ORGANIC LETTERS

2012
Vol. 14, No. 18
4882–4885

Expansion of Enzymatic Friedel–Crafts Alkylation on Indoles: Acceptance of Unnatural β-Unsaturated Allyl Diphospates by Dimethylallyl-tryptophan Synthases

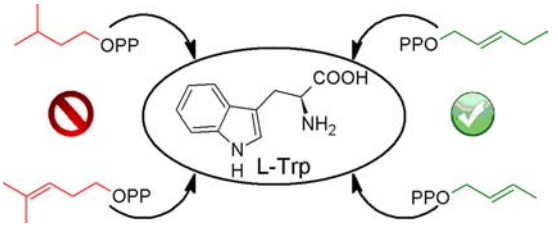
Mike Liebhold,[†] Xiulan Xie,[§] and Shu-Ming Li*[,†]

Institut für Pharmazeutische Biologie und Biotechnologie, Philipps-Universität Marburg, Deutschhausstrasse 17a, 35037 Marburg, Germany, and Fachbereich Chemie, Philipps-Universität Marburg, Hans-Meerwein-Strasse, 35032 Marburg, Germany

shuming.li@staff.uni-marburg.de

Received August 8, 2012

ABSTRACT



Prenyltransferases of the dimethylallyl-tryptophan synthase (DMATS) superfamily catalyze Friedel–Crafts alkylation with high flexibility for aromatic substrates, but the high specificity for dimethylallyl diphosphate (DMAPP) prohibits their application as biocatalysts. We demonstrate here that at least one methyl group in DMAPP can be deleted or shifted to the δ-position. For acceptance by some DMATS enzymes, however, a double bond must be situated at the β-position. Furthermore, the alkylation position of an analogue can differ from that of DMAPP.

Prenyltransferases are a large family of enzymes found in many living organisms such as bacteria, fungi, and plants, which are responsible for transfer of a prenyl moiety (nxC5) to another prenyl residue, a protein, or an aromatic substrate.[1] The reactions catalyzed by the last enzyme group, known as "aromatic prenyltransferases", are often Friedel–Crafts alkylations on different aromatic systems such as indoles, naphthalenes, flavonoids, or phenylpropanoids, resulting in the formation of biologically active metabolites.[1,2] One special group of the aromatic prenyltransferases shares a significant sequence similarity with dimethylallyl-tryptophan synthase (DMATS) from *Claviceps*,[3] and they are therefore classified as enzymes of the DMATS superfamily.[2a] The members of this family are involved in the biosynthesis of diverse important natural products including ergot alkaloids and usually catalyze the transfer of a dimethylallyl moiety from dimethylallyl diphosphate (DMAPP) to different positions of the indole ring of L-tryptophan and derivatives thereof.[1c] They showed high flexibility toward their aromatic substrates and accepted even hydroxynaphthalenes, flavonoids, xanthones, and tetracyclic napthacenediones as prenylation substrates.[2b,c,4] In contrast, these enzymes are usually highly specific toward DMAPP and did not accept other prenyl diphosphates as prenyl donors.[2a] One exception was found

[†] Institut für Pharmazeutische Biologie und Biotechnologie.
[§] Fachbereich Chemie.

(1) (a) Heide, L. *Curr. Opin. Chem. Biol.* **2009**, *13*, 171–179. (b) Yazaki, K.; Tsurumaru, Y. *Phytochemistry* **2009**, *70*, 1739–1745. (c) Li, S.-M. *Nat. Prod. Rep.* **2010**, *27*, 57–78.
(2) (a) Yu, X.; Liu, Y.; Xie, X.; Zheng, X.-D.; Li, S.-M. *J. Biol. Chem.* **2012**, *287*, 1371–1380. (b) Yu, X.; Xie, X.; Li, S.-M. *Appl. Microbiol. Biotechnol.* **2011**, *92*, 737–748. (c) Yu, X.; Li, S.-M. *ChemBioChem* **2011**, *12*, 2280–2283. (d) Ozaki, T.; Mishima, S.; Nishiyama, M.; Kuzuyama, T. *J. Antibiot.* **2009**, *62*, 385–92.
(3) Tsai, H. F.; Wang, H.; Gebler, J. C.; Poulter, C. D.; Schardl, C. L. *Biochem. Biophys. Res. Commun.* **1995**, *216*, 119–125.
(4) (a) Sanchez, J. F.; Entwistle, R.; Hung, J. H.; Yaegashi, J.; Jain, S.; Chiang, Y. M.; Wang, C. C.; Oakley, B. R. *J. Am. Chem. Soc.* **2011**, *133*, 4010–7. (b) Chooi, Y. H.; Wang, P.; Fang, J.; Li, Y.; Wu, K.; Wang, P.; Tang, Y. *J. Am. Chem. Soc.* **2012**, *134*, 9428–37.

10.1021/ol302207r
Published on Web 09/07/2012

for the newly characterized DMATS-type prenyltransferase VrtC, which only utilizes GPP.[4b] This feature prohibits their usage as catalysts for Friedel–Crafts alkylation. We expand in this study their potential application for alkylation with unnatural alkyl diphosphates.

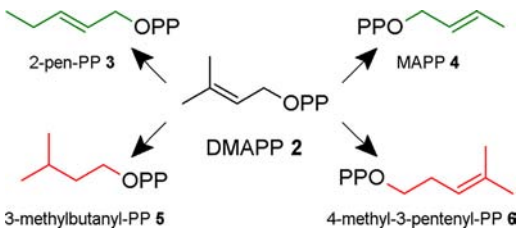


Figure 1. DMAPP and its analogues.

For this purpose, four DMAPP analogues (Figure 1) were successfully synthesized according to the method described for GPP by Woodside et al.,[5] which were confirmed by ^{1}H and ^{31}P NMR analyses. The four DMAPP analogues were used as alkyl donors for enzyme assays with FgaPT2 and 7-DMATS from *Aspergillus fumigatus* as well as with 5-DMATS from *Aspergillus clavatus*, which catalyzed prenylation of L-tryptophan at C-4, C-7, and C-5, respectively.[2a,6] Enzyme assays with DMAPP were used as positive controls (Figure 2). HPLC analysis of the incubation mixtures revealed an almost complete conversion of L-tryptophan to its prenylated products in the three enzyme assays with DMAPP after incubation with 5 μg of protein at 37 °C for 16 h (Figure 2). Shifting one methyl group of DMAPP from C-3 to C-4, as in the case of 2-pentenyl diphosphate (2-pen-PP), strongly reduced the activity of 7-DMATS. No product formation was detected in the incubation mixture under assay conditions (Figure 2C). In contrast, FgaPT2 and 5-DMATS still accepted 2-pen-PP well as a substrate (Figure 2A and 2B) and conversion yields of 37% and 91% from the respective assays with DMAPP were achieved under these conditions.

For structure elucidation, the enzyme products **3a** and **3b** were isolated from the incubation mixtures of FgaPT2 and 5-DMATS, respectively, and subjected to NMR and MS analyses. HR-MS data clearly showed the alkylation of both compounds by detection of $[M]^{+}$ ions at m/z 272.1518 (**3a**) and 272.1509 (**3b**), which are 68 Da larger than that of tryptophan.

In the ^{1}H NMR spectrum of **3a**, signals of a 2-pentenyl moiety proved the alkylation at a C-atom in a regular pattern.[2a]

The four aromatic protons of the indole moiety appeared, according to their chemical shifts and coupling pattern, in the same order as those of C5-prenylated indole derivatives obtained from enzyme assays of 5-DMATS

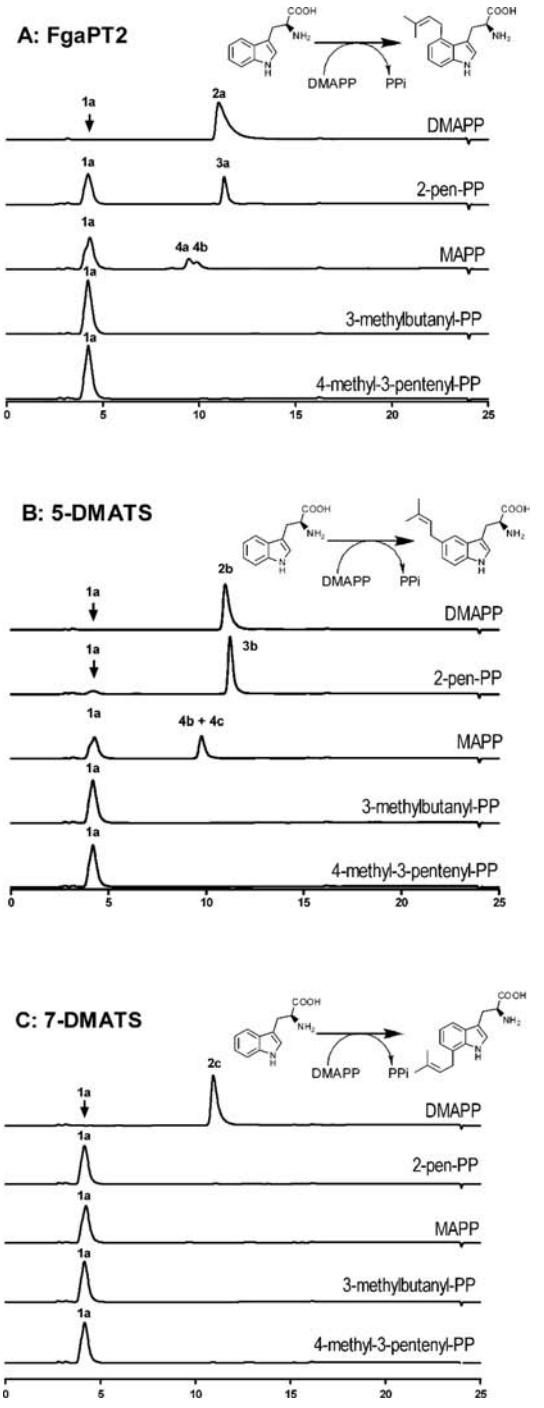


Figure 2. HPLC analysis of L-tryptophan after incubation with DMAPP or its analogues at 37 °C and 16 h.

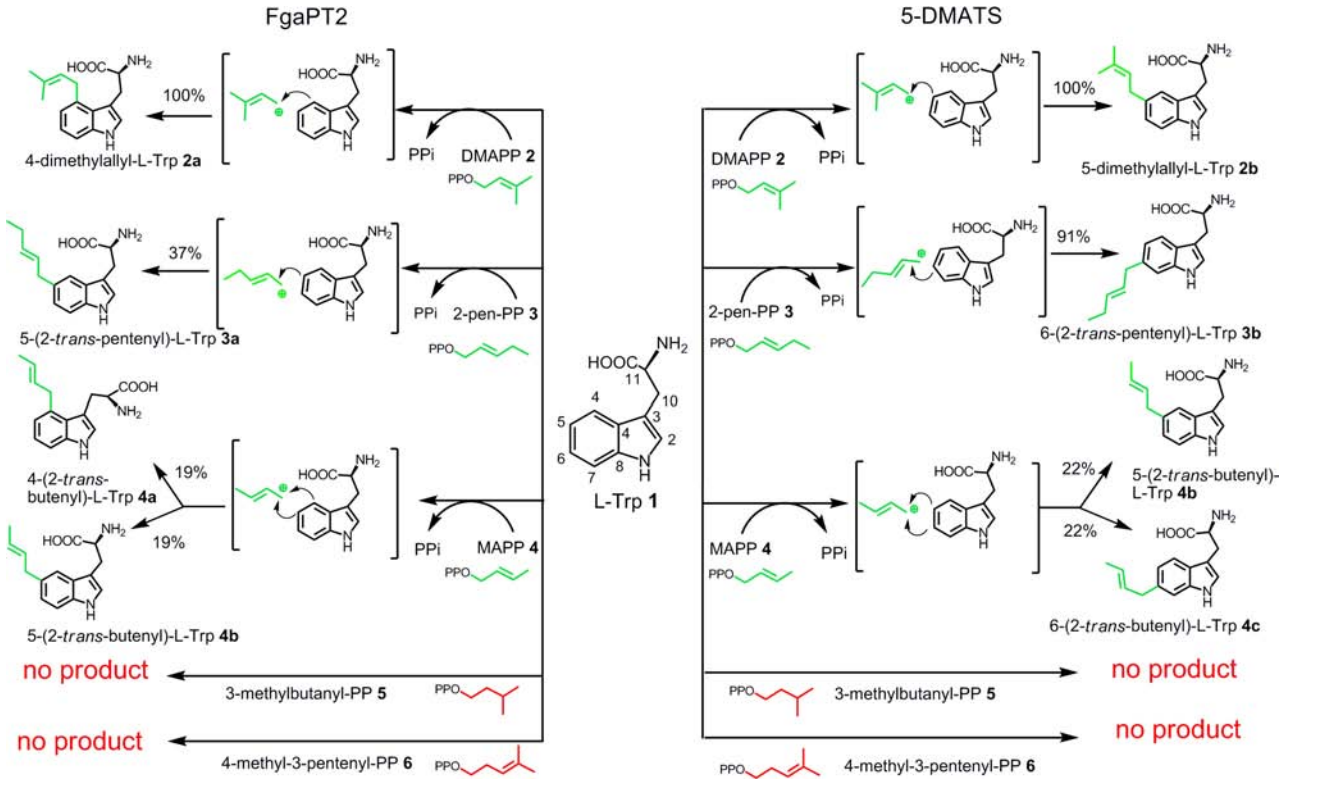
with DMAPP[2a] and therefore can be assigned to H-4, H-7, H-2, and H-6.

This indicated a C5-alkylation of L-tryptophan by FgaPT2 in the presence of 2-pen-PP and is somewhat unexpected for a C4-prenyltransferase with its natural substrate DMAPP (Scheme 1).[6b] Therefore, we obtained various 2D NMR spectra for **3a** to confirm the structure. In the HMBC spectrum, connectivities were clearly observed for H-4 of

(5) Woodside, A. B.; Huang, Z.; Poulter, C. D. *Org. Synth.* **1988**, *66*, 211–215.

(6) (a) Kremer, A.; Westrich, L.; Li, S.-M. *Microbiology* **2007**, *153*, 3409–3416. (b) Unsöld, I. A.; Li, S.-M. *Microbiology* **2005**, *151*, 1499–1505.

Scheme 1. Proposed Reaction Mechanisms and Enzyme Products of FgaPT2 and 5-DMATS with DMAPP and Analogues



the indole ring with C-3 and C-8 as well as with C-1′ of the alkyl moiety, proving unequivocally the C5-akylation of L-tryptophan in **3a**.

In the ^{1}H NMR spectrum of **3b**, similar signals to those of **3a** were also observed for a 2-pentenyl moiety. However, the ^{1}H NMR spectrum of **3b** differed clearly from that of **3a**, especially in the aromatic region, although the same number and similar coupling patterns were observed in both spectra. The signals of the four aromatic protons in **3b** corresponded very well to those of C6-prenylated indole derivatives obtained from enzyme assays of IptA with DMAPP.[7] This means that the alkylation of L-tryptophan by 5-DMATS was shifted from C-5 in the presence of DMAPP to C-6 in the presence of 2-pen-PP (Scheme 1).

The alkylation shifts from C-4 to C-5 for FgaPT2 and from C-5 to C-6 for 5-DMATS could be explained by the longer chain length and thus deeper extension of 2-pen-PP into the reaction chamber in comparison to that of DMAPP, so that C-1′ of the alkyl residue was closer to C-5 than C-4 in FgaPT2 and to C-6 than C-5 in 5-DMATS. To prove the influence of chain length of the alkyl diphosphate on the enzyme activity and alkylation position, the *cis*-methyl group of DMAPP was deleted to obtain methylallyl diphosphate (MAPP) (Figure 1), which was then used as an alkyl donor for enzyme assays of L-tryptophan with FgaPT2, 5-DMATS, and 7-DMATS. Again, 7-DMATS showed no detectable conversion (Figure 2C).

The enzyme activities of FgaPT2 and 5-DMATS were reduced, in comparison to those of DMAPP and 2-pen-PP, but still clearly detectable. Two and one product peaks were detected in the HPLC chromatograms of the incubation mixtures of L-tryptophan and MAPP with FgaPT2 and 5-DMATS, respectively. A conversion yield of 29% was calculated for FgaPT2, and 44%, for 5-DMATS, respectively. For structure elucidation, all three peaks were isolated and subjected to NMR and MS analyses. HR-MS data confirmed the presence of a butenyl moiety in their structures by detection of $[M+H]^+$ at m/z 259.1471 (**4a**) and 259.1434 (**4b**) or $[M]^+$ ion at 258.1388 (**4b**+**4c**), which are 54 Da larger than that of tryptophan.

The ^{1}H NMR spectra of **4a** and **4b** are similar to each other in the region of olefinic and aliphatic protons. Signals for a 2-*trans*-butenyl moiety were detected for both **4a** and **4b**. The spectra however differed clearly from each other by the chemical shifts and coupling pattern of the four aromatic protons in the spectrum of **4a** and **4b.**

The features of the aromatic protons of **4a** indicated an alkylation at C-4 or C-7. The HMBC spectrum clearly showed connectivities of H-1′ of the butenyl residue[8] to C-2′ (δ_C 131.2) and C-3′ (δ_C 125.0) as well as to C4 (δ_C 132.7) and C5 (δ_C 119.2) of the indole moiety. The alkylation position at C-4 of L-tryptophan was confirmed

(7) Takahashi, S.; Takagi, H.; Toyoda, A.; Uramoto, M.; Nogawa, T.; Ueki, M.; Sakaki, Y.; Osada, H. *J. Bacteriol.* **2010**, *192*, 2839–2851.

(8) The two protons at H-1′ of the butenyl residue differ from each other, probably due to the steric interaction with the side chain of tryptophan. δ_H 3.75 (1H, dd, 15.4, 6.3, H-1′) and δ_H 3.63 (1H, dd, 15.4, 6.3, H-1′).

by detection of additional connectivities in the indole moiety.[9]

The coupling pattern of the four aromatic protons in **4b** indicated a substitution at C-5 or C-6. The order of these coupling protons was found to be identical to those in **3a** and other C5-prenylated derivatives.[2a] The alkylation at C-5 of L-tryptophan in **4b** (Scheme 1) was also confirmed by the observation of connectivities in its HMBC spectrum.

Inspection of the ^{1}H NMR spectrum of the product peak isolated from the incubation mixture of L-tryptophan and MAPP with 5-DMATS revealed the presence of two compounds with a ratio of 1:1. Unfortunately, it was difficult to separate them from each other by HPLC under different conditions. Fortunately, it was yet possible to elucidate their structures based on the obtained spectra, because one of them can be easily identified as **4b** by comparison of their ^{1}H NMR spectra. The second one, **4c**, showed similar signals and identical order in the aromatic ranges to **3b** and other C6-prenylated indole derivatives[7] and was therefore identified as a C6-alkylated derivative. It seems that the smaller methylallyl group was placed in the reaction site with a similar distance from its C-1′ to C-4 and C-5 in FgaPT2 or to C-5 and C6 in 5-DMATS, so that attacking from both positions was possible.

To test the importance of the double bond at the β-position to diphosphate, we prepared 3-methylbutanyl diphosphate **5** and 4-methyl-3-pentenyl diphosphate **6** (Figure 1) and used them as alkyl donors for FgaPT2, 5-DMATS, and 7-DMATS. As shown in Figure 2, no product formation was detected for any of the enzyme assays. This proved that the double bond at the β-position is essential for the alkylation. Based on the protein structures of several prenyltransferases, it was proposed that a carbocation will be first created with the help of several basic amino acids.[10] This carbocation must be then stabilized by several amino acid residues, e.g. tyrosine, as well as by the aromatic ring system of the substrate itself to protect its reaction with undesired nucleophiles.[10a] Our data in this study showed that stabilization of the carbocation by itself, *via* a β-unsaturated C−C bond, is also essential for a successful Friedel−Crafts alkylation. It would be of great interest to create enzyme derivatives, e.g. by site directed mutagenesis, which can stabilize such ions and accept also saturated alkyl diphosphates as alkyl donors.

To compare the biochemical properties of FgaPT2 and 5-DMATS toward DMAPP and its analogues, kinetic parameters were calculated from Hanes−Woolf and Eadie−Hofstee transformations (Table 1). All the reactions apparently followed Michaelis−Menten kinetics. FgaPT2 accepted its natural substrate DMAPP with a K_M value of 4 μM, much better than MAPP and 2-pen-PP with K_M values at 40 and 56 μM, respectively. In contrast, 5-DMATS has no significant preference and even a higher affinity to MAPP than to DMAPP. The turnover numbers of FgaPT2 and 5-DMATS toward unnatural alkyl diphosphates were found to be less than 3% of those with DMAPP but are still within a realized range for use in chemical reactions. For both enzymes, 2-pen-PP was accepted with a higher reaction velocity than MAPP.

Table 1. Kinetic Parameters of FgaPT2 and 5-DMATS with DMAPP and Analogues as Alkyl Donors

enzyme	donor	K_M (μM)	k_{cat} (s^{-1})	k_{cat}/K_M ($s^{-1}\cdot M^{-1}$)
FgaPT2	DMAPP[a]	4	0.37	92 500
	MAPP	40	0.009	232
	2-pen-PP	56	0.011	198
5-DMATS	DMAPP[b]	76	1.3	17 105
	MAPP	40	0.005	136
	2-pen-PP	131	0.023	174

[a] The data were adopted from ref 6b. [b] The data were adopted from ref 2a.

In conclusion, the structure of the natural alkylation reagent DMAPP can be modified and used as an alkyl donor for some members of the DMATS superfamily, which were successfully used for the prenylation of diverse aromatic substrates including indoles, hydroxynaphthalenes, flavonoids, and tetracyclic napthacenediones.[1c,2b,2c,4b] One methyl group in DMAPP can be deleted or shifted, but the double bond at the β-position seems to be essential for the stability of the formed carbocation. In comparison to DMAPP, the alkylation of the smaller MAPP is in part retained and in part shifted to one position, i.e. from C-4 to C-5 by FgaPT2 and from C-5 to C-6 by 5-DMATS. With 2-pen-PP as an alkyl donor, the alkylation position was completely shifted for one position. The acceptance of the modified prenyl donors by prenyltransferases expanded significantly the potential use of these enzymes as catalysts in the chemoenzymatic synthesis. It would be interesting to test the acceptance of the DMAPP analogues by other prenyltransferases.

Acknowledgment. We thank Dr. Laufenberg and Lena Ludwig (Philipps-Universität Marburg) for acquiring mass spectra and for the synthesis of DMAPP, respectively. This work was supported in part by Deutsche Forschungsgemeinschaft Grant Li844/4-1 (to S.-M.L.).

Supporting Information Available. Experimental procedures, HR-MS, NMR data and spectra. This material is available free of charge via Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

(9) Position C-5 (δ_C 119.2) is confirmed by connectivities of H-5 (δ_H 6.74) to C-7 (δ_C 109.5) and C-9 (δ_C 124.7) as well as connectivities of H-10 (δ_H 3.29 and 2.90) and H-2 (δ_H 7.16) to C-9.

(10) (a) Metzger, U.; Schall, C.; Zocher, G.; Unsöld, I.; Stec, E.; Li, S.-M.; Heide, L.; Stehle, T. *Proc. Natl. Acad. Sci. U.S.A* **2009**, *106*, 14309–14314. (b) Lik, L. Y. P.; Tanner, M. E. *J. Am. Chem. Soc.* **2009**, *131*, 13932–13933.