Biosci. Biotechnol. Biochem., **72** (9), 2456–2458, 2008

Note

Conversion of 5-Hydroxytryptophan into Serotonin by Tryptophan Decarboxylase in Plants, *Escherichia coli*, and Yeast

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Received April 4, 2008; Accepted May 19, 2008; Online Publication, September 7, 2008
[doi:10.1271/bbb.80220]

The L-tryptophan decarboxylase (TDC) gene of rice was heterologously expressed in various organisms. Transgenic rice overexpressing TDC showed accumulation of serotonin upon 5-hydroxytryptophan treatment, which was consistent with the *in vitro* 5-hydroxytryptophan decarboxylase enzyme activity of purified recombinant rice TDC in a pyridoxal phosphate-dependent manner. Recombinant yeast harboring *TDC* produced serotonin at the expense of the endogenous 5-hydroxytryptophan levels.

Key words: tryptophan decarboxylase; 5-hydroxytryptophan decarboxylase; 5-hydroxytryptophan; serotonin

L-Tryptophan decarboxylase (TDC) catalyzes tryptophan into tryptamine. In plants, TDC is involved in the biosynthesis of several types of secondary metabolites, including terpenoid indole alkaloids, serotonin derivatives, and serotonin.[1,2] Since the first isolation of a *TDC* gene was reported, from *Catharanthus roseus*,[3] only three other *TDC* genes have been characterized at the biochemical and molecular levels.[1,4] In addition, the substrate specificities of TDC proteins as purified enzymes and in heterologous expression systems have not been characterized, except for a study in which a partially purified TDC from tomato was also found to catalyze 5-hydroxytryptophan (5-OH Trp) into serotonin, as shown by a simple paper chromatography analysis.[5] Subsequently, there have been no further reports as to whether TDC can catalyze 5-OH Trp into serotonin or synthesize serotonin *via* 5-OH Trp *via* its 5-hydroxytryptophan decarboxylase activity (5-OHTDC) in heterologous expression systems. Here, in the present study, we carried out functional analyses of TDC, focusing on the availability of 5-OH Trp as a substrate and using rice plants, *E. coli*, and yeast as heterologous systems.

Recombinant TDC was purified from *E. coli* harboring pET28b-TDC, in which a full-length rice *TDC* gene (GenBank accession no. AK069031) was constructed in-frame with codons for six additional histidine residues at the carboxy terminus, as described previously.[4] The purified TDC protein was used to determine whether rice TDC would catalyze 5-OH Trp into serotonin and would prove to be dependent on pyridoxal phosphate. As shown in Fig. 1A, TDC activity was 8 nkat mg^{-1} protein even in the absence of cofactor pyridoxal phosphate, whereas the addition of pyridoxal phosphate to the reaction medium increased TDC activity by 9-fold, suggesting that rice TDC requires pyridoxal phosphate as a cofactor. In contrast, its 5-hydroxytryptophan decarboxylase (5-OHTDC) activity was 8 times lower than TDC activity in the absence of pyridoxal phosphate, but the addition of pyridoxal phosphate caused an 18-fold increase in 5-OHTDC activity. The optimum pyridoxal phosphate concentration for the activity of both TDC and 5-OHTDC was about 0.1 mM.

The *in vivo* activity of 5-OHTDC was further confirmed in transgenic rice constitutively expressing a rice *TDC* gene.[4] Three-week-old rice leaves were employed. One-cm leaf squares were excised with a razor blade and placed them in 6-cm diameter polystyrene Petri dishes containing various concentrations of 5-OH Trp. The tissues were then incubated in a growth chamber at 25 °C for 12 h. The levels of serotonin were quantified by HPLC and compared.

The leaves of the transgenic rice plants accumulated 28 and 46 μg of serotonin per g fresh weight (fw) when treated with 100 and 500 μM 5-OH Trp respectively (Fig. 1B). In contrast, the wild-type produced 2.7 and 17 μg of serotonin per g fw in response to 100 and 500 μM 5-OH Trp respectively. The transgenic leaves produced 10-fold higher serotonin than the wild-type

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Abbreviations: 5-OH Trp, 5-hydroxytryptophan; 5-OHTDC, 5-hydroxytryptophan decarboxylase; TDC, tryptophan decarboxylase

A

Pyridoxal phosphate (mM)	Decarboxylase activity (pkat mg^{-1}protein)	
	Tryptophan	5-OH tryptophan
0.0	8060 ± 30	970 ± 12
0.1	61672 ± 60	17800 ± 50
0.2	64382 ± 85	17545 ± 65
0.5	64002 ± 55	17500 ± 35

B

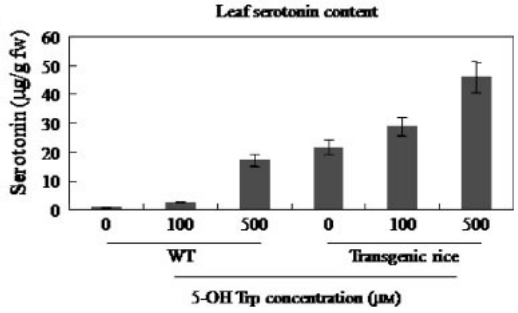

Fig. 1. Substrate Specificity for Tryptophan and 5-OH Tryptophan of TDC (A) and Serotonin Production in Transgenic Rice Plants Overexpressing a Rice *TDC* Gene in Response to 5-OH Trp (B).

Recombinant purified TDC (1 μg) was used in enzyme activity measurements. Homozygous T_2 transgenic rice plants (line 10) were analyzed for serotonin synthesis upon treatment with 5-OH Trp. The data are mean values of two replicates ± SD.

leaves under 100 μM 5-OH Trp treatment, but following 500 μM 5-OH Trp treatment, the transgenic leaves showed 2.6-fold higher serotonin synthesis than the wild type. These results indicate that TDC overexpression in rice plants leads to increased synthesis of serotonin in the presence of 5-OH Trp due to its 5-OHTDC enzyme activity. The basal level of serotonin (about 20 μg/g fw) in the transgenic rice without the extraneously added 5OH-Trp was produced *via* tryptamine by TDC overexpression, since the enhanced tryptamine is readily converted into serotonin by tryptamine 5-hydroxylase, which is expressed constitutively.[4)]

Next we examined to determine whether the expression of TDC in *E. coli* would result in serotonin production. The same *E. coli* strain used in the affinity purification of TDC (pET28b-TDC) was employed. Recombinant *E. coli* expressing TDC was grown to $OD_{600} = 1.0$ at 37 °C, treated with 0.5 mM isopropyl β-D-thiogalactoside (IPTG) and 3 mM tryptophan or 5-OH Trp, and incubated at 28 °C for various durations. The medium fractions of the cultures were collected by centrifugation for 1 min in a microfuge, and the supernatants were extracted with ethyl acetate. Next, the ethyl-acetate fractions were evaporated and dissolved in MeOH, and the solutions were subjected to HPLC analysis for tryptamine and serotonin, as described previously.[4)] Upon treatment with 3 mM tryptophan, tryptamine accumulation in the recombinant *E. coli* (pET28b-TDC) increased strongly during the 24-h incubation time, reaching a maximum level of 180 mg/l culture, whereas the control *E. coli* (pET28b) produced no tryptamine (Fig. 2A). Similarly, serotonin production in the presence of 3 mM 5-OH Trp increased to 35 mg/l culture in the recombinant *E. coli*, but this level was 5-fold lower than that of tryptamine (Fig. 2B). These relatively low levels of serotonin production were

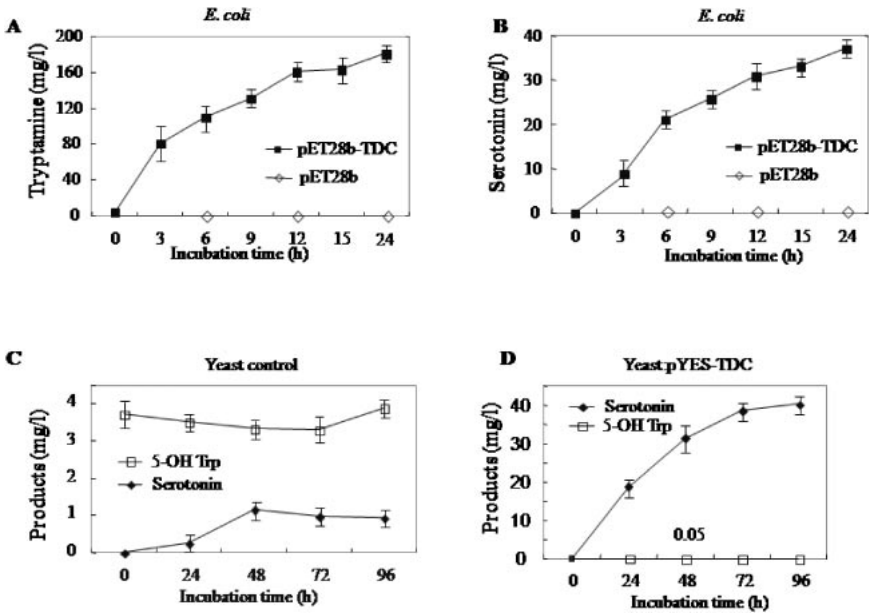

Fig. 2. Heterologous Expression of TDC and Production of Serotonin in *Escherichia coli* and Yeast.

Production of tryptamine (A) and serotonin (B) in control *E. coli* harboring the pET28b vector only and recombinant *E. coli* harboring pET28b-TDC in the presence of 3 mM tryptophan and of 3 mM 5-OH Trp. Production of serotonin and 5-OH Trp in control *S. cerevisiae* INV*Sc*1 (C) and recombinant *S. cerevisiae* INV*Sc*1 harboring pYES-TDC (D). The data are mean values of two replicates ± SD.

probably associated with the low catalytic activity of 5-OHTDC relative to that of TDC.

To determine whether the expression of TDC in yeast also caused serotonin production, we introduced the *TDC* gene into *Saccharomyces cerevisiae* strain INV*Sc*1 following the manufacturer's instructions (EasyComp™ Transformation Kit, Invitrogen, La Jolla, CA). Recombinant pYES-TDC yeast ($OD_{600} = 2$) was induced with SC medium (0.67% yeast nitrogen base, 0.192% yeast medium (Ura^-), 2% galactose) and the cells were incubated at 28 °C for varying durations. Medium extraction and serotonin analysis were performed as described for the *E. coli* system. The results are shown in Fig. 2C and D. The control yeast produced 5-OH Trp constitutively at about 4 mg/l, and no serotonin was detected (Fig. 2C). Upon galactose treatment, the 5-OH Trp levels decreased slightly up to 72 h, and thereafter increased back to their initial level by 96 h. In contrast, serotonin levels began to increase at 24 h and reached a peak level of 1.17 mg/l at 48 h, and the levels remained steady through 96 h. However, recombinant yeast pYES-TDC showed a dramatic increase in serotonin synthesis, to 40 mg/l, even without 5-OH Trp treatment (Fig. 2D). At the same time, endogenous 5-OH Trp dropped to negligible levels, averaging 0.05 mg/l. Thus serotonin production occurred at the expense of endogenous 5-OH Trp.

Serotonin was long ago reported to be synthesized in yeast in response to UV treatment,[6] but no studies on serotonin synthesis caused by TDC overexpression or the detection of 5-OH Trp in yeast have been reported. This study of TDC in yeast indicates that the enzymatic step from 5-OH Trp to serotonin is rate-limiting in yeast. Our data also suggest for the first time, to our knowledge, that serotonin can be overproduced in yeast at up to 40 mg/l through ectopic expression of TDC.

Acknowledgments

This work was supported by a grant from the Agricultural Plant Stress Research Center (R11-2001-092-03001-0) and by a grant from the BioGreen 21 Program funded by the Rural Development Administration, Republic of Korea.

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