ChemPubSoc Europe

DOI: 10.1002/cbic.201600304

CHEMBIOCHEM Full Papers

# Characterization of the Aminotransferase ThdN from Thienodolin Biosynthesis in *Streptomyces albogriseolus*

Daniela Milbredt, Eugenio P. Patallo, and Karl-Heinz van Pée*[a]

In *Streptomyces albogriseolus* the indolethiophen alkaloid thienodolin is derived from tryptophan. The first step in thienodolin biosynthesis is the regioselective chlorination of tryptophan in the 6-position of the indole ring. The second step is catalyzed by the aminotransferase ThdN. ThdN shows sequence homology (up to 69% similarity) with known pyridoxal 5′-phosphate-dependent aminotransferases of the aspartate aminotransferase family from Gram-positive bacteria. *thdN* was heterologously expressed in *Pseudomonas fluorescens*, and the enzyme was purified by nickel-affinity chromatography. ThdN is a homodimeric enzyme with a mass of 90600 kDa and catalyzes the conversion of L-tryptophan and a number of chlorinated and brominated L-tryptophans. The lowest $K_M$ values were found for 6-bromo- and 6-chlorotryptophan (40 and 66 μM, respectively). For L-tryptophan it was 454 μM, which explains why thienodolin is the major product and dechlorothienodolin is only a minor component. The turnover number ($k_{cat}$) for 7-chlorotryptophan (128 $min^{-1}$) was higher than that for the natural substrate 6-chlorotryptophan (88 $min^{-1}$).

## Introduction

The alkaloid thienodolin is a unique secondary metabolite and was first isolated in the early 1990s from the soil bacterium *Streptomyces albogriseolus* MJ286-76F7 during screening for new plant growth-regulating substances in microorganisms;[1] it was recently also detected in two other Streptomyces strains.[2,3] Structure elucidation of thienodolin (6-chloro-8*H*-thieno[2,3-*b*]indole-2-carboxamide) by spectroscopic and X-ray crystallographic analyses revealed its unusual tricyclic indole S-hetero scaffold and chlorination at position 6 of the indole ring.[4] The total chemical synthesis was accomplished in three steps starting from 6-chlorooxindole.[5]

Thienodolin was the first actinomycete metabolite to show concentration-dependent growth-regulating activity in rice seedlings. Growth was promoted at low concentrations (1.2–12 mM), but inhibited at higher concentrations (>40 mM).[4] It is of significant pharmaceutical interest, because recently it was demonstrated that thienodolin exhibits anti-inflammatory and anti-tumor activity through suppression of nitric oxide synthases.[3]

In a previous paper, we described the identification of the thienodolin biosynthetic gene cluster.[6] The first step in thienodolin biosynthesis is the flavin-dependent chlorination of the precursor tryptophan to 6-chlorotryptophan, catalyzed by ThdH (formerly Thal), a flavin-dependent tryptophan 6-halogenase;[7] the final step is the ATP-dependent conversion of 6-chlorothieno-[2,3-*b*]indole-2-carboxylic acid into thienodolin, catalyzed by the amidotransferase ThdD. For the conversion of 6-chlorotryptophan into 6-chlorothieno-[2,3-*b*]indole-2-carboxylic acid, the incorporation of a sulfur atom followed by ring formation/closure and oxidation building the thienyl ring is necessary. Activation of the α-carbon of 6-chlorotryptophan, catalyzed by an aminotransferase to form 6-chloroindole-3-pyruvate (Scheme 1), was suggested to facilitate sulfur incorporation. The gene for a potential aminotransferase was found in the thienodolin biosynthetic cluster. Disruption of this gene, *thdN*, in the thienodolin producer *S. albogriseolus* led to loss of thienodolin formation.[6] This was confirmed by Wang et al., who recently described the isolation of the thienodolin biosynthetic gene cluster from *Streptomyces* sp. FXJ1.172 and showed the in vitro aminotransferase activity of ThnJ (homologue of ThdN).[2] Here, we characterized the in vitro aminotransferase activity of ThdN and its involvement in thienodolin biosynthesis.

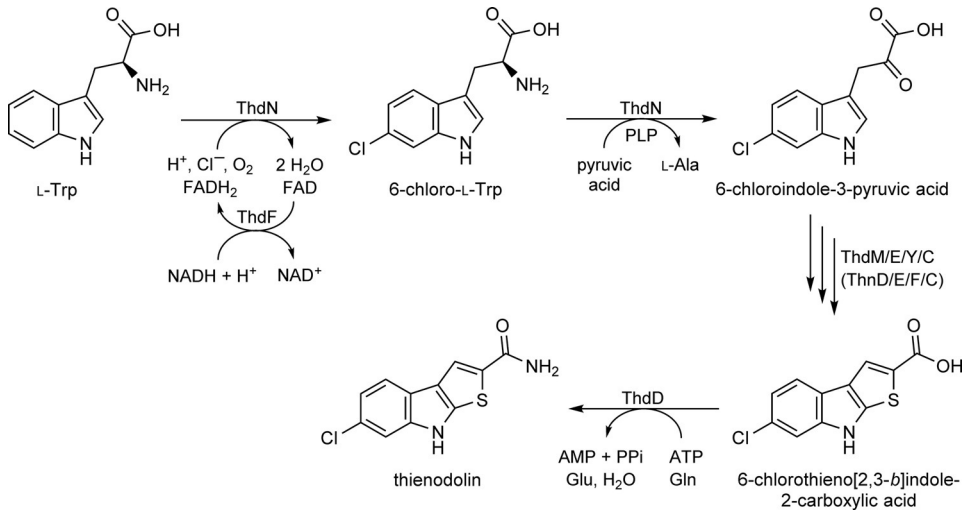
## Results

### Sequence comparison of ThdN and in silico analysis

The gene *thdN* (1152 nt) has a high overall GC content (71%). Analysis of the predicted amino acid sequence (383 aa) indicated that ThdN belongs to the aspartate aminotransferase family (AAT, fold type I) of pyridoxal phosphate (PLP)-dependent enzymes. It is homologous (up to 69% similarity, 57% identity) to a variety of histidinol-phosphate aminotransferases, aromatic amino acid aminotransferases, and other unspecified aminotransferases from different Gram-positive actinobacteria, and was therefore suggested to be an aminotransferase (Scheme 1). ThdN shows 59% identity to ThnJ from *Streptomy-*

[a] Dr. D. Milbredt, Dr. E. P. Patallo, Prof. K.-H. van Pée
Professur für Allgemeine Biochemie
Fachrichtung Chemie und Lebensmittelchemie,
Technische Universität Dresden
01062 Dresden (Germany)
E-mail: karl-heinz.vanpee@chemie.tu-dresden.de

Supporting information and ORCID(s) from the author(s) for this article can be found under http://dx.doi.org/10.1002/cbic.201600304.




**Scheme 1.** Proposed biosynthetic pathway to thienodolin. The first two steps, catalyzed by ThdH and ThdN, and the final amidation by ThdD have been verified experimentally.[1,3]

*ces* sp. FXJ1.172.[2] The next-highest homology was with the tryptophan aminotransferase Ind8, identified to catalyze the conversion of L-tryptophan into indole-3-pyruvate during indolmycin biosynthesis in *Streptomyces griseus* subsp. *griseus*.[8,9] Sequence alignment with some other aminotransferases (Figure S1 in the Supporting Information) showed that the amino acids important for the formation of the tertiary and the quaternary structure of the protein (determined by protein BLAST against conserved domains) are highly conserved among all these proteins. Thus, the PLP-binding site of ThdN might be formed by G115, S116, G117, F141, N189, Y219, T245, S247, K248, and R256, with K248 as the catalytic residue, whereas G118, S151, V212, G254, L255, R256, and G283 seem to constitute the homodimer interface. This indicates that ThdN, like the great majority of aminotransferases with resolved crystal structures, such as *Arabidopsis thaliana* L-tryptophan aminotransferase (PDB ID: 3BWN; involved in auxin biosynthesis)[10] and *Escherichia coli* histidinol-phosphate aminotransferase (PDB ID: 1GEW),[11] might be a homodimeric protein.

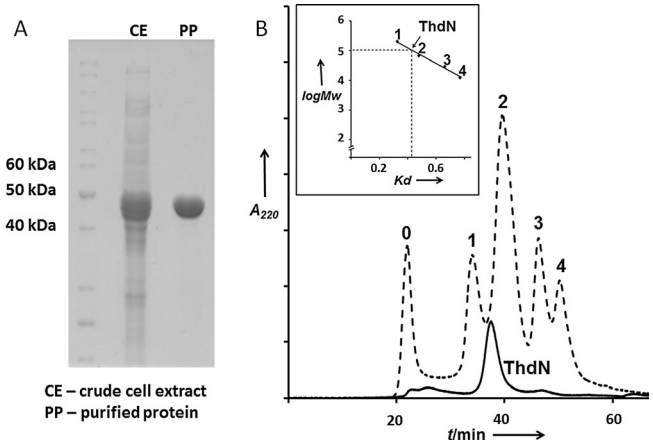



**Figure 1.** A) SDS-PAGE of ThdN in crude cell extract of *P. fluorescens* BL915 ΔORF1-4 harboring pCIBhis*thdN* (CE) and after purification (PP). B) Size-exclusion chromatography for molecular mass determination of native ThdN (MWGF200 markers (Sigma–Aldrich): 0 (2000 kDa) 1, (200 kDa), 2 (66 kDa), 3 (29 kDa), 4 (12.4 kDa). The calibration for determining the molecular mass is shown as an insert.

### Heterologous expression and purification of ThdN

*thdN* was expressed in *Pseudomonas fluorescens* BL915 ΔORF1-4[12] by using the *E. coli–Pseudomonas* shuttle vector pCIBhis.[13] Growing the strain in lysogeny broth (LB) at 30 °C for 72 h resulted in the formation of soluble ThdN carrying a $His_6$ tag and an enterokinase cleavage site at its N terminus (Figure 1 A, sample "CE"). Purification to homogeneity was achieved by immobilized nickel-chelating affinity chromatography. Approximately 30 mg of purified ThdN was obtained per liter of bacterial culture.

ThdN (including the $His_6$ tag and enterokinase site) was determined to be about 45 kDa in size (Figure 1 A), which is in agreement with the theoretical mass (45 298 Da; ProtParam). According to size-exclusion chromatography with a Superdex 200 10/300 GL column (GE Healthcare), the mass of native ThdN was about 100 kDa, thus indicating that ThdN is a homodimer (Figure 1 B). This is in agreement with the existence of a homodimer interface in the predicted tertiary structure (Figure S1).

### Determination of aminating activity, optimal reaction parameters, and enzyme kinetics

Because of the involvement of the aminotransferase ThdN in thienodolin biosynthesis, 6-chloro-L-tryptophan is likely the natural amino group donor substrate. ThdN also accepted L-tryptophan, 5-fluoro-L-tryptophan, 5-chloro-L-tryptophan, 5-bromo-L-tryptophan, 6-bromo-L-tryptophan, 7-chloro-L-tryptophan, and 7-bromo-L-tryptophan as substrates; L-tyrosine was a very poor substrate (data not shown). L-Phenylalanine and L-histidine (the other aromatic amino acids) were not accepted. Enzyme assays performed with L-tryptophan and D-tryptophan

showed that ThdN only converts the L-enantiomer into the corresponding α-keto acid. Of the two compounds tested as co-substrates, α-ketoglutarate and sodium pyruvate, only pyruvate was accepted as amino group acceptor.

The optimal reaction conditions for ThdN were determined to be 40 °C, 3 mM sodium pyruvate, and 10–20 μM PLP in 20 mM Tris·HCl buffer containing 50 mM NaCl at pH 8 (Figure 2). Transaminase kinetic values ($K_M$, $k_{cat}$, and $k_{eff}$) were determined under these conditions for the eight amino acid substrates converted by ThdN, by applying the least-squares method (Table 1 and Figure 3; Michaelis–Menten and Lineweaver–Burk plots in Figures S2–S9).

ThdN showed excellent long term stability. After purification, dialysis against 20 mM Tris·HCl (pH 8.0) with 50 mM NaCl, and filter-sterilization, the enzyme could be stored at 4 °C for at least 4 months without significant loss of aminating activity.

**Table 1.** Kinetic data for the conversion of halogenated tryptophans into the corresponding keto acids by ThdN.

| Substrate | $K_M$ [μM] | $k_{cat}$ [$min^{-1}$] | $k_{eff}$ [$\mu M^{-1} min^{-1}$] |
|---|---|---|---|
| 5-fluoro-L-tryptophan | 586 | 40.7 | 0.069 |
| 5-chloro-L-tryptophan | 355 | 54.0 | 0.152 |
| 5-bromo-L-tryptophan | 552 | 53.0 | 0.096 |
| 6-chloro-L-tryptophan | 66 | 88.0 | 1.342 |
| 6-bromo-L-tryptophan | 40 | 55.5 | 1.401 |
| 7-chloro-L-tryptophan | 207 | 128.4 | 0.619 |
| 7-bromo-L-tryptophan | 133 | 95.5 | 0.718 |
| L-tryptophan | 454 | 100.6 | 0.221 |

## Discussion

### Enzyme characterization

Because of the high GC content of *Streptomyces* genes (thus potential expression problems in lower GC content bacteria like *E. coli*, *thdN* was expressed in *Pseudomonas fluorescens* BL915 ΔORF1-4, which has been used for overexpression of other *Streptomyces* genes and has resulted in high amounts of soluble and functional enzyme.[6,12,13,14] As the N-terminal $His_6$ tag did not seem to influence the aminating activity of the enzyme, it was not necessary to cleave the fusion protein for investigation of enzyme activity and kinetic measurements. Wang et al. expressed the *thdN* analogue *thnJ* in *E. coli*, but did not specify the amount of active enzyme obtained.[2] According to size-exclusion chromatography, native ThdN exists as a homodimer. ThdN was quite stable: several months at 4 °C in 20 mM Tris·HCl (pH 8) containing 50 mM NaCl. This is quite unusual, as other tryptophan aminotransferases are only stable for shorter periods.[9,15]

To characterize the aminating activity of ThdN, we used an enzymatic assay based on the detection of indole-3-pyruvic acid formation. However, because of the instability of indole-3-pyruvic acid,[16] it was not possible to quantify the product directly and reliably by HPLC. In order to overcome this problem, derivatization of the product followed by HPLC or GC analysis is normally used.[17] We used a spectrophotometric assay based on the absorbance of the enol form of indole-3-pyruvic acid complexed with borate.[18] The enol–borate complex is stable and allowed reproducible measurements (UV spectra of pyruvate and complex in Figure S10). ThdN is specific for L-tryptophan (not D-tryptophan), as described for other tryptophan aminotransferases.[9,15,19–22]

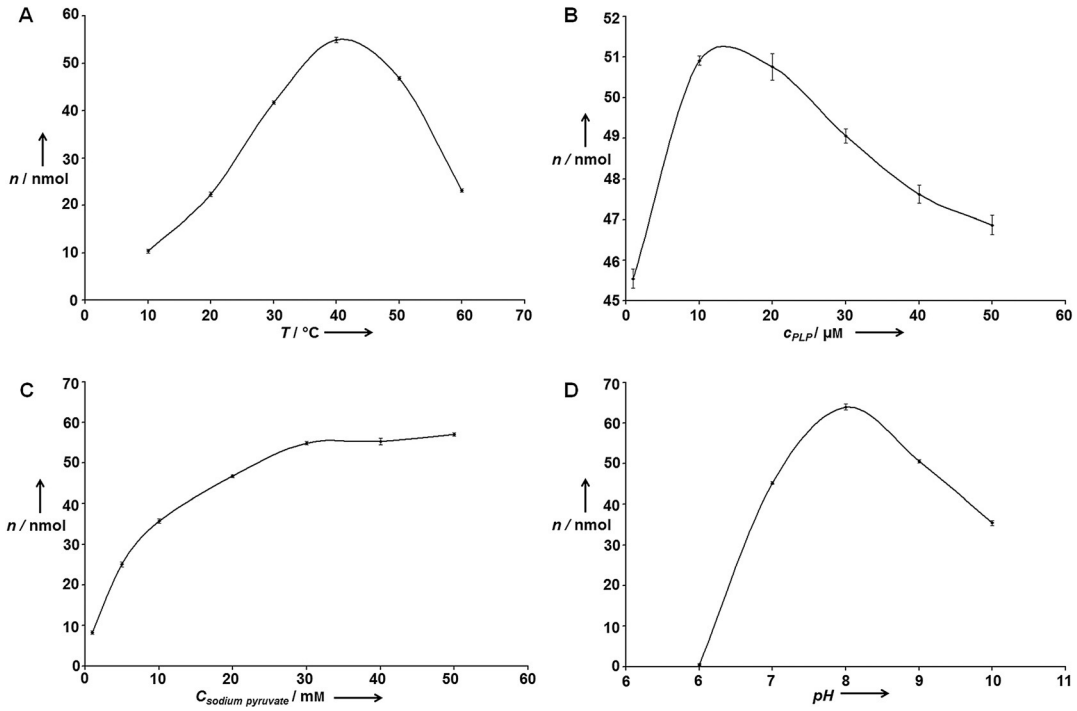



**Figure 2.** Optimization of A) temperature, B) PLP concentration, C) sodium pyruvate concentration, and D) pH for the conversion of 6-chloro-L-tryptophan into 6-chloroindole-3-pyruvate. *n*: amount of formed 6-chloroindole-3-pyruvate.

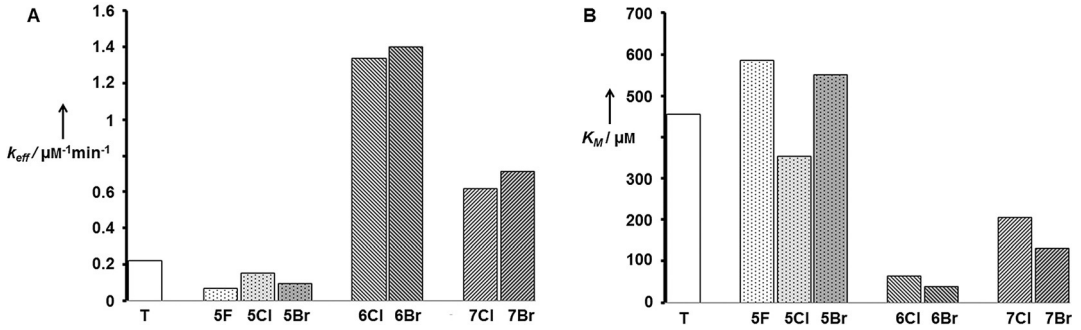



**Figure 3.** Kinetic values A) $k_{eff}$ and B) $K_M$ for the conversion of amino donor substrates into thei corresponding keto acids by ThdN. T: L-tryptophan, 5F: 5-fluoro-L-tryptophan, 5Cl: 5-chloro-L-tryptophan, 5Br: 5-bromo-L-tryptophan, 6Cl: 6-chloro-L-tryptophan, 6Br: 6-bromo-L-tryptophan, 7Cl: 7-chloro-L-tryptophan, 7Br: 7-bromo-L-tryptophan.

ThdN is the first aminotransferase to use 6-chloro-L-tryptophan as the natural substrate. Thus, the optimal reaction parameters for its aminating activity were determined for the conversion of 6-chlorotryptophan into 6-chloroindole-3-pyruvic acid. The highest activity was at pH 8.0; this is consistent with specifications for other tryptophan aminotransferases (optimal pH in the slightly alkaline range).[15,21,23–26] The temperature optimum for characterized tryptophan aminotransferases from different organisms varies significantly from 20 to 60 °C.[15,9,24] The optimal temperature for ThdN was around 40 °C, which is quite high compared to the optimal temperature for growth and thienodolin production (28 °C for *S. albogriseolus*).[1]

The $K_M$ values for tryptophan aminotransferases with L-tryptophan vary significantly between different organisms (from 300 μM for mung bean seedlings[21] to low-millimolar for bacterial aminotransferases).[15,19,22] For ThdN, a $K_M$ of 454 μM was determined; this is similar to ThnJ (330 μM) but in the lower range for other tryptophan aminotransferases with L-tryptophan.[2] The $K_M$ values for the natural substrate (6-chloro-L-tryptophan) and its brominated analogue were found to be significantly lower (66 and 40 μM, respectively); this is consistent with thienodolin being the major and dechlorothienodolin only a minor product.[2,3] However, Wang et al. reported a $K_M$ value of 2.62 mM for ThnJ from the thienodolin producer *Streptomyces* sp. FXJ1.172 with 6-chloro-L-tryptophan. In their assays, substrate saturation was not reached even at a substrate concentration of 6 mM.[2] ThdN also accepts tryptophan halogenated at the positions 5 and 7 as substrates (Table 1), but with higher $K_M$ than for 6-chloro- or 6-bromotryptophan. The $k_{cat}$ values for L-tryptophan and halogenated tryptophans were quite similar (41 to 128 min$^{-1}$). The $k_{eff}$ data show that tryptophan chlorinated or brominated in position 6 is the best substrate. Tryptophan was also a much better substrate when halogenated at position 7 than at position 5. Interestingly, all brominated tryptophans were better substrates; this indicates that thienodolin producers might have originated from the marine environment, as the thienodolin producer *Streptomyces* sp. FXJ1.172 was isolated from Chilean marine sediment.[3] The brominated derivative of thienodolin can be obtained by adding 6-bromotryptophan to the growth medium of *S. albogriseolus*, but 5-chloro- or 7-chlorotryptophan in the culture medium did not lead to formation of the respective thienodolin derivatives. Obviously, the substrate specificity of an enzyme downstream of ThdN is too high.[6]

## The role of ThdN in thienodolin biosynthesis

Thienodolin biosynthesis starts with the conversion of tryptophan into 6-chloro-tryptophan by ThdH, a flavin-dependent tryptophan 6-halogenase.[6] This reaction is analogous to halogenation in the biosyntheses of other chlorinated bacterial secondary metabolites, such as pyrroindomycin B from *S. rugosporus*,[13] the kutznerids produced by *Kutzneria* sp.,[27,28] pyrrolnitrin produced by various *Pseudomonas* strains and other microorganisms,[14,29–33] and rebeccamycin from *Lechevalieria aerocolonigenes*;[34,35] these chlorinate the precursor tryptophan at positions 5, 6 and/or 7. We have also reported the final step of thienodolin biosynthesis: ATP-dependent conversion of 6-chlorothieno[2,3-*b*]indole-2-carboxylic acid into thienodolin catalyzed by the amidotransferase ThdD.[6] Conversion of 6-chlorotryptophan into 6-chlorothieno[2,3-*b*]indole-2-carboxylic acid has an unknown number of biosynthetic steps; we postulate the activation of the α-carbon of 6-chlorotryptophan to facilitate incorporation of a sulfur atom, followed by ring formation/closure and oxidation building the thienyl ring, thus leading to 6-chlorothieno[2,3-*b*]indole-2-carboxylic acid.[6]

In secondary metabolite biosynthesis, activation of the α-carbon of L-amino acids is often realized by an L-amino acid oxidase (EC 1.4.3.2), a flavin-dependent enzyme that catalyzes oxidative deamination of L-amino acids to the corresponding α-keto acids via an α-imino acid intermediate. Well-characterized examples for the conversion of L-tryptophan (or 7-chlorotryptophan) into the corresponding indole-3-pyruvic acid were isolated from staurosporine biosynthesis in *Streptomyces* sp. TP-A0274 (StaO),[36] violacein biosynthesis in *Chromobacterium violaceum* (VioA),[37] and rebeccamycin biosynthesis in *Lechevalieria aerocolonigenes* (RebO).[38]

We suggested that the proposed carbon activation during thienodolin biosynthesis is accomplished by substitution of the amino group of 6-chloro-L-tryptophan against an oxygen atom by ThdN to form 6-chloroindole-3-pyruvic acid (resulting in increased reactivity of the α-carbon atom).[6] This is strongly supported by our in vitro experiments and by Wang et al.[2] Further transformation of 6-chloroindole-3-pyruvic acid into 6-

chlorothieno[2,3-*b*]indole-2-carboxylic acid (the final intermediate before thienodolin) was recently suggested to be catalyzed by ThnD, E, F, and C (ThdM, E, Y, and C in *S. albogriseolus*, Scheme 1), although in vitro experimental evidence is lacking.[2,6]

## Conclusion

We have expressed *thdN* of the thienodolin biosynthetic gene cluster of *S. albogriseolus*, and characterized the enzyme as an aminotransferase. ThdN was shown to be a 6-chloro-L-tryptophan:pyruvic acid aminotransferase. It is also able to convert nonhalogenated L-tryptophan and other halogenated L-tryptophans (but not D-tryptophan) into their respective α-keto acids in the presence of pyruvate as the amino group acceptor. The optimal reaction conditions for the transamination reaction and the kinetic data for the different substrates were determined. ThdN shows the highest $k_{cat}$ values for the conversion of 6-bromo- and 6-chlorotryptophan, entirely consistent with its biological function in thienodolin biosynthesis. With the identification of ThdN as an aminotransferase catalyzing the formation of 6-chloroindole-3-pyruvic acid from 6-chloro-L-tryptophan, the second step of thienodolin biosynthesis could now be elucidated.

## Experimental Section

**Strains, plasmids and culture conditions:** *E. coli* TG1 (Stratagene) was used for cloning and *Pseudomonas fluorescens* BL915 ΔORF1–4[12] was used for heterologous gene expression.[14] Both were cultivated in lysogeny broth (LB) at 37 °C (*E. coli*) or 30 °C (*P. fluorescens*) with agitation (150 rpm);[39] where necessary, the medium was supplemented with ampicillin (100 μg mL$^{-1}$) and tetracycline (15 μg mL$^{-1}$) for *E. coli* and tetracycline (30 μg mL$^{-1}$) and kanamycin (50 μg mL$^{-1}$) for *P. fluorescens*. The plasmids were pLitmus28[40] (NEB) for general cloning and pCIBhis[13] (containing a T7 promotor) for constitutive gene expression in *P. fluorescens*.

**Genetic manipulation and PCR:** Standard molecular biology procedures were performed according to Sambrook et al. (1989).[39] DNA restriction, dephosphorylation, ligation, and PCR were performed by following the manufacturers' protocols: Thermo Scientific/NEB PCR conditions were: 94 °C for 5 min, 35 cycles at 94 °C for 30 s, 55 °C for 1 min, 72 °C for 3 min, and final extension at 72 °C for 5 min. Pfu polymerase (Thermo Fisher) and DMSO (10%) were used. Oligonucleotide primers were purchased from Eurofins MWG Operon (Ebersberg, Germany).

**Heterologous expression of *thdN*:** *thdN* was expressed as a $His_6$-tagged fusion protein in *P. fluorescens* BL915 ΔORF1–4. The gene was amplified by PCR with primers ThdN_for (5′-ATGGC TCGAG GATGA CGGCA CGCCA AGTCA CG-3′; XhoI restriction site underlined) and ThdN_rev (5′-ATATA AGCTT TCACA CCGAC AGGAA GTCCT TC-3′; HindIII site underlined). The PCR product was double-digested with XhoI and HindIII and ligated into pCIBhis linearized with the same enzymes. The resulting plasmid (pCIBhis*thdN*) was transferred into *P. fluorescens* BL915 ΔORF1–4 by electroporation (12.5 kV cm$^{-1}$, 5 ms). After growing on LB agar containing kanamycin (50 μg mL$^{-1}$) and tetracycline (30 μg mL$^{-1}$) for 48 h at 30 °C, colonies were selected for protein analysis. One clone showing significant ThdN production was selected for further experiments.

**Protein purification:** A BioRad chromatography system (ES-1 Econo System Controller, Model 1327 Econo Recorder, Model 2128 Econo Fraction Collector, EG-1 Econo Gradient Monitor, EP-1 Econo Pump, and EM-1 Econo UV-Monitor) was used. *P. fluorescens* BL915 ΔORF1–4 harboring pCIBhis*thdN* was grown in LB medium (30 mL) containing antibiotics for 24 h at 30 °C. This starter culture was used to inoculate fresh LB medium (3×1 L) and cultivated for 72 h. Cells were separated from the culture medium by centrifugation (8200 *g*, 10 min), washed with saline (2 mL per gram of cells), and then resuspended (2 mL buffer per gram of cells) in cold loading buffer (Tris·HCl (20 mM, pH 8.0) with NaCl (50 mM)). The suspension was sonicated (6 min; amplitude 65%; pulse 5 s, pause 10 s) and centrifuged (48000 *g*, 1 h, 4 °C). The resulting crude extract was filtered through a Millex-GP sterile filter (0.22 μm; Merck Millipore) and then applied to a 50 mL chromatography column packed with Chelating Sepharose Fast Flow (GE Healthcare) loaded with $Ni^{2+}$ (40 mL, 200 mM) and equilibrated with at least 5 column volumes of loading buffer. The column was washed with loading buffer until no further change in absorption at 280 nm could be detected. Bound protein was eluted with a linear gradient of imidazole (up to 0.5 M in loading buffer) over six column volumes at 0.5 mL min$^{-1}$. The eluate was collected as 6 mL fractions. Fractions showing absorbance at 280 nm were analyzed by SDS-PAGE. Fractions containing homogeneous ThdN (45.3 kDa) were pooled, dialyzed against Tris·HCl (20 mM, pH 8.0) containing NaCl (50 mM) to remove imidazole, filter-sterilized, and stored at 4 °C.

**Gel filtration:** Gel filtration was carried out on a Superdex 200 10/300 GL column (GE Healthcare) in Tris·HCl (20 mM, pH 8.0) with NaCl (50 mM), at a flow rate of 0.4 mL min$^{-1}$. A kit for molecular weights 12000–200000 (MWGF200-1KT; Sigma–Aldrich) was used to calibrate the column.

**In vitro activity of ThdN:** In vitro aminating activity of purified ThdN was evaluated with an assay reported for phenylalanine aminotransferase activity.[41] The assay mixture consisted of PLP, substrate, co-substrate, and enzyme (1.1 μM) in Tris·HCl (20 mM, pH 8.0) with NaCl (50 mM). The reaction product was quantified by the spectrophotometric method of Lin et al.[18] Substrates L-tryptophan, D-tryptophan, halogenated tryptophans, L-tyrosine, L-phenylalanine, and L-histidine were tested. The assay mixture consisted of substrate (500 μM), co-substrate (3 mM; α-ketoglutarate or sodium pyruvate), PLP (10 μM), and ThdN (1.1 μM) in Tris·HCl (200 μL, 20 mM, pH 8.0) with NaCl (50 mM). All components were combined and incubated at 28 °C. The reaction was stopped by adding TCA (40 μL, 20%). Precipitated protein was removed by centrifugation (16000 *g*, 10 min), and the supernatant (200 μL) was incubated with arsenate-borate buffer (1 mL, 1 M, pH 6.5: sodium arsenate (1 M) in boric acid (1 M)) at 25 °C for 20 min. The formed enol-borate complex was quantified by measuring UV absorption ($\varepsilon_{332}$ = 12,700 M$^{-1}$ cm$^{-1}$) against a control (pyruvate omitted). For determination of the optimal reaction conditions, reactions were performed with 6-chloro-L-tryptophan (Santa Cruz Biotechnology), sodium pyruvate (0.1–5 mM), and PLP (1–50 μM). Temperature and pH were varied (10 to 60 °C, pH 6 to 10). All assays were performed in triplicate.

**Kinetic studies:** Assays to determine the kinetic data of the amination reaction were performed under optimal reaction conditions (above). Reaction mixtures comprised PLP (20 μM), substrate (25–750 μM), sodium pyruvate (3 mM) and ThdN (0.138–1.1 μM) in Tris·HCl, (200 μL, 20 mM, pH 8.0) with NaCl (50 mM). All components except sodium pyruvate were mixed and incubated at 40 °C for 5 min. Sodium pyruvate was added to start the reaction, and then incubated for 10 min at 40 °C. The reaction was stopped by

addition of TCA (40 μL, 20%). After 10 min at 4 °C, the mixture was centrifuged (16000 *g*, 10 min), and the supernatant (200 μL) was transferred to a new tube. Arsenate-borate (1 mL, 1 M) was added, incubated at room temperature for 20 min, and the formed indole-borate complex was quantified by measuring absorbance (332 nm) against a control (identical, but without sodium pyruvate). All assays were performed in triplicate.

## *Acknowledgements*

*We thank Dr. Rebecca J. M. Goss (University of St Andrews, UK) for kindly providing us with 5-chloro-L-tryptophan, 5-bromo-L-tryptophan, 5-fluoro-L-tryptophan, 6-bromo-L-tryptophan, 7-chloro-L-tryptophan, and 7-bromo-L-tryptophan.*

**Keywords:** aminotransferases · chlorotryptophan · enzyme catalysis · natural products · thienodolin

[1] K. Kanbe, M. Okamura, S. Hattori, H. Naganawa, M. Hamada, Y. Okami, T. Takeuchi, *Biosci. Biotechnol. Biochem.* **1993**, *57*, 632 – 635.
[2] Y. Wang, J. Wang, S. Ju, F. Wang, H. Ma, C. Yue, M. Liu, Z. Deng, Y. Huang, X. Qu, *ChemBioChem* **2016**, *17*, 799 – 803.
[3] E.-J. Park, J. M. Pezzuto, K. H. Jang, S.-J. Nam, S. A. Bucarey, W. Fenical, *Nat. Prod. Commun.* **2012**, *7*, 789 – 794.
[4] K. Kanbe, M. Okamura, S. Hattori, H. Naganawa, M. Hamada, Y. Okami, T. Takeuchi, *Biosci. Biotechnol. Biochem.* **1993**, *57*, 636 – 637.
[5] R. Engqvist, A. Javaid, J. Bergman, *Eur. J. Org. Chem.* **2004**, 2589 – 2592.
[6] D. Milbredt, E. P. Patallo, K.-H. van Pée, *ChemBioChem* **2014**, *15*, 1011 – 1020.
[7] C. Seibold, H. Schnerr, J. Rumpf, A. Kunzendorf, C. Hatscher, T. Wage, A. J. Ernyei, C. Dong, J. H. Naismith, K.-H. van Pée, *Biocatal. Biotransform.* **2006**, *24*, 401 – 408.
[8] Y.-L. Du, L. M. Alkhalaf, K. S. Ryan, *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 2717 – 2722.
[9] M. K. Speedie, U. Hornemann, H. G. Floss, *J. Biol. Chem.* **1975**, *250*, 7819 – 7825.
[10] Y. Tao, J.-L. Ferrer, K. Ljung, F. Pojer, F. Hong, J. A. Long, L. Li, J. E. Moreno, M. E. Bowman, L. J. Ivans, Y. Cheng, J. Lim, Y. Zhao, C. L. Ballaré, G. Sandberg, J. P. Noel, J. Chory, *Cell* **2008**, *133*, 164 – 176.
[11] K. Haruyama, T. Nakai, I. Miyahara, K. Hirotsu, H. Mizuguchi, H. Hayashi, H. Kagamiyama, *Biochemistry* **2001**, *40*, 4633 – 4644.
[12] K.-H. van Pée, J. M. Ligon, *Appl. Environ. Microbiol.* **1997**, *63*, 2147 – 2154.
[13] S. Zehner, A. Kotzsch, B. Bister, R. D. Süssmuth, C. Méndez, J. A. Salas, K.-H. van Pée, *Chem. Biol.* **2005**, *12*, 445 – 452.
[14] S. Keller, T. Wage, K. Hohaus, M. Hölzer, E. Eichhorn, K.-H. van Pée, *Angew. Chem. Int. Ed.* **2000**, *39*, 2300 – 2302; *Angew. Chem.* **2000**, *112*, 2380 – 2382.
[15] J. Preuss, W. Hort, S. Lang, A. Netsch, S. Rahlfs, G. Lochnit, E. Jortzik, K. Becker, P. A. Mayser, *Exp. Dermatol.* **2013**, *22*, 736 – 741.
[16] Y. Y. Tam, J. Normanly, *J. Chromatogr. A.* **1998**, *800*, 101 – 108.
[17] X. Liu, A. D. Hegeman, G. Gardner, J. D. Cohen, *Plant Methods* **2012**, *8*, 1 – 17.
[18] E. C. C. Lin, B. M. Pitt, M. Civen, W. E. Knox, *J. Biol. Chem.* **1958**, *233*, 668 – 673.
[19] P. Schneider, M. Weber, K. Rosenberger, D. Hoffmeister, *Chem. Biol.* **2007**, *14*, 635 – 644.
[20] C. J. Balibar, A. R. Howard-Jones, C. T. Walsh, *Nat. Chem. Biol.* **2007**, *3*, 584 – 592.
[21] T. A. Truelsen, *Physiol. Plant.* **1972**, *26*, 289 – 295.
[22] J. Koga, K. Syōono, T. Ichikawa, T. Adachi, *Biochim. Biophys. Acta Protein Struct. Mol. Enzymol.* **1994**, *1209*, 241 – 247.
[23] R. M. Simpson, H. M. Nonhebel, D. L. Christie, *Planta* **1997**, *201*, 71 – 77.
[24] T. Koshiba, N. Mito, M. Miyakado, *J. Plant Res.* **1993**, *106*, 25 – 29.
[25] Y. Minatogawa, T. Noguchi, R. Kido, *J. Neurochem.* **1976**, *27*, 1097 – 1101.
[26] T. Noguchi, S. Hayashi, *J. Biol. Chem.* **1980**, *255*, 2267 – 2269.
[27] D. G. Fujimori, S. Hrvatin, C. S. Neumann, M. Strieker, M. A. Marahiel, C. T. Walsh, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 16498 – 16503.
[28] J. R. Heemstra Jr., C. T. Walsh, *J. Am. Chem. Soc.* **2008**, *130*, 14024 – 14025.
[29] D. H. Lively, M. Gorman, M. E. Haney, J. A. Mabe, *Antimicrob. Agents Chemother.* **1966**, *6*, 462 – 469.
[30] R. P. Elander, J. A. Mabe, R. H. Hamill, M. Gorman, *Appl. Microbiol.* **1968**, *16*, 753 – 758.
[31] K. Gerth, W. Trowitzsch, V. Wray, G. Höfle, H. Irschik, H. Reichenbach, *J. Antibiot.* **1982**, *35*, 1101 – 1103.
[32] N. El-Banna, G. Winkelmann, *J. Appl. Microbiol.* **1998**, *85*, 69 – 78.
[33] P. Knauth, H. Reichenbach, *J. Antibiot.* **2000**, *53*, 1182 – 1190.
[34] C. Sánchez, I. A. Butovich, A. F. Braña, J. Rohr, C. Méndez, J. A. Salas, *Chem. Biol.* **2002**, *9*, 519 – 531.
[35] E. Yeh, S. Garneau, C. T. Walsh, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 3960 – 3965.
[36] M. Kameya, H. Onaka, Y. Asano, *Anal. Biochem.* **2013**, *438*, 124 – 132.
[37] C. J. Balibar, C. T. Walsh, *Biochemistry* **2006**, *45*, 15444 – 15457.
[38] T. Nishizawa, C. C. Aldrich, D. H. Sherman, *J. Bacteriol.* **2005**, *187*, 2084 – 2092.
[39] J. Sambrook, E. F. Fritsch, T. Maniatis, *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press, New York, **1989**.
[40] P. D. Evans, S. N. Cook, P. D. Riggs, C. J. Noren, *Biotechniques* **1995**, *19*, 130 – 135.
[41] M. Fujioka, J. Morino, H. Wada, *Methods Enzymol.* **1970**, *17A*, 585 – 596.

Manuscript received: May 26, 2016
Final article published: August 17, 2016