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Synthetic applications of tryptophan synthase

Robert S. Phillips*

Departments of Chemistry and of Biochemistry and Molecular Biology, University of Georgia, Athens, GA 30602-2556, USA

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Abstract—Tryptophan synthase catalyzes the final two steps in L-tryptophan synthesis in bacteria and plants. The α-subunit catalyzes the reversible retroaldol cleavage of indole-3-glycerol phosphate to give indole and D-glyceraldehyde-3-phosphate, and the β-subunit catalyzes the condensation of indole with L-serine to give L-tryptophan. The α-reaction has been used for the synthesis of indole-3-glycerol phosphate, but has not been investigated extensively for synthesis of analogues. The β-reaction has been used for the preparation of a wide range of analogues of L-tryptophan, including chloro and azido benzene ring-substituted tryptophans, as well as aza, thia, and selena heterocyclic analogues. In addition, *S*-alkyl and *S*-aryl cysteines, *Se*-alkyl selenocysteines, as well as β-nitrogen substituted alanines have been prepared.

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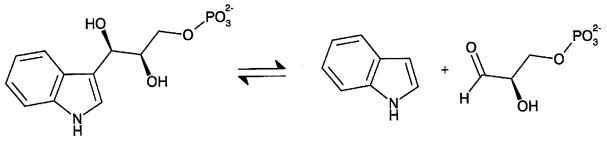
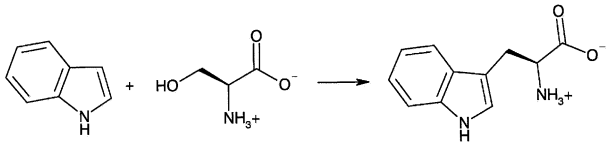
1. Properties of tryptophan synthase

Tryptophan synthase is a bienzyme complex that catalyzes the final two steps in the biosynthesis of L-tryptophan in bacteria and plants.[1] The enzyme is an $\alpha_2\beta_2$ tetramer of 147 kD, arranged as two α-subunits connected to the opposite ends of a β_2 dimer.[2] The reaction catalyzed by the α-subunit is the reversible retroaldol cleavage of indole-3-glycerol phosphate to give indole and D-glyceraldehyde-3-phosphate (Eq. 1), and the reaction catalyzed by the β-subunit is the condensation of indole with L-serine to give L-tryptophan (Eq. 2). The physiological reaction of tryptophan synthase is the combination of the two half reactions of Eqs. (1 and 2), conversion of indole-3-glycerol phosphate and L-serine to L-tryptophan and D-glyceraldehyde-3-phosphate



(1)



(2)

(Eq. 3). Indole is not produced as a free intermediate in solution, but is transferred intramolecularly through

* Fax: +1 706 542 9454; e-mail: rsphillips@chem.uga.edu

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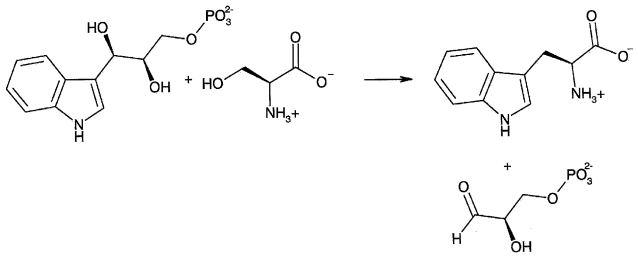


(3)

a 30 Å long tunnel, which connects the active sites of the α- and β-subunits.[3–5]

The structure of the functional unit of tryptophan synthase, the αβ dimer, is shown in Figure 1, with the α-chain in yellow, and the β-chain in magenta. In this structure, the product, L-tryptophan, can be seen bound to the pyridoxal 5′-phosphate co-factor in the β-active site, and the catalytically essential aspartates are shown as space-filling models in the α-active site. The coupling of the α- and β-reactions is controlled by a complex set of allosteric interactions. Binding of indole-3-glycerol phosphate to the α-site activates the β-site to bind L-serine.[3] Subsequent elimination of water from serine at the β-site produces an aminoacrylate intermediate, which then activates the α-site to produce indole.[5,6] The retro-aldol reaction is catalyzed by general acid–base catalysis

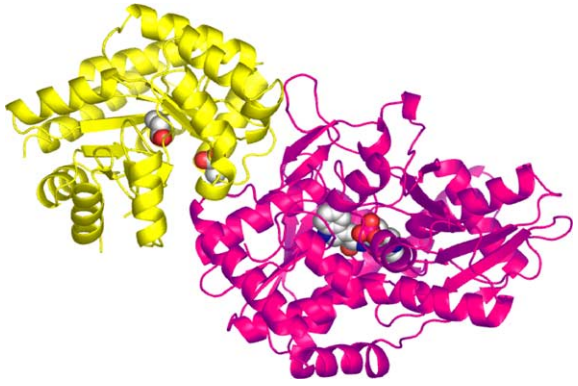


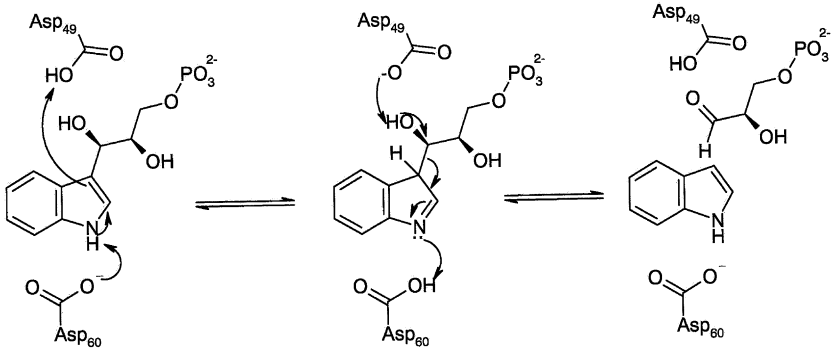
Figure 1. Structure of tryptophan synthase αβ dimer from *Salmonella typhimurium*.

with αAsp49 and αAsp60[7] (Scheme 1). Indole traverses the intramolecular tunnel to undergo a Michael reaction with the conjugated aminoacrylate at the β-site, forming a resonance-stabilized quinonoid carbanion (E_{Q1} in Scheme 2). The stereospecificity of inhibition of tryptophan synthase by the diastereomers of 2,3-dihydrotryptophan suggests that the reaction occurs on the *si*-face of indole to produce the (*S*)-indolenine.[8] Deprotonation of the indolenine at C-3, and subsequent protonation of the α-carbon of the quinonoid intermediate, E_{Q2}, produces the Schiff's base of L-tryptophan, $E_{EA\text{-}Trp}$, which releases L-tryptophan (Scheme 2). The allosteric interactions, which control the reaction of tryptophan synthase are affected by a wide range of variables, including monovalent cations,[9–13] pH,[14] organic co-solvents,[15,16] and temperature.[14] Many mutant forms of tryptophan synthase have been expressed and characterized, in order to understand the reaction mechanism and the allosteric interactions, and some of these mutant enzymes have enhanced ability to catalyze synthesis of unnatural amino acids from L-serine and nucleophiles other than indole.

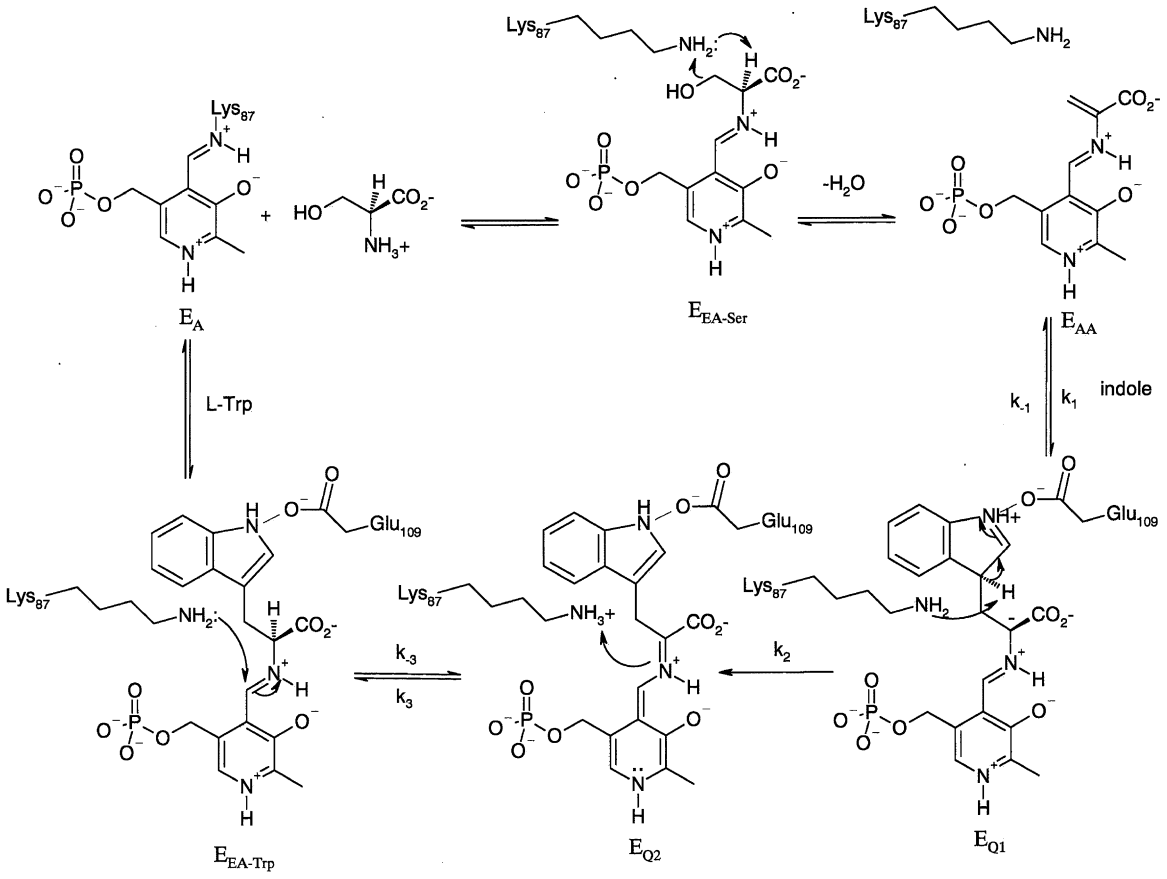
2. Synthetic applications of tryptophan synthase

2.1. α-Reaction

Although the α-reaction of tryptophan synthase is readily reversible, and thus makes it possible to synthesize indole-3-glycerol phosphate and analogs from indole and aldehydes, there have been relatively few synthetic investigations. Since indole-3-glycerol phosphate is biosynthetically derived from D-ribose, the configuration at both C-1 and C-2 of the glycerol unit, which come from carbons 3 and 4 of D-ribose-5-phosphate, is *R*. The synthesis of indole-3-glycerol phosphate from D-glyceraldehyde-3-phosphate by the reverse of the α-reaction thus creates a new chiral center at C-1 of the product with high stereoselectivity. Schwarz et al. used a coupled reaction of fructose-1,6-bisphosphate aldolase and triose phosphate isomerase to prepare D-glyceraldehyde-3-phosphate, coupled with the $\alpha_2\beta_2$ complex of tryptophan synthase from *Escherichia coli*, to synthesize indole-3-glycerol phosphate in a continuous hollow-fiber reactor.[17] The enzyme activity was gradually lost over a period of 17 days of operation, with tryptophan



Scheme 1. Mechanism of the α-reaction of tryptophan synthase.



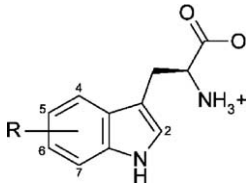
Scheme 2. Mechanism of the β-reaction of tryptophan synthase.

synthase having higher thermal stability than the aldolase and triose phosphate isomerase. High concentrations of indole (>11 mM) were found to cause partial denaturation of the enzymes, but tryptophan synthase is less sensitive to indole than is aldolase and triose phosphate isomerase. Kirschner et al. described the preparation of 6-nitroindole-3-glycerol phosphate for use in mechanistic investigations from 6-nitroindole and D-glyceraldehyde-3-phosphate,[5] an indication that the reaction may show broad specificity for the nucleophilic indole component of the aldol reaction. The specificity for the electrophilic aldehyde component of the aldol reaction has not been investigated. In this regard, it is interesting that a series of indole-3-alkanol phosphates were examined as competitive inhibitors, and indole-3-butanol phosphate was found to be a better inhibitor than indole-3-propanol phosphate,[18] even though indole-3-propanol phosphate is the deoxyanalog of indole-3-glycerol phosphate. This suggests that longer chain analogs of D-glyceraldehyde-3-phosphate may be substrates.

2.2. β-Reaction

The β-reaction of tryptophan synthase has been utilized for the preparation of a wide variety of amino acids. A wide range of substituted indoles have been found to undergo reaction to make the corresponding L-tryptophans (Table 1), although some (e.g., 5-nitroindole and 5-bromoindole) react extremely slowly.[5] Methylindoles

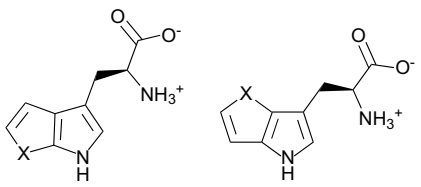
Table 1. Tryptophan analogs prepared with tryptophan synthase



Substituent	Reference
5-F	19
6-F	19
5-OH	19
2-Me	19
4-Me	19
5-Me	18,19
6-Me	18
7-Me	18,19
5-OMe	19
6-OMe	19
4-N_3	20
5-N_3	20
6-N_3	20
7-N_3	20
4-Cl	22
5-Cl	22
6-Cl	22
7-Cl	22
6-CHF_2	23

were first reported to be substrates of *Neurospora crassa* tryptophan synthase by Hall et al. in 1962.[19] In 1974, Wilcox used tryptophan synthase from *E. coli* to prepare 5- and 6-fluorotryptophan, 5-hydroxytryptophan, 5- and 6-methoxytryptophan, and 2-, 5-, and 7-methyltryptophan from the corresponding indoles.[20] Saito and Rilling prepared 4-, 5-, 6-, and 7-azidotryptophans from the azidoindoles and L-serine using tryptophan synthase from *E. coli*.[21] The availability of radioactively labeled serine made this reaction practical to prepare ^{14}C-6-azidotryptophan, useful for photoaffinity labeling studies of tryptophan synthase.[22] Similarly, all four benzene ring substituted chloro-L-tryptophans were synthesized from the respective chloroindoles and L-serine with *S. typhimurium* tryptophan synthase.[23] In this latter case, the low solubility of some of the chloroindoles in aqueous solution required the use of a two-phase reaction, with a toluene co-solvent. Woolridge and Rokita examined the reaction of 6-difluoromethylindole and found that 6-difluoromethyl-L-tryptophan is produced, but at a 5-fold slower rate than 6-methyl-L-tryptophan.[24] The substituted tryptophan analogs synthesized with tryptophan synthase are summarized in Table 1. All of these reactions take place with very high stereospecificity, since the reprotonation of the quinonoid intermediate (E_{Q2} in Scheme 2) at Cα takes place exclusively on the *re*-face of the imine.

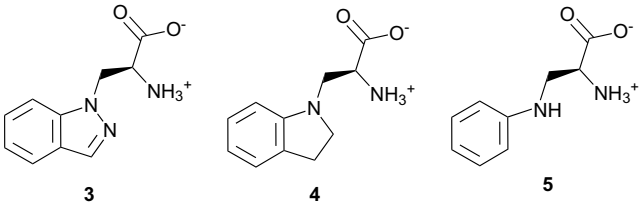
In addition to 7-aza-L-tryptophan, initially reported by Wilcox,[20] the other aza-L-tryptophans (4-, 5-, and 6-aza) were also synthesized with *S. typhimurium* tryptophan synthase from the corresponding azaindoles and L-serine.[25] The much lower nucleophilicity of the azaindoles necessitated relatively long reaction times of 1–2 weeks before completion. Other novel heterocyclic tryptophan analogs have been prepared with tryptophan synthase. Thiatryptophans **1a** and **1b** were prepared from thieno[2,3-*b*]pyrrole and thieno[3,2-*b*]pyrrole, respectively, and L-serine,[26] and later selenatryptophans **2a** and **2b** were prepared from selenolo[2,3-*b*]pyrrole and selenolo[3,2-*b*]pyrrole.[27,28] The pronounced acid sensitivity of these compounds would make their preparation by total chemical synthesis problematic. The thiatryptophans and selenatryptophans can be substituted for tryptophan in proteins,[28,29] and are potentially useful as spectroscopic probes. The selenatryptophans may be applicable as heavy atom derivatives for phasing in protein crystallography.[30] β-(Selenolo[3,2-*b*]pyrroyl)-L-alanine **2b** was quantitatively incorporated into annexin V and barstar in vivo using a tryptophan auxotroph of *E. coli*. The substituted proteins were crystallized and proved to be isomorphous with the native proteins.



1a: X=S
2a: X=Se

1b: X=S
2b: X=Se

The conjugated aminoacrylate intermediate (E_{AA} in Scheme 2) in the reaction of tryptophan synthase is highly electrophilic, so other good nucleophiles would be expected to undergo reaction with tryptophan synthase to prepare amino acids. Thus, not surprisingly, Esaki and co-workers found thiols and selenols to react readily with L-serine and tryptophan synthase to form substituted L-cysteines and L-selenocysteines, respectively.[31–33] Indazole was originally examined as a substrate by Wilcox,[29] who thought that 2-azatryptophan (tryptazan) was the reaction product, but Tanaka et al. found the product arises from reaction at N-1, rather than at C-3, of indazole to form a novel compound, β-(1-indazolyl)-L-alanine **3**.[34] Indoline reacts, as expected, on N-1, to form dihydroisotryptophan **4**, but the reaction with wild-type tryptophan synthase is very slow, primarily because the quinonoid intermediate is formed rapidly in the Michael reaction, but it is protonated very slowly at Cα, so the product release is extremely slow.[35] A mutant tryptophan synthase, E109D, in which βGlu109 to mutated to aspartic acid, was found to synthesize dihydroisotryptophan at a 5-fold higher rate.[36] Another mutant form, D305A, which has βAsp305 mutated to alanine, has a more open active site and shows broader substrate specificity towards nucleophiles.[37] Small nitrogen nucleophiles like *N*-methylhydroxylamine, methoxyamine, as well as phenylhydrazine and aniline, were shown to react with the aminoacrylate spectroscopically, and some of the new amino acid products were detected by TLC. This mutant enzyme forms β-anilino-L-alanine **5**, which is not formed by wild-type tryptophan synthase, and also forms dihydroisotryptophan **4** from indoline much more rapidly (14-fold) than the wild-type enzyme. As is the case with tryptophan, these unnatural amino acids are formed stereospecifically.



The majority of tryptophan synthase reactions have been performed with L-serine as the source of the amino acid component. Continuous production of L-tryptophan has been performed with immobilized whole cells of *E. coli* B 10, which contain a high concentration of tryptophan synthase, and which could be used for up to 50 days.[38] However, a coupled reaction with amino acid racemase was used to prepare L-tryptophan from indole and DL-serine using a mixture of whole cells of *E. coli* with tryptophan synthase and *Pseudomonas putida* containing amino acid racemase.[39] Other amino acids besides serine, with suitable leaving groups on the β-carbon, such as *S*-alkyl-L-cysteines, *O*-alkyl-L-serines, and β-chloro-L-alanine, are good substrates for the tryptophan synthase reaction[31,40] and can be used in synthesis, but have not seen much application. Esaki et al. have used *O*-methylserine, *O*-acetylserine, *S*-methylcysteine, *S*-ethylcysteine, and β-chloroalanine as sub-

strates in the synthesis of *S*-benzyl-L-cysteine from thiobenzylalcohol.[31]

Tryptophan synthase has been used for the preparation of stable isotopically labeled amino acids as well. Malthouse et al. prepared L-[1, 2-^{13}C, ^{15}N]-tryptophan from L-[1, 2-^{13}C, ^{15}N]-serine and indole with tryptophan synthase.[41] We recently prepared N^1-[^{15}N]-L-tryptophan from N^1-[^{15}N]-indole and L-serine with tryptophan synthase.[42] This latter compound was used for ^{15}N HSQC NMR studies of tryptophan synthase.

3. Future outlook

The range of indoles which have been used successfully for synthesis by the β-subunit of tryptophan synthase is somewhat limited at present. It is likely that the indole specificity can be expanded in the future by random mutagenesis and directed evolution. Furthermore, tryptophan synthases from other organisms, especially hyperthermophilic archea, and psychrophilic bacteria, may have different substrate specificities, but this has not been investigated. One advantage of tryptophan synthase, but also a limitation, is the high stereospecificity for L-amino acids. It would be particularly interesting if the stereospecificity could be changed by directed evolution to allow synthesis of D-amino acids. The scope of aldol reactions catalyzed by the α-subunit of tryptophan synthase needs further investigation. Directed evolution may also be useful to expand the reaction specificity of the α-subunit.

4. Conclusions

The β-subunit has been investigated extensively for synthesis, and it is a versatile catalyst for the synthesis of analogs of L-tryptophan and L-cysteine, as well as other unnatural amino acids. In contrast, although the α-subunit of tryptophan synthase can catalyze aldol reactions, it has primarily been used for the synthesis of the natural substrate, indole-3-glycerol phosphate.

Acknowledgments

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