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# SUCROSE METABOLISM IN SUGAR CANE GROWN UNDER VARYING CLIMATIC CONDITIONS: SYNTHESIS AND STORAGE OF SUCROSE IN RELATION TO THE ACTIVITIES OF SUCROSE SYNTHASE, SUCROSE–PHOSPHATE SYNTHASE AND INVERTASE

SURESH K. BATTA and RANGIL SINGH

Department of Biochemistry, Punjab Agricultural University, Ludhiana-141004, Punjab, India

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**Key Word Index**—*Saccharum officinarum*; Gramineae; sugar cane; sucrose synthesis; sucrose storage; invertase; sucrose synthase; sucrose–phosphate synthase; $^{14}$C-sugar incorporation.

**Abstract**—The contents of sucrose and hexoses in relation to the activities of sucrose synthase, sucrose–phosphate synthase and soluble invertase in leaf blade and of soluble and wall-bound invertase in stem tissues of sugar cane growing under naturally varying weather conditions were determined. In leaf blades, sucrose synthase, sucrose–phosphate synthase and soluble invertase were most active at tillering. Sucrose synthase was relatively more active than sucrose–phosphate synthase throughout the growth of the cane. In stem tissues the activity of soluble acid invertase was highest at the stem elongation stage and then declined, whereas the activities of soluble neutral invertase and wall-bound acid invertase increased with age. With the ageing of the cane, sucrose accumulation in the stem gradually increased with a concomitant fall in hexose content. A narrow ratio of sucrose in the basal to top portion of the cane relates to maturity of the cane. On feeding uniformly labelled sucrose and hexoses to storage tissue for 4 hr, *ca* 85–90% of the $^{14}$C in intracellular sugars was present in sucrose. Synthesis of sucrose from glucose in leaf disks was stimulated by $Mg^{2+}$ and $Mn^{2+}$.

## INTRODUCTION

Sucrose synthesized in sugar cane leaves is translocated to the stem where it is hydrolysed by cell wall invertase before the hexose moieties are taken up by the storage cells [1, 2]. The wall invertases from sugar cane are thought to participate in the apoplasmic–symplasmic translocation of sucrose [3, 4]. The extra-protoplastic hydrolysis of translocated sucrose has also been reported in the growing region of pea epicotyls [5, 6]. In Punjab State (Northern India), sugar cane experiences varied weather conditions, namely hot and dry (May, June), hot and humid (July, August), and cool and dry (November, December, January), during its growth and development. In this region, the ranges of temperature during tillering, stem elongation, ripening and maturity of cane are 40–45, 35–40, 20–25 and 10–15°, respectively. The pertinent question, therefore, is how these marked climatic changes, with special reference to temperature, influence the activities of sucrose-hydrolysing and sucrose-synthesizing enzymes and thereby affect the synthesis and movement of sucrose from source to sink tissues and its accumulation in the latter. Hatch *et al.* [7] studied the activities of soluble acid- and neutral-invertases in relatively immature, maturing and mature storage tissues of about 3-month-old cane at the stem elongation stage. However, their study was confined to only one particular temperature. It is now known that even diurnal temperature variation has been reported to influence greatly sucrose metabolism in sugar cane [8, 9].

The present paper reports on the synthesis and storage of sucrose in relation to the activities of the enzymes involved in its synthesis and hydrolysis in leaf and stem tissues of sugar cane grown through widely varying naturally occurring climatic conditions. By using isolated leaf and stem disks, the *in vivo* incorporation of $^{14}$C from exogenously supplied $^{14}$C-sugars into sucrose has also been studied.

## RESULTS

### *Endogenous sugars and starch*

Sucrose, glucose and fructose were the only free sugars detected in leaf (source) and stem (sink) tissues of sugar cane at all the stages of its growth. The sucrose content (fr. wt basis) of the leaf blade was 18 mg/g at tillering, i.e. 105 days after planting (DAP), but dropped by 35% at the stem elongation stage (135 DAP). Whereas the relative level of sucrose in the leaves at tillering was *ca* 59%, this value rose to *ca* 80% in the mature leaves. The contents of free glucose and fructose in this tissue were also high at tillering. With the advancement in growth, the contents of both these sugars fell. Compared to the leaf blade, the contents of sucrose, glucose and fructose were higher in the leaf sheath at all stages of growth (Table 1).

In each part of the stem, i.e. top (immature), mid (maturing) and bottom (mature), the sucrose content increased gradually from stem elongation stage to maturity (Table 2). This increase was *ca* 14-, 6- and 3-fold in top, mid and bottom parts of the stem, respectively. The relative levels of sucrose from the stem elongation stage to maturity varied between *ca* 18 and 99, 37 and 99, and 63 and 99% in top, mid and bottom portions of the stem, respectively. At each stage of growth, the sucrose content

Table 1. Free sugars (mg/g fr. wt) in leaf at various stages of cane growth

| Growth stage | Leaf blade | | | Leaf sheath | | |
|---|---|---|---|---|---|---|
| | Sucrose | Glucose | Fructose | Sucrose | Glucose | Fructose |
| Tillering | 18.0 ± 0.7 (59) | 5.3 ± 0.5 (17) | 7.5 ± 0.8 (24) | 28.6 ± 1.2 (60) | 11.5 ± 0.5 (24) | 7.9 ± 0.7 (16) |
| Stem elongation | 11.7 ± 0.8 (66) | 0.6 ± 0.1 (3) | 5.5 ± 0.4 (31) | 12.6 ± 0.6 (39) | 8.1 ± 0.6 (25) | 11.5 ± 0.9 (36) |
| Ripening | 24.8 ± 0.9 (87) | 0.8 ± 0.1 (3) | 2.9 ± 0.2 (10) | 35.6 ± 1.4 (78) | 4.9 ± 0.3 (11) | 5.1 ± 0.3 (11) |
| Maturity | 24.1 ± 0.9 (81) | 1.2 ± 0.2 (4) | 4.6 ± 0.5 (15) | 48.2 ± 1.5 (81) | 3.6 ± 0.2 (6) | 7.4 ± 0.5 (13) |

Values in parentheses (Tables 1–5) represent the percentage of the total.

Table 2. Free sugars and starch (mg/g fr. wt) in stem at various stages of cane growth

| Growth stage | Carbohydrate component | Stem part | | |
|---|---|---|---|---|
| | | Top | Mid | Bottom |
| Stem elongation | Sucrose | 12.4 ± 0.8 (18) | 29.2 ± 1.0 (37) | 71.7 ± 2.5 (63) |
| | Glucose | 30.2 ± 1.0 (43) | 26.1 ± 0.9 (33) | 22.4 ± 0.9 (19) |
| | Fructose | 27.4 ± 1.0 (39) | 24.3 ± 0.9 (30) | 20.5 ± 0.8 (18) |
| | Starch | 1.7 ± 0.2 | 1.5 ± 0.2 | 1.1 ± 0.2 |
| Ripening | Sucrose | 134.1 ± 4.3 (90) | 182.8 ± 5.7 (99) | 189.5 ± 6.3 (99) |
| | Glucose | 8.9 ± 0.7 (6) | 1.8 ± 0.2 (< 1) | 1.6 ± 0.2 (< 1) |
| | Fructose | 5.6 ± 0.5 (4) | 0.5 ± 0.1 (< 1) | 0.3 ± 0.1 (< 1) |
| | Starch | 10.3 ± 0.6 | 8.4 ± 0.5 | 6.2 ± 0.5 |
| Maturity | Sucrose | 172.5 ± 3.3 (99) | 188.1 ± 5.0 (99) | 190.5 ± 5.5 (99) |
| | Glucose | 1.8 ± 0.3 (< 1) | 1.9 ± 0.2 (1) | 1.2 ± 0.1 (< 1) |
| | Fructose | 0.3 ± 0.1 (< 1) | Traces | Traces |
| | Starch | 7.6 ± 0.3 | 5.2 ± 0.2 | 3.8 ± 0.3 |

in the three portions of stem was in the order top < mid < bottom. The ratios of sucrose content in bottom to top portions of the cane at stem elongation, ripening and maturity stages of growth were 5.8, 1.4 and 1.1, respectively. In contrast to the gradual increase in sucrose content, the contents of glucose and fructose continuously decreased with the advancement in the growth of the stem. The relative levels of glucose plus fructose which were *ca* 82, 63 and 37% in top, mid and bottom parts of the stem, respectively, at stem elongation stage fell to *ca* 1% at maturity. In leaf blades at all stages of cane growth, the fructose level was higher than that of glucose (Table 1) but this trend was reversed in stem tissues (Table 2).

At each physiological stage of cane growth, the starch content in the three stem portions was in the order top > mid > bottom (Table 2). Regardless of the stem portion, the starch content peaked at ripening and declined thereafter. In all three portions of the stem, the starch content was *ca* 6 and 4 times more at ripening and maturity stages, respectively, compared to its level at the stem elongation stage.

*Sucrose synthase, sucrose–phosphate synthase and invertase in leaf*

In leaf blade, the activity of sucrose synthase (EC 2.4.1.13) was much higher than that of sucrose-phosphate synthase (EC 2.4.1.14) at all stages of cane growth (Fig. 1a). The activity ratio in leaf blade of sucrose synthase to that of sucrose–phosphate synthase was *ca* 3:1 at tillering. At maturity, the activities of sucrose synthase and sucrose–phosphate synthase were *ca* 75 and 30% respectively, of their activities at tillering. Both in leaf blade and sheath, the activity of soluble invertase (pH 5.4) was highest at tillering and gradually declined with further growth of the cane (Fig. 1b).

*Invertase in stem*

Soluble invertase (EC 3.2.1.26) exhibited two pH optima, namely 5.4 and 7.1. The activity of soluble acid invertase was much higher in the top than the mid and bottom portions at the stem elongation stage (Fig. 2a). With the advancement of cane growth, this activity fell but more drastically in the top part of the stem. The activity of soluble neutral invertase was, however, higher in the bottom than the mid and top portions, and markedly increased in the bottom part of the mature cane (Fig. 2b).

The activity of wall-bound invertase (pH 4.0) during stem elongation stage was low in each portion of the stem and increased with the maturity of the cane (Fig. 3). In the top part of the cane the activity of this enzyme was much higher throughout the period of cane development.

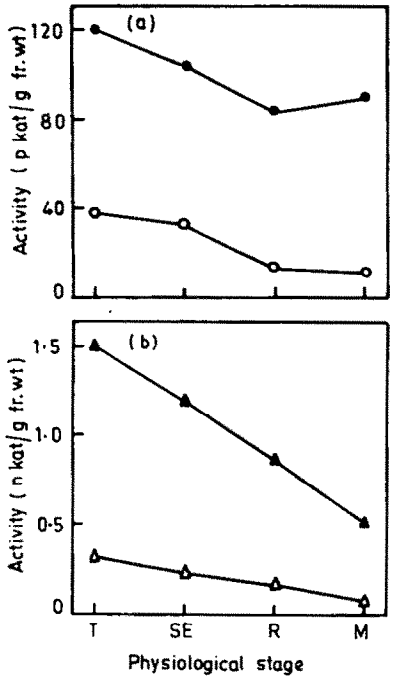


Fig. 1. Activities of (a) sucrose synthase (●) and sucrose-phosphate synthase (○) in leaf blade; (b) soluble acid invertase in leaf blade (▲) and leaf sheath (△) at tillering (T), stem elongation (SE), ripening (R) and maturity (M) stages of cane growth.

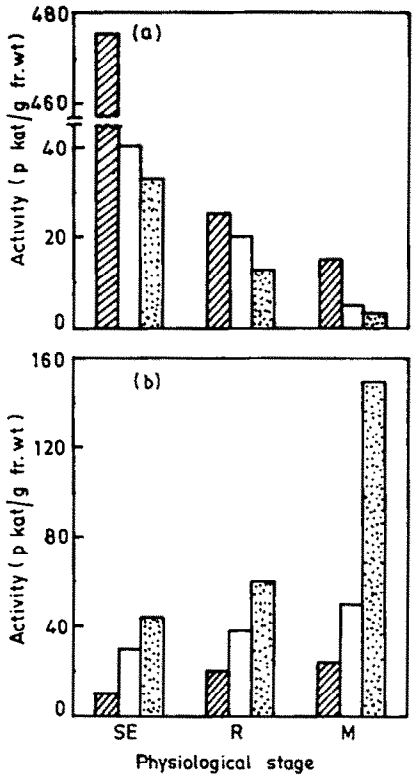


Fig. 2. Activities of soluble acid (a) and neutral (b) invertase in top (▨), mid (□) and bottom (▩) parts of stem at stem elongation (SE), ripening (R) and maturity (M) stages of cane growth.

In vivo *incorporation of* $^{14}C$ *from exogenously supplied* $^{14}C$*-sugars into free sugars in leaf blade*

When the leaf blade disks were incubated in [U-$^{14}C$]glucose solution for 2 hr, *ca* 30% of the total ethanol-soluble $^{14}C$ activity of the leaf appeared in sucrose (Table 3). However, when [U-$^{14}C$]sucrose was supplied exogenously, *ca* 77% of this $^{14}C$ activity was incorporated into sucrose. The ratio of $^{14}C$ distribution between the glucose and fructose moieties of extracted sucrose was *ca* 1.0 when the $^{14}C$-sugar supplied was either glucose or sucrose (Table 3). Feeding [$^{14}C$]glucose (1 mM) in the presence of cold fructose (1 mM) and [$^{14}C$]fructose (1 mM) in the presence of cold glucose (1 mM) to leaf blade disks (at stem elongation) for 2 hr at 28°, resulted in an increase in $^{14}C$ incorporation into the ethanol-soluble fraction by *ca* 10 and 19%, respectively. $Mg^{2+}$ and $Mn^{2+}$ supplied exogenously to the leaf disks stimulated the incorporation of $^{14}C$ from [U-$^{14}C$]glucose into the ethanol-soluble fraction and its relative incorporation into sucrose was enhanced with the increasing concentration of these divalent ions in the bathing solutions (Table 4).

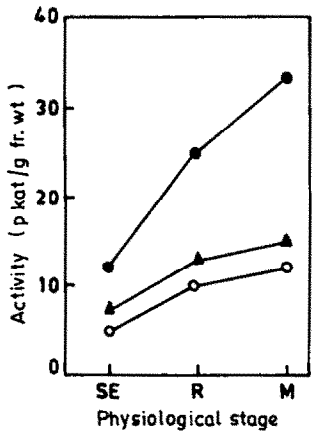


Fig. 3. Activity of wall-bound invertase in top (●), mid (○) and bottom (▲) parts of stem at stem elongation (SE), ripening (R) and maturity (M) stages of cane growth.

In vivo *incorporation of* $^{14}C$ *from exogenously supplied* $^{14}C$*-sugars into free sugars in stem*

After 4 hr incubation of stem disks with labelled sugar solutions (details in Table 5), the $^{14}C$ incorporation into the ethanol-soluble fraction from glucose and fructose was *ca* 8- and 5-fold, respectively, more than that from sucrose in immature storage tissue. In mature storage tissue, this incorporation was *ca* 2-fold more than that from sucrose. Whether the supplied labelled sugar was glucose, fructose or sucrose, more than 85% of the $^{14}C$ of the accumulated sugars, in both mature and immature storage tissues, appeared in sucrose alone and the two moieties of sucrose were equally labelled.

Table 3. Relative incorporation of $^{14}C$ from [U-$^{14}C$]-labelled sugars into free sugars and other ethanol-soluble products of leaf blade at stem elongation stage of cane growth

| [U-$^{14}C$]-Sugar | $^{14}C$-Activity (Bq) in EtOH-soluble products | | | | | | [$^{14}C$]Glucose/[$^{14}C$] fructose in extracted sucrose |
|---|---|---|---|---|---|---|---|
| | Sucrose | Glucose | Fructose | $UIP_1$ | $UIP_2$ | Total | |
| Glucose | 330 ± 10.8 (30) | 563 ± 14.9 (51) | 156 ± 5.3 (14) | 34 ± 2.4 (3) | 17 ± 1.4 (2) | 1100 | 1.1 |
| Sucrose | 1249 ± 20.0 (77) | 112 ± 2.2 (7) | 239 ± 5.6 (15) | 23 ± 1.3 (1) | — | 1623 | 1.0 |

Fresh leaf disks (0.5 g) were incubated in 1 ml 0.5% $^{14}C$-sugar solution (5 $\mu$Ci) for 2 hr at 28°. $UIP_1$, Unidentified product ($R_G$ 0.41); $UIP_2$, unidentified product ($R_G$ 0.54).

Table 4. Effect of $Mg^{2+}$ and $Mn^{2+}$ on the incorporation of $^{14}C$ from [U-$^{14}C$]glucose into free sugars and other ethanol-soluble products of leaf blade at stem elongation stage of cane growth

| Metal ion | Concentration (mM) | $^{14}C$-Activity($B_q$) in EtOH-soluble products | | | | |
|---|---|---|---|---|---|---|
| | | Sucrose | Glucose | Fructose | $UIP_1$ | Total |
| Control | — | 215 ± 5.0 (55) | 112 ± 3.0 (29) | 24 ± 1.8 (6) | 37 ± 1.8 (10) | 388 |
| $Mn^{2+}$ | 2 | 229 ± 4.9 (55) | 122 ± 3.2 (30) | 24 ± 1.6 (6) | 37 ± 1.5 (9) | 412 |
| | 5 | 273 ± 5.4 (56) | 86 ± 2.6 (18) | 61 ± 2.0 (13) | 62 ± 2.5 (13) | 482 |
| | 10 | 712 ± 10.5 (73) | 111 ± 2.9 (11) | 51 ± 2.5 (5) | 111 ± 2.9 (11) | 985 |
| $Mg^{2+}$ | 80 | 650 ± 10.2 (75) | 100 ± 2.5 (11) | 30 ± 1.8 (4) | 87 ± 2.9 (10) | 867 |
| | 200 | 978 ± 15.3 (76) | 143 ± 3.8 (11) | 66 ± 2.5 (5) | 102 ± 2.5 (8) | 1289 |
| | 400 | 1555 ± 25.0 (80) | 236 ± 4.8 (12) | 80 ± 2.5 (4) | 76 ± 2.0 (4) | 1947 |

Fresh leaf disks (0.5 g) were incubated in 1 ml 1 mM [U-$^{14}C$]glucose solution (4 $\mu$Ci) with or without metal ion for 2 hr at 28°.

## DISCUSSION

The fall in the content of sucrose in leaf blade from tillering to the stem elongation stage (Table 1) suggests translocation of some sucrose from leaves to stem tissue. Since sucrose translocation declines with the fall in ambient temperature [10], the higher content of this disaccharide in leaf blade at ripening and maturity stages of cane growth may be a reflection of this effect and of the concomitant decrease in the activity of acid invertase (Fig. 1b). High levels of invert sugars (Table 1) and soluble acid invertase (Fig. 1b) in this tissue at tillering indicate hydrolysis of some sucrose *in situ*. Unlike some other $C_4$ plants, like sorghum, where the activity of sucrose–phosphate synthase has been shown to be higher than that of sucrose synthase [11], we observed the reverse in sugar cane (Fig. 1a). That sucrose–phosphate synthase operates at lower rates than sucrose synthase in sugar cane leaf has also been reported by Hawker [12].

The high levels of soluble acid invertase activity (Fig. 2a) and invert sugars (Table 2) in immature (top) storage tissue at stem elongation are an indication of a rapid hydrolysis of sucrose during internodal expansion. At this stage of cane development, the soluble neutral invertase activity is low (Fig. 2b). This cytoplasmic enzyme has been reported to regulate the sugar flux in mature storage tissue [13]. High levels of soluble-acid, neutral invertase in immature and mature storage tissues, respectively, of sugar cane have also been reported by Hatch and Glasziou [7]. The wide ratio of sucrose content in bottom to top portion of the cane at stem elongation stage tends to decrease towards the maturity phase of cane development (Table 2). This ratio, which attains constancy in the post-maturity period of the cane [14], can, therefore, be taken as an index of the cane maturity.

Since both moieties of extracted sucrose were equally labelled when the leaf disks at stem elongation stage were fed with [U-$^{14}C$]glucose (Table 3), it appears that UDPG and fructose are derived from glucose through hexose phosphates at more or less equal rates (Fig. 4). This symmetrical labelling of sucrose also shows that a higher content of free fructose (*ca* 9 ×) over free glucose in the

Table 5. Relative incorporation of $^{14}C$ from [U-$^{14}C$]-labelled sugars into free sugars of storage tissue

| $^{14}C$-Sugar | Immature storage tissue | | | | | Mature storage tissue | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $^{14}C$-Activity (Bq) in intracellular sugar | | | | [$^{14}C$]Glucose/[$^{14}C$]fructose in extracted sucrose | $^{14}C$-Activity (Bq) in intracellular sugar | | | | [$^{14}C$]Glucose/[$^{14}C$]fructose in extracted sucrose |
| | Sucrose | Glucose | Fructose | Total | | Sucrose | Glucose | Fructose | Total | |
| Glucose | 984 ± 16.0 (94) | 50 ± 2.0 (5) | 13 ± 0.8 (1) | 1047 | 1.0 | 370 ± 4.5 (88) | 44 ± 1.8 (10) | 7 ± 0.2 (2) | 421 | 1.2 |
| Fructose | 599 ± 10.0 (89) | 11 ± 0.8 (2) | 63 ± 2.2 (9) | 673 | 0.8 | 344 ± 4.0 (85) | 18 ± 0.9 (5) | 41 ± 1.5 (10) | 403 | 1.0 |
| Sucrose | 122 ± 2.8 (87) | 7 ± 0.7 (5) | 11 ± 0.7 (8) | 140 | 1.1 | 207 ± 3.0 (89) | 15 ± 0.6 (6) | 11 ± 0.5 (5) | 233 | 1.0 |

Storage tissue disks (5 × 0.5 mm) were washed in running tap water for 1 hr to remove free-space sugars, gently blotted and 0.5 g incubated aseptically in 1% $^{14}C$-sugar solution (5 $\mu$Ci) for 4 hr at 28°.

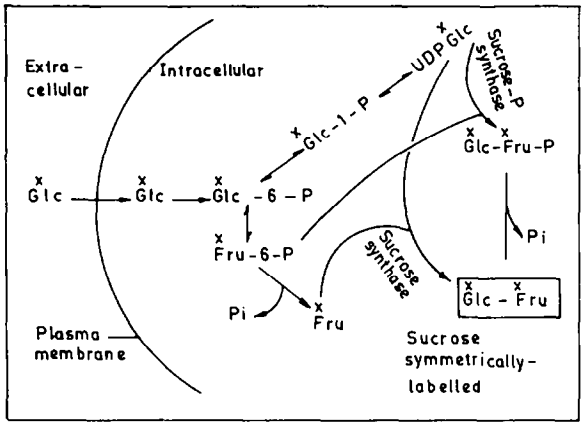


Fig. 4. Proposed pathway from exogenously supplied [U-$^{14}C$]glucose to sucrose in leaf blade. ×, $^{14}C$-Labelled.

endogenous free sugar pool of leaf blade at the stem elongation stage (Table 1) does not lower the specific activity of labelled fructose.

Although there is some additive effect of cold fructose (1 mM) on the $^{14}C$-incorporation into the ethanol-soluble fraction from $^{14}C$-glucose (1 mM) and vice versa, it is difficult to say on the basis of these results if the two hexoses are carried by the same or different carriers across the plasmalemma in the leaf blade. An increase in the relative incorporation of $^{14}C$ from the ethanol-soluble fraction into sucrose as a result of addition of $Mn^{2+}$ (2–10 mM) and $Mg^{2+}$ (80–400 mM) in the bathing solutions (Table 4) reveals that these divalent cations, which have been reported to be *in vitro* activators of sucrose synthase and sucrose–phosphate synthase [15, 16], stimulate sucrose synthesis *in vivo*.

The appearance of over 85% of the $^{14}C$ of intracellular sugars in sucrose after 4 hr incubation of the storage tissue indicates a rapid synthesis of sucrose in this tissue (Table 5). Symmetric labelling of sucrose extracted from the storage tissue disks fed with labelled glucose or fructose (Table 5) indicates that in this tissue $^{14}C$ movement from hexose to sucrose occurs through rapid formation and equilibration of hexose phosphates. In view of the evidence that sucrose is first inverted by an acid invertase in the outer space of sugar cane storage tissue and the resulting hexoses are transported across plasmalemma [17], the observed diminished incorporation of $^{14}C$ from supplied sucrose over hexoses into intracellular sugars of the storage tissue disks (Table 5) may be an effect of this invertase barrier.

## EXPERIMENTAL

*Chemicals.* [U-$^{14}C$]-Labelled glucose (210 mCi/mmol), fructose (107 mCi/mmol) and sucrose (480 mCi/mmol) were obtained from BARC, Trombay, Bombay, India, UDPG, fructose-6-phosphate, glucose oxidase, peroxidase and invertase were acquired from Sigma, St. Louis, MO, U.S.A.

*Plant samples.* The sugar cane crop (an early maturing cv. CoJ 64) was grown in the field under recommended cultural practices. Whole plant samples were collected during the tillering, stem elongation, ripening and maturity stages of growth and their leaf blade, sheath and stem separated. The third and fourth leaves, i.e.

those maximally active in photosynthesis, were taken as the leaf samples. For stem samples, the stem was cut into three equal parts, namely top, mid and bottom, representing relatively immature, maturing and mature storage tissues, respectively.

*Extraction and determination of free sugars and starch.* The pH of fresh juice extracted from the storage tissues of sugar cane, collected at different stages of growth, varied between 5 and 5.6. The stability of the sucrose at pH 4.5 and above was confirmed by placing authentic sucrose solns, buffered at pH 4, 4.5, 5.5 and 6, in boiling $H_2O$ for 30 min. However, to avoid even the slightest breakdown of sucrose, hot EtOH was employed for the extraction of sugars. The free sugars were quantitatively extracted from the leaf and rind-free storage tissues first with hot 80% EtOH (× 3) followed by extraction (× 2) with hot 70% EtOH. The extract was freed from alcohol at 40° under red. pres. The aq. extract was clarified as in ref. [18] and the content of glucose plus fructose determined (as reducing sugar fraction) by reaction with Nelson's arsenomolybdate reagent [19]. Free glucose was determined by glucose oxidase [20]. The difference in the amounts of reducing sugars and free glucose was taken as the content of free fructose. Sucrose was estimated [21] after destroying free fructose with alkaline $NaBH_4$ [11]. Starch was extracted from the air-dried sugar-free residue of storage tissue with 52% $HClO_4$ and assayed by reaction with anthrone as in refs [22, 23].

*Enzyme extraction and assay.* For the extraction of sucrose synthase and sucrose–phosphate synthase from leaf blade, fresh tissue (200 g) was homogenized and extracted at 2–3° with 0.1 M Tris–HCl buffer (pH 7.6) containing 0.3 M mannitol, 0.01 M $MgCl_2$, and 0.02 M each of EDTA, cysteine–HCl and DIECA [12]. In addition, the extraction buffer also contained 1% Triton X-100 [11]. The homogenate was filtered through two layers of cheesecloth. The residue was re-extracted (× 2) with the same buffer and filtered through the same cheesecloth, and finally washed on the cheesecloth with more buffer. The filtrates were pooled and centrifuged at 15000 *g* for 15 min. The pellet was washed by resuspending in buffer and repeating the centrifugation step. From the combined supernatants, the proteins were fractionally precipitated at 0–30, 30–60 and 60–80% $(NH_4)_2SO_4$ satn. By this procedure, the enzymes were quantitatively extracted/precipitated and there was no loss of their activity in the residue. The ppts were dissolved in minimum vol. of the extraction buffer and dialysed against 4–5 l. of the same buffer for 24 hr at 3–4°. The dialysates were tested for enzymic activity. The activities of both the enzymes were detected only in the 30–60% $(NH_4)_2SO_4$ fraction [24] and were found to be the same as those obtained in the dialysed crude extract. This fraction was, therefore, taken for enzymic assay. The composition of the reaction mixture for sucrose synthase was 100 μl 0.04 M UDPG, 100 μl 0.08 M fructose, 100 μl 1 M Tris–HCl buffer (pH 8.2) containing 0.01 M $MgCl_2$ and 200 μl enzyme preparation (10–18 μg protein/μl). For sucrose–phosphate synthase the reaction mixture contained 100 μl 0.04 M UDPG, 100 μl 0.08 M fructose-6-phosphate, 100 μl 1 M Tris–HCl buffer (pH 7.4) containing 0.01 M $MgCl_2$ and 0.02 M NaF, and 200 μl enzyme preparation (10–18 μg protein/μl). The reaction mixtures were incubated at 37° for 20 min and the amount of sucrose (P) formed was determined [11, 21]. The protein concn of the dialysate used for enzymic assay was determined according to ref. [25].

Soluble and wall-bound invertases were extracted essentially by the method of ref. [26]. The enzymes were assayed using the reaction mixtures: 50 μl 0.2 M sucrose, 100 μl 0.2 M NaOAc buffer (pH 5.4)/NaPi buffer (pH 7.1) and 50 μl enzyme preparation (0.5–10 μg protein/μl) for soluble invertase; and 50 μl 0.2 M sucrose, 100 μl 0.2 M NaOAc buffer (pH 4.0) and 50 μl cell-wall preparation for wall-bound invertase. The reaction mixtures were incubated at 37° for 20 min and the amounts of reducing sugars produced were determined [19]. The corresponding amount of sucrose hydrolysed was calculated by multiplying the amount of reducing sugars by a factor of 0.95.

In preliminary assays, optimum pH, temperature and conditions for linear rate with respect to substrate concn and time were determined for all the enzymes studied.

*Metabolism of $^{14}C$-sugars in leaf-blade and storage tissue disks.* The leaf and storage tissue disks at stem elongation stage were incubated in labelled sugar solns (see details under Tables 3–5). After incubation, the disks were washed with chilled $H_2O$ for 1 hr to remove free-space sugars [3, 27]. The radio-labelled sugars were then extracted from the washed disks with hot 80% EtOH, as already described and resolved by descending PC using *n*-BuOH–HOAc–$H_2O$ (4:1:5) as the irrigating solvent [28] and $AgNO_3$–NaOH as the stain [29]. Resolution of $^{14}C$ sugars was also confirmed as follows: 5 mm strips, starting from the base line, were cut from the dried unstained paper chromatogram of the labelled EtOH-soluble products and their $^{14}C$ activity measured in a toluene-based scintillant. Total $^{14}C$ activity of the strips covering each resolved sugar/EtOH-soluble product was then calculated. At the same time, resolved [$^{14}C$]sucrose was eluted from the chromatogram, hydrolysed with invertase (Sigma), rechromatographed and the distribution of $^{14}C$ in the hexose moieties of sucrose ascertained as described.

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