How a purine salvage enzyme singles out the right base

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Two phosphoribosyltransferases in the purine salvage pathway exhibit exquisite substrate specificity despite the chemical similarity of their distinct substrates, but the basis for this discrimination was not fully understood. Ozeir et al. now employ a complementary biochemical, structural, and computational approach to deduce the chemical constraints governing binding and propose a distinct mechanism for catalysis in one of these enzymes, adenine phosphoribosyltransferase. These insights, built on data from an unexpected finding, finally provide direct answers to key questions regarding these enzymes and substrate recognition more generally.

The fundamental understanding of enzyme–substrate specificity was introduced through the “Schlüssel-Schloss-Prinzip” (lock and key principle) by Emil Fischer in 1894. Mechanistic understanding of enzyme function has grown beyond this uncompromising theory beginning with Pauling’s seminal proposal of preferential binding of the transition state to the enzyme to today, where much is known about the determinants of substrate specificity and the mechanistic details of catalysis. This information adds to the knowledge base and is integral to the development of analogs that can serve as drugs with extensive implications in human health and disease, motivating continuing efforts even in well-trodden areas. For example, phosphoribosyltransferases have been studied extensively, yet critical questions still remain unanswered. This list of questions has just been shortened, however, by a new work from Ozeir et al. (1), in which a crystal structure that defined the odds has helped to demonstrate the specific chemical and conformation features that determine the substrate specificity and reaction mechanism of adenine phosphoribosyltransferase (APRT).²

Phosphoribosyltransferases reversibly catalyze the formation of a glycosidic bond, transferring a ribose phosphate moiety from 5-phospho-α-D-ribose 1-diphosphate (PRPP) to an acceptor molecule (purine, pyrimidine, ATP, quinolinate, nicotinamide, etc.). APRT and hypoxanthine-guanine phosphoribosyltransferase (HGPRT) are both involved in the purine salvage pathway, in which purines acquired from the environment are converted to mononucleotides that feed into the overall nucleotide pool. These enzymes are thus vital for actively metabolizing cells of protozoan parasites and cancer tissues, and their deficiencies are causative of physiological disorders such as Lesch–Nyhan syndrome, gouty arthritis, and urolithiasis. HGPRT and APRT have distinct specificities for either 6-oxopurines (i.e. guanine and hypoxanthine) or 6-aminopurine (i.e. adenine), respectively. However, as these purines differ only in the functional groups at the C2 and C6 carbons, the basis for selectivity is unclear. Moreover, the extensive literature available (2–9) has demonstrated variations in reaction mechanism; for example, different HGPRTs have been shown to employ S₈,1 or S₈,2 mechanisms. The mechanism for APRT was not known.

The new study from Ozeir et al. (1) takes on both of the mysteries surrounding APRT. The authors first report differential scanning fluorimetry data and biochemical assays that suggest hypoxanthine might not bind at all to hAPRT (1). Despite this information, they crystallize the protein in the presence of high concentrations of the purine, and remarkably, they get a crystal structure of the complex of human APRT (hAPRT) with hypoxanthine, PRPP, and Mg²⁺ in the active site. Similar experiments with guanine failed to trap this ligand in the active site. Comparing the hypoxanthine structure with a previous hAPRT–adenine–PRPP complex allowed them to determine molecular features responsible for conferring substrate specificity in this enzyme (Fig. 1). Compared with adenine, hypoxanthine shows relatively poorer electron density, as expected from its very weak affinity to the enzyme. Examination of the contacts of N1 and N3 of hypoxanthine with Arg-27 and Arg-67 in the ternary complex indicates that they are hydrogen bond acceptors, suggesting that the purine is present as the enol tautom. The contact of C6O with the backbone carbonyl of Val-25 and the side chain of the catalytic base Glu-104 similarly points to the enol form. In contrast, in HGPRTs, the interaction with the side chain of an invariant lysyl residue ensures binding of the predominant keto form of the 6-oxopurine base (10) (Fig. 1A). The authors suggest that the destabilization effect of the enol form of hypoxanthine prevents phosphoribosylation. This may also suggest a general distinguishing principle for the two families of enzymes, one with specificity for oxopurines (keto form) and another with specificity for aminopurines.

What about the reverse reaction? Unlike hypoxanthine and guanine, IMP and GMP do show moderate binding affinities with hAPRT, both in earlier inhibition studies and new differential scanning fluorimetry reported by Ozeir et al. (1). To understand why hAPRT can bind but not turn over these substrates, the

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2 The abbreviations used are: APRT, adenine phosphoribosyltransferase; hAPRT, human APRT; PRPP, 5-phospho-α-D-ribose 1-diphosphate; HGPRT, hypoxanthine-guanine phosphoribosyltransferase.

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authors obtained structures bound to IMP and GMP and compared these with the AMP-bound structure. Interestingly, in both IMP and GMP complexes, the oxopurine base appears to be in the keto form, meaning that other factors have impeded the reverse reaction from proceeding. The keto tautomers alter key hydrogen bonds with the purine nitrogens, leading to different energetics and different conformations within the ligand binding site. The AMP N1 interacts with the Arg-27 amide NH, whereas the protonated IMP N1 interacts with the Arg-27 backbone carbonyl, resulting in the displacement of the oxopurine ring that in turn reorients the Arg-67 side chain into the diphosphate-binding region, thereby occluding it from binding and catalysis. This diphosphate occlusion was observed in the GMP structure as well.

Finally, the authors use QM/MM calculations to explore the mechanism of hAPRT’s two reactions. Their approach provides a highly accurate description of the electronic structure of atoms in the QM region using hybrid density functionals. Similar QM/MM calculations were used to determine the mechanism of another phosphoribosyltransferase, HG(X)PRTase, as following the DNAN (SN2) mechanism for the same reaction in hAPRT, as the DNAN route is shown to involve an intermediate with a high energy of 100 kJ/mol over the reactant state, unlike the ANDN mechanism, whose intermediate is 7 kJ/mol more stable than the reactants.

It will be interesting to see what new information can be obtained from additional calculations that explicitly consider changes in the configurational entropy along the reaction coordinate, although the unfavorable DNAN intermediate suggests that these methods would not significantly alter the mechanism from the one proposed. Similarly, corroboration of these QM/MM studies with investigations involving kinetic isotope effect and spectroscopic methods should be performed to confirm the SN2 type mechanism in human APRT as proposed in this paper. Finally, it will be exciting to see how these new insights into hAPRT and HGPR T discrimination inform inhibitor design strategies, with the hope of disrupting parasites and cancer and treating human disorders.

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