Introduction

Aminoacyl-tRNA synthetases play an essential role in protein synthesis by producing charged tRNAs: an amino acid is first condensed with an ATP molecule to form a stable aminoacyl-adenylate intermediate, then the amino acid is transferred onto a cognate tRNA to form the desired product. Because the synthetases play such an essential role, compounds that specifically inhibit bacterial aminoacyl-tRNA synthetases could become potent antibacterial drugs. This concept is proven by the success of the broad-spectrum antibacterial drug mupirocin, which targets bacterial isoleucyl-tRNA synthetases (IleRS). Multidrug-resistant bacteria are becoming more prevalent and present a major threat to public health. For instance, multiple-resistant Staphylococcus aureus causes serious hospital-acquired infections that are difficult to treat. With today's technology, it is possible to obtain large amounts of aminoacyl-tRNA synthetases, especially those from virulent strains, such as S. aureus, and use them to generate inhibitors via high-throughput screening of compound libraries. Studying aminoacyl-tRNA synthetases will not only enrich our fundamental understanding of this class of essential enzymes, but also provide us with new insights into combating bacterial infections. In this study, we work with the tyrosyl-tRNA synthetase (TyrRS) from S. aureus and obtain crystal structures of the enzyme-inhibitor complexes to aid the design of better inhibitors.

Results and Discussion

We have determined the crystal structures of the S. aureus tyrosyl-tRNA synthetase in complex with numerous inhibitors (Fig. 1). TyrRS is a member of the class I synthetases, characterized by a Rossmann fold in the catalytic domain and the so-called HIGH and KMSKS motifs for ATP binding. TyrRS also has an α-helical domain, linked to the catalytic domain via residues 221-247, and a C-terminal domain that is disordered in most of the structures. The catalytic domain contains a six-stranded parallel β-sheet and a deep active-site cleft that binds ligands such as tyrosine. The tyrosine amino group forms hydrogen bonds with Tyr169 OH, Asp78 OD1 and Gln173 OE1, the phenolic hydroxyl group forms hydrogen bonds with Asp176 OD1 and Tyr34 OH, and the carboxyl group interacts with Lys82 side chain via a water molecule. All these polar interactions are well conserved in the tyrosyl- and tyrosinyl-adenylate complexes. In the adenylate complexes, the α-phosphate group interacts with Asp38 N, the 2’-hydroxyl group of ribose interacts with the Asp194 carboxylate and Gly192 N, the 3’-hydroxyl group interacts with a tightly bound water, while the adenine moiety makes nonpolar contacts with the enzyme at Leu222, Val223 and Gly47, which is part of the HIGH motif. It has been postulated that Thr40 and His45 (part of the HIGH motif) interact with the γ-phosphate of ATP and are essential for the formation of tyrosyl-AMP. Our inhibitors bind to TyrRS using the aforementioned binding sites. We also made a truncation mutant of the enzyme, which allowed us to extend the resolution of the TyrRS structures about 2 Å. These structures offer a structure-based strategy for inhibitor design.

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