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Tryptophan synthase, an allosteric molecular factory

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Tryptophan synthase (TrpS) is a pyridoxal phosphate-containing bifunctional enzyme that catalyzes the last two steps in the biosynthesis of L-tryptophan. Indole, an intermediate generated at the active site of the α-subunit is channeled via a 25 Å long tunnel to the β-active site where it reacts with an aminoacrylate intermediate derived from L-serine. The two reactions are kept in phase by allosteric interactions between the two subunits. The recent development of novel α-site ligands and α-reaction transition state analogs combined with kinetic and crystal structure analysis of *Salmonella typhimurium* tryptophan synthase has provided new insights into the allosteric regulation of substrate channeling, the reaction mechanisms of the α and β active sites, and the influence of structural dynamics.

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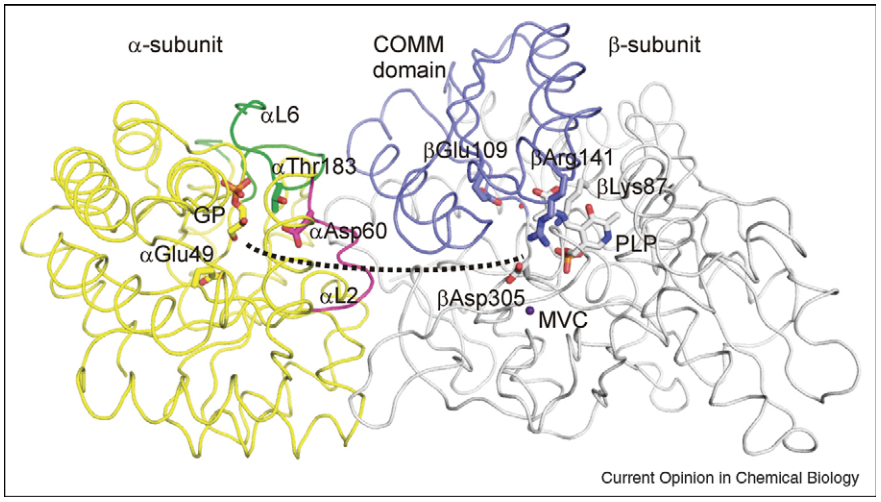
Imagine being a chemist having to perform a tricky synthesis using just one catalyst, involving a reaction between two intermediates that are difficult and costly to prepare and that can easily escape from the system or take part in unwanted side reactions. Ideally, one would prepare each molecule of the two intermediates precisely at the moment it is required, that is, producing one molecule of the one intermediate and keeping it sequestered until a molecule of the other intermediate has been prepared, and then bringing the two molecules together immediately so that side reactions and material losses are virtually excluded. Although such a system would seem impossible, bacterial tryptophan synthases have evolved to do just this.

Tryptophan synthase (TrpS) is a bienzyme complex that catalyzes the last two steps in the biosynthesis of L-Trp. TrpS has an α- and a β-active site in an αβ heterodimer that is part of an $\alpha_2\beta_2$ oligomer, connected by a 25 Å long tunnel (Figure 1). The α-site cleaves 3-indole-D-glycerol 3′-phosphate (IGP) to give indole and D-glyceraldehyde 3-phosphate (G3P) (Figure 2). The tryptophan-generating β-reaction requires a pyridoxal phosphate (PLP) cofactor and occurs in two stages (Figure 2): In Stage I, the external aldimine of L-Ser undergoes a β-elimination reaction to give the external aldimine of α-aminoacrylate, E(A-A); in Stage II, indole reacts with E(A-A) to give L-Trp. In order to prevent escape of indole by diffusion through the cell membranes, indole is channeled from the α-site to the β-site via the tunnel (for reviews see [1,2,3••]). In order to keep the two reactions in phase, the α-reaction and β-reaction regulate each other through reciprocal allosteric interactions. An important feature in TrpS allosteric regulation is the shuttling between open, low-activity, and closed, high-activity conformations of the active sites, with the equilibrium depending on the ligands bound to the α-active site and the covalent intermediates formed at the β-active site. For instance, production of the aminoacrylate at the β-site activates the α-site, ensuring timely production of indole [1,3••,4–7]. TrpS is a model system for substrate channeling, allosteric communication, and PLP biochemistry. Thus, a wealth of structural, kinetic, and mechanistic data on TrpS has been gathered over decades, and a great deal is known about the overall mechanisms of the α-reaction and β-reaction (see e.g. [3••,8,9]). Nevertheless, as summarized here, in the past couple of years, significant progress has been made in understanding the structural basis of allosteric regulation of TrpS, the mechanisms of the α-reaction and the β-reaction, and the roles played by conformational change in modulating the activities of the two catalytic sites. These advances have been made possible by new IGP analogs, α-reaction transition state analogs generated *in situ*, active site mutants, advanced kinetic studies, and crystal structures of reaction intermediates, in particular of the β-site quinonoid intermediate. Since a recent in-depth review had a strong emphasis on the kinetic aspects of these studies [3••], the current review will mainly focus on recently developed insights into the structural determinants of TrpS catalysis and allosteric regulation.

Non-cleavable IGP mimics and transition-state analogs

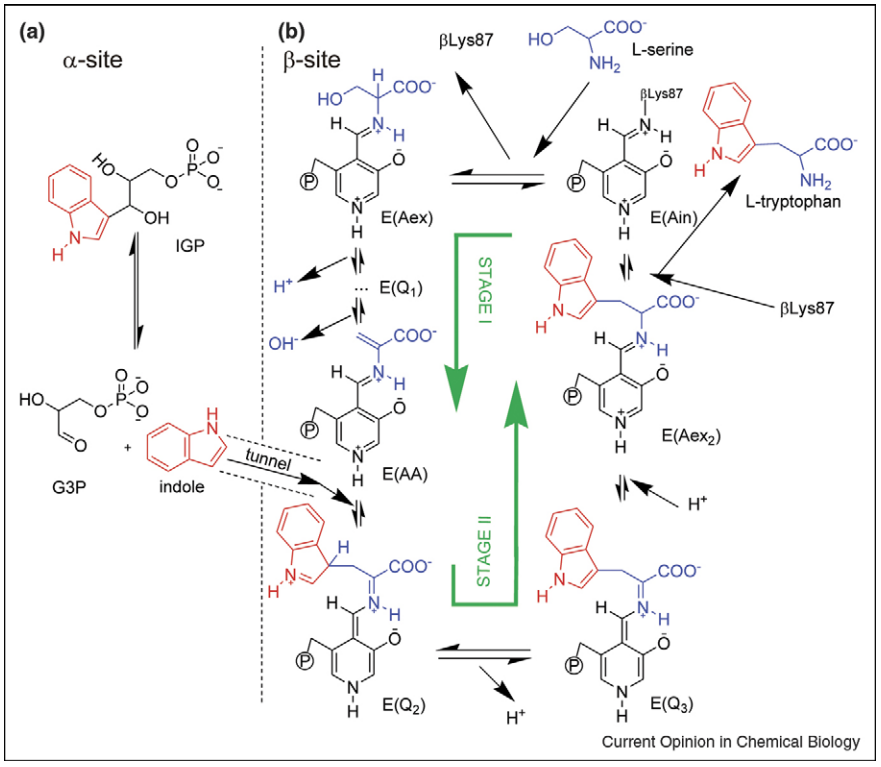
The α-site cleaves the C3–C3′ bond of IGP to yield G3P and indole. Recently, several novel synthetic analogs of IGP have been introduced [10,11•]. It has been shown that some of these α-site ligands (ASLs) promote the closed form of the α-subunit and are potent allosteric

Figure 1



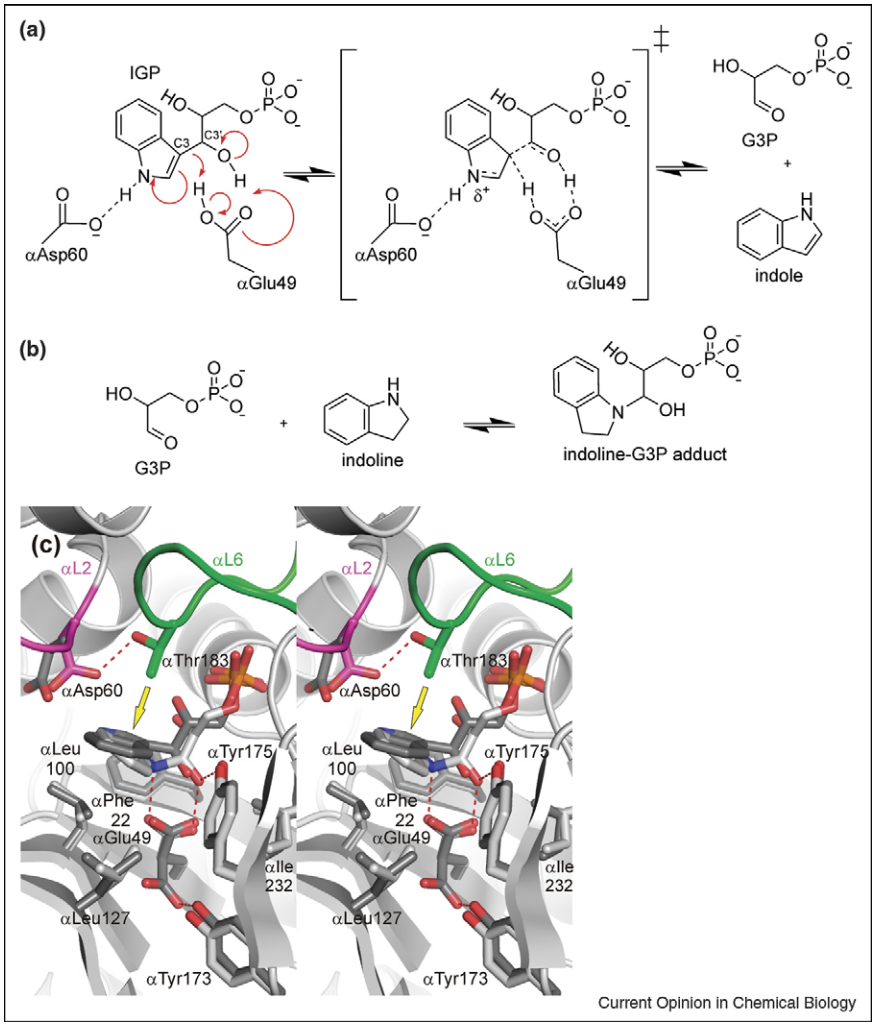
Overview of a TrpS αβ-dimer. The structure of the glyceraldehyde-3-phosphate/aminoacrylate complex (pdb code 2JX9 [12••]) was used. The α-subunit is shown in yellow. Loop αL2 is colored green, αL6 is colored magenta. The β-subunit is shown in gray with a blue COMM domain. The monovalent cation site (MVC) [45,46] is indicated by a magenta sphere. The PLP cofactor, glyceraldehyde-3-phosphate and selected amino acids are indicated. The approximate path of the tunnel connecting the α and β active sites is indicated by the dashed line. Molecular graphics were prepared using PyMol [47].

Figure 2



Overview of reactions catalyzed by TrpS. The α-site splits indole glycerol phosphate (IGP) into glyceraldehyde 3-phosphate (G3P) and indole. Indole diffuses through a tunnel to the β-site. The β-reaction is shown counterclockwise, starting with the covalent PLP-enzyme complex, the internal aldimine E(Ain) which is shown in the upper right corner. Reaction with serine results in the external aldimine of serine E(Aex). This is in equilibrium with its dehydration product, the external aldimine of aminoacrylate E(AA), the production of which concludes Stage I of the β-reaction. Indole emerging from the tunnel reacts with E(AA) in Stage II of the β-reaction, resulting in the quinonoid intermediates $E(Q_2)$ and $E(Q_3)$ and the external aldimine of tryptophan $E(Aex_2)$, from which tryptophan is cleaved, resulting in the internal aldimine and restoration of the β-site. For clarity, the geminal diamine intermediates occurring between internal aldimines and external aldimines and the quinonoid intermediate $E(Q_1)$ [39] occurring on the pathway between the external aldimine and the aminoacrylate are omitted.

Figure 3



The α-site **(a)** Reaction mechanism of the α-reaction. αGlu49 transfers a proton from C3′-OH to C3 using a push–pull mechanism, while αAsp60 stabilizes the developing positive charge on N1. **(b)** In the α-site, indoline reacts with glyceraldehyde 3-phosphate to form an indoline-G3P adduct that is a transition-state mimic. **(c)** Stereo figure showing a close-up of the α-sites of the indoline-G3P adduct/quinonoid complex (light gray, pdb code 3CEP [15••]) and the IGP complex (dark gray, pdb code 2RH9). Loop αL2 is colored green, αL6 is colored magenta. Two conformations of the catalytic αGlu49 exist, an active one, oriented toward the indoline-G3P adduct and interacting with the atoms corresponding to C3 and the C3′-OH, and an inactive one, hydrogen bonded to αTyr173. αGlu49 resides in a hydrophobic pocket formed by αLeu127, αLeu100, αPhe22 and αIle232. Closure of the active site involves a hydrogen bond between αAsp60 on loop αL2 and αThr183 on loop αL6. The methyl group of the αThr183 side chain pushes the indole group of IGP toward the catalytic αGlu49 and helps the C3 atom attain a tetrahedral configuration that is required in the transition state.

effectors, affecting the E(Aex)/E(AA) equilibrium in the β-site and increasing the apparent L-Ser affinity of TrpS as do IGP and G3P [12••]. Since these synthetic ASLs are non-cleavable, they can be used to probe allosteric interactions with the early intermediates in Stage I of the β-reaction. In this way it was shown that in the internal aldimine E(Ain) state the β-site switches from a low-affinity to a high-affinity state in response to ASL binding [13••].

In the transition state of the α-reaction, the IGP C3 atom is sp3-hybridized (Figure 3a). By allowing G3P to react in the α-site with a nucleophilic indole analog like *o*-aminophenol or indoline, two α-reaction transition state analogs were prepared *in situ*, which each have an sp3-hybridized atom at the C3-equivalent position [12••,14,15••] (Figure 3b). Crystal structures showed that like the binding of IGP, G3P, indole propanol phosphate (IPP) [1,6,16–19] and the novel non-cleavable ASLs [12••,20•], these transition state analogs induce the closed conformation of the α-site in which loop αL2 (α53 to α60) moves toward the active site and loop αL6 (α179 to α193) becomes ordered (Figure 3b), binding to αL2 via a crucial hydrogen bond between αThr183 on αL6 and αAsp60 on

αL2 [14,15••]. This orients αAsp60 so that it can stabilize the charge developing on the indole N as the indole is cleaved off. The C3′–OH proton is transferred to C3 by the catalytic αGlu49 in a push–pull mechanism, probably involving a neutral αGlu49 given the apolar surroundings of this residue [3••,14,15••] (Figure 3c). Crystal structures show two different conformations for αGlu49: an inactive one pointing away from the substrate [21], and an active one pointing toward the positions of the C3′–OH and C3 moieties [14,15••,17,22]. Switching between these conformations of αGlu49 is probably an important factor in turning the α-site on and off [14].

β-Site reaction specificity

Whereas the α-site produces indole from IGP, the β-site produces aminoacrylate from serine, using a PLP cofactor to activate it by binding it as an external aldimine. PLP can stabilize negative charges, making substrates bound to PLP liable to many kinds of reactions including β-elimination and decarboxylation [23,24]. The reaction specificity in a PLP enzyme can be understood using the Dunathan hypothesis (Figure 4a): Stereoelectronic considerations predict that of the three bonds to the α-carbon atom bound to PLP, the one that is perpendicular to the plane of the PLP π-system will be most easily cleaved [25–27]. In TrpS, the conserved loop βL3 (β109–β115) on the COMM domain forms a highly specific binding site for the carboxylate group of the substrate and various intermediates of the β-reaction. This places the bond to the Hα of the serine external aldimine perpendicular to the plane of the PLP π-system, setting up the system for removal of the α-proton and subsequent β-elimination. Mutations to loop βL3 have been studied to further the understanding of the reaction mechanism and specificity of TrpS [20•,28,29]. The βThr110Val mutation, which replaces a hydrogen bonding partner to the substrate carboxylate with a methyl group severely decreased the ability to form the aminoacrylate external aldimine E(AA) and consequently severely impaired β-reaction and αβ-reaction. This is probably due to a widening of the β-active site and a reorientation of the communication or COMM domain (see below) as demonstrated in the crystal structure. The βGln114Asn mutation, which shortens the βGln114 side chain connecting the βL3 loop with the rest of the protein, resulted in two fractions of enzyme; a yellow one with wild-type-like properties and – surprisingly – a red one inactivated by a side reaction typical for PLP enzymes [30]: Just like the substrate and product external aldimines, the aminoacrylate external aldimine can be cleaved off via a geminal diamine intermediate. In the E(AA) case this leads to the formation of an internal aldimine with βLys87 and highly reactive, free aminoacrylate (Figure 4b) as in for example tyrosine phenol-lyase [31]. Like in other PLP enzymes [30,32], the aminoacrylate external aldimine is not as easily cleaved as the substrate or product external aldimines [20•]. Nevertheless, unbound aminoacrylate may form and if not released to the solvent to give pyruvate and ammonia, can attack the internal aldimine with its acrylate C3 atom to form a C–C bond at C-4′ of the PLP moiety resulting in the inactivation of the tightly bound cofactor and thus the enzyme [33,34]. This side reaction is enhanced in the βGln114Asn mutant because in the mutant the closed state of the β-site is stabilized, presumably keeping unbound aminoacrylate in the β-site long enough for inactivation to occur. Thus, in determining the reaction specificity at the β-site not only the orientation of the scissile bond (as in Dunathan's hypothesis) but also the rate of interconversion between the opened and closed states is crucial [20•]. This phenomenon was hinted at by other mutants that exhibited altered reaction specificity, for example, βAsp305Ala [35].

Communication in TrpS: a switchboard in the COMM domain

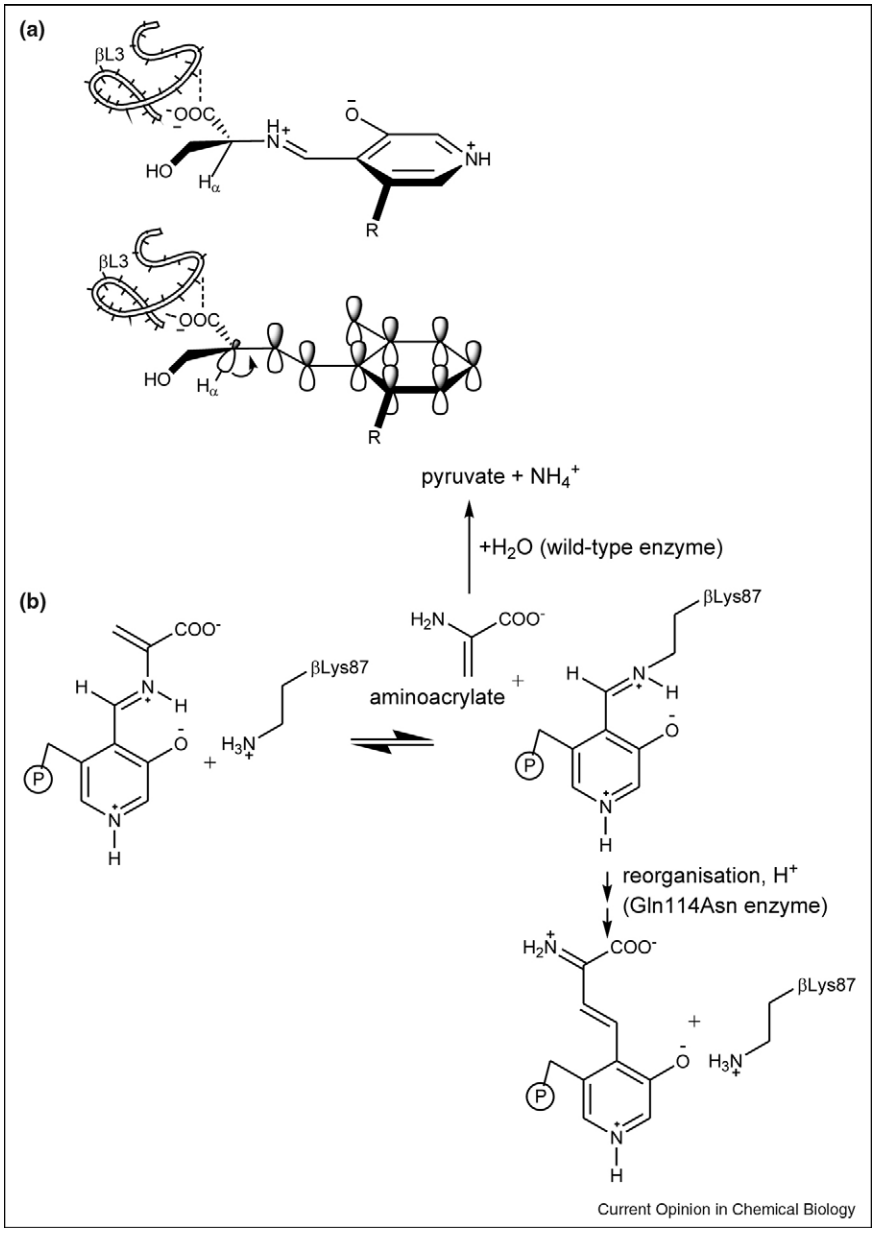
The key structural element that coordinates the activities of the α-sites and β-sites is a single domain formed by residues β102–β189, which has been called the movable [16] or COMM domain [6]. The positioning of this domain defines the open state and closed state of the β-subunit: In the open state PLP is accessible to the solvent [21], whereas in the closed state, the COMM domain moves toward PLP and forms crucial interactions with the rest of the enzyme [12••,15••,20•].

Within the COMM domain, helix βH6 (β165–β181) is emerging as the switchboard for allosteric communication within the enzyme [3••]: Comparing structures of open and closed α-sites and β-sites shows that in the open conformation, βAsn171 on βH6 interacts with the catalytic αAsp60 on αL2, whereas in the closed conformation βH6 is displaced by one turn owing to the closure of αL6, placing βArg175 in contact with αAsp60 (and αPro57) and a hydrogen bond is formed between αGly181 on αL6 and βSer178 on βH6 (Figure 5). Mutations of βSer178 severely affect allostery [19,36,37]. Moreover, the network of interactions between βH6, αL2, and αL6 in the closed state probably assists in the closure of the α-site. Also, substitution of βLeu169 and βCys170 on βH6 occludes the tunnel leading to indole accumulation [38]. Thus, βH6 is at the center of allosteric communication, functioning as a hub for communication involving the α-active sites and the β-active site and possibly also the tunnel.

Reaction of indole with aminoacrylate: structure of a quinonoid intermediate

The next step in the enzymatic sequence is the reaction of the aminoacrylate external aldimine with indole. The formation of E(AA) leads to ~30-fold activation of the α-site [5,28]. As soon as indole has been produced, it diffuses through the tunnel and reacts with the

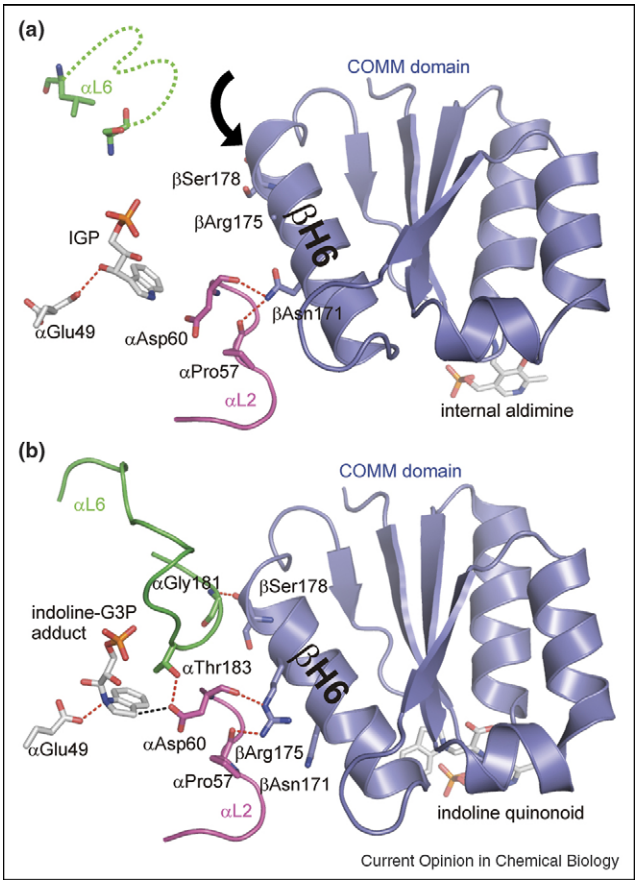
Figure 4



β-site reaction specificity. **(a)** Application of Dunathan's hypothesis [25–27] to TrpS. Loop βL3 fixes the position of the substrate's carboxylate group, placing the Cα–Hα bond perpendicular to the PLP π-system. Upon removal of the α-proton, the bonding electrons can thus be easily accommodated in the PLP π-system, and this destabilizes the Cα–Hα bond. **(b)** Cleavage of the aminoacrylate external aldimine results in the formation of aminoacrylate and E(Ain). When released to the solvent, aminoacrylate is converted to pyruvate and ammonia, but when kept inside the β-site may react with E(Ain) with its C3 atom resulting in inactivation of the cofactor [20•,33,34].

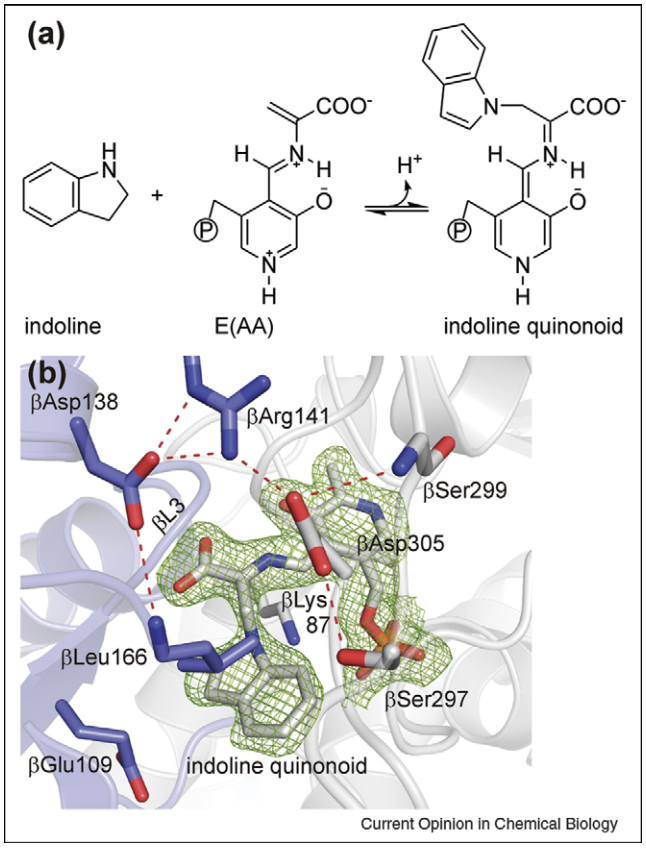
aminoacrylate to first form a quinonoid intermediate E(Q_2), which is deprotonated to form quinonoid intermediate E(Q_3) [39,40]. Quinonoid intermediates play a key role in the catalytic mechanism of PLP-dependent enzymes. Despite this importance, a high-quality structure of a PLP-quinonoid has been determined only recently. In TrpS, lifetime and occupancy of the fleeting quinonoid species can be increased significantly by α-subunit ligands and monovalent cations [41,42]. Exploiting this, a close structural mimic of the E(Q_2) intermediate was trapped in the crystal using the indole analog indoline (Figure 6a) [15••]. The 2.1 Å resolution electron density map of this indoline quinonoid shows the expected flat configuration of the conjugated system (Figure 6b). Moreover, the β-site has adopted the closed conformation, causing the formation of an H-bond

Figure 5



Communication in TrpS. **(a)** IGP/E(Ain) structure of TrpS (pdb code 1QOQ). The α-site and β-site are in the open conformation. IGP (white) is bound to αGlu49 for which two conformations are observed. Loop αL6 (green) is disordered and loop αL2 (magenta) interacts with helix βH6of the COMM domain (blue) via a hydrogen bond to βAsn171. **(b)** In the indoline-G3P adduct/indoline quinonoid structure (pdb code 3CEP) [15••] the α site and β site are in the closed conformation. Loop αL6 is ordered, loop αL2 has moved toward the α-site ligand, and helix βH6 has shifted by one turn, placing βArg175 in contact with αL2.

Figure 6



Trapping of a quinonoid intermediate. **(a)** Reaction of indoline with E(AA) produces the indoline quinonoid E(Qind). **(b)** Crystal structure of the E(Qind) form of TrpS (pdb code 3CEP [15••]). The COMM domain (blue) has adopted the closed conformation, and an extensive hydrogen bond network (see text) has formed. βGly109 is positioned close to the indoline ring.

network between βArg141, βAsp305, βSer297, βSer299, βAsp138, and βLeu166 [6,12••,16,35,43]. Given its position with respect to the indoline moiety, βGlu109 could, after a small side chain rotation, act to stabilize the charges developing on the indole N during the reaction with E(AA), as had been proposed before (see [12••] and [44••] and references therein). This shows that βGlu109 plays a dual role: It facilitates dehydration of E(Aex) to form the aminoacrylate [12••,29,44••] and stabilizes the charges developing upon the formation of the indole quinonoid. Moreover, βLeu166 moves into the active site to complete the indole-binding pocket, which it forms together with βPhe306 and βThr190. Also, the structure implicates βLys87 as an acid/base catalyst in the conversion of $E(Q_2)$ to $E(Q_3)$. Thus, the quinonoid structure allows for a detailed study of Stage II of the β-reaction.

Conclusion

The past few years have seen the accumulation of important new kinetic and structural data on catalysis and allosteric regulation in TrpS, thanks in no small part to the development of powerful new tools like non-cleavable ASLs that allow control over the state of the α-site and thus of the allosteric signals sent by it. Transition state analogs prepared *in situ* have shed light on the α-reaction mechanism and associated signaling, and the *in situ* preparation of a quinonoid species in the β-site has allowed a structural study of Stage II of the β-mechanism and its allostery. Also, mutations to the β-site have revealed a new layer of complexities associated with β-site reaction specificity, in particular its structural dynamics. As TrpS is a paradigm for two central themes in protein biochemistry, allosteric regulation and catalysis by PLP, the recent

findings have implications for a broad range of enzymatic systems.

Acknowledgements

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- • of special interest
- •• of outstanding interest

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