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Note

Determination of the activity of sucrose phosphate synthase by measurement of the release of UDP by h.p.l.c.

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The synthesis of sucrose in leaves of higher plants is catalysed by the enzymes SPS (sucrose phosphate synthase: UDP-glucose:D-fructose 6-phosphate 2-glucosyl transferase, EC 2.4.1.14) and SS (sucrose synthase: UDP-glucose:D-fructose 2-glucosyl transferase, EC 2.4.2.13), and involves the reactions

$$\text{UDP-glucose} + \text{D-fructose 6-phosphate} \overset{\text{SPS}}{\rightleftharpoons} \text{sucrose-p} + \text{UDP}$$

$$\text{UDP-glucose} + \text{D-fructose} \overset{\text{SS}}{\rightleftharpoons} \text{sucrose} + \text{UDP}$$

Several methods are currently employed to determine the activity of these enzymes; these include chemical analysis of the sucrose and sucrose phosphate[1,2], measurements of the incorporation of labelled D-fructose 6-phosphate into sucrose phosphate or of D-fructose into sucrose[3,4], and the determination of UDP enzymically[5]. However, the results obtained may be inaccurate because of hydrolysis of sucrose phosphate and sucrose by alkaline phosphatases and invertases present in the enzyme extracts[6].

The enzymic method[7] currently used for the determination of UDP is complicated, time consuming, and requires highly purified enzymes. A more convenient method is now described for determining SPS and SS activity based on the separation of UDP and UDP-glucose by h.p.l.c.

A UDP-calibration curve was linear (Fig. 1) in the range 0–0.75 μg. H.p.l.c. of the reaction mixture revealed compounds with retention times of 13.38 and 21.28 min, corresponding to UDP-glucose and UDP, respectively (Fig. 2). SPS activity was determined from the difference between the peak areas of UDP in the reaction mixtures in the presence (Fig. 2b) and in the absence (Fig. 2a) of D-fructose 6-phosphate. The activity of SPS as a function of the concentration of D-fructose 6-phosphate is shown in Fig. 3. The data show that the enzyme activity is saturated at 11.5mM D-fructose 6-phosphate. This method is also applicable to the assay of

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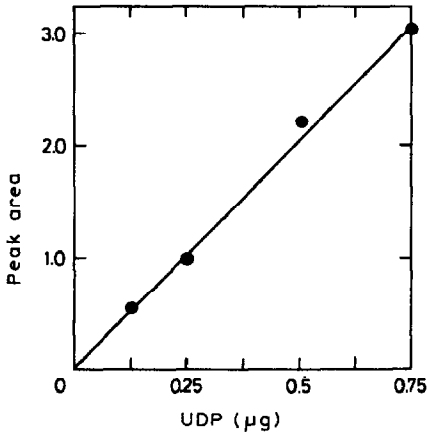

Fig. 1. Calibration curve of UDP, 0–0.75 μg per 5-μL injection.

sucrose synthase, using D-fructose rather than D-fructose 6-phosphate as the substrate.

A comparison between the enzyme activity as measured by the determination of sucrose using the resorcinol method[2,8] and by determination of UDP using h.p.l.c. showed a difference of only 2.8%, with the latter method giving the higher result. Thus, although the h.p.l.c. method gives a more accurate indication of the enzyme activity, a comparison of the two methods is recommended in order to show up possible discrepancies that may arise from the low stability of UDP. In 21 separate treatments each in triplicate, the average standard error was <7%.

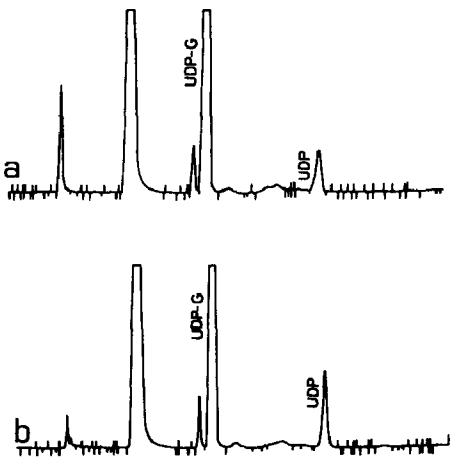

Fig. 2. H.p.l.c. analysis of UDP-glucose and UDP in rose-leaf extracts: (*a*) in the absence of D-fructose 6-phosphate and (*b*) in the presence of D-fructose 6-phosphate.

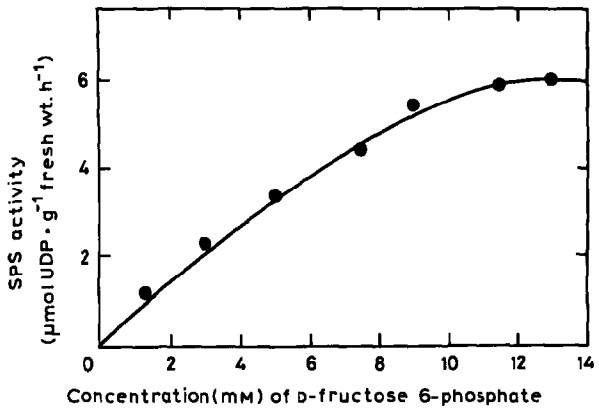

Fig. 3. Effect of the concentration of D-fructose 6-phosphate on SPS activity in rose-leaf extracts.

EXPERIMENTAL

Plant material. — Rose plants (*Rosa hybrida* cv. "Golden Times") were grown[9] under greenhouse conditions at a minimum day and night temperature of 18°. SPS activity was measured in the third 5-leaflet leaf from the top of a branch on which the sepals of the terminal bud had not yet opened.

Enzyme extraction and assay. — Leaf tissue (3 g) was ground by hand in liquid nitrogen and homogenised in 25 mL of extraction medium containing 50mM Hepes–NaOH buffer (pH 7.5), 0.5mM $MgCl_2 \cdot H_2O$, mM Na_2EDTA, 2mM diethyl dithiocarbamate, 2.5mM 1,4-dithio-DL-threitol (DDT), 1% of bovine serum albumin (BSA), and 2% of polyvinylpolypyrrolidone (PVPP). The extract was filtered through six layers of cheesecloth and centrifuged for 20 min at 20,000*g*. The supernatant solution was passed through a column (4 × 350 nm) of Sephadex G-25, and assayed for enzymic activity. A 70-μL aliquot of tissue extract was incubated for 30 min at 37° with 70 μL of reaction mixture prepared according to Rufty and Huber[2], with minor modifications. The reaction mixture contained 15mM UDP-glucose, 15mM D-fructose 6-phosphate (except when the effect of D-fructose 6-phosphate on SPS activity was being measured), 0.5mM $MgCl_2 \cdot 6\ H_2O$, 5mM NaF, 5mM $Na_2MoO_4 \cdot 2\ H_2O$, and 50mM Hepes–NaOH buffer (pH 7.5). The reaction was terminated by the addition of 70 μL of M NaOH (for sucrose determination) or by placing the tubes in boiling water for 1 min (for UDP determination). Sucrose was determined by the resorcinol colorimetric method[2].

H.p.l.c.. — After termination of the enzymic reaction, 20 μL of the mixture was separated at 32.5° on a column (250 × 4.6 mm) of Partisil-10 SAX (Whatman) by elution at 1 mL/min with a buffer gradient from 20mM KH_2PO_4–HCl (pH 4.1) and 500mM KH_2PO_4–KOH (pH 4.6). The latter was added to the former by 2 linear gradients, reaching 6% at 6 min and 75% at 30 min. Concentrations were quantified by peak area integration of absorption at 262 nm and compared with a standard

curve derived under the same conditions, using 5-μL aliquots containing between 0 and 0.75 μg of UDP (Sigma).

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