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OCCURRENCE OF SUCROSE PHOSPHATASE IN VASCULAR AND NON-VASCULAR PLANTS

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Abstract—Green plants including representatives of angiosperms, gymnosperms, ferns, mosses, liverworts and green algae were shown to contain a specific sucrose phosphatase, the last enzyme in the pathway of sucrose synthesis. The enzyme from all species required Mg^{2+} for activity and it was partially inhibited by sucrose. It was not detected in a red alga, brown algae, or mushroom species which contain little or no sucrose.

INTRODUCTION

Sucrose synthesis in plants proceeds by a pathway involving sucrose phosphate [1], the synthesis of which is catalysed by sucrose phosphate synthase (EC 2.4.1.14). A specific enzyme, sucrose phosphatase (EC 3.1.3.24) has been found in several species of higher plants [2]. It has been partially purified from carrot roots and immature stem tissue of sugar cane. The enzyme requires Mg^{2+} and is inactive in the presence of EDTA. Sucrose, maltose, turanose and melezitose inhibit the enzyme, whereas glucose and fructose do not. Crude enzyme extracts can be assayed for sucrose phosphatase in a medium designed to maximize activity and minimize the effects of tannins, phenols and low pH [2, 3]. With the low concentrations of plant extract and sucrose phosphate used, and at pH 6.7, there is little interference by other phosphatases. Specificity is further checked by the addition of EDTA, sucrose and maltose [2].

Although sucrose phosphatase activity has been demonstrated in several species of higher plants, its presence in other vascular plants and in non-vascular plants has not been shown. The present paper describes the presence of the enzyme in a wider range of angiosperms and in lower plants which synthesize sucrose. Some lower plants which do not contain sucrose lacked sucrose phosphatase.

RESULTS

Phosphatase activities in crude spinach leaf extracts are shown in Table 1. The hydrolysis of 60 μM sucrose phosphate is compared with the hydrolysis of 2.5 mM *p*-nitrophenyl phosphate. The relatively different rates of activity at pH 6.0 and 6.7 with the two substrates, the lack of inhibition by EDTA and the absence of an effect of sucrose with *p*-nitrophenyl phosphate as substrate, all point to the presence of sucrose phosphatase in the extract. Furthermore, at pH 6.7 and with the small concentration of sucrose phosphate used, little interference would have been caused by the non-specific phosphatase (see also [2, 3]).

Table 2 shows the activities of sucrose phosphatase previously reported in angiosperms with the effects of EDTA, sucrose and maltose. In Table 3 lower plants including a green alga, a liverwort, mosses, a fern, gymnosperms and an extended range of angiosperms are now listed, with the activity of sucrose phosphatase and its inhibition by EDTA, sucrose and maltose reported. In all except three species, EDTA caused 99 or 100% inhibition and considerable inhibition was observed with either sucrose or maltose or both, indicating that the activity was not due to non-specific phosphatase. The relative magnitudes of the inhibition by sucrose and maltose varied between species as has been observed previously in other species (Table 2).

Extracts of three brown algae, a red alga, mushroom, yeast and sheep rumen fluid (containing a mixture of previously dried hay, living bacteria and living protozoa), hydrolysed *p*-nitrophenyl phosphate, but did not hydrolyse sucrose phosphate under the conditions used (Table 4). Mixtures of maize leaf tissue and either mushroom cap, mushroom stalk or yeast were extracted together. The hydrolysis of sucrose phosphate by these extracts was

Table 1. Comparison of hydrolysis of sucrose phosphate and *p*-nitrophenyl phosphate by spinach leaf extract

Substrate	pH	Activity (μmol/min g fr. wt)	Compound: EDTA (30 mM) % Inhibition	Compound: Sucrose (100 mM) % Inhibition
Sucrose phosphate	6.0	0.29	99	49
(60 μM)*	6.7	0.25	99	49
p-Nitrophenyl phosphate	6.0	1.37	24	0
(2.5 mM)	6.7	0.16	21	0

*Assuming a K_m for sucrose phosphate of 0.1 mM, approximate maximum velocities can be calculated by multiplying the values by 2.7.

Table 2. Occurrence and properties of sucrose phosphatase in plants—previous studies (1966–76)

Enzyme source	Activity* (μmol/min. g fr. wt) pH 6.7	Compound			Reference
		EDTA (30 mM)	Sucrose (100 mM)	Maltose (100 mM)	
			% Inhibition		
Angiosperms					
Broad bean seed					
Cotyledon, young	1.1				[4]
Cotyledon, mature	14.1	100	82	89	[4]
Castor bean					
Endosperm	1.35				[4]
Cotyledon	3.25	100	74	90	[4]
Whole young seed	0.52				[4]
Maize seeds					
Scutellum	6.1	100	73	85	[4]
Endosperm	0.7				[4]
Maize leaves					
Mesophyll cells	2.16				[6]
Bundle sheath cells	1.33				[6]
Rice, dry seeds	0.03				[4]
Sugar cane, leaf laminae	0.94	100	74	45	[3, 5]
Tobacco leaf			33	72	[5]
Carrot root	0.10	100	90	87	[3, 5]
Rye leaf	3.35				[7]

*Assuming a K_m for sucrose phosphate of 0.1 mM, approximate maximum velocities can be calculated by multiplying the values by 2.7.

equivalent to the hydrolysis of sucrose phosphate by extracts of maize leaf alone.

DISCUSSION

Sucrose phosphate is likely to be an intermediate in sucrose synthesis in most, if not all, tissues of plants [1, 8, 9]. It has also been suggested as an intermediate in sucrose accumulation in sugar cane stem tissue [10] and in sugar movement into grape berry vacuoles [11, 12]. Sucrose phosphatase might act as a sucrosyl transferase in this process [13].

The presence in plants of a specific sucrose phosphatase with greatest activity between pH 6.5 and pH 7.5, allows hydrolysis of sucrose phosphate in the cytoplasm without the concurrent hydrolysis of other phosphorylated compounds as would occur with a non-specific phosphatase. An alternative pathway of sucrose synthesis involving the hydrolysis of fructose-6-phosphate by a specific enzyme followed by the action of sucrose synthase (EC 2.4.2.13) is most unlikely because a specific fructose-6-phosphatase has not been found. Even if one did occur it could result in excess conversion of glucose-1-phosphate, glucose-6-phosphate and fructose-6-phosphate to fructose. Also the available evidence suggests that sucrose synthase is involved in sucrose hydrolysis rather than synthesis [1, 14].

The current work shows that sucrose phosphatase activity occurs in a wide range of both vascular and non-vascular plants and the effects of EDTA, sucrose and maltose, by comparison with partially purified specific sucrose phosphatase from other species, suggests that the activity in all cases is due to a specific enzyme. The enzyme is found in roots, stems, leaves, fruits and seeds. It usually accompanies sucrose phosphate synthase at a level 10-foid in excess of that enzyme and both sucrose phosphate synthase and sucrose phosphatase activities are low in plant tissues not rapidly synthesizing sucrose [1, 4]. Amongst the angiosperms, the enzyme occurs in mono- or dicotyledonous plants, C-3 plants, both mesophyll and bundle sheath cells of C-4 plants, herbaceous annual, and woody perennial plants, latex-containing plants and sorbitol-translocating plants (Tables 2 and 3). It is not surprising that apple and pear leaves contain sucrose phosphatase because evidence from work with apples suggests that sorbitol supplements sucrose but does not replace it as the translocated form of carbon [15].

Brown algae, red algae and mushrooms do not contain sucrose [1, 16] and presumably do not synthesize sucrose. Although extracts of these tissues contained appreciable phosphatase activity as detected with *p*-nitrophenyl phosphate as substrate, no sucrose phosphate was hydrolysed using the normal assay conditions for sucrose phosphatase (Table 4). By comparison, extracts of *Ulva australis* (a green alga) hydrolysed 0.16 μmol of *p*-nitrophenyl phosphate/min.g fr.wt and also contained easily measurable activities of sucrose phosphatase (Table 3). Brown algae and mushrooms contain tannins which could inactivate enzymes, but extracts of these tissues did not affect maize sucrose phosphatase activity. Also many of the plants listed in Table 3, e.g. apple [15] and grape [11, 17], in which sucrose phosphatase was detected, contain tannins. Not only does Table 4 show that some species of lower plants do not contain significant amounts of sucrose phosphatase, but it is also further proof that activity towards sucrose phosphate in other species (Table 3) is not due to the presence of non-specific phosphatases.

Table 3. Occurrence and properties of sucrose phosphatase in plants—present work

Enzyme source	Activity* (μ mol/min g fr. wt) pH 6.7	Compound		
		EDTA (30 mM)	Sucrose (100 mM)	Maltose (100 mM)
		%Inhibition		
Green alga				
Ulva australis Aresch.	0.07	100	51	6
Liverwort				
Lunularia truciata (L.) Dun. (Gametophyte)	0.11	99	41	46
Mosses				
Funaria hygrometrica Hedw. (Gametophyte)	0.19	99	29	12
Bryum sp. Hedw. (Gametophyte)	0.19	97	31	17
Bryum sp., (Sporophyte)	0.35	99	30	21
Fern				
Nephrolepsis cordifolia, leaf (L.) Presl.	0.16	99	29	18
Gymnosperms				
Pinus radiata, D. Don.				
Leaf	0.30	99	24	26
Thuja orientalis L.				
Leaf	0.52	100	19	28
Angiosperms				
Hordeum vulgare L. (barley)				
Leaf	0.40	99	35	
Triticum aestivum L. (wheat)				
Leaf	0.38	99	42	
Spinacea oleracea L. (spinach)				
Leaf	0.25	99	49	50
Beta vulgaris L. (sugar beet)				
Storage root	0.11	100	33	
Citrullus lanatus (Thunb.) Matsum. and Nakai (watermelon)				
Flowers				
Stigma	0.66	99	55	
Style	1.28	99	59	
Ovary	0.40	98	41	
Malus domestica Borkh. (apple)				
Leaf	0.36	98	28	38
Pyrus communis L. (pear)				
Leaf	0.55	99		
Manihot esculenta Crantz (cassava)				
Leaf	0.84	100	50	54
Latex plants				
Callotropis procera (Wild) R. Br.				
Leaf	0.61	100		
Euphorbia lathyris L.				
Leaf	0.63	89		
Asclepias rotundifolia Mill.				
Leaf	1.37	99	50	32
Vitis vinifera L. (grapevines)				
Leaf				
cv Cabernet Sauvignon	0.17	100	64	94
cv Sultana	0.57	100	41	90
Berry				
cv Cabernet Sauvignon	0.07	100	41	84
cv Sultana	0.10	99	22	83

*Assuming a K_m for sucrose phosphate of 0.1 mM, approximate maximum velocities can be calculated by multiplying the values by 2.7.

Table 4. Tissues lacking sucrose phosphatase

Tissue	Hydrolysis of substrate (μmol/min. g fr. wt)	
	Sucrose phosphate (60 μM) pH 6.7	*p*-Nitrophenyl phosphate (2.5 mM) pH 6.0
Brown algae		
Ecklonia radiata C.Ag.J.Ag.	0*	0.03
Cystophora subsarcinata (Mert.) J.Ag.	0	0.14
Hormosira banksii (Turn.) Decaisne	0	0.07
Red alga		
Asparagopsis taxiformis (Delile.) Coll. Hew.	0	0.002
Agaricus campestris L. mushroom		
Cap	0	1.45
Stalk	0	1.26
Rumen fluid	0	0.026
Saccharomyces cerevisiae Hansen yeast.	0	0.074

*Hydrolysis < 1 nmol/min. g fr. wt.

The fact that sucrose phosphatase usually occurs in at least a 10-fold excess over sucrose phosphate synthase had led to the suggestion that it is unlikely to play a role in the regulation of sucrose synthesis [7, 8, 18]. However, sucrose is a partially competitive inhibitor of sucrose phosphatase, at least in some tissue [5], and this property could act as a control mechanism limiting the concentration of sucrose. This would not preclude the involvement of other control mechanisms such as modification of the activities of cytoplasmic fructose-1,6-bisphosphatase or sucrose phosphate synthase [8, 18, 19] or changes in the amount of sucrose phosphate synthase present [20, 21].

In addition and possibly more important, the inhibition of sucrose phosphatase by sucrose shows that the enzyme has affinity for both sucrose phosphate and sucrose which would allow it to act as a sucrosyl transferase in sugarcane stems, grape berries and other plant tissues. It has recently been shown [22] that sucrose accumulates in the vacuoles of barley mesophyll protoplasts during photosynthesis. Possibly sucrose phosphatase is involved in this transfer of the sucrosyl moiety from the cytosol to the vacuolar compartment.

EXPERIMENTAL

Plant material. Healthy mature tissue was used from growing green plants and mushrooms at Adelaide in glasshouses, shadehouses or the field. Algae were collected at low tide in May–July from Aldinga Reef (near Adelaide) and maintained in aerated sea water for up to 2 days before use. Baker's yeast was purchased locally. Fresh sheep rumen fluid was obtained from Dr. A. R. Egan, Waite Agricultural Research Institute.

Preparation of enzyme extracts. Tissue (1 g) was ground in a Potter-Elvejhem homogenizer in 4 ml of 0.35 M Tris–HCl buffer, pH 8.5, containing 15 mM EDTA, 10 mM Na diethyldithiocarbamate, 15 mM cysteine–HCl and 6% Carbowax 4000 at 0–4°. The homogenate was centrifuged at 30 000*g* for 10 min. The ppt. in some cases was suspended in 2 ml medium. Preliminary tests showed that the ppt. from several species contained less than 10% of the phosphatase activity and this fraction was consequently not routinely assayed. The three brown algae (Table 4) were ground (1 g in 10 ml) in the above medium in a mortar and pestle with acid-washed sand to achieve cell breakage.

Assay of sucrose phosphatase. Supernatant diluted up to 100-fold with Tris–maleate–bovine serum albumin mixture was assayed for sucrose phosphatase activity, routinely at pH 6.7 and 30° in Tris–maleate buffer containing bovine serum albumin, $MgCl_2$ and (fructosyl-^{14}C) sucrose phosphate as described previously [2]. Conditions of assay were adjusted to give rates of hydrolysis of sucrose phosphate so that initial rates could be calculated [2]. Maximum velocities were also calculated assuming a K_m for sucrose phosphate of 0.1 mM, a value near that found for the enzyme from several species [3, 4, 23].

Hydrolysis of p-nitrophenyl phosphate. Supernatant diluted with 0.1 M Na citrate buffer was incubated with an equal vol. (0.1 ml) of buffer containing 5 mM *p*-nitrophenyl phosphate at a final pH of 6.0 and at 30°. The reaction was stopped by the addition of 2.8 ml 5% Na_2CO_3 and the absorbance of the released yellow *p*-nitrophenol was read at 410 nm. Activities of phosphatase were calculated using a molar extinction co-efficient of 18 300.

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