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cDNA cloning and characterization of tryptophan synthase alpha subunit from *Polygonum tinctorium*

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Abstract

Polygonum tinctorium is a cultivated plant that produces indigo, a natural blue dye. Its leaves contain a large amount of indican (indoxyl-beta-D-glucoside), a colorless precursor of indigo. The enzyme beta-glucosidase, which degrades indican, is present in leaf cells. If the leaves are scratched because of some external factors, indican is enzymatically degraded into indoxyl and glucose. Because of the chemical instability of indoxyl, it is immediately oxidized to indigo by atmospheric oxygen. Beta-glucosidase is located in chloroplasts, whereas the substrate indican is stored in vacuoles. Therefore, indigo is only produced if leaf cells are physically broken. The insoluble indigo may have a negative effect on infectious fungi and bacteria as well as on invasive insects and other animals. We hypothesize that the physiological role of indican as a secondary metabolite is of a defense system against predators.

In a previous study, we have shown that indican is synthesized from indoxyl and UDP-glucose by the catalysis of UDP-glucosyltransferase. The substrate indoxyl is probably produced by the hydroxylation of indole catalyzed by cytochrome P450. Indole is an intermediate product in tryptophan synthesis, which is the final step of the shikimic acid pathway, a primary metabolic pathway. The tryptophan synthase consists of four subunits: two alpha subunits (TSA) and two beta subunits (TSB). Only TSA catalyzes the synthetic reaction of indole. Subsequently, indole is converted to tryptophan by the action of TSB. The purpose of this study is to uncover the complete indican synthetic pathway and to provide insight into the switching mechanism from primary to secondary metabolism.

Here, we report on the cDNA cloning, expression, and characterization of TSA from *P. tinctorium*. Transcriptome analysis using mRNA from *P. tinctorium* leaf tissue resulted in a one-fragment sequence that has homology with sequences from other plant TSAs. Based on this sequence, the RACE method was used to get the complete length of the TSA cDNA. The obtained cDNA consisted of 1,469 bp encoding a polypeptide of 315 amino acids. The primary structure contained the consensus sequences of TSAs and the regions for interaction with beta subunits. *P. tinctorium* TSA, which we named as ptTSA1, showed high homology to some enzymes from plants; this was the case particularly with TSA from *Isatis tinctoria*, another indigo plant, which showed 95.7% homology to ptTSA1. To analyze the properties and functions of ptTSA1, the recombinant protein was expressed in *Escherichia coli*. In addition, the ptTSA1 cDNA was used to examine whether ptTSA1 could complement a TSA deletion in *E. coli*. ptTSA1 protein expression and mRNA levels in various tissues of *P. tinctorium* were examined by the Western blot analysis and semi-quantitative RT-PCR. These expression patterns were also compared with those of TSBs. Here, we will further discuss regarding the analysis of ptTSA1 and the interaction between TSA and TSB.

Footnotes

This abstract is from the Experimental Biology 2016 Meeting. There is no full text article associated with this abstract published in The FASEB Journal.