Plant Physiol. (1987) 84, 1096–1101
0032-0889/87/84/1096/06/$01.00/0

Light Modulation and Localization of Sucrose Phosphate Synthase Activity between Mesophyll Cells and Bundle Sheath Cells in C_4 Species[1]

Received for publication February 5, 1987 and in revised form April 13, 1987

RYU OHSUGI[2] AND STEVEN C. HUBER*
United States Department of Agriculture, Agricultural Research Service, and Department of Crop Science and Botany, North Carolina State University, Raleigh, North Carolina 27695-7631

ABSTRACT

Experiments were conducted with several *Panicum* species (representing the different C_4 subtypes) to examine the light modulation of sucrose phosphate synthase (SPS) activity and the effect of illumination on the distribution of SPS activity between mesophyll cells (MC) and bundle sheath cells (BSC). Activity of SPS in the light decreased in the order: C_4 > C_3-C_4 intermediate > C_3. In illuminated leaves, SPS activities were similar among the three C_4 subtypes, but SPS activity was higher for NAD-malic enzyme (NAD-ME) species with centripetal chloroplasts in BSC (NAD-ME(P) species) than for NAD-ME species with centrifugal chloroplasts in BSC (NAD-ME(F) species). Transfer of plants into darkness for 30 minutes resulted in decreased SPS activity for all species tested except *Panicum bisulcatum* (C_3 species) and *Panicum virgatum* (NAD-ME(P) species) which showed little or no change. All C_4 subtypes had some SPS activity both in MC and BSC. In the light, SPS activity was mainly in the MC for NADP-ME, NAD-ME(F) and phosphoenolpyruvate carboxykinase species, while it was mainly in the BSC for NAD-ME(P) species. In the dark, for all C_4 subtypes, SPS activity in the MC was decreased to a greater extent than that in the BSC. It is intriguing that NAD-ME(F) and NAD-ME(P) species differed in the activity and distribution of SPS activity between MC and BSC, although they are otherwise identical in the photosynthetic carbon assimilation pathway. Diurnal changes in SPS activity in the MC and BSC were also examined in maize leaves. SPS activity in the MC in maize leaves was high and relatively constant throughout the middle of the light period, dropped rapidly after sunset and increased again prior to the light period. On the other hand, SPS activity in the BSC was lower and changed more coincidently with light intensity than that in the MC. The results suggested that light activation of SPS activity located in the BSC may require higher irradiance for saturation than the SPS in the MC. We conclude that SPS may function in both MC and BSC for sucrose synthesis in the light, particularly at high light intensity, while in the dark, the major function may be in the BSC during starch degradation.

Sucrose is the primary transport form of photosynthetically reduced carbon and SPS,[3] which catalyzes the formation of sucrose-P from fructose-6-P and UDP-glucose, plays a key role in the regulation of sucrose biosynthesis (10). In C_3 species, sucrose biosynthesis occurs in the cytoplasm of mesophyll cells and the biochemical mechanism of sucrose formation has been relatively well established (28). In C_4 species, however, research has concentrated almost exclusively on maize which is an NADP-ME species. The biochemical mechanism involved in the regulation of photosynthetic carbohydrate formation is considered to be more complex than in C_3 species since two different cell types (*i.e.* MC and BSC) are involved in the photosynthetic carbon assimilation.

In maize leaves, the MC is considered to be the main site of sucrose biosynthesis since SPS and other enzymes involved in sucrose formation are thought to be located largely, if not exclusively, in the MC (5, 29). However, limited studies with other NADP-ME species did not show such a strict compartmentation of SPS activity: 90% of the whole leaf SPS activity was in the BSC for *Digitaria pentzli* (15), and almost an equal distribution between MC and BSC was reported for *Cyperus rotundus* (2) and *Digitaria sanguinalis* (3). These reports suggest that the distribution of SPS activity between cell types may vary among NADP-ME species. The compartmentation of SPS activity between MC and BSC in the other C_4 subtypes has not yet been examined.

Light modulation of SPS activity has also been identified in some C_3 monocots, *i.e.* barley (25) and *Lolium temulentum* (22). SPS activity in maize leaves was reported to be activated by light (26) and in a natural environment whole leaf SPS activity paralleled changes in light intensity (12). It is not known, however, whether the light modulation of SPS activity is characteristic of C_4 species and whether the compartmentation of SPS activity between MC and BSC changes during a day/night cycle.

Panicum is an appropriate genus to examine diversity in the mechanism of sucrose biosynthesis since it has C_3 species, C_3-C_4 intermediate species, and all C_4 subtypes (6, 7, 18). Recently, some NAD-ME species in the *Dichotomiflora* group of *Panicum* were found to have centrifugally arranged chloroplasts in BSC (designated NAD-ME(F) species), although most NAD-ME species have centripetally arranged chloroplasts in BSC (17, 20) (designated NAD-ME(P) species).

By using *Panicum* species as well as maize, therefore, experiments were conducted in the present study to: (a) measure the light modulation of SPS activity for many species which differ in photosynthetic carbon assimilation pathways, (b) examine the distribution of SPS activity between MC and BSC both in the light and in the dark for all C_4 subtypes, and (c) examine the diurnal change of SPS activity in MC and BSC in maize leaves.

[1] Cooperative investigations of the United States Department of Agriculture, Agricultural Research Service, and the North Carolina Agricultural Research Service, Raleigh, NC 27695-7601. Paper No. 10994 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh, NC 27695-7601.

[2] Permanent address: Pasture Plants Division, National Grassland Research Institute, Nishinasuno, Tochigi, Japan, 329-27.

[3] Abbreviations: SPS, sucrose phosphate synthase; BSC, bundle sheath cell; MC, mesophyll cell; ME, malic enzyme; PEPC, phosphoenolpyruvate carboxylase; PEP-CK, phosphoenolpyruvate carboxykinase; PGA, 3-phosphoglycerate; RuBPC, ribulose 1,5-bisphosphate carboxylase.

MATERIALS AND METHODS

Plant Culture. C_4 subtypes of the species used in this study have already been determined (6, 17, 20). NAD-ME species were divided into NAD-ME(F) species which have centrifugal chloroplasts in BSC, and NAD-ME(P) species which have centripetal chloroplasts in BSC. *Panicum virgatum* has peripherally arranged chloroplasts in BSC and therefore could not be included in either NAD-ME(F) or NAD-ME(P) species based on chloroplast location in the BSC. However, leaf anatomical characteristics were similar to NAD-ME(P) species rather than NAD-ME(F) species (19), and it was therefore included in the NAD-ME(P) group in this study. Plants were grown in 8 inch pots in the greenhouse in a medium containing Peat-Lite,[4] perlite, and sterile sand (2:2:1). Plants were watered daily and at one month intervals a commercial slow release fertilizer (Sta-Green Pro Start, 13-6-6, Brawley Seed Co., Mooresville, NC) was incorporated at the recommended rate.

Experimental Protocol. The experiment involving light modulation of SPS activity was conducted in March 1986, with the *Panicum* species listed in Table I. Fully expanded, uppermost leaves of vegetative plants were harvested at noon on a sunny day and again 30 min after transferring plants into a darkened growth chamber whose temperature approximated that in the greenhouse. Leaves were immediately frozen in liquid N_2 and stored at −80°C prior to enzyme assay.

The distribution of SPS activity between MC and BSC in the light and in the dark was examined in three separate experiments using representative species of each of the C_4 subtypes listed in Table II. The three experiments were conducted at different times of the year with plants at different stages of development. Experiment 1 was done early in summer (June 24 and 25 for the light experiment and July 6 and 7 for the dark experiment) when plants were at the early reproductive stage. Experiment 2 was done in mid-summer (July 26 and 27 for the light and dark treatments) when plants were at the vegetative stage after cutting. Experiment 3 was done in Autumn (October 31 for the light and dark treatments) when plants were at the early reproductive stage. Each experiment was conducted on consecutive sunny days. However, experiment 3 was done on a single day and supplementary lights (which provided 500 μmol photons $m^{-2}s^{-1}$ PAR) were used in the greenhouse to extend the photoperiod to 15 h to prevent floral induction. In all experiments, fully expanded, uppermost leaves were harvested at noon and at 23:00 h. The central midrib was removed and leaf tissue was separated into two parts. One half of the leaf blade (0.2–0.3 g) was rolled with a test tube to extract leaf sap and was assayed for some enzymes as described below. The other half of the blade was frozen in liquid N_2 and stored at −80°C prior to enzyme assay in whole leaf extracts.

Diurnal changes in SPS activity in MC and BSC were studied in mature leaves of maize (*Zea mays* L. cv Pioneer 3184). Two separate experiments were conducted. Experiment 1 (Fig. 1) was done from 08:00 h to 21:00 h on August 29 when it was sunny all day long. Experiment 2 (Fig. 2) was done from 21:00 h on September 7 to 12:00 h on September 8 (it was cloudy on the second day). All measurements were made on the third leaf from the top which was leaf 5 (acropetally numbered) in experiment 1, and leaf 7 in experiment 2. At 2 to 3 h intervals, a 7 cm section of the leaf blade, starting 10 cm from the leaf tip, was divided into two parts from midrib. One half of the leaf blade was immediately rolled with a test tube as described below and the other half of the blade was frozen and stored at −80°C prior to enzyme assay.

Extraction of Leaf Sap. The extraction of mesophyll sap was basically as described by Leegood (14). A leaf segment, without the midrib (0.2–0.3 g), was placed on a cooled glass plate with 0.5 ml extraction buffer (50 mM Hepes-NaOH [pH 7.5], 10 mM $MgCl_2$, 1 mM EDTA, 2.5 mM DTT, 0.5% [w/v] BSA, and 0.02% (v/v) Triton X-100). The leaf segment was rolled several times with a chilled test tube and washed with 0.5 ml extraction buffer. Extruded leaf sap was collected with a Pasteur pipette, desalted by centrifugal gel filtration (8) and assayed for enzymes as described below.

Whole Leaf Extraction and Enzyme Assays. Frozen leaf samples (0.2–0.3 g) were ground in a chilled mortar with 0.1 g sea sand and eight volumes of extraction buffer. The extract was filtered through Miracloth (Behring Diagnostics, LaJolla CA) and centrifuged at 10,000*g* for 10 s in a Beckman Microfuge. The extract was then desalted by centrifugal gel filtration (8). SPS was measured at 25°C in a fixed point assay as described elsewhere (12). PEPC was assayed at room temperature according to Huber *et al.* (11) except that the assay was done with 5 mM PEP at pH 8.0. RuBPC was assayed at room temperature according to Lilley and Walker (15).

Estimation of the Percentage of SPS Activity in MC. Based on the activities of marker enzymes (PEPC for MC and RuBPC for BSC) and SPS, the fraction of SPS activity in MC was estimated by the following equation:

$$S(P_{PEP}) + (1\text{-}S)\,(P_{RuBP}) = P_{SPS}$$

where P_X is the fraction of the respective enzyme in the leaf 'rollate,' compared to a whole leaf extract on a fresh weight basis, and S is the fraction of SPS activity distributed in MC. The calculation assumes that 100% of the PEPC activity is in MC and 100% of the RuBPC activity is in BSC, and that the activities of the three enzymes were restricted to photosynthetic tissues, *i.e.* MC and BSC. SPS activity in each cell type was calculated based on the fraction of SPS activity in the MC and the total activity in the whole leaf extract. In preliminary experiments with maize, it was verified that freezing of leaf tissue had no significant effect on measured enzyme activities. Further, activity in the rollate plus activity extracted from the remaining tissue generally equalled the activity in the whole leaf extract.

RESULTS

Light Modulation of SPS Activity. Table I shows the light modulation of SPS activity in several *Panicum* species. In the light, the single C_3 species tested showed the lowest activity of SPS. It was low compared with the other C_3 species such as soybean or spinach (9, 24). The C_3-C_4 intermediate species had higher activity than the C_3 *Panicum* species and was close to those in the C_4 subtypes. Among the C_4 subtypes in this experiment, the NAD-ME(P) species showed the highest activity and the other subtypes had lower but similar activities to each other.

Transfer of plants to a darkened growth chamber for 30 min resulted in decreased SPS activity in all species examined except for *Panicum bisulcatum* (C_3 species) and *P. virgatum* (NAD-ME(P) species) which showed little or no change in activity. The single NADP-ME species studied showed the greatest decrease of SPS activity with a light/dark transition. NAD-ME species in the *Dichotomiflora* group are identified with asterisks in Table I. NAD-ME(P) species in the *Dichotomiflora* showed a greater decrease in SPS activity in darkened leaves compared to NAD-ME(F) species in the same group.

Distribution of SPS Activity between MC and BSC. Table II shows the activities of marker enzymes in whole leaf extracts and in leaf rollate samples prepared from representative species of the C_4 subtypes. In both the light and dark, whole leaf extracts

[4] Mention of a trademark or proprietary product does not consititute a guarantee or warranty of the product by the United States Department of Agriculture or the North Carolina Agricultural Research Service and does not imply its approval to the exclusion of other products that may also be suitable.

Table I. *Sucrose Phosphate Synthase Activity in Leaves of Various Panicum Species in Light and after 30 min of Darkness*

Sucrose phosphate synthase activity was measured at 12:00 h (light) and 30 min after transferring plants to a darkened chamber (dark). Each value is the mean of three replicate experiments. Asterisks (*) designate the species in the *Dichotomiflora* group. NAD-ME(F) and NAD-ME(P) species have centrifugal and centripetal chloroplasts in bundle sheath cells, respectively.

Species	SPS Activity		Light/Dark
	Light	Dark	
	$\mu mol\ g^{-1}\ fresh\ wt \cdot h^{-1}$		*ratio*
C_3			
P. bisulcatum	14.4	12.6	1.14
C_3–C_4 intermediate			
P. milioides	50.4	31.8	1.59
C_4, NADP-ME			
P. antidotale	73.2	13.8	5.26
C_4, NAD-ME(F)			
P. coloratum (cv Kabulabula)*	56.7	33.9	1.67
P. coloratum (cv Solai)*	53.4	42.0	1.27
*P. dichotomiflorum**	85.8	43.8	1.96
*P. laevifolium**	69.6	40.8	1.69
Mean ± SE	66.4 ± 7.4	40.1 ± 2.2	1.64 ± 0.14
C_4, NAD-ME(P)			
P. coloratum (cv Klein)*	118.2	42.6	2.78
P. coloratum var *makarikariense**	85.2	30.6	2.78
*P. stapfianum**	130.2	45.6	2.22
P. miliaceum	176.4	78.6	2.22
P. virgatum	71.4	78.6	0.91
Mean ± SE	116.3 ± 18.4	55.2 ± 9.9	2.18 ± 0.34
C_4, PEP-CK			
P. maximum	44.4	15.0	2.94
P. texanum	69.6	27.0	2.56

Table II. *Activity of PEPC and RuBPC in Whole Leaf Extracts and in Rollate Samples*

The experiment was the same as Experiment 1 in Table III. Leaves were harvested at 12:00 h (light) and at 23:00 h (dark). Each value is the mean of two replicate experiments. Enzyme activities were measured in desalted whole leaf extracts and in rollate samples obtained as described in "Materials and Methods."

Species		PEPC Activity		RuBPC Activity	
		Leaf	Rollate	Leaf	Rollate
		$\mu mol\ g^{-1}\ fresh\ wt \cdot h^{-1}$			
P. antidotale (NADP-ME)	Light	4591	1262	591	2
	Dark	5528	1437	185	1
P. coloratum (cv Kabulabula) (NAD-ME(F))	Light	1572	354	280	7
	Dark	984	403	359	14
P. coloratum var *makarikariense* (NAD-ME(P))	Light	2067	475	306	1
	Dark	1703	613	254	1
P. maximum (PEP-CK)	Light	2065	289	969	7
	Dark	808	153	503	2

contained high activities of both PEPC and RuBPC as expected. The leaf rollates, in contrast, contained only trace activities of RuBPC, which is considered to be confined to the BSC, but substantial activities of PEPC, which is located predominantly in the MC. These results indicate that the leaf rollates consist primarily of MC sap with only slight contamination of BSC contents for all the C_4 subtypes examined. Thus, the rollate technique of Leegood (13) provided a highly pure MC extract for quantifying the distribution of SPS activity between the cell types in a range of C_4 species including maize.

Table III shows the whole leaf activity and the percentage of SPS activity in the MC in representative C_4 species in the light and dark. In experiment 1, in the light, 60 to 80% of the whole leaf SPS activity was in the MC for NADP-ME, NAD-ME(F) and PEP-CK species, although only 33% of the activity was in the MC for NAD-ME(P) species. In the dark, on the other hand, the percentage of SPS activity in the MC for all subtypes was lower than 40%. In experiment 2, in the light, SPS activity was distributed almost evenly between MC and BSC for NAD-ME(F), NAD-ME(P) and PEP-CK species, although NADP-ME species showed a slightly lower percentage of the activity in the MC. In the dark, SPS activity was found almost exclusively in the BSC except for NAD-ME(P) species which still showed 36% of the activity in the MC. Experiment 3 was conducted only with NAD-ME(F) and NAD-ME(P) species. Essentially all of the SPS activity was present in the MC in the light and the percentage dropped to 16% in the dark for NAD-ME(F). Conversely, only 25% of the activity was in the MC in the light and 40% in the dark for NAD-ME(P) species.

Therefore, for NADP-ME, NAD-ME(F), and PEP-CK species, SPS activity was observed mainly in the MC in the light. In the dark, the activity in the MC decreased to a greater extent than that in the BSC. Accordingly, SPS activity was observed mainly in the BSC. On the other hand, for NAD-ME(P) species, SPS activity was observed mainly in the BSC in the light and the distribution did not change greatly with a light/dark transition, indicating that in this subtype, SPS activity in both cell types was modulated to the same extent by light. In most species examined, the percentage of SPS activity in the MC, both in the light and in the dark, was lower in experiment 2 than in experiments 1 and 3. For NADP-ME species, especially, the percentage in the MC in illuminated leaves dropped from 76 to 31%. Possible explanations for this change include differences in stage of plant development (reproductive versus vegetative) and/or light environment under which plants were grown. In experiment 2, plants were in the vegetative stage and optimal environmental conditions (*e.g.* high temperature and irradiance) promoted more rapid growth than in experiments 1 and 3.

Diurnal Change in SPS Activity in MC and BSC in Maize Leaves. Figure 1A shows the diurnal change in the percentage of SPS activity in the MC in maize leaves. Approximately 85% of the whole leaf activity was present in the MC early in the morning. The percentage activity decreased in the morning hours and then stabilized during the afternoon. Figure 1B shows the diurnal change in SPS activity in MC and BSC. SPS activity in the MC was high and constant throughout the day and decreased after sunset. On the other hand, SPS activity in the BSC was low early in the morning, increased until midday and then dropped to a similar activity at 21:00 h (*i.e.* early night period) as that observed early in the morning.

In another experiment, the changes in SPS activity in MC and BSC during the night and early in the photoperiod were examined in more detail. As shown in Figure 2A, the percentage of SPS activity in the MC was the lowest at the beginning of the dark period, increased constantly during the night and reached a maximum level of almost 100% early in the subsequent photoperiod, which was quite cloudy. Whole leaf SPS activity was constant throughout the night and increased about twofold during the light period (not shown). However, SPS activities in the MC and BSC changed in different ways (Fig. 2B). SPS activity in the MC was the lowest at the beginning of the night and

Table III. *Sucrose Phosphate Synthase Activity in Whole Leaf Extracts and Percentage of Activity Located in MCs in Different C_4 Subtypes*

Each experiment was conducted in a different season: early summer (experiment 1), mid-summer (experiment 2) and autumn (experiment 3). Activity was measured at 12:00 h (light) and at 23:00 h (dark) and is expressed as μmol g^{-1} fresh weight·h^{-1}. Percentage of the whole leaf SPS activity located in mesophyll cells was assessed using the rollate technique described in "Materials and Methods." Each value is the mean of two replicate experiments.

		Experiment 1 (Early Reproductive)		Experiment 2 (Vegetative)		Experiment 3 (Early Reproductive)	
		Activity in leaf	Percentage in MC	Activity in leaf	Percentage in MC	Activity in leaf	Percentage in MC
P. antidotale (NADP-ME)	Light	151	76	101	31	[a]ND[a]	ND
	Dark	27	36	35	5	ND	ND
P. coloratum (cv Kabulabula) (NAD-ME(F))	Light	105	58	111	62	70	114
	Dark	93	1	49	−7	42	16
P. coloratum var *markarikariense* (NAD-ME(P))	Light	138	33	127	50	158	25
	Dark	89	23	78	36	120	41
P. maximum (PEP-CK)	Light	137	77	136	50	ND	ND
	Dark	31	16	43	−5	ND	ND

[a] ND = not determined.

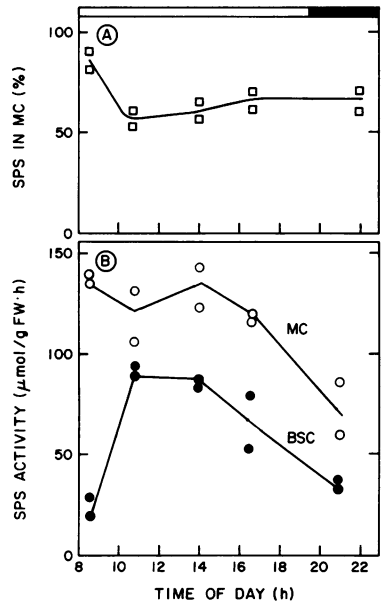

FIG. 1. Diurnal change in (A) percentage of the whole leaf SPS activity in MCs and (B) SPS activities in MCs (○) and BSs (●) of mature maize leaves. Cellular compartmentation of SPS activity was assessed using the rollate technique described in "Materials and Methods." Experiments were conducted on a sunny day from early in the morning to the beginning of the dark period. The light and dark periods are indicated by the bar at the top of upper figure.

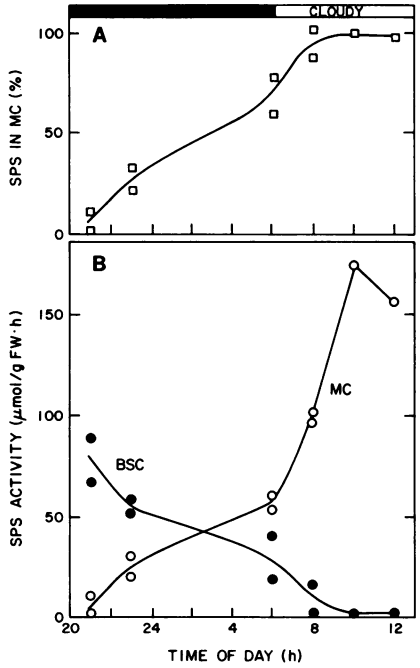

FIG. 2. Diurnal change in (A) a percentage of the whole leaf SPS activity in MCs and (B) SPS activities in MCs (○) and BSs (●) of maize leaves. Cellular compartmentation of SPS activity was assessed using the rollate technique described in "Materials and Methods." Experiments were conducted from the beginning of dark period to the morning of the following day when it was cloudy. The light and dark periods are indicated by the bar at the top of upper figure.

gradually increased throughout the night and sharply increased the following morning. Conversely, SPS activity in the BSC was highest at 21:00 h and decreased during the night. After sunrise, SPS activity in the BSC approached zero.

Similar results were obtained in a third study which spanned a complete 24 h period (data not shown). However, in this case, maximum SPS activity in the BSC at midday was about 15% of the whole leaf activity. Immediately after the light-dark transition, SPS activity in the MC continued to decrease while BSC activity increased until midnight. Thereafter, the patterns reversed, exactly as shown in Figure 2B. Thus, changes in compartmentation of SPS activity early in the dark period may explain why the percentage of activity in the MC at the beginning of the night was high in one experiment (Fig. 1) but low in another (Fig. 2).

DISCUSSION

Light Modulation of SPS Activity. The results obtained in the present study indicate that light modulation of SPS activity occurred in a variety of *Panicum* species which differed in photosynthetic carbon assimilation pathway. The only excep-

tions were *P. bisulcatum* (C_3 species) and *P. virgatum* (NAD-ME(P) species) which showed little or no change in SPS activity during a light/dark transition. Among the species which showed light modulation, the extent to which SPS activity responded to a light/dark transition was variable. Among the C_4 subtypes, SPS activity in NAD-ME(F) species was activated by light to the least extent (Table I). It is possible that SPS activity in the NAD-ME(F) species (and *P. bisulcatum* and *P. virgatum*) changes more slowly in response to a light/dark transition compared with the other species. In maize leaves, the dark deactivation of SPS occurs within 30 min (12), which was the basis for the exposure time used in the present study.

Distribution of SPS Activity between MC and BSC. In all of the C_4 species examined in the present study, evidence was obtained for SPS activity both in the MC and BSC. However, the distribution of SPS activity between the MC and BSC varied somewhat among the C_4 subtypes and with light and dark. It can be concluded, therefore, that both cell types may participate to some extent in the biosynthesis of sucrose. It is important to note that the distribution of SPS activity between the cell types was measured in plants in the light at midday and early in the normal dark period; *i.e.* not after a rapid light/dark transition as used to survey for light modulation of whole leaf SPS activity (Table I). In general, the MC contained the highest percentage of the whole leaf SPS activity during the day and the lowest percentage at night (Table III). Thus, the primary function of the MC may be to synthesize sucrose during photosynthesis, whereas the primary function of the BSC may be in sucrose formation at night during starch mobilization. However, both cell types contain significant activities of SPS during the light period and thus, involvement of the BSC in photosynthetic sucrose formation cannot be discounted.

In NADP-ME species in the light, transport of PGA from the BSC to the MC for reduction is thought to be unavoidable because of a deficiency of PSII activity in the BSC chloroplasts; hence, sucrose biosynthesis in the MC of this subtype is considered to be reasonable (28). NAD-ME and PEP-CK species generate enough NADPH in the BSC chloroplasts to allow all the PGA formed in the Calvin cycle to be reduced. However, some of the PGA may be transported to the MC in these C_4 subtypes, since a high activity of phosphoglycerate kinase and NADP-glyceraldehyde-3-P dehydrogenase is characteristically observed in the mesophyll chloroplasts for all C_4 subtypes (29). It is not surprising, therefore, that in the light some sucrose formation may occur in the MC for NAD-ME and PEP-CK species as well as NADP-ME species. The only consistent difference among the C_4 subtypes examined was between NAD-ME(F) and NAD-ME(P) species. The NAD-ME(F) species behaved similarly to the other subtypes as described above. However, the NAD-ME(P) species showed a different trend in which SPS activity was present mainly in the BSC and the distribution between MC and BSC did not change substantially with light versus dark. Thus, the sensitivity of SPS activity to light modulation was similar in the two cell types. The difference in the distribution of SPS activity between NAD-ME(P) and the other C_4 subtypes appears not to be related to the photosynthetic carbon assimilation pathway since NAD-ME(F) and NAD-ME(P) species have the same basic photosynthetic mechanism (21). Rather, some leaf anatomical characteristics, such as chloroplast location in the BSC may be related to this difference. NADP-ME, NAD-ME(F) and PEP-CK species have centrifugal chloroplasts in the BSC, while NAD-ME(P) species have centripetal chloroplasts in the BSC. The functional significance of chloroplast location in BSC remains unclear at present. However, NAD-ME(F) species have higher $\delta^{13}C$ values than NAD-ME(P) species, suggesting that the former may be more 'tight' than the latter in terms of CO_2 leakage from BSC and it is possible that the difference in chloroplast location in BSC may affect CO_2 leakage (16). Therefore, it is possible that chloroplast location in the BSC may be related to the difference in the distribution and light modulation of SPS activity in the MC and BSC.

Among C_4 subtypes, NAD-ME species are considered to be highly adapted to arid environments (1, 4). However, NAD-ME(F) species seem to be adapted to higher moisture conditions than NAD-ME(P) species, based on natural habitat distribution and the effect of soil moisture on growth (16, 18). A recent report (23) supports this postulate since NAD-ME(F) species were also found in *Eragrostis* and were distributed largely in areas which had higher rainfall or were more humid, compared with NAD-ME(P) species in Australia. It is intriguing that NAD-ME(F) and NAD-ME(P) species differ in several fundamental regards; it is possible that the difference in SPS compartmentation and light modulation may be directly related to some of these factors. One of us (R. O.) has succeeded in the hybridization of NAD-ME(F) and NAD-ME(P) species and studies of the biochemical mechanism of sucrose biosynthesis will be undertaken with the F_1 hybrids.

Diurnal Change in SPS Activity in MC and BSC in Maize Leaves. This study provides new information about the diurnal change of SPS activity in MC and BSC in a natural (greenhouse) environment. SPS activity in whole leaf extracts of maize generally paralleled changes in light intensity during the day and was low and constant during the night (12). Because SPS activity tracks irradiance during the day, the light activation process may require a high light intensity for saturation. In the present study, SPS activity in the MC was activated early in the photoperiod and remained relatively constant throughout the middle of the day. On the other hand, SPS activity in the BSC was near zero at the beginning of the photoperiod but then increased coincident with light intensity (Fig. 1); SPS activity in the BSC was not detected during the day when it was cloudy (Fig. 2). These results suggest that SPS activity in the BSC may require a higher light intensity for saturation than that in the MC. In different experiments, the highest percentage of the whole leaf SPS activity contained in the BSC during the photoperiod varied between 15% (data not shown) and 40% (Fig. 1). During the night, whole leaf SPS activity remains constant, but the activity in the BSC declines while the MC activity increases (Fig. 2). Thus, the BSC contains the highest percentage of the whole leaf SPS activity early in the dark period (around midnight, Figs. 2 and 3). It is noteworthy that a similar generalization emerged from the studies with the C_4 subtypes, although detailed diurnal profiles were not obtained (Table III). The physiological significance of changes in activation status of SPS in the cell types during the dark period is not known. However, the decline in BSC-SPS activity appears to parallel the loss of starch (which is contained primarily in the BSC chloroplasts). Thus, the BSC may function to synthesize sucrose in the dark during starch mobilization.

The biochemical mechanism of SPS light modulation is unknown. However, it is thought that the process may be controlled to some extent by metabolite levels. If the increase in the MC-SPS activity in the dark (Fig. 2) occurs via the same mechanism responsible for increased activity in the light, it is clear that the effect of light is indirect rather than direct. Barley (a C_3 species) leaf SPS activity is also light modulated and it has been reported that after an initial decrease with darkening, SPS activity increases slowly throughout the night (26). These results lead Sicher and Kremer (26) to conclude that the activation state of the enzyme may be modulated by the presence or absence of precursors for sucrose biosynthesis. A similar phenomenon may be occurring in maize leaves, but reciprocal change in SPS activity in the MC and BSC masked the effect. If metabolites modulate the activation (or deactivation) of SPS, it is possible that the observed changes in SPS activity reflect slow changes in the

compartmentation of the metabolite(s) between the cell types during the night period.

Our results appear to contradict recent reports that SPS activity (and sucrose biosynthesis) occurs exclusively in the MC of maize leaves. However, reasonable explanations can be forwarded to resolve the apparent contradictions. For example, the enzymatic digestion procedure used by Usuda and Edwards (29) to separate mesophyll protoplasts from BSC takes several hours and SPS activity might be easily lost especially in the BSC (due to possible high light requirement of this enzyme). In the present study, we avoided this potential problem by using the 'rollate' technique, which provides leaf sap exclusively from the MC, and can be assayed within minutes after extraction. Light intensity under which plants were grown and experiments were conducted might be the difference between our work and the study by Furbank *et al.* (5). Activation of the BSC-SPS apparently requires high irradiance, and thus would be undetected under low light conditions used in their study (5).

For maize leaves, evidence has also been obtained for the primary localization of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (27) and cytoplasmic fructose-1,6-bisphosphatase (5) activities in the MC. However, a strict compartmentation (as in the case for carboxylating enzymes) has not been established for any of these enzymes and it is certainly possible that 10 to 40% of the whole leaf activities could be contained in the BSC. We attempted to examine the distribution of fructose-1,6-bisphosphatase, UDPG pyrophosphorylase and 6-phosphofructo-2-kinase activities between MC and BSC using the rollate technique. UDPG pyrophosphorylase was evenly distributed between the MC and BSC which is generally consistent with the findings of Usuda and Edwards (29). However, fructose-1,6-bisphosphatase and 6-phosphofructo-2-kinase activities were substantially higher in the leaf 'rollate' samples than in whole leaf extracts (data not shown). The whole leaf extract contained some inhibitory factor(s) which erroneously reduced the activities of these enzymes, and thus, the rollate technique could not be used to assess intercellular compartmentation. However, the distribution of SPS and UDPG pyrophosphorylase activity between MC and BSC strongly suggests that both cell types have the biochemical potential to function in sucrose biosynthesis, although some division of labor may occur with light versus dark conditions.

Acknowledgments—The authors thank Mr. Mark Bickett and Dr. Willy Kalt-Torres for excellent technical assistance.

LITERATURE CITED

1. BROWN WV 1977 The Kranz syndrome and its subtypes in grass systematics. Mem Torrey Bot Club 23: 1–97
2. CHEN TM, P DITTRICH, WH CHAMPBELL, CC BLACK 1974 Metabolism of epidermal tissues, mesophyll cells, and bundle sheath strands resolved from mature nutsedge leaves. Arch Biochem Biophys 163: 246–262
3. EDWARDS GE, CC BLACK 1971 Photosynthesis in mesophyll cells and bundle sheath cells isolated from *Digitaria sanquinalis* (L.) Scop. leaves. *In* MD Hatch, CB Osmond, RD Slatyer, eds, Photosynthesis and Photorespiration. Wiley Interscience, New York, pp 153–168
4. ELLIS RP, JC VOGEL, A FULS 1980 Photosynthetic pathways and the geographical distribution of grasses in South West Africa/Namibia. S Afr J Sci 76: 307–314
5. FURBANK RT, M STITT, CH FOYER 1985 Intercellular compartmentation of sucrose synthesis in leaves of *Zea mays* L. Planta 164: 172–178
6. GUTIERREZ M, VE GRACEN, GE EDWARDS 1974 Biochemical and cytological relationship in C_4 plants. Planta 119: 279–300
7. HATCH MD, T KAGAWA, S CRAIG 1975 Subdivision of C_4-pathway species based on differing C_4 acid decarboxylating systems and ultrastructural features. Aust J Plant Physiol 2: 111–128
8. HELMERHORST E, GB STOKES 1980 Microcentrifuge desalting: a rapid quantitative method for desalting small amounts of protein. Anal Biochem 104: 130–135
9. HUBER SC, PS KERR, TW RUFTY 1985 Diurnal changes in sucrose phosphate synthase activity in leaves. Physiol Plant 64: 81–87
10. HUBER SC, DC DOEHLERT, PS KERR, W KALT-TORRES 1985 Regulation of photosynthetic sucrose formation in leaves. *In* PW Ludden, JE Burris, eds, Nitrogen Fixation and CO_2 Metabolism. Elsevier, New York, pp 399–407
11. HUBER SC, T SUGIYAMA, T AKAZAWA 1986 Light modulation of maize leaf phosphoenolpyruvate carboxylase. Plant Physiol 82: 550–554
12. KALT-TORRES W, PS KERR, H USUDA, SC HUBER 1987 Diurnal changes in maize leaf photosynthesis. I. Carbon exchange rate, assimilate export rate and enzyme activities. Plant Physiol 83: 283–288
13. LEEGOOD RC 1985 The intercellular compartmentation of metabolites in leaves of *Zea mays* L. Planta 164: 163–171
14. LILLEY R, DA WALKER 1974 An improved spectrophotometric assay for RuBP carboxylase. Biochim Biophys Acta 358: 226–229
15. MBAKU SB, GJ FRITZ, G BOWES 1978 Photosynthetic and carbohydrate metabolism in isolated leaf cells of *Digitaria pentzli*. Plant Physiol 62: 510–515
16. OHSUGI R 1986 Relationship between leaf anatomy and photosynthetic characteristics in C_4 grasses with special reference to *Panicum* species in the *Dichotomiflora* group. PhD thesis, University of Tokyo, Tokyo, Japan
17. OHSUGI R, T MURATA 1980 Leaf anatomy, post-illumination CO_2 burst and NAD-malic enzyme activity of *Panicum dichotomiflorum*. Plant Cell Physiol 21: 1329–1333
18. OHSUGI R, T MURATA 1985 C_4 photosynthetic characteristics of *Panicum* species in the *Dichotomiflora* group (Graminae). J Agric Res Q Jpn 19: 125–131
19. OHSUGI R, T MURATA 1986 Variations in the leaf anatomy among some C_4 *Panicum* species. Ann Bot 58: 443–453
20. OHSUGI R, T MURATA, N CHONAN 1982 C_4 syndrome of the species in the *Dichotomiflora* group of the genus *Panicum* (Gramineae). Bot Mag, Tokyo 595: 339–347
21. OHSUGI R, T MURATA, S MUTO, S MIYACHI 1987 Photosynthetic $^{14}CO_2$ fixation products and the distribution of enzyme activities related to C_4 photosynthesis in the mesophyll cells of NAD-malic enzyme species with centrifugal chloroplasts in the Kranz cells. Photosynthetica. In press
22. POLLOCK CJ, TL HOUSLEY 1985 Light-induced increase in sucrose phosphate synthetase activity in leaves of *Lolium temulentum*. Ann Bot 55: 593–596
23. PRENDERGAST HDW, PW HATTERSLEY, NE STONE, M LAZARIDES 1986 C_4 acid decarboxlation type in *Eragrostis* (Poaceae): patterns of variation in chloroplast position, ultrastructure and geographical distribution. Plant Cell Environ 9: 333–344
24. RUFTY TW, PS KERR, SC HUBER 1983 Characterization of diurnal changes in activities of enzymes involved in sucrose biosynthesis. Plant Physiol 73: 428–433
25. SICHER RC, DF KREMER 1984 Changes of sucrose-phosphate synthase activity in barley primary leaves during light/dark transitions. Plant Physiol 76: 910–912
26. SICHER RC, DF KREMER 1985 Possible control of maize leaf sucrose-phosphate synthase activity by light modulation. Plant Physiol 79: 695–698
27. SOLL J, C WOTZEL, BB BUCHANAN 1985 Enzyme regulation in C_4 photosynthesis: identification and localization of activities catalyzing the synthesis and hydrolysis of fructose-2,6-bisphosphate in corn leaves. Plant Physiol 77: 999–1003
28. STITT M, SC HUBER, PS KERR 1987 Control of photosynthetic sucrose formation. *In* PK Stumpf, NE Tolbert, EE Conn, eds, The Biochemistry of Plants. Academic Press, New York. In press
29. USUDA H, GE EDWARDS 1980 Localization of glycerate kinase and some enzymes for sucrose synthesis in C_3 and C_4 plants. Plant Physiol 65: 1017–1022