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Correlation of tyrosinase activity and betacyanin biosynthesis induced by dark in C_3 halophyte *Suaeda salsa* seedlings

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Abstract

The role of tyrosinase (EC 1.14.18.1 and EC 1.10.3.1) in the secondary metabolism of plants still remains unclear, and the involvement of both hydroxylation and oxidation activities of tyrosinase in betalain biosynthesis has been proposed, but has not yet been shown conclusively. Betalains are an important class of water-soluble pigments, which comprise the red–violet betacyanins and the yellow betaxanthins, and accumulate in most families of the Caryophyllales and some higher fungi. In this article, the role of tyrosinase in dark-induced betacyanin biosynthesis in *Suaeda salsa* was examined. Seeds of the halophyte *Suaeda salsa* were cultured in the dark for 3 days and betacyanin accumulation was induced significantly in cotyledons of *Suaeda salsa* seedlings. Accumulated betacyanin in cotyledons of the dark-grown seedlings decomposed with time in light. As the betacyanin content declined, the hydroxylation and oxidation activities of tyrosinase extracted from cotyledons of the dark-grown seedlings decreased with time in light. The apparent molecular mass of tyrosinase in cotyledons of *Suaeda salsa* seedlings was about 60 kDa estimated by SDS-PAGE, enzyme activity staining and Western blotting. Furthermore, the tyrosinase only synthesized in the cotyledons of dark-grown *Suaeda salsa* seedlings, and declined with time in light, which was paralleled by the decreases of tyrosinase activity and betacyanin content in light. These results suggest that the specific tyrosinase is positively correlated with betalain biosynthesis, and betacyanin biosynthesis is induced by dark via synthesis of tyrosinase in *Suaeda salsa*.

Keywords: Betacyanin; Dark; Light; *Suaeda salsa*; Tyrosinase; Western blotting

1. Introduction

Betalains are water-soluble nitrogen-containing pigments, which comprise the red–violet betacyanins and the yellow betaxanthins. Betalains accumulate in flowers, fruits and occasionally in vegetative tissues of plants in most families of the Caryophyllales and some higher fungi [1]. Interest in betalains has grown since their antiradical activity was characterized, and they are widely used as additives for food, drugs and cosmetic products because of their natural colorant properties and absence of toxicity [2–4]. In addition, betalains are important chemotaxonomical markers and have never been found jointly with anthocyanins in the same plant. Gain and loss of anthocyanins or betalains during plant evolution still remain a mystery [1,5].

The Chenopodiaceae *Suaeda salsa* is one of the most important halophytes in China and even grows in the intertidal zone of the Yellow River Delta [6]. As a halophyte, physiological and molecular responses of *Suaeda salsa* to salinity stress have been extensively studied in our laboratory [7–9]. However, nothing is known about the biosynthesis regulation of the red pigments in *Suaeda salsa*. Recently, our primary analysis showed that the red pigments in *Suaeda salsa* were not anthocyanins but betacyanins, and their contents were regulated by light (and/or dark). Dark was one of the most important environmental factors to induce betacyanin biosynthesis, while light promoted the degradation of betacyanins in *Suaeda salsa* [10–12].

The tyrosinase (or polyphenol oxidase) belongs to the group of type-3 copper proteins that not only exhibit monophenol monooxygenase activity (EC 1.14.18.1), but also exhibit *o*-diphenol oxidase activity (EC 1.10.3.1). The involvement of

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tyrosinase in the biosynthetic pathway of the betalains has been suggested [13–16], but has not yet been shown conclusively. The hydroxylation of tyrosine to DOPA (dihydroxyphenylalanine) and the further oxidation of DOPA, both catalyzed by tyrosinase, are considered as the first step in the proposed biogenesis pathway of betalains. Furthermore, DOPA undergoes an enzymatic extradiol cleavage leading via 4, 5-seco-DOPA to betalamic acid, which likewise reacts with cyclo-DOPA spontaneously to form the betanidin, or reacts with various amino acid and amine spontaneously to form the betaxanthin (Fig. 1) [17]. The involvement of DOPA dioxygenase in the DOPA extradiol ring-cleavage has been partially characterized from *Portulaca grandiflora* [18]. In recent work, the tyramine-betaxanthin (miraxanthin III) and dopamine-betaxanthin (miraxanthin V) have been reported as new natural substrates for tyrosinase in the betalain biosynthesis pathway [15,16].

To understand the detailed role of tyrosinase in betalain biosynthesis, studies on betalain biosynthesis regulated by different environmental factors are needed. In the present study, the C_3 halophyte *Suaeda salsa* was introduced as a model plant, in which betacyanin accumulated in dark and decomposed in light, to study the role of tyrosinase in betacyanin biosynthesis. We detected tyrosinase synthesized in cotyledons of *Suaeda salsa* seedlings formed in the dark, but degraded or deactivated in light, which was in agreement of the decreases of tyrosinase activity and betacyanin content in light. These results provide an evidence that the specific tyrosinase is positively correlated with betalain biosynthesis in *Suaeda salsa*.

2. Materials and methods

2.1. Plant materials and experimental conditions

Following the method of Wang and Liu [11], seeds of *Suaeda salsa* were collected from the Yellow River Delta of China. After being sterilized with 0.5% $HgCl_2$ for 10 min, seeds were washed and germinated in plastic plates filled with sand and watered with 1/2 MS solution containing 100 mmol L^{-1} NaCl. In the growth cabinet, seeds were divided into two groups, one was cultured in dark (24 h dark) and the other one was cultured in light (14 h light/10 h dark). Temperature was 28 °C, relative humidity was 60%, photon density was 200 μmol m^{-2} s^{-1}. Three days later, tyrosinase was extracted from cotyledons of the seedlings, and used for non-denaturing SDS-PAGE and tyrosinase activity staining.

Seedlings obtained from seeds cultured in the dark for 3 days as described above were transferred from dark to light (14 h light/10 h dark), the other culture conditions were the same as previously described. Betacyanin content, tyrosinase activity and Western blotting were determined at 1, 2, 3, 4, 5 day thereafter. Seedlings grown in continuous dark were used as the control. Each measurement was taken and performed in triplicate.

2.2. Isolation and analysis of betacyanins

Betacyanin of *Suaeda salsa* seedlings was extracted and measured as we previously described [19]. The absorbance at 538 nm of betacyanin solution was determined, and the

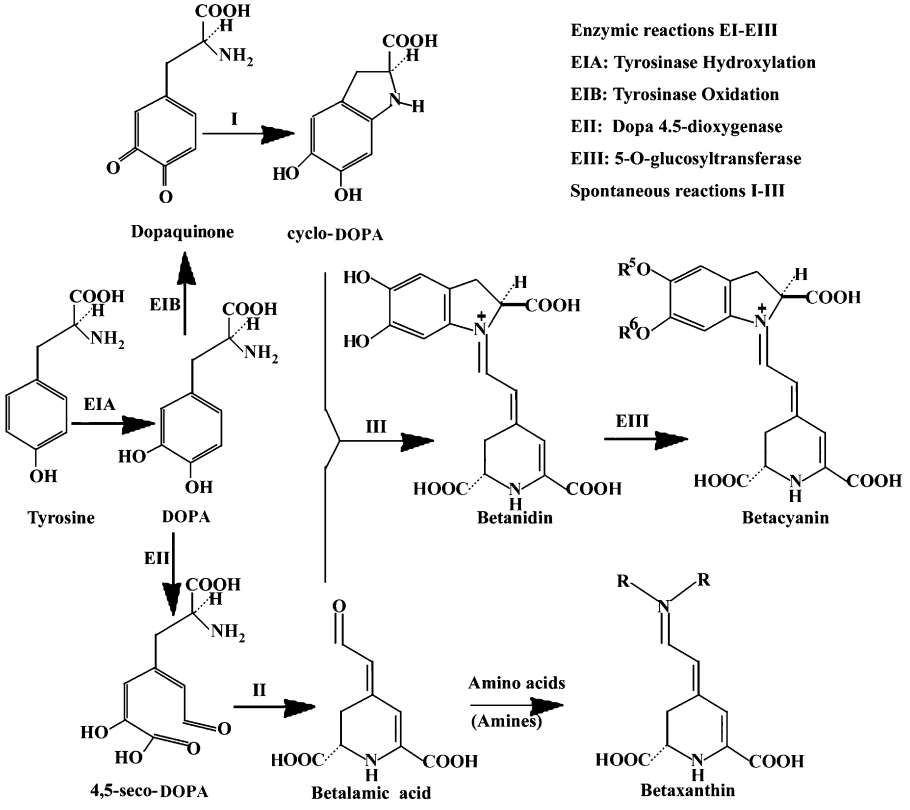

Fig. 1. Biosynthetic scheme of betacyanin and betaxanthin.