Supplementary material for

L-Enantiomers of Transition State Analogue Inhibitors Bound to Human Purine Nucleoside Phosphorylase

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Materials and Methods

Determination of inhibition constants. Kinetic studies of the interactions between L-ImmH and L-DADMe-ImmH and human PNP were carried out by the methods previously reported.[1] Inhibitor and inosine concentrations were determined spectrophotometrically using ε_{261} = 9.54 mM^{-1} cm^{-1} and ε_{260} = 7.1 mM^{-1} cm^{-1}, respectively.[2,3] Inhibition constants were determined from nonlinear fitting to the

$$v_i = \frac{v_o[S]}{[S] + K_m\left(1 + {[I]}/{K_i}\right)}$$

equation for competitive inhibition,

where v_i and v_o are initial (for K_i) or final (for K_i*) steady-state rates in the presence and absence of inhibitor, respectively, and K_m = 40 μM for inosine.[4]

Crystallization of human PNP in complex with L-ImmH and L-DADMe-ImmH. The protein was expressed and purified using the same procedure as described before.[5] The purified protein (conc. ~30 mg/mL) was mixed with 1 mM inhibitor and 1 mM KH_2PO_4 before crystallization trials. The human PNP•L-ImmH•PO_4 complex was crystallized using sitting drops that were equilibrated by vapor diffusion at 18 °C against an 80-μL reservoir containing 1.8 M ammonium dihydrogen phosphate and 100 mM sodium citrate, pH 5.0, where 1 μL of the protein solution was mixed with 1 μL of the reservoir solution. The PNP•L-DADMe-ImmH•PO_4 complex was crystallized in a similar manner against a 1-mL reservoir containing 2.3 M ammonium dihydrogen phosphate and 100 mM sodium citrate, pH 5.0, where 3 μL of the protein solution was mixed with 3 μL of the reservoir solution.

Data collection. Crystals were soaked in mother liquor supplemented with 20% glycerol and flash cooled to –178 °C prior to data collection. Diffraction is consistent

with the space group H32 with one molecule in the asymmetric unit. The Matthews coefficient was 4.8 Å^3/Da, which corresponds to a solvent content of 76%. Diffraction data were collected to a resolution of 2.9 Å for the L-ImmH complex and 2.1 Å for the L-DADMe-ImmH complex at beamline X29A at the National Synchrotron Light Source, Brookhaven National Laboratory using an ADSC Quantum 315 detector. The HKL2000 suite[6] was used for integration and scaling of the data (see Table 2 in main text).

Structure determination and refinement. The complex structures were solved by molecular replacement using the human PNP structure 1RR6.pdb as a template. Molecular replacement with MOLREP[7] and refinement with REFMAC5[8] were carried out using the CCP4i package.[9] COOT[10] was used for molecular modeling, and figures were generated using PyMOL.[11]

References

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