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Anthranilate phosphoribosyltransferase: Binding determinants for 5′-phospho-alpha-D-ribosyl-1′-pyrophosphate (PRPP) and the implications for inhibitor design

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ABSTRACT

Phosphoribosyltransferases (PRTs) bind 5′-phospho-α-D-ribosyl-1′-pyrophosphate (PRPP) and transfer its phosphoribosyl group (PRib) to specific nucleophiles. Anthranilate PRT (AnPRT) is a promiscuous PRT that can phosphoribosylate both anthranilate and alternative substrates, and is the only example of a type III PRT. Comparison of the PRPP binding mode in type I, II and III PRTs indicates that AnPRT does not bind PRPP, or nearby metals, in the same conformation as other PRTs. A structure with a stereoisomer of PRPP bound to AnPRT from *Mycobacterium tuberculosis* (*Mtb*) suggests a catalytic or post-catalytic state that links PRib movement to metal movement. Crystal structures of *Mtb*-AnPRT in complex with PRPP and with varying occupancies of the two metal binding sites, complemented by activity assay data, indicate that this type III PRT binds a single metal-coordinated species of PRPP, while an adjacent second metal site can be occupied due to a separate binding event. A series of compounds were synthesized that included a phosphonate group to probe PRPP binding site. Compounds containing a "bianthranilate"-like moiety are inhibitors with IC_{50} values of 10–60 μM, and K_i values of 1.3–15 μM. Structures of *Mtb*-AnPRT in complex with these compounds indicate that their phosphonate moieties are unable to mimic the binding modes of the PRib or pyrophosphate moieties of PRPP. The AnPRT structures presented herein indicated that PRPP binds a surface cleft and becomes enclosed due to re-positioning of two mobile loops.

1. Introduction

Phosphoribosyltransferases (PRTs) catalyze phosphoribosylation of a variety of nucleophilic nitrogenous compounds [1]. The majority of PRTs use 5′-phospho-α-D-ribosyl-1′-pyrophosphate (PRPP) as a source for the phosphoribosyl moiety (PRib) and produce inorganic pyrophosphate (PP_i) [2]. Anthranilate PRT (AnPRT, EC 2.4.2.18; *trpD*) represents a structurally distinct class of these enzymes that does not contain the 13-residue sequence known as the "PRPP-binding motif" found in type I PRTs [3,4]. AnPRT has been shown to be promiscuous, catalyzing phosphoribosylation with nucleophiles other than its native substrate, anthranilate, including methylated and fluorinated anthranilates [5], as well as enamines such as 2-aminocrotonate, 2-amino-2-pentenoate, and 2-aminoacrylate [6]. This promiscuousity has been shown to have *in vivo* role in thiamine biosynthesis for *Escherichia coli* [7].

The PRT family contains at least four distinct structural folds. Type I PRTs act on purines and pyrimidines as part of salvage pathways (*e.g.*

Abbreviations: AnPRT, anthranilate phosphoribosyltransferase; IC_{50}, absolute IC_{50}, that is the concentration of inhibitor which decreased enzyme activity by 50%; K_m, Michaelis-Menten constant; K_i, inhibition constant; *Mtb*, *Mycobacterium tuberculosis*; PP_i, pyrophosphate; PRA, *N*-(5′-phosphoribosyl)anthranilate; PRAI-InGPS, PRA isomerase-indoleglycerol phosphate synthase; PRib, phosphoribosyl; PRPP, 5′-phospho-α-D-ribosyl-1′-pyrophosphate; 1PR5PP, 1′-phospho-α-D-ribosyl-5′-pyrophosphate; PRT, phosphoribosyltransferases; RMSD, root mean square difference; *Sso*, *Sulfolobus solfataricus*

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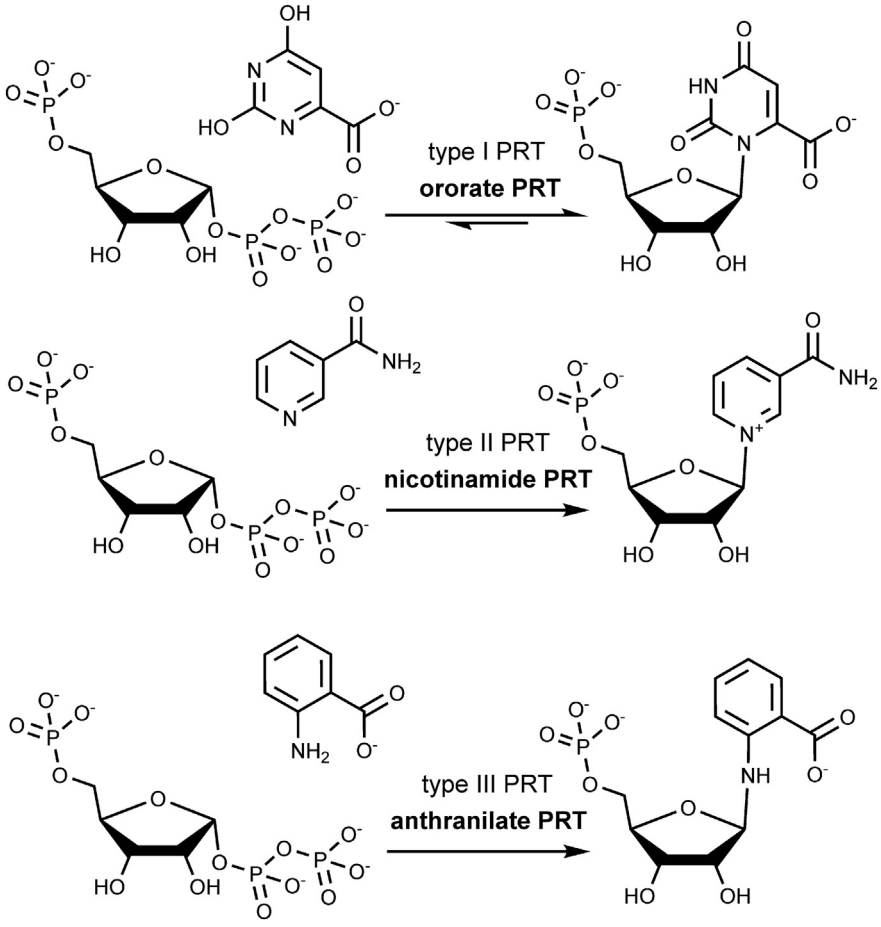

Fig. 1. Reactions catalyzed by type I, II and III PRTs.

adenine PRT, hypoxanthine PRT, uracil PRT) or in the case of orotate PRT as part of *de novo* pyrimidine biosynthetic pathway [8]. Quinolate PRT, nicotinate PRT and nicotinamide PRT are type II PRTs [8], and the only type III and IV PRTs identified to date are AnPRT [3,9] and ATP PRT [10], involved in tryptophan and histidine biosynthesis, respectively. Type III PRTs phosphoribosylate 6-carbon ring nucleophiles, as do some type I and II PRTs (Fig. 1). For type I PRTs, kinetic isotope effect (KIE) analysis has indicated that their substrate nucleophile is a weak participant in their transition state [8].

ATP, pyrophosphate (PP_i) and other phosphate-rich ligands can form complexes with Mg^{2+} and these ligand:Mg^{2+} complexes, rather than their unchelated equivalents, are the active substrates for many enzyme classes *e.g.* ATP synthases and phosphatases. PRPP has two phosphate-containing moieties linked by a sugar, with each moiety able to chelate Mg^{2+} [11]. For type I PRTs, PRPP binding sites have been found to involve one or two magnesium ions [8], and Mg^{2+} has been hypothesized to shield approaching nucleophiles from the negative charge of PRPP [12] and/or make PP_i a better leaving group [13]. For type III PRTs, PRPP and two magnesium ions are observed in the same position in multiple structures that have been determined for AnPRT from *Mycobacterium tuberculosis* (*Mtb*) [5,14–17]. The binding mode of Mg^{2+} and PRPP in AnPRT from the thermophile *Sulfolobus solfataricus* (*Sso*) is less clear (based on examination of the electron density at the active site in crystal structures of PDB entries 1ZYK and 1ZXY, [18] and analysis of these structures with the CheckMyMetal web server [19]).

We previously determined the X-ray crystal structure of AnPRT from *Mtb* strain H37Rv, and found it to be a two-domain protein that exists as a homodimer in solution [17]. We also screened a targeted library of anthranilate-like compounds, among which the best hit (ACS172; 2-(2-carboxyphenylamino)benzoic acid) had a IC_{50} value of 40 ± 2 μM [14,16]. We determined the structure of *Mtb*-AnPRT in complex with ACS172, PRPP and Mg^{2+}, showing that PRPP bound in the bottom of a tunnel, 15 Å from the tunnel's entrance (PDB ID: 3QQS; [14]). Comparison of *Mtb*-AnPRT structures indicate that two loops near the PRPP binding site re-arrange upon PRPP binding (*e.g.* complexes with ACS172 in the presence and absence of PRPP; PDB IDs: 3QQS [14] and PDB 4IJ1 [16], respectively). Most amino acids involved in binding PRPP are part of these two mobile loops (encompassing residues 107–117 and 138–146; loops I and II). Loop I contains a sequence GTGGD that is invariant among AnPRTs [17].

AnPRT catalyzes the second step in the biosynthesis of tryptophan. The failure of tryptophan auxotroph strains of *Mtb* H37Rv to cause disease in mice or survive in human macrophages [20,21], indicates the importance of tryptophan biosynthesis during infection. Recently, inhibitors of tryptophan synthase, which catalyzes the final step of the synthesis, have been identified as effective anti-mycobacterial compounds, both *in vitro* and in animal models of disease [22]. Thus *Mtb*-AnPRT is also target of interest in developing novel anti-tuberculosis treatments [5,14,16,17]. This work highlights unique features of metal and PRPP-binding in type III PRTs and considers the challenge they pose for designing compounds that will compete for the PRPP binding site. We have also synthesized and tested phosphonate-containing compounds designed to probe the PRPP binding site, and determined protein:ligand complex structures for them.

2. Materials & methods

2.1. Materials

Unless otherwise stated, all chemicals were obtained from Sigma-Aldrich, Scharlau, or Pure Science. The substrates of *Mtb*-AnPRT, anthranilate and PRPP, were obtained from Sigma-Aldrich. Protein