The Functional Basis of Mycophenolic Acid Resistance in *Candida albicans* IMP Dehydrogenase

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*Candida albicans* is an important fungal pathogen of immunocompromised patients. In cell culture, *C. albicans* is sensitive to mycophenolic acid (MPA) and mizoribine, both natural product inhibitors of IMP dehydrogenase (IMPDH). These drugs have opposing interactions with the enzyme. MPA prevents formation of the closed enzyme conformation by binding to the same site as a mobile flap. In contrast, mizoribine monophosphate, the active metabolite of mizoribine, induces the closed conformation. Here, we report the characterization of IMPDH from wild-type and MPA-resistant strains of *C. albicans*. The wild-type enzyme displays significant differences from human IMPDHs, suggesting that selective inhibitors that could be novel antifungal agents may be developed. IMPDH from the MPA-resistant strain contains a single substitution (A251T) that is far from the MPA-binding site. The A251T variant was 4-fold less sensitive to MPA as expected. This substitution did not affect the *k*<sub>cat</sub> value, but did decrease the *K*<sub>m</sub> values for both substrates, so the mutant enzyme is more catalytically efficient as measured by the value of *k*<sub>cat</sub>*<sub>k</sub>/*K*<sub>m</sub>. These simple criteria suggest that the A251T variant would be the evolutionarily superior enzyme. However, the A251T substitution caused the enzyme to be 40-fold more sensitive to mizoribine monophosphate. This result suggests that A251T stabilizes the closed conformation, and this hypothesis is supported by further inhibitor analysis. Likewise, the MPA-resistant strain was more sensitive to mizoribine in cell culture. These observations illustrate the evolutionary challenge posed by the gauntlet of chemical warfare at the microbial level.

*Candida albicans* is an important fungal pathogen of immunocompromised patients (1, 2). Amphotericin B, 5-fluorocytosine, azoles, and echinocandins are currently used to treat candidiasis, although toxicity, limited availability, and the emergence of resistance create an urgent need for new drugs (3, 4). *C. albicans* is sensitive to mycophenolic acid (MPA) and mizoribine when cultured in vitro (5, 6). Both of these compounds inhibit IMP dehydrogenase (IMPDH), so this enzyme presents a novel target for antifungal chemotherapy. In addition, the IMPDH gene *IMH3* is a useful genetic marker for *C. albicans* because multiple copies confer MPA resistance (7). *MPA*<sup>a</sup>, an *IMH3* allele encoding an MPA-resistant IMPDH, confers drug resistance with a single gene copy (8–10). A second MPA-resistant allele of *C. albicans* IMPDH that carries mutations I47V, S102A, and G482D has been reported, although the functional effects of these mutations on enzyme activity have not been characterized (11, 12).

IMPDH catalyzes the rate-limiting step in guanine nucleotide metabolism: the oxidation of IMP to XMP with concomitant reduction of NAD<sup>+</sup> (Fig. 1). IMPDH is a target for the immunosuppressive drugs mycophenolate mofetil (CellCept<sup>®</sup>) and mizoribine; the active metabolites of these drugs are MPA and mizoribine monophosphate, respectively (Fig. 1) (13, 14). The mechanism of the IMPDH reaction is complex, with important consequences for inhibitor selectivity (Fig. 1). IMPDH undergoes a large conformational change in mid-catalytic cycle that converts the enzyme from a dehydrogenase to a hydrolase (15). In the first half of the reaction, substrates bind, and hydride transfer occurs to form the covalent XMP<sup>+</sup> intermediate. NADH departs, and the mobile flap folds into the NADH site. This conformational change positions the conserved Arg-Tyr dyad to activate water, and E-XMP<sup>+</sup> is hydrolyzed to XMP (16). MPA traps E-XMP<sup>+</sup> by competing with the flap for the NADH site (18, 19). In contrast, mizoribine monophosphate (MZP) binds to the IMP site and induces the flap to close (15). Thus, the equilibrium between open and closed conformations controls drug sensitivity: the open conformation favors MPA binding, whereas the closed conformation favors MZP binding.

To explore the potential of *C. albicans* IMPDH as a chemotherapeutic target and to understand the mechanism of MPA resistance, we have characterized the wild-type enzyme as well as the enzyme encoded by *MPA*<sup>a</sup>. The *MPA*<sup>a</sup> enzyme contains a single substitution (A251T) that is outside of the MPA-binding site. Here, we show that this mutation stabilizes the closed conformation, causing MPA resistance, but increasing MZP sensitivity.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AY864854.

The on-line version of this article (available at http://www.jbc.org) presents a novel target for antifungal chemotherapy. In addition, the IMPDH gene *IMH3* is a useful genetic marker for *C. albicans* because multiple copies confer MPA resistance (7). *MPA*<sup>a</sup>, an *IMH3* allele encoding an MPA-resistant IMPDH, confers drug resistance with a single gene copy (8–10). A second MPA-resistant allele of *C. albicans* IMPDH that carries mutations I47V, S102A, and G482D has been reported, although the functional effects of these mutations on enzyme activity have not been characterized (11, 12).

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1 The abbreviations used are: MPA, mycophenolic acid; IMPDH, IMP dehydrogenase; MZP, mizoribine monophosphate; MIC<sub>50</sub>, minimum concentration needed to inhibit cell growth by 50%.
from clones EC352 and EC551 and found that they contain the same and pEC551, respectively. We sequenced both plasmid inserts derived primer pair and cloned the amplicons into pBluescript, yielding pEC352 C. albicans clones (EC352 and EC551) using the IMH3RXF/IDHBXR

TCTAGAACTCAGTATATC-3

GGATC- GTATT

GAATTC, with the intro-

amplicon with EcoRI and BamHI, followed by cloning into pBluescript derived from the respective strains were generated by cutting the transformations yielded MPA-resistant

tance. We re-amplified the

M9CA medium with suitable supplements.

Dithiothreitol was purchased from Research Organics, Inc. Glycerin, cloning and plasmid isolation.

enous IMPDH, was obtained from the

strain CAI4 were obtained by incubating 1010

strain CAI4. Both

MPAR1

CL1M7, ∆imh3::URA3 flipper/IMH3

Table I

C. albicans strains used in this study

| Strain          | Parent/genotype | Ref/source |
|-----------------|-----------------|-----------|
| SC5314          | Wild-type strain| 39        |
| CAI1            | SC5314; ∆ura3::imm434/∆ura3::imm434 | 40        |
| EC3             | MPA-resistant CAI4 | This work |
| EC5             | MPA-resistant CAI4 | This work |
| EC352           | CAI4; IMH3/MPAR^r | This work |
| EC551           | CAI4; IMH3/MPAR^r | This work |
| CL1M7           | CAI4; ∆imh3::URA3 flipper/IMH3 | This work |
| MPA1            | CL1M7, ∆imh3::URA3 flipper/MPA^r | This work |
| MPA2            | CL1M7, ∆imh3::URA3 flipper/MPA^r | This work |

point mutations. The sequence of the MPA-resistant IMH3 allele MPA^r has been deposited in the GenBank/EMBL Data Bank with the accession number AF268854.

Generation of IMH3 Mutants—A heterozygous imh3/IMH3 mutant was generated using the URA3 flipper method (20) in C. albicans strain CAI4. For this purpose, plasmid p44F was constructed by amplification of a 2.7-kb DNA fragment containing the IMH3 gene plus the flanking regions using the IMH3RFX/IDHBXR primer pair, followed by insertion of a EcoRI BamHI-cut ampiclon into pBlueScript. Subsequently, the SacI and NotI restriction sites of p44F were removed. The resulting plasmid was used as template for divergent PCR with primers KO-

CAI4 SC5314; IMH3/MPAR^r

MPAR1 CL1M7, ∆imh3::URA3 flipper/MPA^r

MPAR2 CL1M7; ∆imh3::URA3 flipper/MPA^r

This work
C. albicans IMP Dehydrogenase

Recombinant Expression of C. albicans IMPDH and MPAR—Both the IMH3 gene (7) and MPAR (8) contain an intron at amino acid 150, which would prevent bacterial expression. Therefore, we isolated poly(A)+ RNA from strains CAI4 and EC3 using an Oligotex mRNA mini kit (Qiagen Inc.). Reverse transcription was carried out with Superscript II (Invitrogen) using primers IMHSF (5'-GCGGCCGC-AATTC-H11032) and primer DTNOT (5'-GAATTC-H11032) (Invitrogen) and primer DTNOT (5'-GAATTC-H11032) (Invitrogen). Reverse transcription was carried out with Superscript II (Invitrogen). The blunt end ligation at the NdeI site and direct sequencing of PCR products. Two transformants (MPAR1 and MPAR2) were chosen for further testing.

Susceptibility Testing—The MPA and mizoribine resistance of C. albicans strains was measured quantitatively in a microtiter plate assay as described previously (7). Growth of the strains in synthetic medium in the presence of serial dilutions of the inhibiting substances was determined by A590 measurements after 48 h using a microplate reader. Experiments were performed in triplicate.

Protein Purification—E. coli H712 cells harboring either the pTAC1_5 or pTAC4_13 vector were cultured in LB medium containing 10 g/liter M9 minimal salts (Invitrogen), 17 mM NaCl, 0.1 mM CaCl₂, 1 mM MgSO₄, 1 g/liter casamino acids, 0.78 g/liter complete supplement mix, 1 g/liter thiamine, 2 g/liter glucose, 1 mM isopropyl 1-thio-D-galactopyranoside, and 50 mg/liter ampicillin) to isolate transformants correctly expressing the IMH3 or MPAR plasmid and complementing the guaB mutation. For further studies, we used plasmids pTAC1_5 and pTAC4_13, which express wild-type IMH3 and MPAR IMPDHs, respectively. The sequences of both plasmid inserts were determined and proved free of errors.

Steady-state kinetics of C. albicans IMPDH: V_max versus NAD. A, wild-type IMPDH; B, A251T IMPDH. IMP concentrations were varied in the presence of fixed NAD concentrations. For the wild-type enzyme, the V_max values determined in Fig. 2 were plotted against the NAD concentration and fit to the equation describing uncompetitive substrate inhibition (Equation 2) to determine the values of K_M for NAD and V_max at saturating substrate concentrations. For the A251T enzyme, the V_max values versus NAD data could be fit to the Michaelis-Menten equation (Equation 1) to determine the values of K_M for NAD and V_max at saturating substrate concentrations.

Fig. 2. Steady-state kinetics of C. albicans IMPDH: velocity versus IMP. A, wild-type IMPDH; B, A251T IMPDH. All assays contained 50 mM Tris-Cl (pH 8.0), 100 mM KCl, 1 mM dithiothreitol, and 3 mM EDTA at 25 °C. For the wild-type enzyme, the velocity versus IMP data were fit to the Michaelis-Menten equation (Equation 1) at each NAD concentration. Surprisingly, the A251T enzyme exhibited IMP substrate inhibition. The data were fit to the equation describing uncompetitive substrate inhibition (Equation 2).

Fig. 3. Steady-state kinetics of C. albicans IMPDH: V_max versus NAD. A, wild-type IMPDH; B, A251T IMPDH. IMP concentrations were varied in the presence of fixed NAD concentrations. For the wild-type enzyme, the V_max values determined in Fig. 2 were plotted against the NAD concentration and fit to the equation describing uncompetitive substrate inhibition (Equation 2) to determine the values of K_M for NAD and V_max at saturating substrate concentrations. For the A251T enzyme, the V_max values versus NAD data could be fit to the Michaelis-Menten equation (Equation 1) to determine the values of K_M for NAD and V_max at saturating substrate concentrations.
human IMPDH type II are from Ref. 41. NA, not applicable.

The concentrations of IMP and NAD were varied for wild-type IMPDH and 200 μM for A251T IMPDH. The Km value for IMP was derived from an initial velocity versus IMP plots using Equation 1 for wild-type IMPDH and Equation 2 for A251T IMPDH and replotting these values against the NAD concentration. Initial velocity data were best fit to the uncompetitive tight-binding inhibition equation (Equation 3).

Initial velocity data were fit to the uncompetitive tight-binding inhibition equation (Equation 3) and the noncompetitive inhibition equation (Equation 4) using SigmaPlot software (SPSS Inc.),

\[
v = k_{cat}[E][S]/(K_m + [S])
\]

where \(v\) is the initial velocity, \(k_{cat}\) is the turnover number, \(K_m\) is the Michaelis constant of IMP or NAD, and \([S]\) is the substrate concentration. The Km values for the wild-type enzyme and Equation 2 for A251T IMPDH, and replotting these values against the NAD concentration. Initial velocity data were best fit to the uncompetitive tight-binding inhibition equation (Equation 3).

\[
v = k_{cat}[E][1 + K_m][S]/(K_m + [S])
\]

**Results**

**Isolation of MPA-resistant Strains of C. albicans**—Strain CA14 is sensitive to MPA, with an MIC\(_{50}\) of 0.25 μg/ml. Electrocopentropic C. albicans strain CA14 was cultured in the presence of sub-lethal concentrations of MPA (3–5 μg/ml) as described under “Experimental Procedures.” After prolonged incubation, several colonies appeared; cells were streaked onto agar plates with an increased concentration of MPA (10 μg/ml); and two strains were isolated (EC3 and EC5). The MIC\(_{50}\) values for both strains EC3 and EC5 as determined by MIC-determination assays were 1.7 μg/ml MPA (Supplemental Fig. S1).

**Identification of the MPA\(_a\) Mutation**—Assuming that a mutant IMH3 allele confers MPA resistance, we amplified the entire coding regions of the IMH3 genes plus the flanking regions of both strains EC3 and EC5. Transformation of strain CA14 with these amplification products yielded MPA-resistant clones, confirming that drug resistance results from mutation(s) in IMH3. Isolation and sequencing of the IMH3 genes from these strains revealed several differences from the published nucleic acid sequence of the IMH3 gene from strain SS
The wild-type enzyme displayed strong NAD$^+$/H$_1$1022/XMP$^*$ inhibition (Figs. 2 and 3). Although the $k_{cat}$ value appears to be similar to that for the wild-type enzyme, the $K_m$ values for both substrates are significantly lower. Surprisingly, high concentrations of IMP inhibited the A251T IMPDH reaction. To our knowledge, this is the first report of IMP substrate inhibition in an IMPDH. This inhibition is competitive versus NAD.

### Inhibition of C. albicans IMPDHs—
We determined the sensitivity of both C. albicans enzymes to well known IMPDH inhibitors. MPA is an uncompetitive inhibitor of IMPDHs from other sources (27); it binds selectively to the NAD$^+$ substrate inhibition (Figs. 2 and 3). Although the $k_{cat}$ value appears to be similar to that for the wild-type enzyme, the $K_m$ values for both substrates are significantly lower. Surprisingly, high concentrations of IMP inhibited the A251T IMPDH reaction. To our knowledge, this is the first report of IMP substrate inhibition in an IMPDH. This inhibition is competitive versus NAD.

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| Inhibitor (mechanism) | C. albicans IMPDH | A251T | Human IMPDH type II |
|-----------------------|------------------|-------|---------------------|
| MPA (UC vs. NAD)      | $K_m$ (nm)       | 11 ± 4| 44 ± 7              |
| Tiazofurin (NC vs. NAD)| $K_m$ (nm)    | 26 ± 8| 40 ± 10             |
|                       | $K_i$ (nm)      | 3.3 ± 0.3| 9 ± 1           |
| ADP (C vs. NAD)       | $K_m$ (nm)      | 34 ± 3| 16 ± 2              |
|                       | $K_i$ (nm)      | 0.7 ± 0.3| 0.32 ± 0.1   |
| MZP (C vs. IMP)       | $K_i$ (nm)      | 0.8 ± 0.2| 0.02 ± 0.01 |
|                       | $K_i$ (nm)      | 0.2 ± 0.1| 0.01 ± 0.04   |
|                       | $k_{cat}$ (M$^{-1}$s$^{-1}$) | $(5 ± 2) × 10^5$| $(1 ± 4) × 10^7$  |
|                       | $k_{cat}$ (M$^{-1}$s$^{-1}$) | $(1.0 ± 0.1) × 10^{-3}$| $(1.2 ± 0.2) × 10^{-4}$  |

* Data taken from Ref. 17.
* Data taken from Ref. 26.
* $K_i$ calculated from $k_{cat}$/$K_m$.

### Characterization of Wild-type and A251T IMPDHs—
Both the wild-type and MPA$^R$ alleles of C. albicans IMPDH were expressed in E. coli by isopropyl 1-thio-β-D-galactopyranoside induction using vector pTACTAC. Both constructs complement the guanine auxotrophy of E. coli strain H712, indicating that active IMPDHs were produced, and both enzymes were purified to >95% homogeneity as described under “Experimental Procedures” (Supplemental Fig. S2).

The wild-type enzyme displayed strong NAD$^+$ substrate inhibition, which is generally attributed to formation of a non-productive E-XMP$^*$-NAD complex. Initial velocity data collected at saturating IMP concentrations suggested that the $K_m$ value for NAD$^+$ is greater than the $K_i$ value, which complicates determination of steady-state parameters. This observation was confirmed when both substrates were varied (Figs. 2 and 3). Therefore, the $k_{cat}$ and $K_m$ values should not be considered well determined. Nevertheless, it is apparent that the kinetic parameters for C. albicans IMPDH are significantly different from those for human IMPDH, which is promising for the development of specific inhibitors. The parameters for human IMPDH type II are shown in Table II for reference; the parameters for human IMPDH type I are very similar to those for human IMPDH type II (25, 26).

In contrast to the wild-type enzyme, A251T IMPDH did not exhibit NAD$^+$ substrate inhibition (Figs. 2 and 3). Although the $k_{cat}$ value appears to be similar to that for the wild-type enzyme, the $K_m$ values for both substrates are significantly lower. Surprisingly, high concentrations of IMP inhibited the A251T IMPDH reaction. To our knowledge, this is the first report of IMP substrate inhibition in an IMPDH. This inhibition is competitive versus NAD.
tiazofurin and ADP inhibited wild-type *C. albicans* IMPDH (Fig. 6 and Table III). This observation suggests that the wild-type enzyme is predominantly in the open conformation. The A251T mutation decreased the $K_i$ value by a factor of 2. We also investigated the MZP inhibition of the *C. albicans* enzymes. Like other IMPDHs, MZP is a slow tight-binding competitive inhibitor versus IMP of wild-type *C. albicans* IMPDH (Fig. 5 and Table III). MZP is a more potent inhibitor of the fungal enzyme, with the $K_i$ value decreased by a factor of 4 relative to that for the human IMPDH (Table III) (26).

Surprisingly, the A251T mutation increased the potency of MZP, with the $K_i$ value decreased by a factor of 40 relative to that for the wild-type enzyme (Fig. 5 and Table III). In *Trichomonas foetus* IMPDH, MZP induces the closed conformation, where the flap has folded into the dinucleotide site (15). Therefore, one possible explanation for the increase in MZP affinity is that the equilibrium between the open and closed conformations has shifted toward the closed conformation.

**Mizoribine Susceptibility of C. albicans**—Surprisingly, A251T IMPDH was more susceptible to inhibition by MZP as described above. Therefore, we tested whether *C. albicans* cells harboring the MPA$^R$ allele are also more susceptible to mizoribine using microdilution assays. In agreement with earlier studies (6), mizoribine is a very potent inhibitor of *C. albicans* growth (>50% inhibition of homozygous IMH3/IMH3 strains such as SC5314 between 1 and 2 μg/ml) (data not shown). For comparison, we constructed heterozygous imh3/IMH3 and imh3/MPAR$^R$ strains and compared their susceptibility to mizoribine. The imh3/IMH3 strain was somewhat more sensitive to MPA than the IMH3/IMH3 strain CA14, as expected given that less of the IMPDH target will be present in this strain. A single copy of MPA$^R$ clearly conferred resistance to MPA as noted previously, but rendered the cells more sensitive to mizoribine (Fig. 7). This growth effect was not as dramatic as the effect on enzyme inhibition (a factor of 3 versus 40, respectively). This diminished effect was expected because drug efficacy will be modulated by substrate and target concentrations as well as the limitations of drug uptake and activation.

Mizoribine sensitivity was decreased by the presence of gua-
nine and guanosine (Supplemental Fig. S3), which indicates that IMPDH is the target of mizoribine in vivo. Xanthine did not protect the cells from mizoribine, as expected because *Candida* does not appear to have the ability to salvage xanthine to bypass a drug block at IMPDH. Moreover, both MPA and mizoribine inhibited *Candida* growth on blood agar plates, and MPA also conferred MPA resistance and mizoribine sensitivity under these conditions (Supplemental Fig. S4). These observations further validate IMPDH as an antifungal target. Surprisingly, uridine and inosine (but not cytidine) protected the cells against mizoribine. Uridine and inosine protection likely results from competition for a common transporter (29, 30). Although mizoribine transport has not been investigated, similar compounds such as ribavirin utilize adenosine/inosine/uridine transporters (31).

**DISCUSSION**

Resistance to MPA can arise from a change in the topology of the MPA-binding site (which is also the nicotinamide subsite of the NAD-binding site) or from a shift in the conformational equilibrium to favor the closed conformation (Fig. 1). These two mechanisms are not mutually exclusive and can be difficult to distinguish. Nevertheless, we believe the A251T mutation acts primarily by shifting the equilibrium to the closed conformation. First, the mutation had similar effects on both MPA and primarily by shifting the equilibrium to the closed conformation (16). These two mechanisms are not mutually exclusive and can be difficult to distinguish. Nevertheless, we believe the A251T mutation acts primarily by shifting the equilibrium to the closed conformation. First, the mutation had similar effects on both MPA and tiazofurin inhibition, increasing the $K_i$ values by factors of 3–4. These inhibitors have very different structures (Fig. 1), so it seems unlikely that a structural change would have similar effects on their potency. In contrast, changes in the open/closed conformational equilibrium should have similar effects on all inhibitors that bind to the open conformation. Second, the affinity of MZP increased ~40-fold; because MZP induces the closed conformation, this increase in affinity can also be explained by a shift in the equilibrium to favor the closed conformation. Finally, the interaction between tiazofurin and ADP was more synergistic in the A251T enzyme as measured by the 2-fold decrease in the interaction constant $\alpha$. This increase in synergy also indicates that the mutation favors the closed conformation (16).

Most importantly, these data are remarkably self-consistent. The 4-fold increase in the $K_i$ value for MPA indicates that the fraction of $E_{\text{open}}$ decreases 4-fold. Similarly, the 40-fold decrease in the $K_i$ value for MZP indicates that $E_{\text{closed}}$ increases 40-fold. Because the other enzyme must always be 1, then $E_{\text{open}} + E_{\text{closed}} = 0.25 E_{\text{open}} + 40 E_{\text{closed}}$. For the wild-type enzyme, $E_{\text{open}} \sim 0.98$ and $E_{\text{closed}} \sim 0.02$; for the A251T enzyme, $E_{\text{open}} \sim 0.2$ and $E_{\text{closed}} \sim 0.8$. If one assumes that tiazofurin and ADP bind independently to the open conformation, then the $\alpha$ value is also an estimate of $E_{\text{open}}$ (16). Both of these assumptions have been verified in experiments with IMPDH from *Trichromonas foetus* (16). Although not well determined, the $\alpha$ values are consistent with the above estimates ($\alpha = E_{\text{open}} \sim 0.7$ and $\sim 0.3$ for the wild-type and A251T enzymes, respectively).

Unfortunately, at present, the structure of *C. albicans* IMPDH has not been solved, and the sequence of the flap is too different from that of the *T. foetus* enzyme to allow reliable modeling of the closed conformation. Therefore, we can only speculate about how the A251T mutation stabilizes the closed conformation. Ala$^{251}$ corresponds to Gly$^{237}$ in *T. foetus* IMPDH (see Supplemental Fig. S5 for alignment) (Fig. 8). Gly$^{237}$ is on the same segment as Arg$^{241}$, which interacts with NAD in the open conformation and with the flap in the closed conformation. With the exception of the side chain of Arg$^{241}$, this segment has the same structure in both the open and closed conformations. It is possible that a substitution at Gly$^{237}$ could propagate to Arg$^{241}$, changing the equilibrium between the open and closed conformations. In addition, Gly$^{237}$ is within 5.0 Å of Asp$^{261}$, which also interacts with NAD in the open conformation and with the flap in the closed conformation. Position 237 can easily accommodate an Ala residue, but Thr creates a potential steric conflict with the $\beta$-carbon of Asp$^{261}$ (Fig. 8). Perhaps the repositioning of Asp$^{261}$ in response to the Thr substitution alters the equilibrium between the open and closed conformations. Interestingly, the IMPDH encoded by *IMD2* in *Saccharomyces cerevisiae* also harbors a Ser residue at the equivalent position (Ser$^{253}$, *S. cerevisiae* numbering). The IMD2 gene is the only member of the IMPDH gene family in *S. cerevisiae* that confers resistance to MPA (note that IMD1 is a pseudogene) (32, 33). Like the wild-type *IMH3* gene in *C. albicans*, the IMPDHs encoded by *S. cerevisiae* IMD3 and IMD4 contain Ala at this position. Whether Ser$^{253}$ in *S. cerevisiae* IMD2p is indeed responsible for the MPA resistance of this enzyme remains to be elucidated.

Evolution is generally presumed to favor the more catalytically efficient enzyme, yet the wild-type *C. albicans* enzyme is less efficient than the A251T enzyme as measured by the value of $k_{\text{cat}}/K_m$. Perhaps IMPDHs evolve in response to inhibitor
pressure rather than to optimize catalysis (34). Unfortunately, we do not know how _C. albicans_ originated or when it adapted to inhabit warm-blooded animals, so we can only speculate on the selective pressures driving its evolution. Both MPA and mizoribine are natural products, and it is likely that the _C. albicans_ progenitor encountered both compounds. MPA is produced by several _Penicillium_ species and has been identified in diverse environments such as sewage and cheese; it has even been suggested that immunosuppression can result from the MPA derived from these sources (35–38). Mizoribine is produced by several _Penicillium_ species and has been identified from soil, grass, water, and dairy products, where _Penicillium_ and _E. brefeldianum_ reside. Nevertheless, the opposing effects of the A251T mutation on MPA and mizoribine suggests that IMPDH may be a new antifungal target.

More antifungal drugs are urgently needed, and our results suggest that IMPDH may be a new antifungal target. _C. albicans_ is highly sensitive to IMPDH inhibitors, which suggests that guanine nucleotides are primarily supplied by _de novo_ biosynthetic pathways. Although MPA resistance can be overcome _in vitro_ by guanine supplementation, guanine levels in blood are too low to support growth, and _C. albicans_ lacks the ability to salvage xanthine. ⁹ Although MZP is a more potent inhibitor of _C. albicans_ IMPDH compared with the human enzyme, it is not sufficiently selective to be useful in chemotherapy. Nevertheless, MZP (as well as ADP) demonstrates that selective inhibition is possible. Furthermore, alignment of the human and _C. albicans_ sequences indicates that the adenine substates are very different. It may be possible to exploit the interactions of the adenine substate to develop fungus-specific IMPDH inhibitors.

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