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The 2.0 Å structure of human hypoxanthine-guanine phosphoribosyltransferase in complex with a transition-state analog inhibitor

Wuxian Shi 1**, Caroline M. Li** 1**, Peter C. Tyler** 2**, Richard H. Furneaux** 2**, Charles Grubmeyer** 3**, Vern L. Schramm** 1**& Steven C. Almo** 1

1. Department of Biochemistry, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, New York 10461, USA.
2. Carbohydrate Chemistry Team, Industrial Research Ltd. , Lower Hutt, New Zealand.
3. Department of Biochemistry and Fels Research Institute, Temple University School of Medicine, Philadelphia, Pennsylvania 19140, USA.
Correspondence should be addressed to S C. Almo. e-mail: almo@aecom.yu.edu and V L. Schramm. e-mail: vern@aecom.yu.edu

Abstract:
The structure of human HGPT bound to the transition-state analog immucillinGP (ImmGP) and Mg 2+ -pyrophosphate has been determined to 2.0 Å resolution. ImmGP was designed as a stable analog with the stereoelectronic features of the transition state. Bound inhibitor at the catalytic site indicates that the oxocarbenium ion of the transition state is stabilized by neighboring-group participation from MgPP iand O5'. A short hydrogen bond forms between Asp 137 and the purine ring analog. Two Mg 2+ ions sandwich the pyrophosphate and contact both hydroxyls of the ribosyl analog. The transition-state analog is shielded from bulk solvent by a catalytic loop that moves ~25 Å to cover the active site and becomes an ordered antiparallel -sheet.

Hypoxanthine-guanine phosphoribosyltransferase (HGPT) (EC 2.4.2.8.), is a central enzyme in purine salvage pathways. It catalyzes the reversible Mg 2+ -dependent transfer of the phosphoribosyl group from -D-5-phosphoribosyl 1-pyrophosphate (PRPP) to hypoxanthine or guanine to form nucleotides IMP or GMP, respectively. In humans, the complete lack of HGPT activity results in Lesch–Nyhan syndrome, a disease characterized by hyperuricemia and neural disorders, whereas the partial deficiency leads to gouty arthritis.

The phosphoribosyltransferases (PRTases) are involved in purine, pyrimidine and amino acid synthesis. Structures of several type I PRTases reveal a common fold called the PRPP motif and highlight a flexible loop. Structural studies of PRTases indicate that substrate binding induces a loop movement in residues corresponding to 100–117 of human HGPT. The loop is proposed to cover and sequester the active site from solvent during catalysis.

We describe here the crystal structure to 2.0 Å resolution of human HGPT complexed with the transition-state inhibitor, ImmGP, and Mg 2+ - pyrophosphate (MgPP). This work provides the first crystal structure of HGPT with components resembling the transition-state complex. The loop forms a well-ordered structure that contacts the bound substrate and immobilizes several water molecules. ImmGP resembles the hypothetical structure of guanine monophosphate at the transition state.

Mg 2+ ion coordination and PP ibinding

Two Mg 2+ ions with octahedral coordination are located in the active site of the human HGPRT–ImmGP–MgPPi complex. The two Mg 2+ ions chelate on opposite sides of PPi to form symmetrical, bidentate interactions with PPi. The first Mg 2+ ion interacts with the two oxygens of PP i(2.5 Å and 2.1 Å), the O2' and O3' of ImmGP (2.3 Å and 2.1 Å) and two water molecules (2.3 Å and 2.2 Å) (Fig. 4a).

The second Mg 2+ ion bound in the active site of the HGPT–ImmGP–MgPP complex has been observed only in the present structure and in the HPRT–formycin-B base–PRPP analog complex from *Trypanosoma cruzi*. Two oxygens of PPi, three water molecules and Asp193 are the closest ligands to the cation (Fig. 4b). Coordination distances to the Mg 2+ ion are 2.2 Å and 2.1 Å to the two oxygens of PPi, 2.0 Å, 2.2 Å and 2.2 Å to three water molecules, and 2.1 Å to the OD1 of Asp 193. The three water molecules liganded to the Mg 2+ also hydrogen bond with N3 of ImmGP, and the side chain of Asp193. Asp193 is the only amino acid in contact with either Mg2+ ion. A human genetic defect at this position (Asp193) causes the Lesch–Nyhan syndrome, an indication of severe HGPRT deficiency. Mg 2+ interactions are central to the spatial arrangement of the inhibitor, PPi and the side chains of the residues in the active site, and reflect its essential role in catalysis.

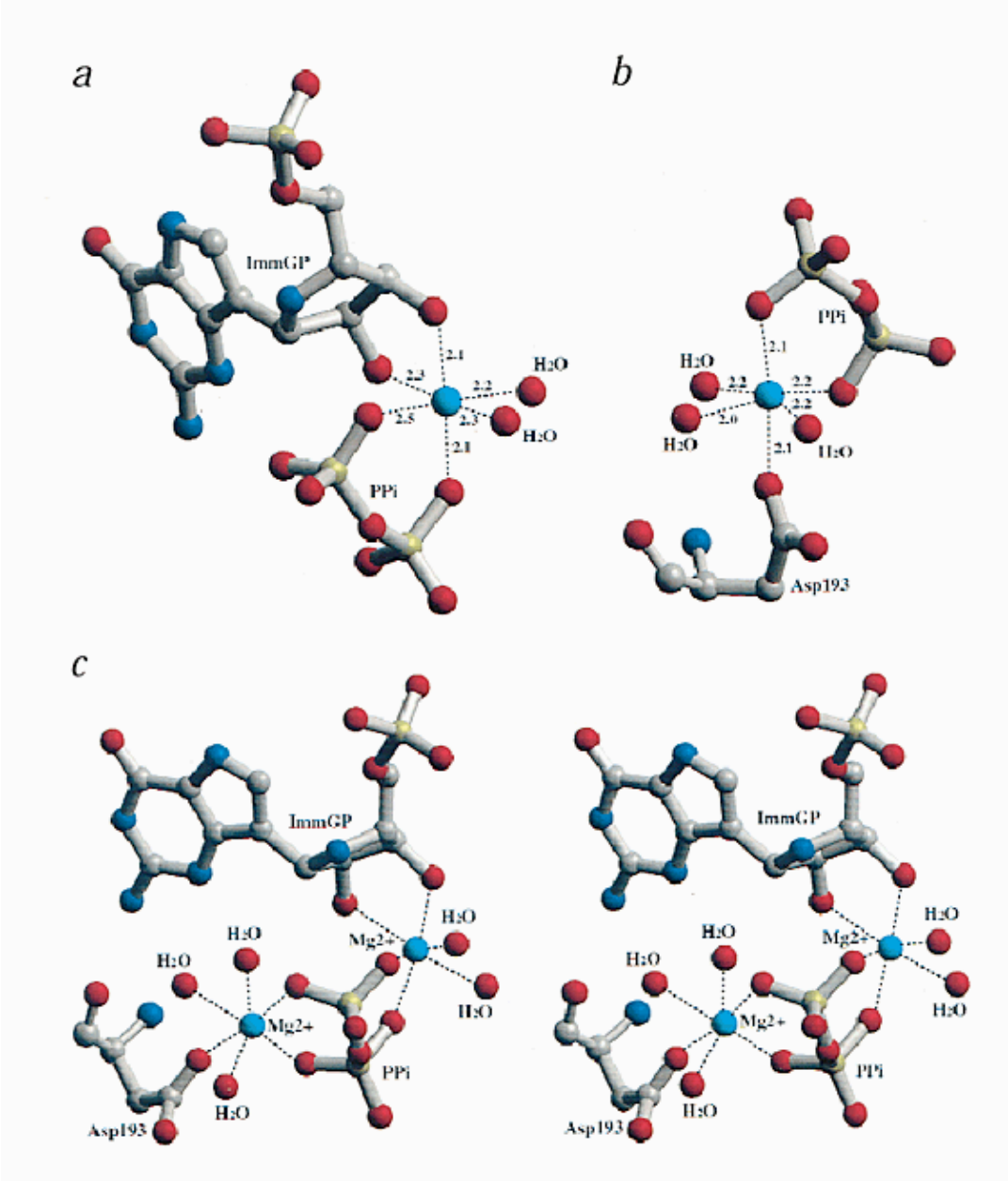

Figure 4: Mg 2+ octahedral coordinations.

a,***b***, Ligands and coordination distances of the two bound Mg 2+ .***c***, A stereo view of the two bound Mg 2+ ions in the HGPT–ImmGP–MgPPi complex.