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Amounts and subcellular localization of stilbene synthase in response of grape berries to UV irradiation

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ABSTRACT

Stilbene synthase (STS) is a pivotal enzyme that catalyzes the biosynthesis of resveratrol and is known to be induced by ultraviolet (UV) irradiation. However, it remains at present unclear about the response of STS to UV radiation at protein level and at subcellular distribution. Here, changes of amounts and subcellular localization of STS in developing grape berries exposure to UV irradiation were investigated using a polyclonal antibody raised against grape berry STS. The analyses of Western blot revealed that the UV-induced increase in STS amount is developmental stage-dependent and time course-dependent, with response of STS being postponed concomitantly with the progressive development of berry. The immuno-localization via immunoglod electron microscopy showed that, on one hand, STS was mainly located on the cell wall, secondary cell wall and chloroplast of the skin tissues during berry development, with only a few particles representing STS present under natural conditions; and on the other hand, STS particles increased when exposure to UV, particularly on the cell wall, the secondary cell wall and chloroplast, while such increase was also developmental stage-dependent. It is thus suggested that the increased STS on the cell wall might be related to grape berry defense against UV irradiation.

1. Introduction

Stilbene synthases (STSs, EC 2.3.1.95) catalyze a condensation of 4-coumaroyl CoA with three C_2 units from malonyl CoA to produce stilbenes. Stilbenes and their derivatives are regarded as phytoalexins that contribute to the defense against predators and pathogens [1]. In addition, these compounds are also shown to accumulate in the epidermal tissues of plant organs where exposure to UV radiation is highest, thereby serving to protect underlying tissues from radiation damage [2]. On the other hand, resveratrol (trans-3,5,4′-trihydroxystilbene), one of the major stilbenes, is known to have advantageous effect for diabetes, constipation, allergies and headaches. It can trigger apoptosis, reduce coronary heart disease mortality and arteriosclerosis, and inhibit low density lipoprotein oxidation and eicosanoid synthesis [3–6]. This compound has been found in at least 72 plant species distributed among 31 genera and 12 families, and a number of these are components of the human diet, for example, grapes, wine, cranberries peanuts, chocolate and cocoa [7]. Grapes and grape products are considered to be the most important human dietary sources of resveratrol. As stilbenes possess dual significances in both plant protection and human health, extensive attention, in recent years, has been attracted to understanding the pharmacological activities, the biological attributes for plant protection and biosynthetic regulation of stilbenes [3,8–10,37].

STSs are often classified into two categories according to the substrate specificity, one is pinosylvin synthase (PSS) in pines (*Pinus sylvestris*, *Pinus strobes*, Pinaceae) and the other is resveratrol synthases (RS) mainly in grapevine (*Vitis vinifera*, Vitaceae) and groundnut (*Arachis hypogaea*, Fabaceae) [11,12]. In grapevine, the level of resveratrol in plant tissues is found to be low under natural growing conditions, but the compound rapidly accumulate to high concentrations when plants are subjected to fungal infection [13], UV light irradiation [14,15], aluminum [16] and ozone exposure [17]. As a pivotal enzyme of the biosynthesis of resveratrol, STS is also found to be activated by various stresses and the inducible stilbene accumulation in developing grape berries is highly regulated at the level of STS gene transcription. Furthermore, the resveratrol biosynthesis induced by UV irradiation in grape berries is closely related to the developmental stage of plant, and only in unripen berries can UV irradiation greatly simulate STS transcript [9,15,18]. Since considersible evidence has supported resveratrol accumulated as a response to UV-C irradiation, it is possible to increase the level of resveratrol through exposure grape berries to UV and consequently to obtain grape products with high level of resveratrol.

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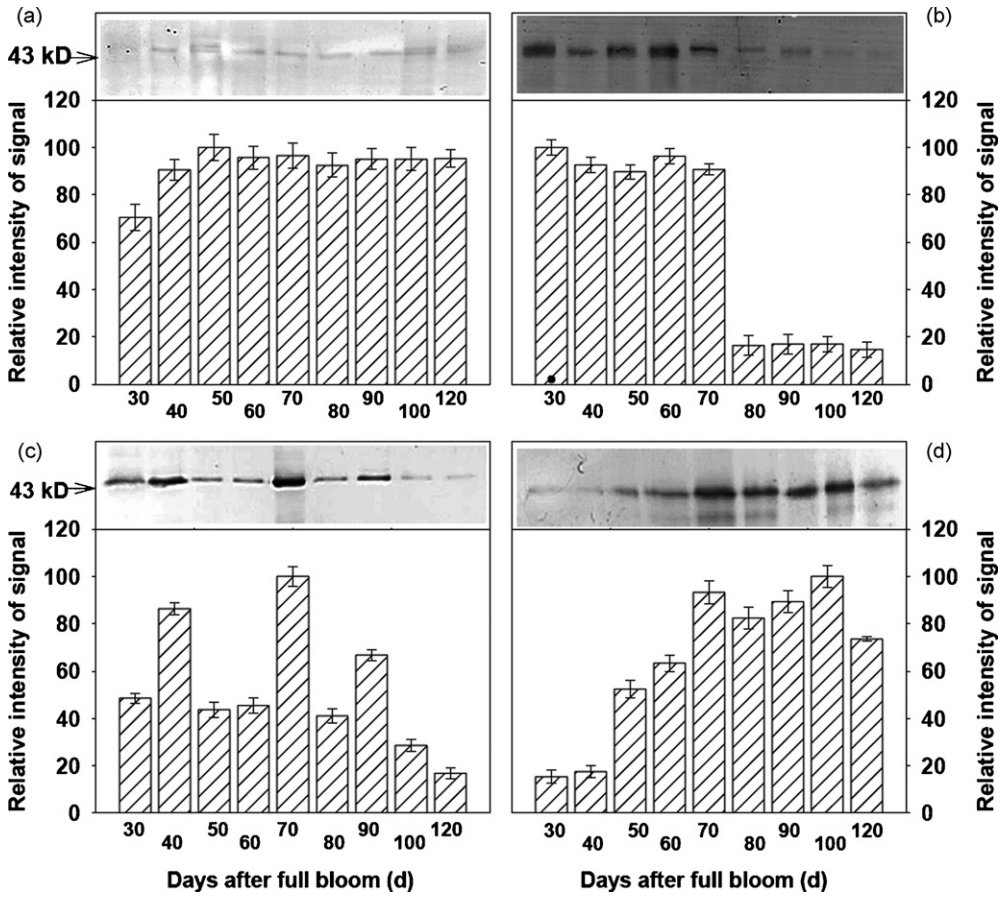

Fig. 1. Comparison of the amounts of stilbene synthease protein among in the skin tissues of grape berries of various developmental stages (from 30 d to 120 d after full bloom), at a given time after UV irradiation. (a) 0 h after UV irradiation, (b) 6 h after UV irradiation, (c) 18 h after UV irradiation and (d) 72 h after UV irradiation. (a–d) The upper shows the result from Western blotting of stilbene synthase and the bottom shows quantitative analyses of each band in the upper panel, which is preformed by Sigmascan software. The relative intensity of the band with the greatest immunosignal in a sheet of membrane of Western blotting is defined as 100. The data in the bottom panel are means ± standard error of three repetitions and these repetitions are from different protein extracts.

The previous studies on the STS almost focused on the cloning of gene and/or cDNAs and the regulation at the transcriptional level. Up to date, STS regulation at translational level has not yet been determined. More recently, the localization of STS in *V. vinifera* L. during grape berry development was addressed, with an antibody specifically developed against grape STS1 enzyme and was shown mainly distributing on the cell wall within exocarp tissues [2,9,18,19]. However, it remains unclear concerning UV irradiation effects on STS at protein and cellular levels. We have previously described the full-length gene encoding STS has been cloned from wine grape berries (Cabernet Sauvigon) and a polyclonal antibody against grape STS has been prepared [20]. Here, the amounts of STS protein were investigated using Western blot and the subcellular localization was detected through immunogold electron microscopy technique in developing grape berries exposed to UV irradiation. The objective of this study is to increase our understanding of how vines respond to UV radiation, and add new insight into plant secondary metabolite biochemistry.

2. Materials and methods

2.1. Plant material

Grape berries (*Vitis vnifera* L. cv. Cabernet SaUVignon) were harvested from 10-year-old trees growing in a commercial vineyard in the Huailai district of Hebei province. Samples were collected every 10 d from 30 d to 120 d after full bloom (DAF). Veraison (about 70 DAF) indicates the onset of berry ripening, at which berry begins to turn color and soften (Fig. 1).

2.2. Irradiation the intact grape berries by UV

Grape samples were directly irradiated for 10 min using a UV-C light (254 nm, Spectroline, Model ZQJ-254, output 300 $\mu W/cm^2$, 10 cm distance from the samples). The berries irradiated for the same time with white light of same irradiation dosage were taken as the control. Following irradiation, both the UV-treated berries and the control samples were transferred into a Robert manual incubation (Model PRX-350D) and then incubated in the dark at 25 °C with relative humidity 80% for 96 h. At each incubation period of 0, 6, 12, 18, 24, 48, 72, 96 h, the skin tissues were collected, peeled, frozen in liquid nitrogen and stored at −40 °C. These skin samples were used for analyses of Western blot. The parts of berry samples at 30, 70, 100 d after full bloom were irradiated for 10 min with UV-C light or white light, and then kept in the dark at 25 °C with relative humidity of 80% for 12 h. The skins of these samples were peeled and immediately used for the preparation of immunogold electron microscopy analysis.

2.3. Protein extraction and Western blot

The proteins were extracted from the berry skins according to the method of Pan et al. [21] with minor modification. The berry