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INVERTASE AND SUCROSE SYNTHASE IN FLOWERS

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Abstract—Sucrose and reducing sugar concentrations in petals of cut carnation flowers, whose life was prolonged up to 7 days by bathing stalks in sucrose solutions, were respectively 3-fold and 2-fold higher than those bathed in water. Reducing sugar concentrations were about 7-fold higher than sucrose concentrations. A study of invertase and sucrose synthase activities in flower petals of carnation and four other species of flowers revealed that both enzymes may be involved in hydrolysis of translocated sucrose. Invertase activity, while being up to 20-fold higher than sucrose synthase activity in some species was approximately comparable in others. More detailed studies on invertase from petals of 3 flower species demonstrated the presence of only the acid form of the enzyme with a K_m value for sucrose of about 2.5 mM.

INTRODUCTION

Addition of sucrose to solutions bathing the stalks of cut flowers is known to prolong flower life [1,2]. It is thought that the sucrose is moved up the flower stalks via either the phloem or xylem, or both, to the flowers where it is metabolised causing increased growth rates, increased respiration rates, and inhibition of ethylene production [3,4]. The reduced rate of ethylene production may be the regulator of flower life since it has been shown that exogenously supplied ethylene causes flowers to wilt [4].

In higher plants, sucrose is hydrolysed by two types of enzymes to provide monosaccharides or sugar nucleotides which are further metabolised [5]. β-Fructofuranosidase (EC 3.2.1.26, commonly called invertase) and sucrose synthase (EC 2.4.1.13) are the two types of enzyme involved and they may occur together or independently according to the tissue [5–8]. Often tissues contain two invertases with pH optima near 5 (acid invertase) and near 7 (alkaline invertase) [9,10]. A brief report of acid invertase and an invertase inhibitor in petals of *Ipomoea purpurea* has been made [11]. The present paper describes activities and some properties of invertases and sucrose synthases from a number of flowers.

RESULTS

Addition of sucrose to solutions containing β-hydroxyquinoline sulphate (a bactericide) resulted in a 6 or 7 day longer life (up to 18 days) of cut carnations under our conditions. Sucrose and reducing sugar concentrations increased 2- to 3-fold in the petals supplied with sucrose, the values for reducing sugar (5 g/100 g fr. wt) being about 7-fold higher than those for sucrose in the later days. These results are in agreement with those of Nicholls and Ho [4] who showed that sucrose moves up flower stalks and is hydrolysed in the petals.

Invertase and sucrose synthase activities and protein content of petals and flower stalks of some commercial species and some Australian native species are shown in Table 1. In petals, invertase activities were higher than sucrose synthase activities ranging from 20-fold in Sturt Pea petals to 1.5-fold in carnation petals. In the 3 varieties of stalks studied, sucrose synthase activity was higher in only one, carnation. In carnation petal extracts the rate of formation of UDPglucose from UDP and sucrose at pH 7 was 2 nkat/g fr. wt. Since it was easier to control interfering reactions in the crude enzyme extracts in the synthesis direction rather than the hydrolysis direction of sucrose synthase, other extracts were assayed only in the synthesis direction. Invertase activity in the unwashed precipitates after centrifugation of homogenates of petals or stalks was less than 10% of the activity in the supernatants except for almond petals. Here the value for the insoluble invertase was 9.7 nkat/g fr. wt compared with 8.5 for the soluble enzyme.

The activity of invertase was greatest at pH values below 5.5 in the 3 petal extracts studied (Table 2). Carnation petal invertase showed most activity at about pH 5

Table 1. Invertase activity, sucrose synthase activity and protein content of flower petals and flower stalks

Plant & plant part	Enzyme activity (nkat/g fr. wt)* Invertase†	Sucrose synthase	Protein* (mg/g)
Carnation petals	9.3	6.1	9.9
Carnation flower stalk	0.2	1.9	6.5
Gladiolus petals	10.5	2.5	2.6
Gladiolus flower stalk	8.4	2.9	2.1
Kangaroo Paw petals	26.9	1.2	6.1
Kangaroo Paw stalk	9.1	0.5	2.8
Sturt Pea petals	10.0	0.5	7.4
Almond petals	8.5	0.5	5.2

* Values are means of determinations on at least 2 samples of each tissue. † Measured at pH 5

Table 2. Invertase activity as a function of pH in desalted petal supernatants

Plant	Invertase activity (relative units), at pH 3.4	3.95	4.45	5.2	5.65	6.5	7.2	7.5	7.95
Carnation petal	48	65	72	100	79	63	38	34	19
Kangaroo Paw petal	100	98	100	86	77	42	17	12	8
Sturt Pea petal	100	85	85	68	43	21	21	14	7

Table 3. K_m values and sugar specificity of invertases* from flower petals

Plant	K_m for sucrose (mM)	Activity (relative units) Sucrose	Raffinose	Melezitose	Maltose
Carnation	2.7	100	23	10	25
Kangaroo Paw	3.3	100	34	9	7
Sturt Pea	2.7	100	38	8	0

* Measured at pH 5.

whereas Kangaroo Paw petals and Sturt Pea petal invertases had broad peaks with activity still at maximum values at pH 3.4. No evidence was obtained for the presence of neutral or alkaline invertases.

Other properties of the invertases were examined for comparison with previously studied enzymes [9]. Both raffinose and melezitose were hydrolysed by the 3 petal extracts, raffinose showing higher rates of hydrolysis than melezitose (Table 3). Carnation petal extracts contained appreciable α-glycosidase activity as evidenced by the hydrolysis of maltose. Kangaroo Paw petal extracts showed much lower activity towards maltose while Sturt Pea extracts did not hydrolyse maltose. The K_m values for sucrose of the invertase extracts from the 3 flower petals were about 2–3 mM (Table 3).

DISCUSSION

The evidence presented shows that at least 3 varieties of flower petals and probably other flower petals and stalks contain high activities of acid invertase. The activities are roughly comparable with those of radish roots, higher than those of carrot roots but lower than those of grape berries [10, 12]. The pH activity curves and rates of hydrolysis of raffinose and melezitose are characteristic of acid invertase and are not the properties of neutral or alkaline invertase [9]. K_m values for sucrose (2–3 mM) for the flower invertase compare with 4 mM for grape invertase [13], 5 mM for radish and pea [10] and 28 mM for carrot root [10]. In addition to acid invertase some of the tissues (e.g. carnation petals) contain high activities of sucrose synthase, another enzyme involved in the breakdown of sucrose *in vivo* [5]. The solubility of plant invertases in relation to their cellular location has been discussed [14], and in this context the ready solubility of flower-petal invertase indicates that its location is likely to be intracellular. Thus the sucrose reaching flower petals via phloem or xylem [3, 4] could be hydrolysed by either acid invertase or sucrose synthase in tissues such as carnation petals, but in Sturt Pea where sucrose synthase activities are relatively low it seems more likely that acid invertase would be the enzyme involved. The current work shows that high acid invertase activities are present in a range of flower petals but that sucrose synthase activities are much more variable between species. More work is required to determine whether the occurrence of acid invertase in flower petals is a general phenomenon and whether it reflects a general mechanism of sucrose metabolism by flower petals.

EXPERIMENTAL

Plant material. Cut flowers of *Dianthus caryophyllus* (carnation) and *Gladiolus* sp. (gladiolus) were obtained from the local florist within 24 hr of picking. Flowers of *Anigozanthos manglesii* (Kangaroo Paw), *Clianthus formosus* (Sturt Pea) and *Prunus dulcis* (almond) were grown in a glasshouse. Three sets of 3 carnation flowers were placed in 200 ml of H_2O, 8-hydroxyquinoline sulphate (a bactericide) (200 mg/l., 8-HQS), or 8-HQS (200 mg/l) plus sucrose (2 g/l.) and kept at 24° in the laboratory.

Enzymes were extracted at 0–4° by grinding 3 g of tissue with 8 ml of 0.25 M Tris–HCl buffer, pH 8.5 containing 20 mM EDTA, 11 mM Na diethyldithiocarbamate and 15 mM cysteine–HCl. The brei was centrifuged at 20000 *g* for 15 min and 3 ml of supernatant was desalted on a 15 ml column of Sephadex G-25 which had been washed with 5 mM Tris–HCl buffer, pH 7. Invertase activity was determined by measuring, at 30°, the appearance of reducing sugar [15] in 0.1 M citrate –Pi or 0.1 M Pi buffers containing 0.1 M sucrose and enzyme. Sucrose synthase activity was measured at 30° by following the incorporation of fructose-{U-^{14}C} into sucrose [16]. The reverse reaction of sucrose synthase of carnation petals was measured by incubating 25 μmol sucrose, 2 μmol UDP, 4 μmol KF and 20 μmol Tris–HCl buffer, pH 7, and enzyme in a final vol of 0.24 ml at 30°. The UDPglucose formed was measured by observing the increase in A at 340 nm of the boiled reaction mixture in the presence of NAD and UDPglucose dehydrogenase at pH 8.5.

Sugar determinations. Reducing sugar [15] and sucrose measurements were made on 20 ml extracts prepared by grinding 0.5 g of petal tissue with boiling 70% EtOH in a glass piston homogeniser on 4 separate samples of petals per treatment. Sucrose was determined by measuring keto-sugars (including sucrose) [18] in extracts which had been treated for 10 min at 100° with 0.2 N NaOH both with and without prior invertase treatment.

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