Structures of Tryptophanyl-tRNA Synthetase II from Deinococcus radiodurans Bound to ATP and Tryptophan

INSIGHT INTO SUBUNIT COOPERATIVITY AND DOMAIN MOTIONS LINKED TO CATALYSIS*

An auxiliary tryptophanyl tRNA synthetase (drTrpRS II) that interacts with nitric-oxide synthase in the radiation-resistant bacterium Deinococcus radiodurans charges tRNA with tryptophan and 4-nitrotryptophan, a specific nitration product of nitric-oxide synthase. Crystal structures of drTrpRS II, empty of ligands or bound to either Trp or ATP, reveal that drTrpRS II has an overall structure similar to standard bacterial TrpRSs but undergoes smaller amplitude motions of the helical tRNA anti-codon binding (TAB) domain on binding substrates. TAB domain loop conformations that more closely resemble those of human TrpRS than those of Bacillus stearothermophilus TrpRS (bsTrpRS) indicate different modes of tRNA recognition by subclasses of bacterial TrpRSs. A compact state of drTrpRS II binds ATP, from which only minimal TAB domain movement is necessary to bring nucleotide in contact with Trp. However, the signature KMSKS loop of class I synthetases does not completely engage the ATP phosphates, and the adenine ring is not well ordered in the absence of Trp. Thus, progression of the KMSKS loop to a high energy conformation that stages acyl-adenylation requires binding of both substrates. In an asymmetric drTrpRS II dimer, the closed subunit binds ATP, whereas the open subunit binds Trp. A crystallographically symmetric dimer lacks no ligands. Half-site reactivity for Trp binding is confirmed by thermodynamic measurements and explained by an asymmetric shift of the dimer interface toward the occupied active site. Upon Trp binding, Asp68 propagates structural changes between subunits by switching its hydrogen bonding partner from dimer interface residue Tyr139 to active site residue Arg30. Since TrpRS IIs are resistant to inactivation by inhibitors of standard TrpRSs, and pathogens contain drTrpRS II homologs, the structure of drTrpRS II provides a framework for the design of potentially useful antibiotics.

The most critical molecular recognition events in the translation of the genetic code involve the error-free attachment of 21 amino acids to tRNAs that bear the appropriate anticodon triplets (1–3). These reactions are catalyzed by aminoacyl-tRNA synthetases (AARSs),¹ which are divided into two classes (I and II) based on their structures and reactivities (4). Class I synthetases attach amino acids to the 2'-hydroxyl group of the 3'-terminal adenosine of tRNA and comprise a catalytic Rossmann fold (RF) domain and a helical tRNA anticodon binding domain (TAB) (5). Two signature sequence motifs (KMSKS and T/H/IGN) that participate in ATP binding typify class Ic synthetases (TrpRS, TyrRS, and PheRS). Some class I synthetases also incorporate an insertion domain (CP1) and participate in additional cellular functions such as RNA splicing, tRNA processing (6) RNA trafficking, and apoptosis (7).

Although most AARSs are usually expressed from single copy genes to avoid the proliferation of synthetase mutations that could corrupt the genetic code, two copies of some synthetases are found in select bacteria and archaea. In particular, Deinococcus radiodurans contains two tryptophanyl-tRNA synthetases (TrpRSs), Whereas D. radiodurans TrpRS (drTrpRS) I has 40% sequence identity to most single copy bacterial TrpRSs, drTrpRS II is more divergent, with only 28% sequence identity (8). We have recently shown that drTrpRS II interacts with D. radiodurans nitric oxide synthase (8, 9) and can charge tRNA with Trp and 4-nitrotryptophan, a specific nitration product of D. radiodurans nitric oxide synthase. drTrpRS II is induced during responses to DNA damage. It contains an unusual 30-residue extension, uncharacteristic of any other known prokaryotic TrpRSs (8) but homologous to the N terminus of the stationary phase stress-response protein, SurE (10).

Extensive crystallographic work by Carter and colleagues (11–13) has established TrpRS from Bacillus stearothermophilus (bsTrpRS) as a paradigm for understanding the structural basis of enzymatic activity for class Ic AARSs. Crystal structures of bsTrpRS in three distinct conformations (open, closed, and closed pretransition state (pre-TS)) provide snapshots of important states in the catalytic cycle (12–14). An open conformation binds either ATP or Trp in a kinetic scheme described as “random bi-bi” (15, 16). Favorable binding interactions provided by the second substrate overcome the stability of the open state and result in domain closure. This high energy closed conformation (inferred in the closely related TyrRS from kinetoplastid studies (17, 18)) engages the conserved KMSKS loop of the TAB domain with the ATP pyrophosphate group and brings the Trp carboxylate in close proximity of the ATP α-phosphate.

¹ The abbreviations used are: AARS, aminoacyl-tRNA synthetase; ADP/PPN, 5'-adenyl-β,γ-mididiphosphate; drTrpRS II, tryptophan tRNA synthetase II from D. radiodurans; bsTrpRS, tryptophan tRNA synthetase from B. stearothermophilus; kTrpRS, tryptophan tRNA synthetase from human; TrpRS, tryptophanyl tRNA synthetase; TS, transition state; RF, Rossmann fold; TAB, tRNA anti-codon binding; r.m.s., root mean square.

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The atomic coordinates and structure factors codes (1YID and 2AAM) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

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Subunit Communication in an Auxiliary TrpRS

The strain generated in the pre-TS drives formation of the acyl-adenylate by destabilizing the enzyme-substrate ground state. More recently, the crystal structure of ligand-free human TrpRS (hTrpRS) has revealed one conformation that resembles the bsTrpRS closed form (19).

Substrate binding and catalytic properties of prokaryotic and eukaryotic TrpRSs share some common features that complement similarity in their overall structures (20). Both the Escherichia coli and bovine TrpRSs bind two Trps per dimer. However, E. coli TrpRS binds both Trps with nearly identical affinity, whereas bovine TrpRS exhibits anticooperative binding (20). Both the E. coli and bovine TrpRS exhibit no cooperativity in ATP binding. This contrasts with ATP binding by bsTrpRS, where the second molecule of ATP binds with a drastically reduced affinity compared with the first (12). However, since all the structures of bsTrpRS are highly symmetric (most due to crystallographic symmetry), little information is available about the mechanism of intersubunit communication in TrpRSs.

The mode of tRNA recognition by bsTrpRS has largely been inferred from the structures of other class I synthetases (such as TyrRS) in complex with tRNA (13, 19). These structures indicate that one tRNA binds both subunits of the dimer simultaneously (21–23). The TAB domain of one subunit binds the anticodon, whereas the RF domain of the adjacent subunit catalyzes aminoaacetylation (12, 23). It was proposed, based on structural homology of bsTrpRS to TyrRS, that adjacent loops comprising residues 219–226 and residues 259–266 of the TAB domain recognize the tRNA anticodon (13). Mutational studies in Bacillus subtilis TrpRS indicate that a region close to the first TAB domain loop (residues 233–237 in bsTrpRS) are involved in binding the anticodon (24), whereas an RF domain helix (αε, residues 106–120, bsTrpRS numbering) recognizes the tRNA acceptor arm.

Tryptophan recognition by bsTrpRS and drTrpRS II differs significantly. Our recent crystal structures of drTrpRS II bound to Trp (Protein Data Bank code 1Y18) or 5-hydroxy-Trp (Protein Data Bank code 1YIA) show that the presence of several residue substitutions (conserved by a subset of TrpRS IIs) alters orientation of the indole ring in the active center and thereby allows binding of Trp moieties derivatized at the 4- and 5-positions (25).

We now report the 2.4 Å crystal structures of drTrpRS II free and in complex with ATP and compare these structures with the Trp-bound enzyme. These structures reveal that Trp and ATP bind to the “closed” and “open” conformers of drTrpRS II, respectively. However, the closed and open conformations of drTrpRS II differ only modestly in TAB domain orientation, which more closely resembles that of hTrpRS than bsTrpRS. Furthermore, no large motions are required for the system to progress to a state competent for adenyl Trp formation. Also, in contrast to bsTrpRS, the conformations that bind substrates involve asymmetric dimers, in which only one subunit occupies ligand. Distinct structural changes propagate effects of ligand binding through the dimer interface to the adjacent subunit. These results explain subunit communication in TrpRS II and indicate that not all TrpRSs require large domain motions to couple protein conformational energy to ligand binding and catalysis.

MATERIALS AND METHODS

Cryostatization, Data Collection, and Structure Determination of drTrpRS II—drTrpRS II was prepared as previously described (20) and concentrated to 40 mg/ml in 50 mM Tris (pH 7.5), 150 mM NaCl, 10 mM Mg-ATP. Diffraction quality crystals were obtained by mixing 2 μl of the protein solution described above with 2 μl of well solution composed of 22% polyethylene glycol 4000, 0.2 M diammmonium hydrogen phosphate. The protein drop was equilibrated against 500 μl of well solution by vapor diffusion in a hanging drop setup at 25 °C. Although 10 mM Mg-ATP was present at all steps of purification and crystallization, crystals were soaked in stabilization buffer (50 mM Tris (pH 7.5), 150 mM NaCl, 22% polyethylene glycol 4000, 0.2 M ammonium phosphate) and 10 mM Mg-ATP 2 h prior to data collection. Higher concentrations of Mg-ATP were precluded by the crystallization of the nucleotide itself in the polyethylene glycol/phosphate buffer where the crystals are stable. Longer soaking times (4, 12, and 16 h) reduced the occupancy of ATP bound to TrpRS II in the crystals. Crystals were briefly soaked in cryoprotectant (22% polyethylene glycol 4000, 0.2 M diammmonium hydrogen phosphate, 10 mM Mg-ATP, 10% methyl pentanediol) and flash frozen in liquid nitrogen prior to data collection. TrpRS II crystals diffracted to 2.4 Å resolution on beamline F1 of Cornell High Energy Synchrotron Source at 100 K. Data were integrated and scaled with HKL2000 (26). The resulting structure of drTrpRS II had one of three subunits bound to ATP. Initial phases were determined by molecular replacement with AmoRe (27) (correlation coefficient = 0.57, initial R-factor = 40.1%) with the previously reported structure of drTrpRS II as the model (25). Although these crystals belong to the same space group (C2) and have the same overall molecular packing arrangement as those of the previously reported structure (25), small changes in packing and cell dimensions required the use of molecular replacement. (Rigid body refinement with the coordinates from the ATP-bound structure of drTrpRS II rendered a high R-factor of 40%.)

RESULTS

Overall Structure—The crystal structure of drTrpRS II resembles known structures of B. stearothermophilus TrpRS (bsTrpRS) and human TrpRS (hTrpRS), with exceptions of striking differences within the tRNA anti-codon binding (TAB) domain and the Trp-binding pocket. Like other TrpRSs, drTrpRS II forms an obligate dimer (Fig. 1, A and B). Two different dimers compose the crystals, but only one and one-half dimers compose the asymmetric unit. Hence, in the first dimer, the two subunits (A and A’) are identical by crystallographic 2-fold symmetry, whereas in the second, the two subunits (B and C) are pseudosymmetric because of changes in relative domain orientations and loop conformations. The three crystallographically unique drTrpRS II subunits have distinctly different recognition properties with respect to substrates.

Two domains comprise the drTrpRS II subunit: a large N-terminal, RF domain (residues 21–202 and 317–351), which has the characteristic αβγ three-layered sandwich of dinucleotide-binding proteins, and a smaller, helical, TAB domain (residues 208–307) (Fig. 1, A and B). The RF and TAB domains connect through two hinge regions that comprise an extended polypeptide segment (residues 203–207) and the junction between two long C-terminal helices (αE1 and αE2) that bracket both domains (Fig. 2). Movement about the hinges results in three different TAB domain orientations for the three molecules in the asymmetric unit (Fig. 3).

Whereas the subunits of bsTrpRS show only subtle asymmetry in all known crystal forms and hTrpRS exclusively forms a crystallographic dimer, the B and C subunits of drTrpRS II are clearly not structurally equivalent (Fig. 3). Whereas the A and
C subunits have a similar "open" TAB domain conformation (r.m.s. deviation of Cα positions between A and C = 1.2 Å), the B subunit adopts a partly closed conformation (r.m.s. deviation of Cα positions between B and C = 2.0 Å). The asymmetry between subunits arises both from a 5° rotation about the hinge regions (residues 300–305) and different loop conformations within the TAB domains themselves (r.m.s. deviation of B and C TAB domain Cα positions = 2.8 Å; Fig. 3). All TAB domain orientations of drTrpRS II are intermediate to the open and closed conformations of bsTrpRS (which differ by a 13° rotation of the TAB domain (12)) but are similar to that of hTrpRS (Fig. 1, C and D). Comparisons of the isolated domain topologies of bsTrpRS and drTrpRS II show that whereas the RF domains are very similar to each other (r.m.s. deviation on Cα positions = 1.6 Å), the TAB domains differ considerably (r.m.s. deviation on Cα positions = 3.4 Å) (Fig. 1D).

The TAB Domain of drTrpRS II and hTrpRS Have Similar Structures and Orientations—Superposition of TAB domains of drTrpRS II and bsTrpRS I reveals striking differences in the loop regions that are implicated in tRNA<sup>Trp</sup> anti-codon recognition. Although three of the four helices adopt conformations similar to those of bsTrpRS, helix α<sub>12</sub> tilts to bring the preceding α<sub>11</sub>-to-α<sub>12</sub> loop closer to the loop connecting α<sub>13</sub> and α<sub>14</sub> (Figs. 2 and 4). The two loops interact with each other through a main-chain hydrogen bond between Gly<sup>280</sup> and Ala<sup>249</sup> (Fig. 4A). These peripheral TAB domain loops have very different conformations from those found in bsTrpRS (Fig. 4A), where the equivalent α<sub>11</sub>-to-α<sub>12</sub> loop extends toward the active center cleft and does not interact with the α<sub>13</sub>-to-α<sub>14</sub> loop. The equivalent loop regions of hTrpRS resemble those of drTrpRS II in that they also retract from the interdomain cleft (Fig. 4B). Since these regions are implicated in anti-codon binding, drTrpRS II and hTrpRS may share aspects of tRNA recognition. In addition, the loop preceding the KMSKS motif is three residues shorter in drTrpRS II and hTrpRS compared with the corresponding loop in bsTrpRS. This could have implications for the activation of adenylation (see below).

Incorporation of ATP into drTrpRS II Crystals—Although drTrpRS II was purified in the presence of 10 mM Mg-ATP and has a K<sub>D</sub> for Mg-ATP of ~10 μM (8), Mg-ATP was found bound in the active center only if the crystals were soaked with 10 mM Mg-ATP 2 h prior to data collection. Higher concentrations of Mg-ATP were precluded in the soaking experiments by crystallization of ATP in the polyethylene glycol/phosphate buffer that stabilizes the crystals. No evidence was found for the binding of the nonhydrolyzable ATP analog ADPβP under similar conditions. A difference electron density map (F<sub>o</sub> - F<sub>c</sub>) calculated with diffraction data from the ATP-soaked crystals and data from crystals grown in the absence of additional ATP shows electron density for the bound nucleotide only in one of the three drTrpRS II subunits contained within the asymmetric unit (Fig. 5). In the most closed B subunit of the asymmetric dimer, the F<sub>o</sub> - F<sub>c</sub> electron density map derived from the refined structure also reveals clear density for the three phosphates, magnesium, and the ribose unit at 2.5 σ but only weak density for the adenine base at this contour level (Fig. 6). Thus, although ATP is present in a substantial fraction of the B subunits, it is either not completely occupied or partially disordered in the binding site. Accordingly, it is difficult to unambiguously differentiate the position of magnesium versus the triphosphate moiety based on the experimental electron density alone. However, the best fit of the nucleotide produces a conformation that is nearly identical to that of Mg-ATP in the active center of bsTrpRS (Fig. 6). The removal of magnesium from the model generates positive electron density peaks in Fourier difference maps surrounding the triphosphate group.

The Closed Conformer of drTrpRS II Binds ATP, whereas the Open Conformer Binds Trp—ATP makes similar contacts with the KMSKS and HLGH motifs of drTrpRS II as observed in the open complex of bsTrpRS with 1 mM ATP (Fig. 6, A and B) (12).
The carbonyl oxygen of Met²¹⁶ is aligned to hydrogen-bond to the amino group of the ATP adenine ring in both structures but is out of hydrogen bonding range in drTrpRS II, perhaps reflecting the weak density of the adenine base in this complex. Ser²¹⁷ and Ser²¹⁹ hydrogen-bond to the \( ^{\text{H9252}} \) and \( ^{\text{H9253}} \)-phosphates with their side-chain hydroxyl groups (as do the equivalent Ser¹⁹⁴ and Ser¹⁹⁶ residues in bsTrpRS). However, in the closed pre-TS conformation of bsTrpRS, the Ser¹⁹⁴ hydroxyl switches to hydrogen-bond with the Lys¹⁹² side chain. Furthermore, the KMSKS loop moves closer to the nucleotide, allowing the main chain nitrogens of Lys¹⁹⁵ and Ser¹⁹⁶ region to coordinate the \( ^{\text{H9252}} \)- and \( ^{\text{H9253}} \)-phosphates (Fig. 6C). In drTrpRS II, His¹⁹⁰ (of the HLGH motif) substitutes for bsTrpRS Asn¹⁸ (of the analogous TIGN motif), but both residues interact with the respective ribose ring oxygen (Fig. 6B). Lys²¹⁵ and Lys²¹⁸ of the KMSKS loop, which bind the \( ^{\beta} \) - and \( ^{\omega} \)-phosphate, respectively, in the bsTrpRS pre-TS, are disordered and project toward solvent in the drTrpRS II ATP complex (Fig. 6B). Most significantly, the indole ring in drTrpRS II tilts by \(-45^\circ\), and the \( ^{\omega} \)-amino and \( ^{\beta} \)-carboxyl moieties exchange positions in the binding

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**FIG. 1.** A, ribbon diagram of the drTrpRS II asymmetric dimer that binds ATP. Each subunit has an RF catalytic domain (B in cyan and C in dark blue) and a TAB domain (B in violet and C in red). ATP binds only the B subunit, which has a more closed conformation than the C subunit. B, ribbon diagram of drTrpRS II asymmetric dimer colored as in A with L-tryptophan bound in the more “open” C subunit (Protein Data Bank code 1YI8). C, superposition of drTrpRS II with bsTrpRS in the open and closed conformations (Protein Data Bank codes 1D2R and 1I6M, respectively). Only equivalent secondary structure elements in the respective RF domains were superimposed. D, superposition of drTrpRS II with human TrpRS (hTrpRS; Protein Data Bank code 1Q5T).
pocket (25). Several substitutions\(^2\) in the active center of drTrpRS II mediate this change in binding orientation (25) (Fig. 7). In particular, Met\(^{129}\), which stacks against the Trp indole and \(\alpha\)-amino group in most other TrpRSs, is replaced by Gln\(^{154}\), which instead hydrogen-bonds to the Trp carboxylate in the flipped orientation (25). Movement of highly conserved Gly\(^{28}\) induced by three other substitutions (Asp\(^{29}\), Arg\(^{47}\), and Val\(^{58}\)) compensates for the tilted indole ring in drTrpRS II. Gln\(^{64}\), which participates in a hydrogen bond network with Arg\(^{30}\) and Gln\(^{154}\), substitutes for a highly conserved Tyr\(^{43}\). Taken together, the unusual residues in the drTrpRS II binding pocket combine to expose the 4- and 5-position of the Trp indole within the active center channel, allowing drTrpRS II to accommodate modified indoles (25). Arg\(^{30}\), a residue conserved only by TrpRS IIIs, interacts with both the ATP \(\alpha\)-phosphate and the Trp amino acid group in the same conformation. This residue then couples the two binding sites and contributes to relative rigidity of TrpRS II.

Superposition of B and C molecules from the crystal structures of drTrpRS II bound to either ATP or Trp shows that only a \(\sim\)1-Å movement of the TAB domain is required to bring the substrates together in a ternary complex (Fig. 8). However, for condensation to adenylation, Trp must flip its side chain to attack the \(\alpha\)-phosphate of ATP. Thus, unlike bsTrpRS, drTrpRS II binds both ATP and Trp in similar conformations that require little further TAB domain motion to engage the substrates. Interestingly, the crystal structures indicate that in

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\(^2\) For an alignment of TrpRS sequences, see supporting information to Ref. 25.
the absence of ATP, the closed conformation of subunit B cannot bind Trp, whereas in the absence of Trp, the open conformation cannot bind ATP. It follows that Trp binding to the open conformation probably precedes ATP interaction with the more closed conformation.

**Residues that Limit Domain Motions in drTrpRS II**—Limited domain motions in drTrpRS II are enforced by interactions supplied by other conserved residues. In bsTrpRS, Phe^{26} and Tyr^{65} serve as a bearing for domain movement; Phe^{65} separates from Tyr^{65} on TAB domain opening and instead contacts Leu^{77} (15). In drTrpRS II, Arg^{47} (which corresponds to Phe^{26} of bsTrpRS), forms a salt bridge with Asp^{29} in all three crystallographically unique subunits. Large relative motions of the RF and TAB domains will result in loss of this salt bridge without an obvious means for stabilizing the liberated charge partners. Furthermore, in bsTrpRS II a salt bridge between Glu^{145} and Arg^{182} reaches across the interdomain hinge to restrict the TAB domain from rotating further in the open state (12, 15). The equivalent residues in drTrpRS II (Asp^{171} and Arg^{206}) also interact in the more compact “open” state of drTrpRS II and thereby also restrict its conformation. Arg^{206} in drTrpRS II projects closer to the RF domain than bsTrpRS Arg^{182} because of an inserted proline residue at position 205. Unlike Arg^{182} of bsTrpRS, Arg^{206} also stacks against the adenine ring of ATP. Taken together, these TrpRS II-conserved residues (including Arg^{20} mentioned above) provide interactions that limit TrpRS II domain motions relative to those of more typical bacterial TrpRSs.

**Inter-subunit Communication in drTrpRS II**—Nearly all TrpRSs exhibit some form of subunit cooperativity on binding substrates (20); nevertheless, all known structures are highly symmetric and thus provide limited insight into how the active centers in adjacent subunits communicate. In contrast, asymmetry found of the BC drTrpRS II dimer results in Trp binding to only the B subunit. Because the crystallographically symmetric drTrpRS II dimer will not bind any ligands, Trp binding appears to require asymmetry between subunits. Half-site reactivity of drTrpRS II is also supported by kinetic and thermodynamic measurements. Steady-state kinetics of tRNA charging indicates that one Trp binds per TrpRS II dimer (n = 0.5) (25). Furthermore, isothermal titration calorimetry of Trp binding to the drTrpRS II dimer gives a binding constant of 30 μM with number of sites, n = 0.5 (Fig. 9).

Differences in loops connecting secondary structural elements near the active site may impact binding of Trp, ATP, and tRNA. In the RF domain, drTrpRS II and hTrpRS have a three-residue longer loop connecting α_{6} and α_{7} (Fig. 2). This
loop projects over the entry to the active center and participates in tRNA acceptor arm recognition in other TrpRSs. In drTrpRs II, the same region is essential to structural communication between Trp binding sites.

Overlaying the symmetric and nonsymmetric dimers of drTrpRS II (Fig. 10A) indicates that negative cooperativity may result from changes in active site residues on Trp binding that propagate to interactions at the dimer interface. Bound Trp in the C subunit interacts with residues on α6, the so-called “substrate specificity helix” (19, 29). Subtle structural changes draw nearby dimer interface helices α6 and α7 toward α8 and the bound ligand (Fig. 10). Asp^{68} of α2 appears key to this transition. Upon Trp binding, Asp^{68} switches the hydrogen bonding partner from Tyr^{139} (on the loop connecting α6 and α7) to active site residue Arg^{30} (Fig. 10B). As a result, the α7-α8 region moves toward the α8 specificity helix and active site in the Trp-bound subunit. To maintain tight interactions at the interface, α6 and α7 on the empty subunit shift toward the Trp-bound subunit and away from α8. The increased gap between α6-α7 and α2 in the empty subunit favors the buttressing hydrogen bond between Asp^{68} and Tyr^{139} (Fig. 10C). Also in response to the α6-α7 shift, the side-chain hydroxyl of Ser^{122} on the empty subunit swivels to hydrogen bond with the peptide carbonyl of Glu^{141} across the dimer interface. Arg^{125} and Glu^{141} of the empty subunit hydrogen-bond with each other near the dimer interface. The position of this salt bridge appears to direct Arg^{125} on the occupied subunit toward the Trp binding site. In all TrpRS II sequences, positions corresponding to 125 and 141 contain a salt bridge pair; however, the acidic side chain can reside at either position. Such compensatory changes indicate that maintenance of an interacting charge pair at residues 125 and 141 has functional importance for TrpRS II.

Binding two Trp residues per dimer would generate strain by causing each subunit to effectively pull on the interface from opposite directions, break both Asp^{68}-Tyr^{139} hydrogen bonds simultaneously, and direct the symmetric Arg^{125}-Glu^{141} salt bridges to the same region. This may be why Trp does not bind the A subunit, which is forced to be symmetric by crystallographic symmetry. Notably, standard bacterial TrpRSs, which do not show negative cooperativity with respect to Trp binding, have a shorter α6-to-α7 loop and do not conserve the same residues in these regions as TrpRS IIs.
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**DISCUSSION**

**TrpRS II Is Conformationally Rigid—Large amplitude domain motions are associated with the mechanism of substrate binding and adenyl-Trp formation by bsTrpRS.** How applicable are these motions to drTrpRS II? ATP binds to two different conformations of bsTrpRS (open and closed), depending on its concentration and the pH (15). Increasing the ATP concentration and lowering the pH closes bsTrpRS to a conformation consistent with the pre-TS proposed for the TyrRS catalytic mechanism (15, 17). This transition results from a 13° rotation of the TAB domain that moves the KMSKS region —4 Å toward the RF domain and increases its interaction with ATP. In contrast, the free and ATP-bound structure of drTrpRS II adopts very similar conformations. In the drTrpRS II structure, the triphosphate moiety of ATP interacts with residues on the RF domain, despite interactions of the KMSKS loop more closely paralleling the open structure of bsTrpRS. Thus, the ATP complex of drTrpRS II shows features of both the bsTrpRS open and pre-TS structure. However, the less than full occupancy for ATP in drTrpRS II despite the high concentrations of nucleotide present may indicate that the conformation observed in the crystal is not optimum for binding ATP. The crystal lattice clearly influences the ability of the subunits to bind substrates, since subunits A and C show no evidence of ATP binding, and subunits A and B will not bind Trp. These subunits are not very different from each other in structure; thus, well ordered binding of ATP to the B subunit probably requires further conformational change either prevented by the lattice or gated by the addition of Trp (see below).

The greater rigidity of drTrpRS II probably stems from differences in sequence conserved by TrpRS II instead of effects of crystal packing. As examples, 1) drTrpRS II Arg⁴⁻³⁰ mediates coupling between sites for ATP and Trp in a manner not replicated by equivalently positioned residues in bsTrpRS; 2) a restricting salt bridge formed between Arg⁴⁻²⁹ and Asp⁴⁻²⁹ replaces the pivot point for domain motion in bsTrpRS generated from Phe⁴⁻²⁶ and Tyr⁴⁻⁶⁵; and 3) Asp⁴⁻¹⁷¹ and Arg⁴⁻²⁰⁶ interact in the drTrpRS II open form much like their analogs Glu⁴⁻¹⁴⁵ and Arg⁴⁻¹⁸² in bsTrpRS, despite the drTrpRS II open state having a more compact structure than that of bsTrpRS. In this latter case, the Asp⁴⁻¹⁷¹ to Arg⁴⁻²⁰⁶ salt bridge forms in drTrpRS II because the hinge region containing Arg⁴⁻²⁰⁶ has an inserted proline residue that places Arg⁴⁻²⁰⁶ closer to the RF domain than its counterpart in bsTrpRS. Thus, key regions that stabilize domain orientations differ in the two types of TrpRSs. However, one mobile region that shows high sequence conservation between drTrpRS II and bsTrpRS is the KMSKS loop, and thus the mechanism by which this region stages the adenylation reaction is also likely to be conserved.

**TrpRS II Is Structurally Closer to hTrpRS than to bsTrpRS—Although the RF domains of drTrpRS II, bsTrpRS, and hTrpRS are topologically quite similar, the TAB domain of drTrpRS II has much higher similarity to the hTrpRS TAB domain than that of bsTrpRS (Figs. 4, A and B). Thus, the mechanism of tRNA recognition by drTrpRS II may be more closely related to that of eukaryotic than to other bacterial TrpRSs. This is somewhat surprising, because prokaryotic and eukaryotic AARSs are usually orthogonal to each other in that they will not aminoacylate the respective tRNA (20). Despite the structural similarity, there is little sequence similarity between the drTrpRS II and hTrpRS TAB domains (Fig. 2), and in fact, the tRNA<sub>Trp</sub> from these organisms are also quite different. In the regions proposed to recognize tRNA, there is considerable sequence homology between drTrpRS I and drTrpRS II, which is consistent with the ability of both synthetases to aminoacylate the same in vitro transcribed D. radiodurans tRNA<sub>Trp</sub> (25).

The Essential Role of the KMSKS Loop in Acyl-adenylation by TrpRSs—The current model for adenyl-Trp formation by bsTrpRS incorporates a high energy pretransition state corresponding to a nearly closed conformation for the TAB domain (12, 15). This places the KMSKS loop in an unfavorable conformation that destabilizes the ground state (12, 15). At high ATP concentrations, the pre-TS of both subunits gains a number of protein-ligand interactions that energetically compensate for the anti-cooperative binding of the second ATP molecule. Under normal circumstances, with only one ATP molecule bound per dimer, the pre-TS forms only after binding both ATP...
and Trp in the same subunit.

All three conformations of drTrpRS II have domain orientations that resemble the pre-TS rather than the open form of bsTrpRS, although interactions between the KMSKS loop and ATP more closely resemble those seen in the open form of bsTrpRS (12). In particular, the main-chain amide nitrogens and lysine side chains of the KMSMS loop have not yet engaged the nucleotide phosphates. Furthermore, the adenine base is disordered or not fully occupied, probably because Lys\textsuperscript{215} has yet to stack against it, and the Met\textsuperscript{216} carbonyl is too far to hydrogen-bond with the adenine amino group.

Unlike bsTrpRS, the ATP complex of drTrpRS II differs little in conformation from the empty structure, and only one subunit of the dimer binds nucleotide. The minimal conformational changes induced upon ATP binding in drTrpRS II correlate with a greater binding affinity of drTrpRS for nucleotide ($K_D\sim10\ \mu M$) (8), compared with bsTrpRS ($K_D=100\ \mu M$) (12). In bsTrpRS, Trp binding induces a coordinated rotation of Tyr\textsuperscript{125}, Gln\textsuperscript{107}, and Gln\textsuperscript{9} (30), which narrow the indole pocket and allow Tyr\textsuperscript{125} to swing down and interact with the Trp $\alpha$-amino group. Thus, Tyr\textsuperscript{125} was proposed to serve as a gate, in that it provides access to Trp in the open conformation but closes down after the substrate has bound (12). Tyr\textsuperscript{125} is conserved in all class I and class Ic synthetases, but its equivalent in drTrpRS II is more homologous to drTrpRS II (50% sequence identity) than typical bacterial TrpRSs. Interestingly, residue substitutions that render TrpRS II more like TrpRS I sensitize $S.\ coelicolor$ TrpRS II to indolmycin (32). Thus, the crystal structures of drTrpRS II provide a structural basis for the development of potentially useful antibiotics against TrpRS IIs.

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