inosine monophosphate dehydrogenase (IMPDH) is an important target for immunosuppressive, antiviral and anticancer therapy. This enzyme catalyzes the key reaction in guanine nucleotide biosynthesis: the conversion of IMP into XMP with the concomitant reduction of NAD. The reaction involves a dehydrogenase step that produces NADH and a covalent E-XMP* intermediate and a hydrolysis step where E-XMP* is converted to XMP. We have solved the structure of the mizoribine monophosphate complex of IMPDH that resembles the transition state for the hydrolysis of E-XMP*. This structure reveals that IMPDH undergoes a large conformational change after NADH departs, transforming the enzyme into a hydrolase. This conformational change positions a mobile flap in the NADH site, with a conserved Arg-Tyr dyad adjacent to E-XMP*. Surprisingly, the Arg-Tyr dyad appears to act as the general base that activates water. The flap competes with drugs such as mycophenolic acid, so that the conformational change also appears to be a major determinant of drug selectivity.

INTRODUCTION
Inosine monophosphate dehydrogenase catalyzes the pivotal step in guanine nucleotide biosynthesis: the conversion of inosine monophosphate (IMP) to xanthosine monophosphate (XMP) with concomitant reduction of NAD. IMPDH controls the guanine nucleotide pool, which in turn controls proliferation and many other important cellular processes. IMPDH inhibitors are antiproliferative and are used as immunosuppressive (mycophenolic acid and mizoribine), antiviral (ribavirin) and anticancer (tiazofurin) chemotherapy (1). IMPDH inhibitors may also be useful antimicrobial agents, although compounds with the appropriate selectivity have not yet been developed.

The IMPDH reaction involves two very different chemical transformations: hydride transfer and formation of E-XMP* (Fig. 1) (2). Substrates bind randomly to form E-IMP•NAD. The active site Cys attacks IMP and a hydride is transferred to NAD, forming the E-XMP• intermediate. NADH must dissociate prior to hydrolysis of E-XMP•; hydrolysis of E-XMP• is at least partially rate-limiting in all of the IMPDHs examined to date. Surprisingly, MZP, the active metabolite of the immunosuppressive drug mizoribine, behaves like a transition state analog for the hydrolysis reaction although its structure does not resemble the transition state (3). All hydrolases have some mechanism to activate water, but this mechanism has proven difficult to identify in IMPDH.

A. Mechanism of IMP dehydrogenase

Hydride transfer and formation of E-XMP*

Hydrolysis of E-XMP*

B. Inhibitors of IMP dehydrogenase

Mycophenolic acid (MPA) Mizoribine monophosphate (MZP)

Fig. 1 The IMPDH reaction and structures of inhibitors.

Mycophenolic acid (MPA) is a potent inhibitor of human IMPDH but a poor inhibitor of microbial enzymes such Tritrichomonas foetus IMPDH. Understanding the mechanistic basis of this selectivity could provide important insights into designing microbial selective IMPDH inhibitors. MPA traps E-XMP• by binding in the nicotinamide half of the NADH site. Interestingly, MPA-resistant IMPDHs have higher values of kcat than MPA-sensitive enzymes. This observation suggests that there is an underlying mechanistic link between drug selectivity and catalysis.
We have discovered that MPA selectivity is determined in part by residues at the MPA site, and in part by a conformational change (4,5). The MPA site of T. foetus IMPDH contains two residues that are different in the human enzyme: Lys310 and Glu431. When these residues are replaced with their human counterparts, the resulting mutant is 20-fold more sensitive to MPA than wild-type, but 20-fold less sensitive than human IMPDH (4). This result demonstrates that drug selectivity is determined by residues outside the MPA binding site. While the IMP site is highly conserved, the adenine half of the NADH site varies dramatically among IMPDHs and thus could be the missing determinant of drug selectivity. The nicotinamide site. The flap docks into the adenosine subsite with a conformational change (43). The MPA site of T. foetus IMPDH contains two residues that are different in the human enzyme. More surprisingly, the mutagenesis experiments suggest that Arg418 is acting as the catalytic base. We make this statement with caution because the high pKa of Arg in solution would seem to preclude such a role.

These experiments indicate that the flap closes into the active site after NADH dissociates from E-XMP*, converting the enzyme into a hydrolase. We believe the equilibrium between the open and closed conformations of E-XMP* controls MPA selectivity. The higher the affinity of the flap for the NADH site, the weaker MPA will bind. The more the closed conformation is favored, the faster E-XMP* will be hydrolyzed and, since the hydrolysis of E-XMP* is rate-limiting, the higher the value of $k_{cat}$. This explains why MPA resistant IMPDHs also have higher values of $k_{cat}$ and accounts for the properties of T. foetus IMPDH. Conversely, the lower the affinity of the flap for the NADH site, the higher the affinity of MPA and the lower the value of $k_{cat}$. These are the properties of human IMPDH.

**RESULTS AND DISCUSSION**

We first proposed that the conformational change involved a mobile flap that is disordered in the human E-XMP*-MPA complex (6), but generally believed to close over the adenine site. We demonstrated that mutations in this flap increase MPA affinity although these residues do not contact MPA. However, the conformational change did not involve simple closing of the flap over the active site as expected (7).

The structure of the E-MZP complex of T. foetus IMPDH solved the mystery of the conformational change (8). This structure is the first of an IMPDH complex with this important immunosuppressive agent. Remarkably, the mobile flap is completely ordered and occupies the NADH site. The flap docks into the adenine subsite with a conserved Arg-Tyr dyad protruding into the nicotinamide subsite (Fig 2). Thus the flap competes with MPA for the nicotinamide site.

This structure also explains the surprising transition state analogy of MZP as well as the mystery of water activation. The active site Cys319, MZP and a water molecule are arranged in a tetrahedral geometry resembling the transition state (Fig 2). Modeling a purine ring over the base of MZP, this structure explains the transition state analogy of MZP as well as the mystery of water activation. The water appears to be activated by the conserved Arg-Tyr dyad selectively disrupt the hydrolysis step. In Arg418Ala, the value of $k_{cat}$ decreases 500x, but the rates of hydride transfer and NADH release are comparable to wild-type. Similarly, in Tyr419Phc, the value of $k_{cat}$ decreases 10x, but again hydride transfer and NADH release are comparable to wild-type. These observations indicate that the conserved Arg-Tyr dyad activates water. More surprisingly, the mutagenesis experiments suggest that Arg418 is acting as the catalytic base. We make this statement with caution because the high pKa of Arg in solution would seem to preclude such a role.

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**REFERENCES**