Phytochemistry 106 (2014) 37–43

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

# Investigation of a substrate-specifying residue within *Papaver somniferum* and *Catharanthus roseus* aromatic amino acid decarboxylases

Michael P. Torrens-Spence, Michael Lazear, Renee von Guggenberg, Haizhen Ding, Jianyong Li *

*Department of Biochemistry, Virginia Tech, Blacksburg, VA, United States*

ARTICLE INFO

*Article history:*
Received 10 March 2014
Received in revised form 2 June 2014
Available online 5 August 2014

*Keywords:*
*Papaver somniferum*
Papaveraceae
*Catharanthus roseus*
Apocynaceae
Aromatic amino acid decarboxylase
Tyrosine decarboxylases
Tryptophan decarboxylases

ABSTRACT

Plant aromatic amino acid decarboxylases (AAADs) catalyze the decarboxylation of aromatic amino acids with either benzene or indole rings. Because the substrate selectivity of AAADs is intimately related to their physiological functions, primary sequence data and their differentiation could provide significant physiological insights. However, due to general high sequence identity, plant AAAD substrate specificities have been difficult to identify through primary sequence comparison. In this study, bioinformatic approaches were utilized to identify several active site residues within plant AAAD enzymes that may impact substrate specificity. Next a *Papaver somniferum* tyrosine decarboxylase (TyDC) was selected as a model to verify our putative substrate-dictating residues through mutation. Results indicated that mutagenesis of serine 372 to glycine enables the *P. somniferum* TyDC to use 5-hydroxytryptophan as a substrate, and reduces the enzyme activity toward 3,4-dihydroxy-L-phenylalanine (dopa). Additionally, the reverse mutation in a *Catharanthus roseus* tryptophan decarboxylase (TDC) enables the mutant enzyme to utilize tyrosine and dopa as substrates with a reduced affinity toward tryptophan. Molecular modeling and molecular docking of the *P. somniferum* TyDC and the *C. roseus* TDC enzymes provided a structural basis to explain alterations in substrate specificity. Identification of an active site residue that impacts substrate selectivity produces a primary sequence identifier that may help differentiate the indolic and phenolic substrate specificities of individual plant AAADs.



## 1. Introduction

Aromatic amino acid decarboxylases (AAADs) are a group of economically important and phylogenetically diverse enzymes that are categorically lined through their pyridoxal 5-phosphate (PLP) dependence and sequence homology. This family of enzymes has been studied extensively in mammals where a single enzyme, 3,4-dihydroxy-L-phenylalanine (dopa) decarboxylase (DDC), catalyzes decarboxylation of both phenolic and indolic amino acids to generate their corresponding aromatic amines. Mammalian DDC is responsible for the decarboxylation of dopa and 5-hydroxytryptophan to yield the neurotransmitters dopamine and serotonin, respectively (Srinivasan and Awapara, 1978; Zhu and Juorio, 1995). Unlike the single mammalian DDC enzyme, plant AAADs have evolved with variations in activity and substrate specificity. The resulting paralogs are formally identified as tryptophan decarboxylases (TDCs), tyrosine decarboxylases (TyDCs) and aromatic acetaldehyde synthases (AASs), respectively. TDCs catalyze decarboxylation of tryptophan and 5-hydroxytryptophan (Noé et al., 1984; De Luca et al., 1988; Lopez-Meyer and Nessler, 1997; Yamazaki et al., 2003; Kang et al., 2007; Park et al., 2009), whereas TyDCs engender decarboxylation of tyrosine and dopa (Facchini and De Luca, 1995; Lehmann and Pollmann, 2009; Torrens-Spence et al., 2012, 2013) and AASs the decarboxylation-oxidative deamination of dopa, tyrosine and phenylalanine (Kaminaga et al., 2006; Gutensohn et al., 2011; Torrens-Spence et al., 2012), respectively.

Differences in substrate selectivity and activity among plant TyDCs, TDCs and AASs enable individual enzymes to generate specific products with unique physiological functions. For example, plant TDCs are required for synthesis of monoterpenoid indole alkaloids that comprise a diverse group of hundreds of pharmacologically active compounds (Meijer et al., 1993; Berlin et al., 1994; Facchini et al., 2000), TyDCs are known to function in several different metabolic pathways including the biosynthesis of simple alkaloids, complex benzylisoquinoline alkaloids, and *N*-hydroxycinnamic acid amides (Leete and Marion, 1953; Ellis,

* Corresponding author. Address: Department of Biochemistry, Engel Hall 204, Virginia Tech, Blacksburg, VA 24061, United States. Tel.: +1 540 321 5779; fax: +1 540 231 9070.
*E-mail address:* lij@vt.edu (J. Li).

http://dx.doi.org/10.1016/j.phytochem.2014.07.007
0031-9422/

1983; Marques and Brodelius, 1988; Trezzini et al., 1993; Facchini et al., 2000) and AAS enzymes catalyze the production of volatile flower scents, floral attractants, and defensive phenolic acetaldehyde secondary metabolites (Kaminaga et al., 2006; Gutensohn et al., 2011; Torrens-Spence et al., 2012). It is clear that the physiological functions of plant AAADs are closely related to their respective activities and substrate specificities; however, due to the subtlety of the enzymatic divergence of plant AAADs, it has historically been difficult to predict the function of any given plant AAAD paralog through sequence comparison. Consequently, being able to distinguish the activity and substrate specificity of individual plant AAADs is of practical significance.

In previous work, it was determined that a single active site residue is capable of dictating the activity of plant TyDCs, TDCs and AASs without altering substrate selectivity. Specifically, the presence of a tyrosine in an active site catalytic loop dictates decarboxylation chemistry while a phenylalanine substitution at the same location dictates aldehyde synthase chemistry (Torrens-Spence et al., 2013). The alteration in enzymatic activity due to a single amino acid substitution suggests that the functional variations in plant AAADs may be dictated by a small number of residues. Such subtle variations in plant AAADs likely explain the difficulty in differentiating plant TyDCs, TDCs and AASs through sequences analysis.

In this study, to further distinguish plant AAADs an attempt to identify specific structural identifiers capable of differentiating between the substrate specificity of TDCs and TyDCs was made. To do so, bioinformatic analyses of characterized plant AAAD enzymes were performed with predicted several target residues that might potentially impact substrate specificity in plant AAADs and these residues assessed through site-directed mutations using a *Papaver somniferum* TyDC and a *Catharanthus roseus* TDC. Results of the biochemical analyses, in conjunction with molecular modeling, provide insights into a residue that impacts plant AAAD indole and benzene substrate selectivity.

## 2. Results

### 2.1. Primary sequence investigation of recombinant characterized TyDC and TDC sequences

To begin the investigation of residues that might impact substrate specificity of plant AAAD enzymes, a primary sequence evaluation of essentially all the characterized plant TyDC and TDC sequences was performed (Supplemental Table S1). Pairwise alignments of these characterized plant AAAD enzymes indicated that inter TDC–TyDC class identities are often greater than that of intra TDC or TyDC classes. For example, despite differences in substrate specificity, the *Capsicum annuum* TDC 2 (Park et al., 2009) shares greater homology with the *Arabidopsis thaliana* TyDC (Lehmann and Pollmann, 2009) (66% identity) than it does with the *Camptotheca acuminata* TDC 2 (Lopez-Meyer and Nessler, 1997) (56% identity). The sequence ambiguity of plant AAADs can be further illustrated in a dendrogram of characterized TyDC and TDC sequences (Fig 1). As this figure illustrates, TyDC and TDC sequences did not appear to exclusively cluster according to their substrate preference. Despite maintaining identical substrate profiles, the characterized TDC sequences appeared to cluster into two groups. Interestingly, TDC sequences from both *Oryza sativa* and *C. annuum* appear in both clusters. This suggests that this clustering is not due to evolutionary divergence of plant species but rather the evolutionary divergence of individual AAAD sequences. In addition to the delocalization of the TDC genes, the dendrogram suggest variable clustering of characterized TyDC sequences. *P. somniferum* TyDC 7, *P. somniferum* TyDC 9 and *Thalictrum flavum* TyDC cluster together while *A. thaliana* TyDC clusters with *O. sativa* TDC 2 and *C. annuum* TDC 2. The sequence and phylogenetic ambiguity of TyDC and TDC enzymes produces significant challenges in plant AAAD gene annotation.

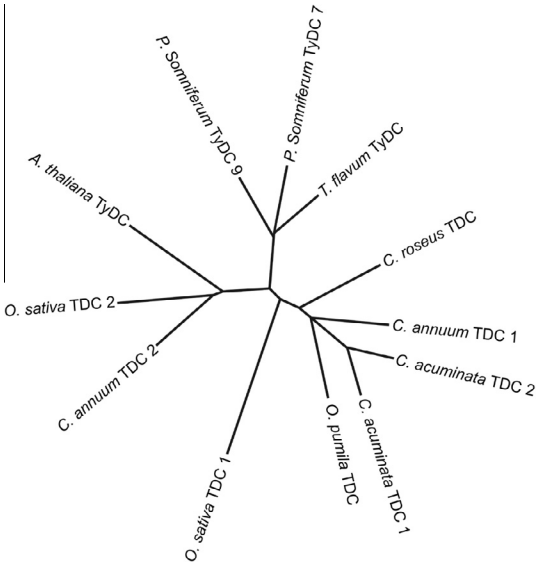


**Fig. 1.** Dendrogram of recombinantly characterized plant TyDC and TDC sequences.

Multiple sequence alignments of characterized TyDC and TDC sequences were used to identify possible substrate specifying residues. Analysis of residues conserved within 9 or more of the 12 characterized TDC and TyDC sequences yielded twenty-two putative substrate-specifying residues. These conserved residues were highlighted in the *P. somniferum* TyDC 9 sequence (subsequently referred to as *P. somniferum* TyDC) (Torrens-Spence et al., 2013) (Fig. 2). Next, the previously characterized *P. somniferum* TyDC was modeled and a carbidopa external aldimine superimposed into the active site. Residues within four angstroms of the carbidopa external aldimine were highlighted to produce an additional 18 residues potentially involved in substrate recognition (Fig. 2). Cross-referencing these active site proximal residues with the conserved TyDC/TDC residues reduced the hypothetical substrate specifying residues down to 5 individual amino acids (Fig. 2). These residues are represented as serine 101, cysteine 170, asparagine 318, alanine 319 and serine 372 within the *P. somniferum* TyDC sequence.

### 2.2. Generation and investigation of putative substrate specifying P. somniferum TyDC mutants

Before evaluating the substrate specifying roles of these select five residues, recombinant *P. somniferum* TyDC was first evaluated to determine the substrate range of the wild type TyDC. The recombinant wild type TyDC was expressed and purified to homogeneity. Activity assay results showed that it efficiently catalyzed the decarboxylation of dopa and tyrosine (at 2 mM substrate concentration, the enzyme showed 5910 ± 200 nmol/min/mg protein to dopa and 5350 ± 200 nmol/min/mg protein to tyrosine), but displayed no activity towards phenylalanine or indolic substrates, including 5-hydroxytryptophan and tryptophan. Next, using the *P. somniferum* TyDC as a model, site directed mutations of S101A, C170A, N318S, A319P and S372G were made based on conserved active site proximal residues.

To test perturbations in substrate specificity, the *P. somniferum* TyDC S101A, C170S, N318S, A319P, and S372G mutant enzymes were cloned, expressed, and purified to homogeneity. The activities of the mutant enzymes were tested using dopa, tyrosine,