내염성 식물 칠면초 (*Suaeda japonica*)가 갖는 자색 색소의 특징

Characteristics of the Purple Pigment Compound in Halophytic Plant, *Suaeda japonica*

정 상 호
목원대학교

Chung sang-ho
Mokwon Univ.

요약

As one of the steps toward understanding how the plant is well adapted to strongly saline habitats, the purple pigment compound that is accumulated in Suaeda japonica was extracted and characterized. The extracted pigment compound exhibited typical characteristics of betacyanin that were represented by water solubility, pH- and temperature-dependent color changes, sensitivity to light, UV-Vis spectra, and gel electrophoretic migration pattern. LC-MS of the extracted pigment compound showed the presence of two major protonated molecular ions ($[M+H]^+$) at m/z 651.1 and m/z 827.1. According to the DPPH assay, it was found to have an antioxidant activity that is linearly increased in proportion to the reaction time for up to 30 min, and the activity was comparable to that of control BHA at 9.0 mg/ml. The cytotoxic activity against several tumor cell lines was also examined following the MTT assay. The significant growth inhibitory effect was observed on two tumor cell lines, SW-156 (human kidney carcinoma) and HEC-1B (human endometrial adenocarcinoma). Probably, the pigment compound may function as an osmolyte to uphold halotolerant physiological processes in saline environment.

Ⅰ. Introduction

Suaeda japonica belongs to the family Chenopodiaceae. As annual herbage it is a representative obligatory halophyte that can grow in high salt concentration. Study on species of Suaeda may give a new insight into a mechanism of the salt resistance as well as the adaptation to terrestrial habitat. Although some mechanisms such as the accumulation of salts in leaf vacuoles were already found, there has been yet no report on the importance of pigment compound that is associated with the salt resistance in Suaeda species. As one of the steps toward understanding how the plant is well adapted to strongly saline habitats, the purple pigment compound that is accumulated in leaves was extracted and examined its characteristics such as the water solubility, pH- and temperature- dependent color changes, sensitivity to light, UV-Vis spectra, gel electrophoretic migration pattern, LC-MS analysis, antioxidant activity, and cytotoxic activity against several tumor cell lines.

Ⅱ. Materials and Methods

The pigment compound was extracted in water as described essentially in Chung and Byun [1]. Electrophoresis of purple pigment compound was carried out in a citrate buffer (pH 3.0) on 3.0% agarose gel together with other already well known water-soluble plant pigments, anthocyanins and betacyanins, as standard references. LC-MS analysis of the extracted pigment compound was performed at the Korea Basic Science Institute (Daejeon, Korea). The free radical scavenging activity of the pigment compound was assayed using a stable DPPH radical, following standard method [2] [3]. The cytotoxicity and the resulting data analysis was performed essentially as described by Choi [4] following the MTT assay.

Ⅲ. Results

To identify what contributes to the purple color extraction, various solvents were attempted to extract from leaf tissues of both seawater- and freshwater-plants. The purple pigment was found to be best extracted in water. No purple pigment was virtually extracted from freshwater-plants.

To find the maximum wavelength absorbed by this purple pigment, the absorption spectrum was measured in both UV and visible light ranges (200 nm to 700 nm). The absorbance maxima occurred in the UV range at near 270 nm and 335 nm as well as in the visible

range at near 545 nm. However, the absorption maximum at 545 nm of purple pigment was disappeared under alkaline condition.

When the purple color of extracted pigment compound was exposed to daylight, it was gradually disappeared and almost completely lost after 14 days.

The variation of color and stability of the compound was also observed when pH or temperature was changed. The pigment color was purple in acidic conditions, but became yellowish in alkaline conditions. As the pH of acidic purple pigment solution was raised, the solution became colorless around pH 8.0. The pigment compound was stable at room temperature under the acidic pH but was unstable at 40℃ or above under the alkaline conditions.

When the standard anthocyanin pigment references (maple, blueberry, morning glory, balloon flower, and begonia) and the standard betacyanin pigment references (red beet, cockscomb, four o'clock, and pokeweed) were separated on an agarose gel, each pigment was migrated to opposite directions; the anthocyanins to cathode and the betacyanins to anode. The purple pigment of S. japonica was moved to anode along with the standard betacyanin references, indicating that this pigment belongs to betacyanin.

LC-MS analysis of the extracted pigment compound was performed. Two major peaks with protonated molecular ions ($[M+H]^+$) at m/z 651.1 and 827.1 were observed.

Antioxidant activity of the extracted pigment compound was determined by DPPH assay. The pigment compound was found to have an antioxidant activity that is linearly increased in proportion to the reaction time for up to 30 min, and the activity was comparable to that of control BHA at 9.0 mg/ml.

The cytotoxic activity against several tumor cell lines was also examined following the MTT assay. The significant growth inhibitory effect was observed on two tumor cell lines SW-156 (human kidney hypernephroma) and HEC-1B (human endometrial adenocarcinoma).

Ⅳ. Conclusion

The extracted purple pigment compound of S. japonica exhibited typical characteristics of betacyanin that were represented by water solubility, pH- and temperature-dependent color changes, sensitivity to light, UV-Vis spectra, and gel electrophoretic migration pattern. LC-MS of the extracted pigment compound showed the presence of two major protonated molecular ions ([M+H]+) at m/z 651.1 and m/z 827.1. Probably, the pigment compound may function as an osmolyte to uphold halotolerant physiological processes in saline environment. This study reveals the presence of the purple betacyanin compound that may enable this plant to tolerate saline environment without any significant harmful effects on plant growth and development and, moreover, shows that this pigment can function as antioxidant and anticancer agents.

▌Reference ▐

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