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Tryptophan Lyase (NosL): Mechanistic Insights from Substrate Analogues and Mutagenesis

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Supporting Information

ABSTRACT: NosL is a member of a family of radical *S*-adenosylmethionine enzymes that catalyze the cleavage of the C_α–C_β bond of aromatic amino acids. In this paper, we describe a set of experiments with substrate analogues and mutants for probing the early steps of the NosL mechanism. We provide biochemical evidence in support of the structural studies showing that the 5′-deoxyadenosyl radical abstracts a hydrogen atom from the amino group of tryptophan. We demonstrate that D-tryptophan is a substrate for NosL but shows relaxed regio control of the first β-scission reaction. Mutagenesis studies confirm that Arg323 is important for controlling the regiochemistry of the β-scission reaction and that Ser340 binds the substrate by hydrogen bonding to the indolic N1 atom.

Nosiheptide is a highly modified polythiazolyl heterocyclic peptide antibiotic active against highly resistant pathogenic strains of *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Clostridium difficile*, and several enterococci.[1] The precursor peptide is ribosomally encoded, and the final molecule contains five thiazoles, a tetrasubstituted pyridine, and an unusual indolic acid (see Figure S1).[2] Recently, the gene cluster involved in the biosynthesis of nosiheptide was sequenced, and NosL was identified as the enzyme required for catalysis of the remarkable transformation of L-tryptophan (**1**) to 3-methyl-2-indolic acid (**2**) as shown in Figure 1.[3,4]

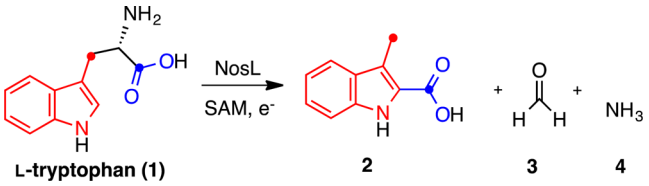

Figure 1. Radical SAM enzyme NosL catalyzes the conversion of L-tryptophan (**1**) to 3-methyl-2-indolic acid (**2**).

NosL is a member of a family of radical *S*-adenosylmethionine (SAM) enzymes that catalyze the cleavage of the C_α–C_β bond of aromatic amino acids. This family includes ThiH, involved in prokaryotic thiamin biosynthesis;[5] HydG, involved in maturation of the H-cluster from FeFe-hydrogenase;[6−9] and CofH, involved in F_{420} biosynthesis.[10] Our mechanistic understanding of these reactions is still at an early stage.[11]

A mechanistic proposal for the NosL-catalyzed reaction is shown in Figure 2. In this mechanism, the 5′-deoxyadenosyl radical (Ado$^\bullet$) abstracts a H atom from the amino group of **1** generating **6**. β-Bond scission of the C_α–C_β bond generates radical **7** and dehydroglycine (**8**). Addition of **7** to **8** gives **10**. A second β-bond scission generates **11**, which then isomerizes to the product **2**. The byproduct, radical **12**, may abstract a hydrogen atom from an unidentified source to form imine **13**, which then undergoes hydrolysis to formaldehyde. This mechanism is supported by labeling experiments identifying the origin of key atoms in the product, by the trapping of radical **7** to give **5**, by a recent structure of the enzyme–substrate complex,[12] and by substrate analogue studies.[13] In this paper, we describe a set of experiments with substrate analogues and mutants to further probe this mechanistic proposal.

The structure of NosL suggests that the 5′-deoxyadenosyl radical abstracts a H atom from the amino rather than from the indolic NH of tryptophan as previously proposed.[12] To test this prediction in a biochemical assay, the NosL reaction was

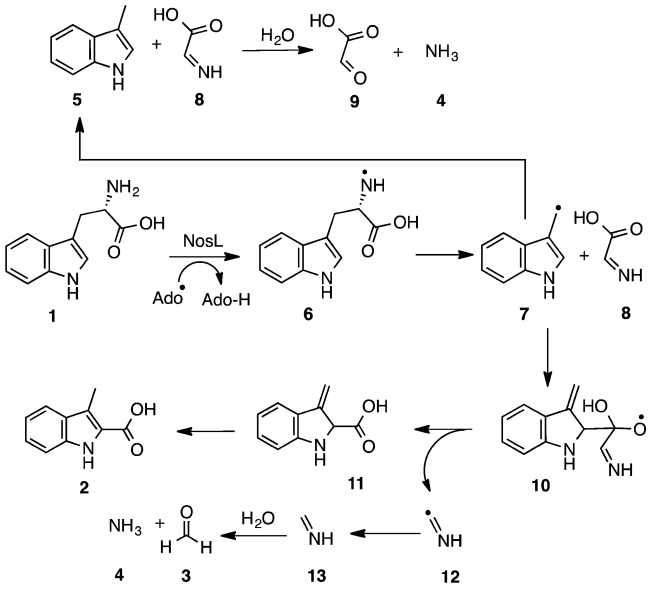

Figure 2. Mechanistic proposal for the NosL-catalyzed reaction.

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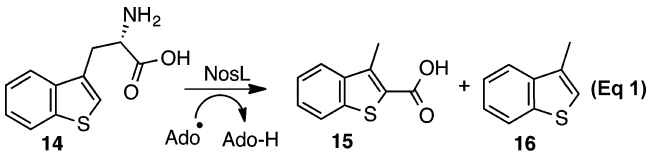
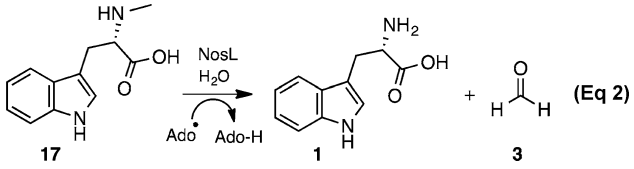
conducted using a tryptophan analogue **14** in which the indole NH group is replaced with sulfur. This reaction generated **15** and **16** as products consistent with abstraction of a hydrogen atom from the amino group (see Figures S4 and S5). A recent study using the benzofuran analogue of tryptophan gave similar results.[13] The NosL-catalyzed reaction also showed incorporation of multiple deuteriums from the solvent into 5′-deoxyadenosine (Ado-H), consistent with Zhang's data[13] (see Figures S6 and S7 and Table S1). This experiment was previously demonstrated, using CofH, to be consistent with formation of the amine radical.[10] N_α-Cyclopropyltryptophan was also investigated as a probe for the nitrogen-centered radical, but this molecule was not a substrate for the enzyme (see Figure S8).



NosL catalyzes the demethylation of N_α-methyl-L-tryptophan (**17**), followed by conversion of L-tryptophan (**1**) to **2** and **5** (see Figure S9). When the reaction was conducted using N_α-methyl-d_3-L-tryptophan, the transfer of deuterium from the methyl group to deoxyadenosine was observed. This demonstrates that the 5′-deoxyadenosyl radical abstracts a H atom from the methyl group instead of the amino group as seen in the native reaction (see Figures S10 and S11). This was unanticipated because in radical SAM enzymes, the 5′-deoxyadenosyl radical is generally assumed to be generated close to the H atom donor to prevent unproductive radical quenching reactions.



Tryptophan radical **6** could in principle undergo three possible β-scission reactions involving decarboxylation, deprotonation, or C_α–C_β bond cleavage. The crystal structure suggests that hydrogen bonding between the amino group and Arg323 is likely to control the regiochemistry of this fragmentation reaction (Figure 4). To test this, we have examined the reaction of NosL with D-tryptophan. With this compound, the amino group and the proton at C_α are interchanged, thus removing the controlling hydrogen bond to Arg323. When D-tryptophan is used as a substrate, all three possible β-scission reactions occur to give 3-methylindole (**5**, C_α–C_β bond cleavage), indole-3-pyruvic acid (**24**, C_α–H bond cleavage), and indole-3-acetaldehyde (**21**, decarboxylation) (see Figures S12 and S13). A mechanistic proposal for the formation of these three products is shown in Figure 3.

A related experiment was conducted using D,L-indole-3-lactic acid generating **21** and **24**. These results are similar to those reported by Zhang et al.[13] (see Figures S14 and S15).

The key interactions between tryptophan and the enzyme are shown in Figure 4. The role of all active site residues in catalysis was probed using mutagenesis (Table S2). Arg323 is hydrogen bonded to the amino and carboxylate groups of L-tryptophan.

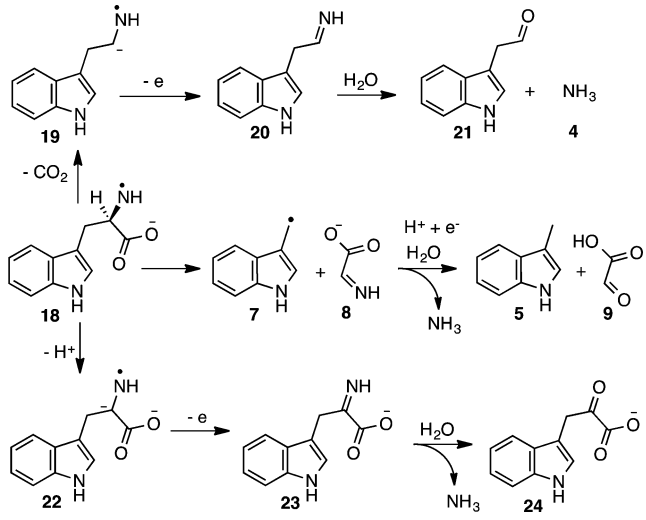


Figure 3. Mechanistic proposal for the fragmentation of D-tryptophan showing loss of regio control of the β-scission reaction.

This interaction is likely to play a role in controlling the regiochemistry of the β-scission reaction and in binding the glycine imine for reaction at C2 of the indole. In support of this, the R323K mutant produces indole-3-pyruvic acid (**24**) instead of **2** and no products arising from C_α–C_β bond scission (see Figure S18). Ser340 is likely to bind to the indolic NH group of tryptophan. This interaction is not essential for the catalytic activity, and the S340A mutant is active. However, N_1-methyl-L-tryptophan (**25**), which is not a substrate for wild-type NosL, is a substrate for the S340A mutant, generating **26** and **27** (see Figure S19). The Y90F and C200A mutants were active (see Figures S16 and S17).

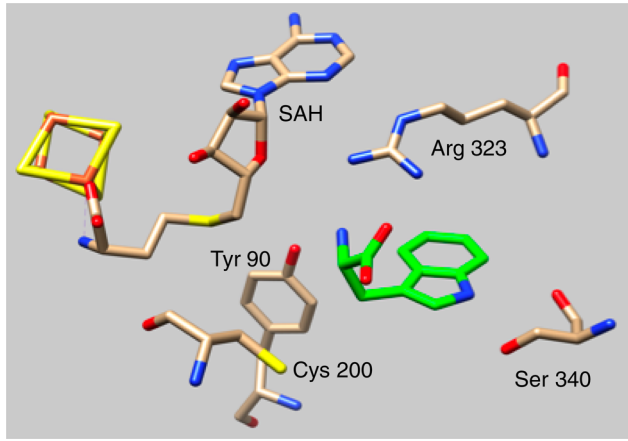
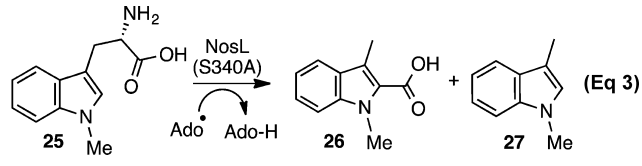


Figure 4. Active site architecture of NosL.



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■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.biochem.5b00764.

Detailed procedures for substrate synthesis, enzyme purification and assays, HPLC and LC−MS chromatograms of all enzymatic reaction products, and NMR spectra of synthesized compounds (PDF)

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Notes

The authors declare no competing financial interest.

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