DOI: 10.1002/cbic.200700703

Structure and Mechanistic Implications of a Tryptophan Synthase Quinonoid Intermediate

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Tryptophan synthase is a pyridoxal 5′-phosphate (PLP)-requiring bienzyme complex, with subunit composition $\alpha_2\beta_2$, that catalyzes the last two steps in the biosynthesis of L-Trp.[1] The active site of the α chain or α site (see Figure S1 in the Supporting Information for an overview of the enzyme's structure) splits 3-indole-D-glycerol 3′-phosphate (IGP; **1**) into indole (**2**) and D-glyceraldehyde 3-phosphate (G3P; **3**; Figure 1 A). The β chain active site or β site replaces the hydroxyl of L-Ser with indole to give L-Trp in the β reaction, which is a two-stage process. In stage I of the β reaction (Scheme 1 A), the external aldimine (**4**) of the substrate L-Ser undergoes a β-elimination reaction to give the external aldimine of α-aminoacrylate, E(A-A) or **5**; in stage II, indole, which is channeled from the α to the β site via a 25 Å long tunnel, reacts with E(A-A) to give L-Trp (Scheme 1 A).[1] The quinonoid intermediates E(Q_{1-3}) are central to the various steps of the β reaction, as the ability of PLP to form these intermediates makes this cofactor a powerful electron sink (Scheme 1 A);

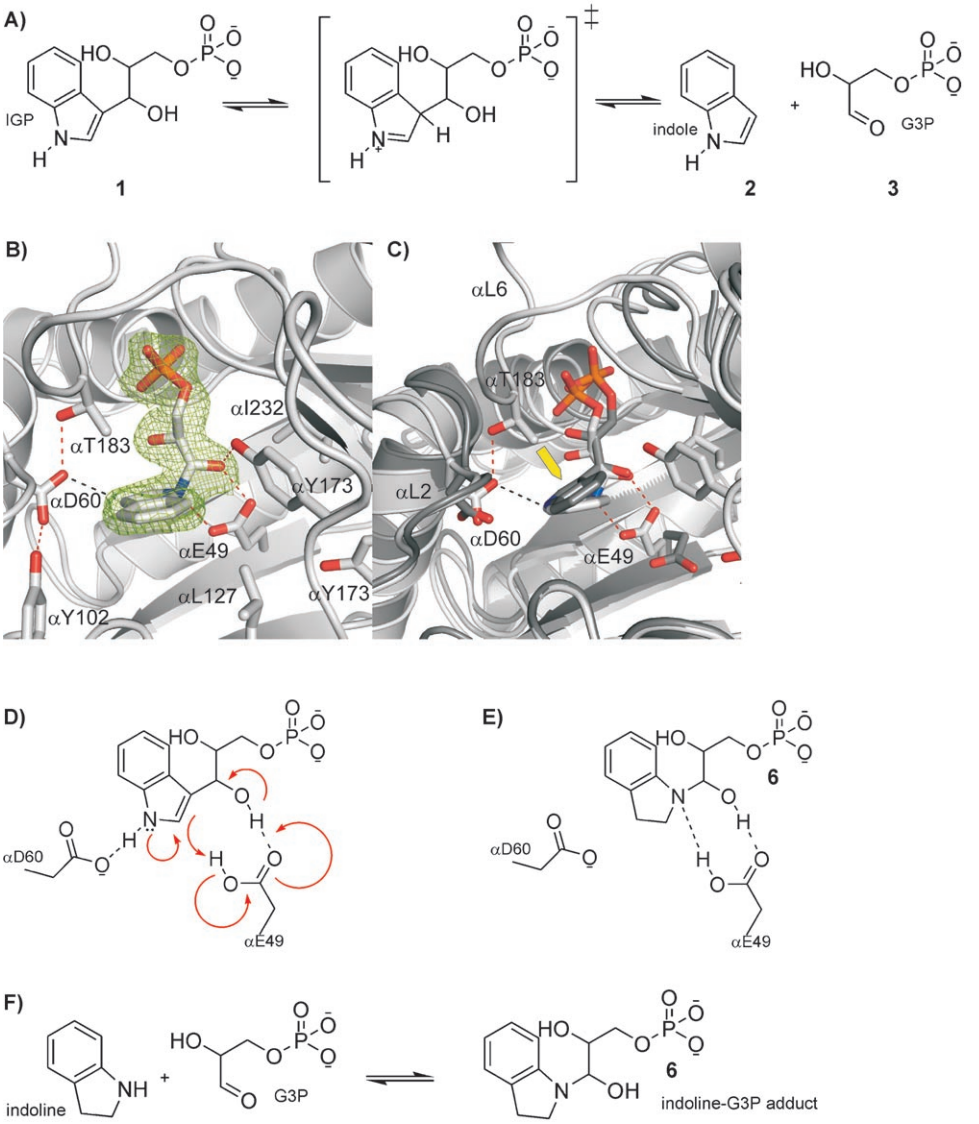

Figure 1. The α active site. A) Chemistry of the α reaction. B) Details of the α site with the indoline–G3P adduct shown with *2Fo-Fc* electron density contoured at 1.0 σ. C) Comparison of the α site and the position of αD60 of the indoline–G3P adduct (light gray) with the IGP complex (PDB ID code 2RHG; dark gray). αT183 pushes the indoline towards αE49 (yellow arrow). D) Interactions and proposed catalytic role of αE49. E) The indoline–G3P adduct closely mimics the α reaction transition state shown in A). F) Formation of the indoline–G3P adduct.

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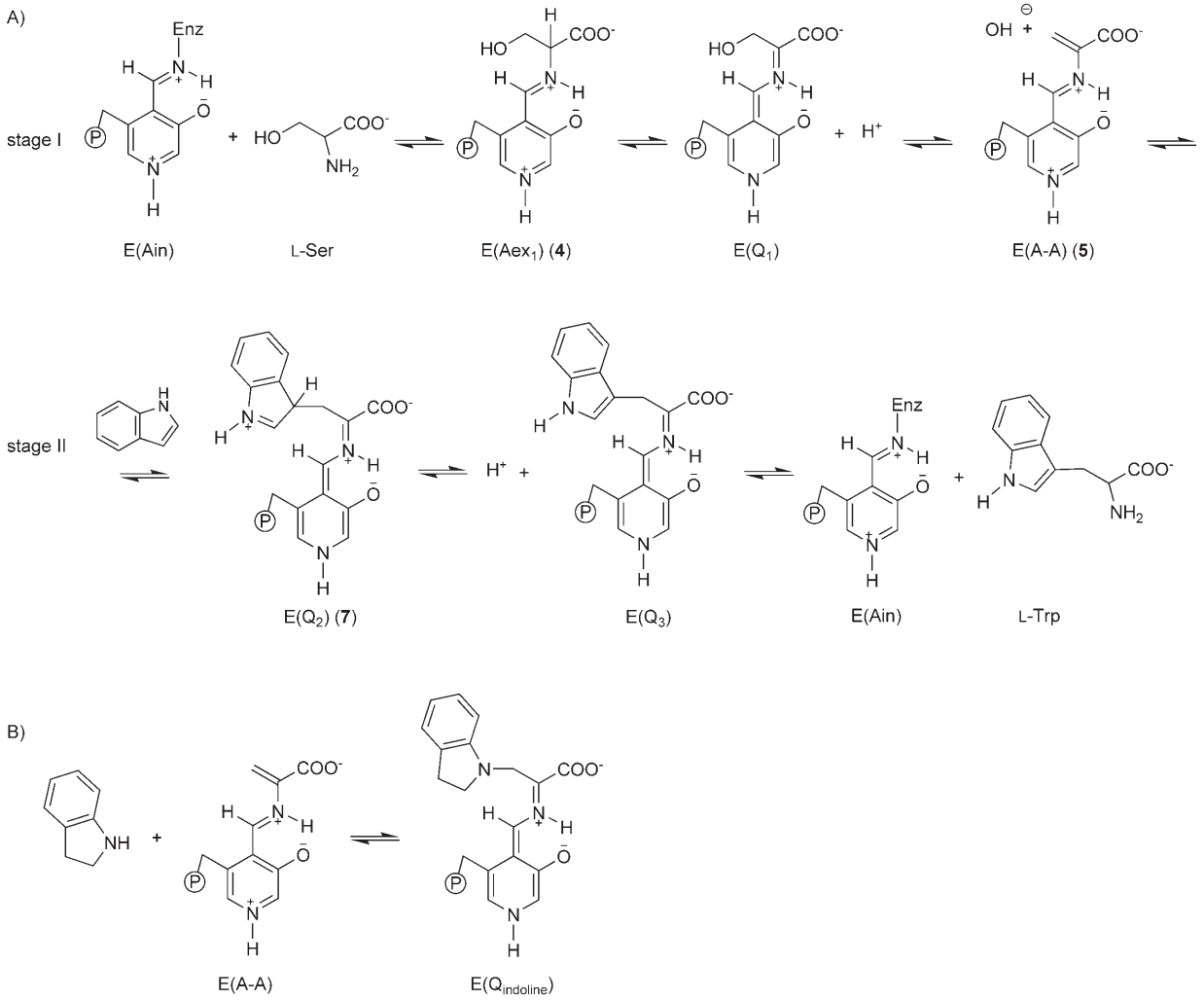
indeed, quinonoid intermediates are central to all PLP-dependent enzyme mechanisms.

In tryptophan synthase, the catalytic cycles of the α and β sites are synchronized by a sequence in which the α site is switched on when E(Aex_1) is converted to E(A-A),[1–3] and



Scheme 1. Chemistry of the β reaction. A) The β reaction occurs in two stages in which quinonoid intermediates play central roles. B) Reaction of indoline with the α-aminoacrylate intermediate to form E($Q_{indoline}$). E(Ain): internal aldimine form of the enzyme; E(Aex_1): external aldimine of L-Ser; E(A-A): external aldimine of α-aminoacrylate; E(Q_{1-3}): quinonoid intermediates; E($Q_{indoline}$): indoline quinonoid intermediate of tryptophan synthase.

switched off after the reaction of indole with E(A-A) and the external aldimine of L-Trp is formed.[1,4] The communication between the α and β sites is performed through the communication or COMM domain, which is formed by residues β102 to β189 (see Figure S1 in the Supporting Information). The COMM domain changes position dramatically as the reactions proceed, and the switch between low- and high-activity states is accompanied by transitions between open and closed conformations of the α and β subunits.[1,5]

To study the mechanisms of the α and β reactions and their allosteric regulation, we determined the 2.1 Å resolution crystal structure (Table S1) of tryptophan synthase with the α site occupied by a transition-state analogue for the cleavage of IGP (see the α reaction, Figures 1 A–F) and the β site occupied by a quinonoid species that mimics the E(Q_2) intermediate (**7**; see the β reaction, Scheme 1 A, Figures 2 A, B). This unusual complex was formed by soaking crystals of the native enzyme with G3P, L-Ser, and indoline. At the α site, indoline reacted with G3P to give the transition-state analogue **6** (Figures 1 E, F); at the β site, indoline reacted with the aminoacrylate intermediate formed in stage I of the β reaction to give the quinonoid species, E($Q_{indoline}$), shown in Scheme 1 B. These quasi-stable, reversibly-formed ligands induce closed conformations of the α and β subunits, respectively, and provide the second example of the closed conformation of the native β subunit.[5] Both complexes are clearly defined by their electron densities (Figures 1 B, 2 A, and Figure S2).

In the physiological reaction, cleavage of IGP has been postulated to occur by a mechanism in which the carboxylate of αD60 stabilizes the development of charge on the indole ring nitrogen, while αE49 plays a catalytic role in the proton transfers at C-3 and at the 3′ hydroxyl of IGP (Figures 1 A, D). The indoline–G3P adduct is a nearly perfect transition-state analogue for the IGP-cleavage reaction (Figures 1 A–E). The carboxylate of αD60 is locked into position by H bonds from αY102 and αT183, and contacts the indoline C-3 (Figure 1 B). The indoline C-3 corresponds to the indole nitrogen in IGP (Figures 1 C, E), and thus αD60 is positioned to stabilize the positive charge that develops on the indole nitrogen during C–C bond cleavage in IGP (Figure 1 D).

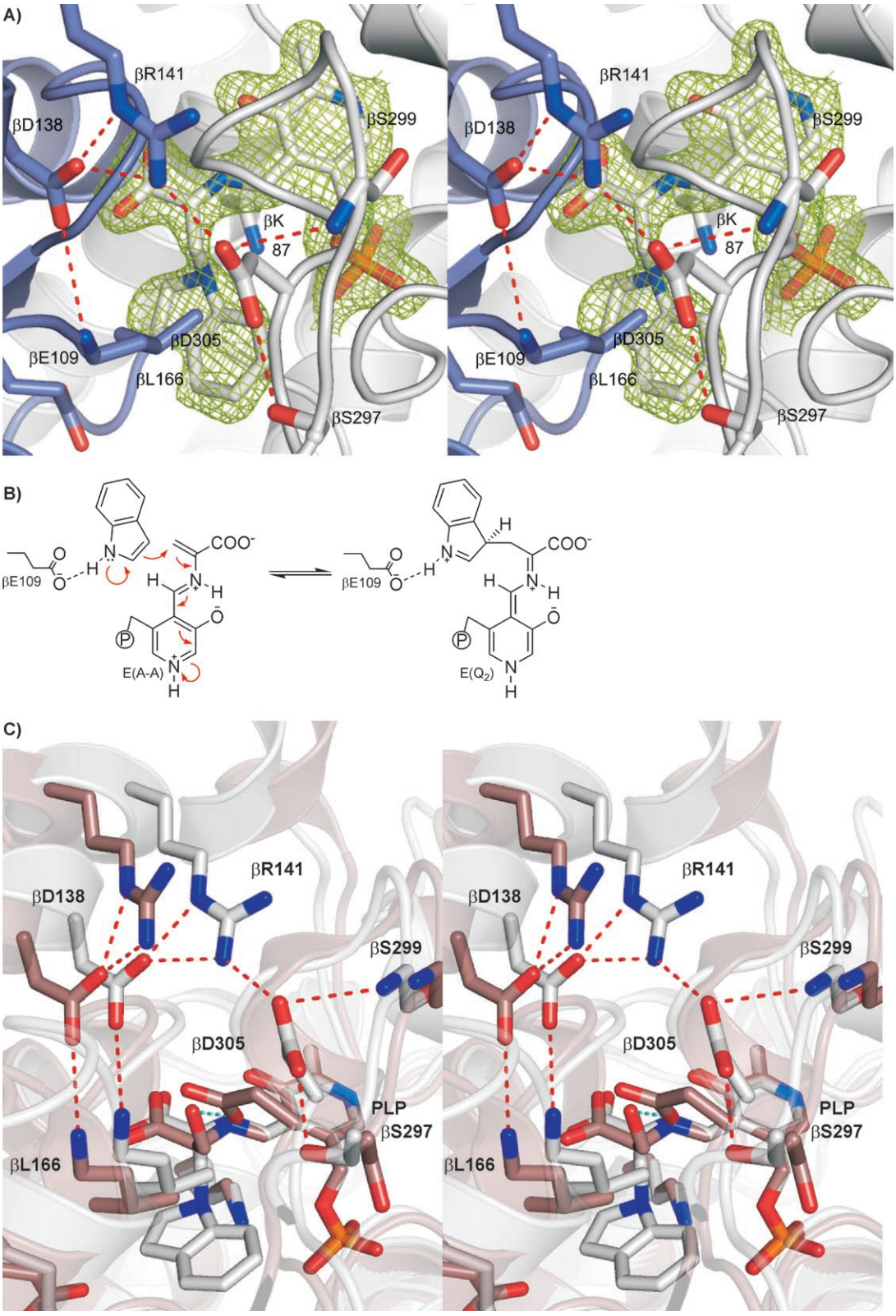


Figure 2. The β active site. A) Stereo figure presenting details of the quinonoid, shown with *2Fo-Fc* electron density contoured at 1.0 σ along with the βR141–βD305 salt bridge and the network of H-bonding interactions that stabilize the closed conformation. B) Proposed charge-stabilization role played by βE109 in stage II of the β reaction. C) Stereo diagram comparing the β sites of the open, inactive F9E(Aex_1) complex (2CLL, brown)[5] with the closed, active (indoline–G3P)E($Q_{indoline}$) complex (gray). The PLP rings are superimposed to show movements relative to the coenzyme. H-bond interactions are shown as dotted lines. The hydrogen bond between the external aldimine hydroxyl and βD305 is shown in cyan. Upon loss of this interaction, βD305 assumes a different conformation; this results in an interaction with βR141, which causes a large conformational change of the COMM domain.

The catalytic αE49 side chain is wedged into a hydrophobic pocket and forms hydrogen bonds to the indoline ring N and to the C-3′ OH of the adduct (Figures 1B, C, E; **6**). This orientation of αE49 supports a concerted push–pull mechanism, in which the bifunctional carboxylic acid group shuttles a proton between the hydroxyl group and the indole C-3 of IGP to initiate C–C bond cleavage (Figure 1D). This alignment of the αE49 carboxylic acid group is only possible when the cavity formed by the αF22, αL100, αL127, αY175, and αI232 side chains expands to accommodate the carboxylic acid moiety. The hydrophobic nature of this cavity tolerates the carboxylic acid, but likely would exclude the carboxylate; this makes a concerted proton transfer more likely than a stepwise transfer. Indeed, in structures with IGP bound to the α site, the side chain of αE49 is either folded away in an inactive conformation, H bonded to the OH of αY173 (see for example ref. [5]), or is H bonded via one carboxylate oxygen to the hydroxyl of IGP, and thus appears to be ionized. These structures correspond to inactive conformations.

Sterically, the E($Q_{indoline}$) complex (Figure 2A) appears to be a close structural analogue for E(Q_2) in stage II of the β reaction (Scheme 1A).[6] The indoline moiety is held between βL166, βF306, and βT190, which would also orient an incoming indole from the tunnel for attack on E(A-A). Figure 2A shows the Nε of βK87 positioned for proton transfer to Cα of the $Q_{indoline}$ moiety; this substantiates a general acid/base catalytic role for βK87, while a small rotation of βE109 would position the carboxylate next to the C-3 of the indoline moiety. This finding strongly corroborates the proposal that βE109 facilitates nucleophilic attack of indole on E(A-A) by stabilizing charge development on the indole ring as the C–C bond is formed to give E(Q_2) as shown in Figure 2B.[7]

Comparison of open, inactive β-subunit structures of E(Aex_1) complexes[5] with the two closed β-subunit conformations available for the wild-type enzyme, the (GP)E(A-A) complex,[5] and the (indoline–G3P)E($Q_{indoline}$) complex presented herein, reveals the structural switch from the open to the closed conformation of the β subunit (Figure 2C). The formation of E(A-A) entails the loss of the hydroxyl group of E(Aex_1). Probably, this causes the conformational switch in the following way: removal of the E(Aex_1) hydroxyl causes the loss of its H bond to the βD305 carboxylate; this results in a repositioning of βD305 to form a salt bridge with βR141, and causes formation of a H-bond network between βS299–βS297–βD305–β141–βD138–βL166 (Figure 2C).[5,8,9] This then leads to a large motion of the COMM domain.[8,9] Moreover, it pulls βL166 into the active site; this completes the binding pocket for the indole, which then, in stage II of the β reaction, is channeled to the β site and reacts with E(A-A) to give the E(Q_2) intermediate (Scheme 1A), possibly assisted by βE109 as explained before.

The switch between open and closed conformations of the β subunit communicates the chemical composition of the β site to the α site through H bonds at the α–β subunit interface, which involves loops αL2 (residues α54–α62) and αL6 (residues α177–α195).[5,9] Importantly, this affects the position of the catalytic αD60 and the position of IGP through the H bond to the indolyl ring (Figure 1C). Depending on its location, which is also affected by closure of the α site through the αD60–αT183 H bond,[10,11] αE49 can act as a proton shuttle to facilitate cleavage of IGP.

In conclusion, the crystal structure of tryptophan synthase complexed with a transition-state analogue at the α site and a quinonoid intermediate at the β site, reveals important new aspects of the structural determinants for the enzyme's allosteric regulation and reaction mechanisms.

Experimental Section

Tryptophan synthase was purified and crystallized as described previously.[9] Life-time and occupancy of the short-lived quinonoid species can be increased by metals, pH, and α-subunit ligands.[12] Nucleophilic analogues of indole, such as 2-aminophenol[13] and indoline, react with G3P at the α site to form covalent adducts. Complex formation at the α- and β active sites was achieved by soaking crystals for 30 min in a solution that contained Bis-Tris-propane, pH 7.8 (50 mM), CsCl (200 mM), PEG 8000 (15%, *w/v*), glycerol (20%), L-Ser (200 mM), G3P (10 mM), indoline (12 mM), and DMSO (10%).[12] The crystals changed color from pale yellow to orange hues. Light exposure of the crystals was kept at a minimum during soaking and mounting. Crystals were cryo-cooled in liquid nitrogen. Diffraction data were collected at Beamline X12C (National Synchrotron Light Source, Brookhaven National Laboratory, USA) with the crystal kept at 100 K. The data were processed with XDS.[14] The starting model for refinement (PDB code 1QOP) was stripped of the coordinates of loops αL2 and αL6, ligands, and water molecules. After the first cycle of simulated annealing refinement with CNS,[15] clear electron density was observed for loops αL2 and αL6, the α-site ligand, and the indoline quinonoid of the pyridoxal phosphate. In particular, the flat geometry of the latter was obvious (Figure S2). Due to the uncertainties associated with the 2.9 Å resolution structure of quinonoid complex of serine hydroxymethyltransferase that contains a mixture of the geminal diamine and the external aldimine/quinonoid complex,[16] geometrical parameters for the quinonoid were obtained by quantum chemical optimization at the DFT level by using the B3LYP/6-31G(d) basis set. The final model displayed good stereochemistry (see Table S1) with R/R_{free} values of 0.198/0.238. Structures were superimposed by using all Cα atoms for each pair of structures except for those that belong to loop αL2, loop αL6, and the COMM domain.

Acknowledgements

Supported by NIH grant GM5574 (M.F.D.). We thank Michael Weyand and Andrea Mozzarelli for initial contributions.

Keywords: allosterism · enzyme catalysis · protein structures · reactive intermediates · structure–activity relationships

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Received: November 18, 2007
Published online on March 20, 2008

