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Biointeraction between precarthamin and cell components in florets of *Carthamus tinctorius* L.

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Abstract

The relative rate of precarthamin extractability and floret protein inactivation by organic solvents were compared. Upon trituration of the floral tissues of *Carthamus tinctorius* L., usually less extractable precarthamin is released at a markedly high level, while the releasability rate is changed conspicuously by macerating the florets in the test solvents of increased concentrations; the amount of releasable precarthamin also varies according to the solvents used. 30 % (v/v) acetone promotes the pigment solubility about twice, whereas, its capacity decreases abruptly in reverse proportion to the increment of acetone content. Methanol accelerates the precarthamin release, but the rate is very low. Ethanol acts in far lesser extent. The data indicate that these varied aspects are more or less attributed to the inactivation of possible factors dissociating precarthamin from cellular components in freezed safflower florets.

Introduction

The floral tissues in most angiosperms produce flavonoids with various structures, which reflect directly or indirectly in the species-specific flower colours. Physiological function and cellular location of these compounds bring about certain appreciably interesting problems, which have been now targeted by many research workers.

It has already been documented that flavonoids in plant tissues act as visible colour, light screen, antioxidants, enzyme regulators, energy transferring compounds, regulators of plant growth and development, *etc.* (McClure 1975). Localization of flavonoids also concerns special interest. Recent investigations suggest the restriction of the pigments to certain cells within tissues (McClure 1975). Several experiments with highly purified and functional organelles indicate that the chloroplasts and plastids may be involved in flavonoid synthesis and accumulation (Saito 1974).

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Saunders *et al.* (1993) obtained similar results with plastids from tomato, carrot and mung bean, which accumulate a radio-labelled flavone. Weissenböck (1973) reported that quantitative and qualitative differences are seen in etioplasts from *Avena sativa* cultivars: different patterns of flavonoids are clearly characterized between plastids and cytoplasmic preparations. More recently, it has been evidenced that certain flavone glycosides are concentrated preferentially in the vacuoles of floral tissues and some others are located in epidermal and/or mesophyll cells of the vegetative parts (Sasse *et al.* 1979, Schulz and Weissenböck 1986). These all are suggestive of the compartmentation of specific flavonoids within plant cells.

In spite of the efforts of many workers, the chemical association of flavonoids with various cell compartments are still obscure. Several authors have declared that hydrogen bonding and/or covalent association may be involved in the state of the 2-phenylchromone compounds within cells (Kreuzaler and Hahlbrock 1973), but their information is rather all speculative.

Previously, we have shown reliable data about the chemical interaction of precarthamin, a chalcoquinoid pigment of safflower (*Carthamus tinctorius* L., Compositae), indicating that it is released markedly by the trituration of the florets through mechanical treatments (Saito and Katsukura 1992), although the pigment is usually less extractable from the intact tissues with organic solvents.

The work presented here is a part of our studies on the chemical association of *Carthamus* pigments, through which additional facts would be revealed, supporting our previous assumption, that precarthamin is linked up with cell components in floral tissues of the flowering plant.

Materials and methods

Plant material: Orange-yellow florets (263.6 g) were harvested from the freshly opened flowering heads of safflower (*Carthamus tinctorius* L.) grown on our experimental field on 27 August 1992, immediately packed in a vinyl bag and kept in a cool container till the experimental use.

Treatment of florets: Cooled florets (0.5 g each) were triturated in 5 ml test solvents with a porcelain pestle and mortar for 5 min in the air at room temperature (23 ± 2 °C). The resulting pastes were filtered on a Büchner funnel by suction. The residues were washed with a 390 ml g^{-1}(f.m.) of the test solvent.

Determination of released precarthamin content: The pooled filtrates were condensed with a rotary evaporator at less than 35 °C. To the resulting solvent free condensate, 0.5 g *Avicel* cellulose was suspended and then 2.5 cm^3 of 1 mM $KMnO_4$ solution mixed dropwise by stirring gently. On standing 5 min, the mixtures were passed through a filter paper on a Büchner funnel and residual *Avicel* washed exhaustively with sufficient amounts of distilled water (approx. 200 cm^3 in total). The reddish *Avicel* cellulose and the filter paper were rinsed in 60 % (v/v) acetone in a beaker for 3 - 5 min and carthamin freed paper was picked out. Then the *Avicel* cellulose was transferred to glass tubes and centrifuged at 4000 *g* for 5 min. At the end of the

centrifugation, the supernatant was pipeted out, new 60 % acetone added, stirred with a glass bar and centrifuged again (4000 *g*, 5 min). The extraction process was repeated further 2 times or more, at each time the old acetone solution was replaced by new one. The pooled acetone layers were messed up to be a net 100 cm^3 and used for the spectrophotometric measurement by a *Hitachi, model U-1100.* The absorbance at 521 nm was monitored and the resulting data from the spectrophotometric reading were consulted with a calibration curve to quantify precarthamin content, which was expressed in terms of carthamin, M_r = 910 (Saito and Yamamoto 1994).

Results and discussion

In one of our previous works (Saito and Katsukura 1992), we reported that precarthamin may be linked up chemically with cellular components. This suggestion was based on the observation that the quinoidchalcone glycoside is released markedly by the trituration of the fresh safflower flowers, while it is usually less solubilized from the intact florets with organic solvents.

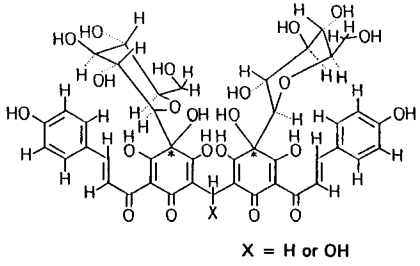

Fig. 1. A postulated structure of precarthamin.

To clarify more details of the chemical association between precarthamin and cellular components within the floral tissues, our previous technique was applied once again in this study after slight modification mainly in the solvent systems and their compositions. In agreement with our recent findings (Saito and Katsukura 1992), the trituration of the fresh materials was obviously stimulative to induce precarthamin liberation (Fig. 1). The freshness of the floral tissues affects directly the rate of precarthamin recovery (Figs. 1 and 2). Acetone is an effective dissociator of precarthamin: its dissociation capacity rises with concentration off to 30 % (v/v) and then decreases. Methanol is less effective: and ethanol inhibited percarthamine liberation. In general, the test solvents tend to reduce the solubility rate of precarthamin at their higher concentrations (Table 1). These indicate clearly that precarthamin release is suppressed through inactivation of certain bio-factor(s) accelerating the pigment liberation.

Presumably, precarthamin is bound tightly in cell compartments of the intact floral tissues under biological control. In this control different mechanisms may operate:

one is a weak association (hydrogen binding) and another is a strong interaction (covalent binding). Upon once tissues being crushed into small fragments by the

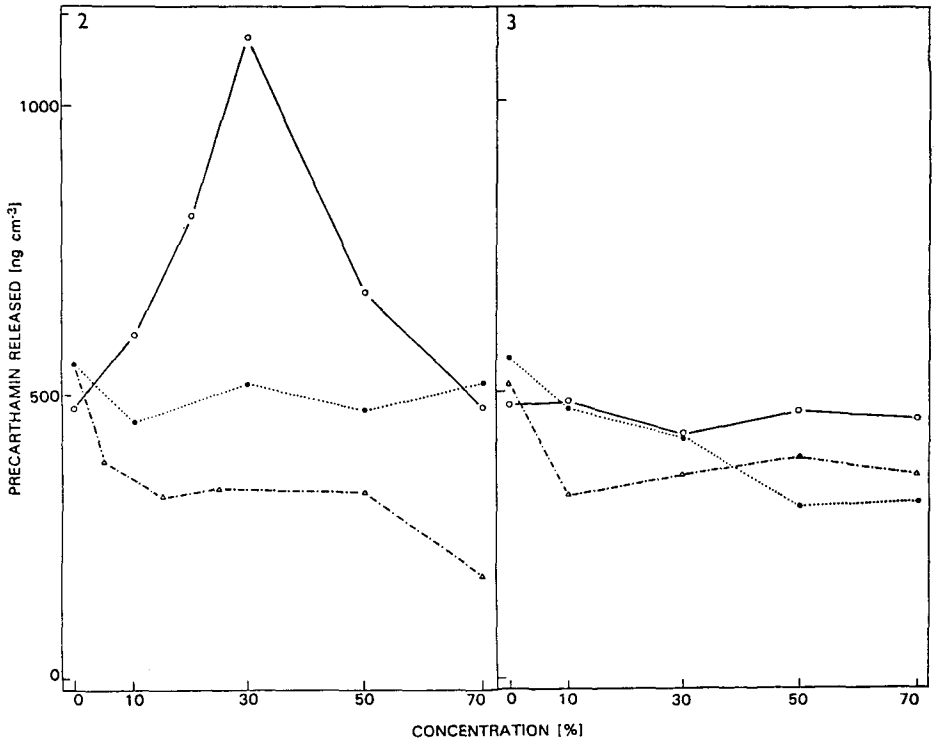

Fig. 2. Effect of organic solvents (acetone - *open circles*, methanol - *closed circles*, ethanol - *open triangles*) on the release of precarthamin from the fresh florets of safflower. Florets (0.5 g each) were used as starting materials. For details of extraction, partial purification and quantitative estimation of precarthamin, see text.

Fig. 3. Effect of organic solvents (acetone - *open circles*, methanol - *closed circles*, ethanol - *open triangles*) on the release of precarthamin from the dried florets of safflower. Dried florets (0.25 g each) were used as starting materials. For details of extraction, partial purification and quantitative estimation of precarthamin, see text. The dried florets were prepared as follows: fresh florets were dipped in glass distilled methanol for 10 d at 4 - 5 °C in the dark. After the solvent was removed, the denatured florets were dried in an air-circulation oven for 24 h at 60 °C and stocked in a freezer at -20 °C just before use.

mechanical treatment, the drastic operation could destroy the normal control mechanism and triggers abrupt physiological changes, by which equilibrated regular metabolic flow could be shifted compulsorily towards abnormal and rapid turnover sites of the quinoidchalcone glycoside. Prior to the initiation of the catabolic process, bound precarthamin is perhaps liberated from the arrested positions within the cell

compartments. At this occasion, hydrolytic enzymes including glucosidases, proteinases, phosphatases, or others must bring their own abilities into full play all at once, through the process of which the feed product can be liberated into cell sap. Some chalcones are known to be accumulating in vacuoles in free state. Of course, they may readily be introduced to the process of the metabolic dissimilation under the drastic treatment.

Table 1. Comparison of precarthamin releasability from the fresh florets of *Carthamus tinctorius* L. after being treated by different solvent systems.

Concentration [%]	Precarthamin released [ng cm^{-3}]		
	Acetone	Methanol	Ethanol
0	2.3	2.3	46.7
10	127.9	17.0	45.6
30	701.6	223.8	64.0
50	226.7	156.7	-51.5*
70	27.2	218.3	-168.2*

*lower than blank test

Apart from precarthamin and its oxidation product, carthamin, other synonymous chalcoquinoid glycoside, safflor yellow A and B (Takahashi *et al.* 1982, 1984) are produced in safflower florets at relatively high levels. They are soluble easily in water or in aqueous organic solvents. At present, it is not clear whether or not these two pigments exist in bound form.

The current works provide us with additional evidence that safflower florets produce two types of chalcoquinoids, the one is bound type (precarthamin) and the other free form (safflor yellow A and B). It is interesting to note that chalcoquinoids composed of similar chemical structures are co-existent in a plant tissues at different states. Studies of the binding mechanism of precarthamin within safflower tissues are under way in our laboratory.

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