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Sucrose Phosphate Synthase and Sucrose Accumulation at Low Temperature[1]

Charles L. Guy*, Joan L. A. Huber, and Steven C. Huber

Department of Environmental Horticulture, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, Florida 32611 (C.L.G.); and United States Department of Agriculture/Agricultural Research Service (S.C.H.) and Departments of Crop Science and Botany, North Carolina State University, Raleigh, North Carolina 27695-7631 (J.L.A.H., S.C.H.)

ABSTRACT

The influence of growth temperature on the free sugar and sucrose phosphate synthase content and activity of spinach (*Spinacia oleracea*) leaf tissue was studied. When plants were grown at 25°C for 3 weeks and then transferred to a constant 5°C, sucrose, glucose, and fructose accumulated to high levels during a 14-d period. Predawn sugar levels increased from 14- to 20-fold over the levels present at the outset of the low-temperature treatment. Sucrose was the most abundant free sugar before, during, and after exposure to 5°C. Leaf sucrose phosphate synthase activity was significantly increased by the low-temperature treatment, whereas sucrose synthase and invertases were not. Synthesis of the sucrose phosphate synthase subunit was increased during and after low-temperature exposure and paralleled an increase in the steady-state level of the subunit. The increases in sucrose and its primary biosynthetic enzyme, sucrose phosphate synthase, are discussed in relation to adjustment of metabolism to low nonfreezing temperature and freezing stress tolerance.

Numerous studies have demonstrated quantitative and qualitative changes in the free saccharide content of plants exposed to low temperature (15, 18, 21, 22, 27, 30, 31, 38). Seasonal fluctuations in the concentration of soluble carbohydrates in temperate perennials are well known in which the content of free sugars in the tissues may increase severalfold in fall and winter (22, 27, 30, 38). The most abundant, as well as the most commonly accumulated free sugar in response to low temperature, is sucrose (10, 15, 30, 31). The increase in sucrose content in some plants can be as high as 10-fold. However, sugar accumulation at low temperature is not limited to only sucrose. Lesser amounts of glucose and fructose are frequently accumulated (10, 17, 22, 31). Also, in temperate grasses, it has been observed that low temperature exposure leads to fructan synthesis (26) that is dependent upon sucrose accumulation. In addition to the fructans, another sucrose-derived oligosaccharide family has been shown to accrue in some species. For example, English ivy will accumulate significant quantities of raffinose and small amounts of stachyose during the winter (22). Neither of these oligosaccharides are present in significant quantities during the summer.

When hardy plants are subjected to low nonfreezing temperatures, alterations in several physiological and metabolic processes affect carbohydrate status. At low temperature, photosynthetic energy capture is reduced but to a lesser degree than the metabolic utilization processes. This leads to surplus-reducing potential in the form of pyridine nucleotides (NADH, NADPH), and ATP production (18), which can be channeled into CO_2 fixation processes. Active growth is almost always reduced or suspended resulting in decreased demand for the products of photosynthesis. Normally, the production of photosynthate in excess of needs would lead to reserve accumulation in the form of starch. Surprisingly, during low-temperature exposure of temperate perennials and many herbaceous species, the antithetical is observed. In winter, starch content typically declines and free saccharides exhibit a direct quantitative increase (26, 27, 30). Although some of these responses to low temperature could be viewed as incidental consequences that lead to shifts in carbohydrate status, the breakdown of starch in many species at precisely the same time other determinants favor sugar accumulation suggests a more important purpose. In less hardy species such as *Solanum* and subtropical *Citrus*, starch accumulates during low-temperature treatment but only in concert with a marked accretion of free sugars (6, 38).

It is rather surprising that, in spite of ample evidence demonstrating a major shift in the carbohydrate status of plants at low temperature, few studies of the enzymology of carbohydrate metabolism of plants at low temperature have been undertaken (9). Pollock and ap Rees (24) examined the effect of cold storage on potato tuber carbohydrate metabolism enzymes. They found that tuber sweetening was not associated with changes in activities of SPS[2], SS, aldolase, or glyceraldehyde phosphate dehydrogenase. When *Chlorella vulgaris* was subjected to a cold shock for 30 h, the concentration of sucrose and raffinose increased by 10-fold (28). The increase in sucrose concentration during the first 20 h

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[2] Abbreviations: SPS, sucrose phosphate synthase; F-2,6-BP, fructose 2,6-bisphosphate; F-1,6-BPase, fructose 1,6-bisphosphatase; SS, sucrose synthase; SST, sucrose sucrose fructosyl transferase.

was paralleled by a doubling in SPS activity, whereas SS and invertase activities declined slightly or remained constant.

Along similar lines, Tognetti and colleagues (35) compared the sucrose and fructan metabolism of four cultivars of wheat differing in freezing tolerance and found a correlation with the ability to accumulate sugars. The greater the sugar accumulation, the greater the apparent hardiness. The ability to accumulate sucrose and fructans was linked to the capacity to increase SPS and SS (35) and SST (14) activities during low-temperature exposure. During deacclimation of wheat, sucrose levels declined along with the activities of SPS, SS, and SST (34). Several groups (7, 19, 20) have shown that the level of SS mRNA (*Ss1*) increased at low temperature in wheat leaf and root tissue. It appears that increased mRNA content is coupled to increased SS protein levels in wheat (7, 20). In contrast, *Ss2* was not responsive to cold shock and the mRNA levels remained constant (19).

Holaday and colleagues (11) surveyed nine enzymes in spinach in a comprehensive study of the influence of low temperature on photosynthetic carbon metabolism. After 10 d at 10°C, Rubisco, F-1,6-BPase, sedoheptulose-1,7-bisphosphatase, phosphoglucoisomerase, SPS, hexokinase, and pyruvate kinase all increased in total activity. Several enzymes, Rubisco, stromal F-1,6-BPase, SPS, and malate dehydrogenase rapidly increased in activation state upon transfer to 10°C from 24°C. These changes in response to low-temperature exposure were considered to be compensatory in nature in an effort to increase the capacity of carbon metabolism to function under conditions causing kinetic constraints (11).

The objective of this study was to examine the influence of low temperature on the activity and synthesis of SPS, the primary biosynthetic enzyme for sucrose, in an effort to clarify the metabolic basis for sucrose accumulation. We show that, at low temperature, not only SPS activity but also subunit abundance and synthesis are all increased.

MATERIALS AND METHODS

Plant Material

Spinach (*Spinacia oleracea*) seed (Stokes Seed Co.) of cv Winter Bloomsdale were sown in a soil mix (Metro-mix, W.R. Grace) and incubated at 25°C for 2 d in the laboratory. Following germination, the flat of seedlings was transferred to a growth chamber. The day/night temperature was 25/25°C, and the photoperiod was for 12 h with an abrupt change from dark to light and light to dark. The irradiance at leaf height was 480 μmol m^{-2} s^{-1} and was provided by incandescent and fluorescent bulbs. The plants were irrigated as needed to avoid the onset of water stress and were fertilized once a week with Hoagland solution. Experiments were initiated when the plants had developed to fourth true leaf stage, about 3 weeks postgermination.

Carbohydrate Analyses

The youngest fully expanded leaves were harvested just before the start of the light period and frozen in liquid nitrogen. The frozen tissue was ground to a fine powder and extracted five times with hot 80% aqueous ethanol. The ethanol was evaporated with a nitrogen stream, the remaining aqueous solution filtered, and the volume adjusted with distilled water. Free sugars were converted to trimethylsilyl derivatives for GLC (33). All samples contained phenyl-β-D-glucopyranoside as an internal standard.

Tissue samples for EM were fixed in 2% glutaraldehyde, 2% formaldehyde in 0.1 M cacodylate buffer, pH 7.2, containing 2 mM $CaCl_2$. Fixed samples were stained with 1% aqueous uranyl acetate.

Radiolabeling

Plants were radiolabeled by applying 10 μL of sterile 0.01% Triton X-100 containing 10 μCi of [^{35}S]methionine to the abaxial side of the same age leaves as used in all other analyses. The label was absorbed through the stomata into the leaf until the droplet evaporated (about 1 h). Label taken up by the leaf was allowed to be incorporated into protein overnight. Leaves were sampled at approximately 8 AM (predawn) the next morning. Unlabeled plants were also sampled at the same time.

Protein Extraction

Proteins were extracted into fresh immunoprecipitation buffer: 50 mM Mops-NaOH (pH 7.5), 10 mM $MgCl_2$, 2.5 mM DTT, 1 mM EDTA, 1 mM methionine, 10% (v/v) glycerol, 0.05% (v/v) Triton X-100. Fresh tissue was weighed and placed in a Ten Broeck glass homogenizer at 0°C. The buffer, in a ratio of 1 mg fresh weight to 5 μL, was added, and the tissue was homogenized. The extract was placed into an Eppendorf tube and centrifuged at 15,000*g* for 10 min at 4°C in a microfuge. The supernatant was removed and the pellet discarded. The amount of TCA-precipitable counts was determined by scintillation counting, and protein concentration was determined by the Bradford method (3). The supernatant was kept on ice until immunoprecipitation was begun.

Immunoprecipitation

Immunoprecipitations were done on samples with equal quantities of tissue or protein from nonradiolabeled tissues or equal TCA-precipitable counts from [^{35}S]methionine-radiolabeled tissues. An SPS-specific monoclonal antibody (36) was used for immunoprecipitations at a ratio of 0.5 mL of culture supernatant per g fresh weight. Immune complexes, following a 2-h incubation at 4°C, were reacted with protein A cells for 1 h at 4°C. Protein A-bound immune complexes were separated from unbound proteins by centrifugation. Protein A-bound material was washed five times with 1 mL of immunoprecipitation buffer. The washed immune complex-protein A pellet was dissolved by boiling for 3 min in 100 μL of SDS extraction buffer. Dissolved proteins were removed from the protein A cells following centrifugation at 42,000*g* for 10 min. Extracts were stored at −20°C. Immunoselected proteins were resolved by electrophoresis through a 10% polyacrylamide SDS gel. After electrophoresis, proteins were stained with Coomassie blue, impregnated with salicylic acid, and dried on Whatman 3 M paper. Fluorography was carried out for 72 h at −80°C using preflashed film. The radioactivity associated with the SPS subunit re-

solved on SDS gels was determined by scintillation counting of the excised band. Because all gels were stained with Coomassie blue, the fluorographic and scintillation data may have underestimated the increased incorporation of label in the acclimated and deacclimated treatments due to quenching.

Enzyme Assay

Extracts were prepared from the youngest fully expanded leaves by grinding frozen tissue in a chilled mortar using a 1:5 tissue to buffer ratio. The extraction buffer contained 50 mM Mops-NaOH (pH 7.5), 10 mM $MgCl_2$, 1 mM EDTA, 5 mM DTT, and 0.1% (v/v) Triton X-100. The crude extracts were centrifuged at 15,000*g* for 30 s, and the supernatants were immediately desalted by centrifugal filtration on Sephadex G-25 columns (1 × 5 cm) equilibrated with extraction buffer minus Triton X-100. SPS was assayed with limiting substrates plus Pi (limiting assay) or with saturating substrates (V_{max} assay). For the limiting assays, reaction mixtures (70 μL) contained 10 mM UDP-Glc, 10 mM Pi (an inhibitor), 3 mM fructose 6-phosphate, 12 mM glucose 6-phosphate (an activator), 50 mM Mops-NaOH (pH 7.5), 15 mM $MgCl_2$, 1 mM DTT, and extract. The V_{max} assay was the same except that Pi was omitted and the concentrations of fructose 6-phosphate and glucose 6-phosphate were 10 and 40 mM, respectively. Reactions were run for 10 min at 25°C and were terminated by addition of 70 μL of 30% (w/v) KOH, followed by a 10-min incubation in a boiling water bath. After the material was cooled, 1 mL of 0.14% (w/v) anthrone in 13.8 M H_2SO_4 was added, and the tubes were incubated at 40°C for 20 min before absorbance was measured at 620 nm.

SS was measured in the synthetic direction by quantitation of sucrose formation from UDP-Glc and fructose using the anthrone method described above. Reaction mixtures were as described for the SPS V_{max} assay, except that 10 mM fructose replaced the hexose-P. Assays for SS, measured in the breakdown direction, and acid and neutral invertase were as previously described (13).

RESULTS

Three major sugars were present in nonacclimated and cold-acclimated spinach leaf tissue, glucose, fructose, and sucrose (Table I). Detectable quantities of raffinose were only found in cold-acclimated tissues. After 14 d at 5°C, the concentrations of the three free sugars showed a steady-state increase in the 10- to 20-fold range, which was similar to published values (17). These were predawn levels and represent what should be the basal levels found in leaf tissue from the various treatments. When cold-acclimated plants were returned to 25°C, the levels of the free sugars were found to be much lower after 7 d. Although all three declined, the greatest relative loss was found in sucrose. In addition to the accumulation of free sugars, EM images showed large increases of chloroplastic starch in the leaves of cold-acclimated plants (Fig. 1).

SPS activity in leaf tissue of cold-acclimated spinach plants showed a clear increase over that found in nonacclimated plants (Table II). The increase observed in spinach leaf tissue in response to 5°C was greater than that found at 10°C (11) and was similar to that reported for *Chlorella* (28) and wheat (35) at low temperature. That the increase in SPS activity was not a universal response of carbohydrate metabolism enzymes in spinach to low temperature was demonstrated by the constancy of SS and invertase activities (Table II). Therefore, the increased SPS activity appears to be rather specific and most like the cold shock response of *Chlorella* (28). Not unexpectedly, increased SPS activity was associated with increased sucrose content in cold-acclimated leaf tissue (Table I). Notably, SS activity in spinach (Table II) was only slightly increased when assayed in the synthetic direction and was not enhanced to the extent found in wheat (34, 35). In other subsequent studies, SS activity assayed in the direction of sucrose breakdown showed no clear trend in response to cold acclimation or deacclimation (not shown). In contrast was the low-temperature response of invertases, for which the activities declined by almost 40%.

Using a monoclonal antibody developed against the native SPS enzyme (36), we immunoprecipitated SPS from leaf extracts of nonacclimated, cold-acclimated, deacclimated spinach. The results of experiments in which immunoprecipitated SPS was resolved by SDS gel electrophoresis and the SPS subunit protein was visualized by Coomassie blue staining are shown in Table III. The relative amount of immunoprecipitated SPS protein for the various treatments was determined by transmission densitometry. The results strongly suggested that the SPS subunit protein was more abundant in cold-acclimated tissue and appeared to correspond well with the observed increase in SPS activity (Table II). This indicated that the increase in SPS activity in tissues exposed

Table I. *Free Sugar Content of the Leaves of Nonacclimated, Cold-Acclimated, and Deacclimated Spinach Harvested at the End of the Dark Period*

Saccharide	Growth Temperature (day/night)								
	25/25°C			25/25 → 5/5°C			25/25 → 5/5 → 25/25°C		
	μmol·g⁻¹ (fresh wt)	%	relative change[a]	μmol·g⁻¹ (fresh wt)	%	relative change	μmol·g⁻¹ (fresh wt)	%	relative change
Fructose	0.42 ± 0.16	10.8	1.0	6.16 ± 1.85	9.7	14.7	1.13 ± 0.99	10.7	2.7
Glucose	0.62 ± 0.27	15.9	1.0	12.94 ± 1.04	20.4	20.1	4.42 ± 4.88	41.9	7.1
Sucrose	2.86 ± 0.01	73.3	1.0	44.26 ± 3.76	69.9	15.5	5.00 ± 0.33	47.4	1.7
Raffinose	ND[b]			Trace			ND		

[a] Standardized to the 25/25°C values. [b] ND, Not detected.

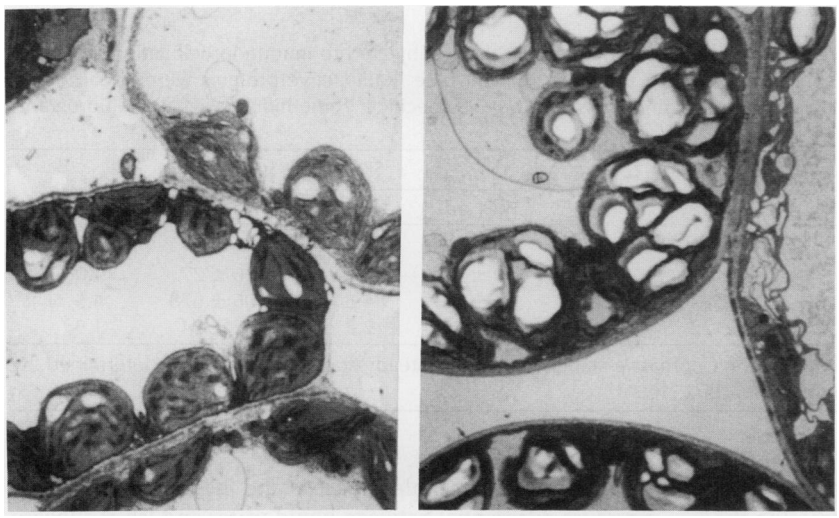

Figure 1. Starch accumulation during exposure to low temperature. Electron micrographs represent leaf mesophyll cells from nonacclimated plants at 25/25°C (left) and 14-d cold-acclimated plants at 5/5°C (right).

to low temperature was associated with a nearly equivalent increase in SPS subunit protein.

When spinach leaf tissue was pulse labeled with [^{35}S] methionine at 25 or 5°C, SPS was radiolabeled. Immunoprecipitation of labeled SPS from extracts containing equal TCA-precipitable counts followed by fluorography of SDS-PAGE-resolved proteins showed a greater percentage of label present in the SPS subunit isolated from labeled plants at 5°C (Fig. 2). Because equal TCA-precipitable counts were subjected to immunoprecipitation, the increase in signal in the SPS subunit in labeled leaf tissue at low temperature suggested that it was being synthesized at a greater rate relative to the total ongoing protein synthesis at 25°C. Unexpectedly, the signal from deacclimated leaf tissue was nearly as strong as that obtained from cold-acclimated tissue and strongly correlated with the elevated SPS activity. Why SPS synthesis remained elevated following cold acclimation is unknown and warrants additional attention. As an additional check on the labeling of the SPS subunit, scintillation counting of the excised 130-kD subunit band from SDS gels confirmed what was evident in the fluorograms (Fig. 2). If we assume no differential effect on turnover of the enzyme, the data indicate that SPS was synthesized at a higher relative rate at low temperature. The increase in labeling at 5°C was between four and five times that of the plants at 25°C. In general agreement with the protein abundance data from Table III, it appears that the increase in protein in leaf tissue at 5°C can be largely explained by increased SPS subunit synthesis.

Plants pulse labeled at different stages of cold acclimation and deacclimation showed that the elevation of SPS synthesis was relatively rapid and stable (Fig. 3). Increased synthesis was observed during the first day of 5°C exposure. Following a 7-d cold acclimation treatment, SPS synthesis remained elevated relative to nonacclimated controls during a 10-d deacclimation treatment.

DISCUSSION

What advantage would sucrose accumulation during low-temperature exposure provide? It could be argued that sucrose is a storage carbohydrate that can be rapidly mobilized as metabolic needs require, and at low temperature, its accumulation is simply for that reason. In this role, sucrose could be rapidly loaded into the translocation stream or quickly used for respiratory energy and anaplerotic metabolite production upon return to more favorable temperatures. One would expect little debate regarding such a contention. The fact that oligosaccharide metabolism seems to depend on sucrose accumulation would also support a role in storage (26). But could an abundance of sucrose provide another benefit? Could the accumulation be a homeostatic response to the reduced kinetic activity of carbohydrate metabolism enzymes at low temperature? Clearly, increasing metabolite concentration might be one way to maintain carbon flux through the various pathways leading to and from sucrose. Utilization of this strategy in plants would suggest that an

Table II. *Influence of Low Temperature on the SPS, SS, and Invertase Activities of Spinach Leaf Tissue Harvested at the End of the Dark Period*

Growth Temperature (°C)	SPS		SS		Soluble Invertase	
	Limiting assay	V_{max} assay	Breakdown	Synthetic	Acid	Neutral
			μmol·g^{-1} (fresh wt)			
25/25	5.8 ± 0.6	50 ± 6	ND[a]	1.8 ± 0.2	4.6 ± 3.0	11.9 ± 2.3
25/25 → 5/5	15.4 ± 2.3	108 ± 7	ND	2.3 ± 0.1	2.9 ± 0.4	7.4 ± 2.0
25/25 → 5/5 → 25/25	17.8 ± 10.5	84 ± 6	ND	1.4 ± 0.4	4.2 ± 1.5	5.6 ± 0.4

[a] ND, Not determined.

Table III. *Quantitative Analyses of SPS Immunoprecipitations*

Equal amounts of tissue extracts, protein, or TCA-precipitable [^{35}S]methionine counts were subjected to immunoprecipitation. Immunoprecipitated proteins were fractionated by SDS-PAGE and stained with Coomassie brilliant blue. Radioactive proteins were visualized by fluorography on preflashed x-ray film. The amount of SPS subunit on stained gels and the SPS-specific [^{35}S]methionine signal on fluorograms was determined by transmission densitometry.

Growth Temperature (°C)	SPS Subunit Abundance[a]				SPS Subunit Synthesis			
	Peak area	Ratio[b]	Peak area	Ratio	Peak area	Ratio	Peak area	Ratio
	g fresh wt		*g protein*		*g fresh wt*		*g protein*	
25/25	11 ± 1	1.0	700 ± 68	1.0	10 ± 1	1.0	426 ± 27	1.0
25/25 → 5/5	28 ± 8	2.5	1500 ± 510	2.1	48 ± 14	4.8	1906 ± 678	4.5
25/25 → 5/5 → 25/25	22 ± 1	2.0	1176 ± 22	1.7	28 ± 2	2.8	808 ± 74	1.9

[a] Steady-state abundance was estimated by densitometry of Coomassie-stained gels, and steady-state synthesis was determined by densitometry of fluorograms. [b] Standardized to the values for 25/25°C.

equally feasible mechanism for dealing with torpid kinetics of certain enzymes at low temperature, that of increasing the amount of the enzyme, would not be sufficient to maintain homeostatic flux (4). However, what little is known about the few enzyme systems in plants acclimated to low temperature would contradict the hypothesis of sucrose accumulation based on a simple kinetic argument (9, 11).

In most cases, enzyme activity seems to be modulated in response to low temperature with increases appearing to result from an increase in the amount of the enzyme (20). Plants may simply overcome the kinetic constraint by making more enzyme. An additional role for sucrose accumulation during cold acclimation is indicated by the fact that sucrose concentration is often correlated with freezing tolerance (18, 27). Justification for a causal relationship between sucrose concentration and freezing tolerance is the well-documented cryoprotectant activity of sucrose (1, 5, 29). As a cryoprotectant, sucrose accumulation would be adaptive and could constitute a pivotal factor in plant hardiness.

Survival of a freeze/thaw cycle, providing intracellular ice crystal nucleation can be avoided, requires a cell to tolerate freeze-induced dehydration (9). Ice typically forms outside of cells in the intercellular spaces, and because of a vapor pressure differential between liquid water and ice at subzero temperatures, water moves out of the cell to the intercellular spaces. Freeze stress unquestionably imposes a water stress. In eukaryotes, striking convergence in osmolyte systems has occurred (37). The major osmolytes are restricted to four classes of low mol wt metabolic products: polyhydric alcohols, free amino acids, amino acid derivatives, and methylamines. Plants subjected to hardening conditions not only exhibit an increase in hardiness but show a substantial parallel change in the osmotic potential of the cell sap (27) that resembles an osmotic adjustment response. The major component responsible for the change in osmotic potential of hardened plants is sugar, and sucrose is often the most abundant sugar (18). Not surprising, sucrose is one of the most universal osmolytes in higher plants (18). In keeping with the dehydrative effect caused by freezing, it is not unexpected that sucrose is the sugar most universally associated with cold acclimation and freezing tolerance. For this reason, we believe there is a strong rationale for an adaptive role on the part of sucrose in freezing tolerance mechanisms. It then logically follows that sucrose metabolism could be a particularly critical component in plant cryobiology.

Would higher SPS activity in plants at low temperature necessarily lead to the accumulation of sucrose? Although the answer is not known, it seems logical that, without an

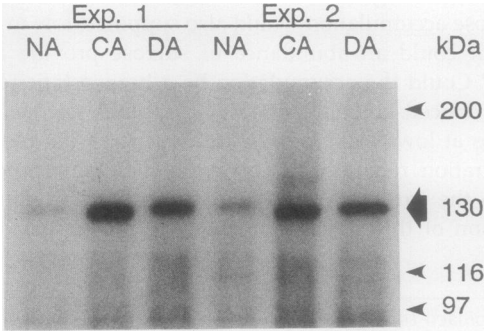

Figure 2. Influence of low temperature on the synthesis of SPS. Radiolabeled proteins were immunoprecipitated with an anti-SPS monoclonal antibody, fractionated by SDS-PAGE, and visualized by fluorography. NA, Nonacclimated, 25/25°C; CA, cold acclimated, 25/25°C → 5/5°C; DA, deacclimated, 25/25°C → 5/5°C → 25/25°C. The duration of 5°C cold acclimation treatment was 14 d and the 25°C deacclimation was 7 d. The arrow at 130 kD is the SPS subunit.

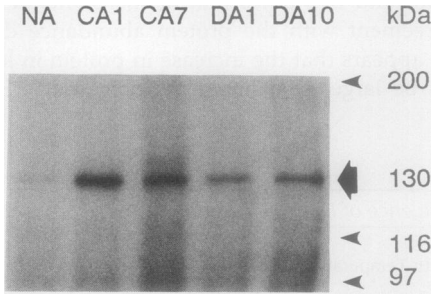

Figure 3. Stability of the elevated synthesis of SPS during cold acclimation and deacclimation. The temperature conditions are the same as in Figure 1. CA1 and CA7 were at 5°C for 1 and 7 d, respectively; DA1 and DA10 were returned to 25°C for 1 and 10 d, respectively.

increase in activity, a given rate of sucrose synthesis could not be maintained under the kinetic constraints imposed by low temperature. However, to drive a given rate of synthesis and the accumulation of sucrose, adequate substrates must be available. Part of the regulation of photosynthesis-driven sucrose synthesis involves fine control by F-2,6-BP (8, 12, 32) and coarse control by modulation of SPS activity (16). When F-2,6-BP concentration is low, the flow of photosynthate is in the direction of sucrose biosynthesis due to relief of the inhibition by F-2,6-BP of F-1,6-BPase and possibly the stimulation of PPi-dependent fructose 6-phosphate phosphotransferase (12). At low temperature, F-2,6-BP concentration decreases dramatically in *Lolium temulentum*, whereas F-1,6-BPase activity increases by 64% (25). Cold storage of pepper fruit has a similar effect on F-2,6-BP (23).

The elevated levels of SPS in leaf tissue exposed to low temperature along with the limited evidence on the effect of low temperature on F-2,6-BP suggests a possible explanation for the increased sucrose levels. If we assume that adequate substrates are available, then greater levels of SPS should, in turn, result in a greater rate of sucrose synthesis. Current evidence is not yet sufficient to determine whether the accumulation of sucrose at low temperature is by design or simply coincidental resulting from the differential influence of low temperature on photosynthate production and utilization (18).

If storage is the primary function of the sucrose accumulation, then why would it be necessary to increase SPS activity? One possibility suggested by Holaday and colleagues (11) is the need for spinach to enhance the capacity of photosynthetic carbon metabolism at low temperature. However, given the marked accumulation of chloroplastic starch, it seems possible that some part of the starch breakdown pathway might be impaired by low temperature, preventing the rapid flow of carbon back into trioses for export to the cytoplasm during the night. Alternatively, starch may have accumulated in spinach leaf chloroplasts because of the massive buildup of free sugars (Table I), which presents a paradox. Which came first at low temperature, sugar or starch accumulation? Our experimental plan did not provide an answer to this question. In any case, it appears that in spinach, sugars are not accumulated as a consequence of starch breakdown but result from a reduction in growth utilization of photosynthate in concert with starch accumulation. The findings of Boese and Huner (2) support this mechanism. They observed an approximate 3-fold reduction in growth kinetics of spinach when the temperature was decreased from 16 to 5°C but less than a 50% reduction in light-saturated CO_2 exchange. Therefore, photosynthate should be produced in excess of growth requirements. In this respect, the accumulation of both starch and sugars at low temperature in spinach appears to behave more like that in citrus (38) than like that in other temperate species (27, 31). Clearly, the prospect that low-temperature exposure leads to a marked accumulation of both starch and sucrose merits further investigation.

Exposure to low temperature leads to an increase in SPS activity in spinach leaf tissue (this report and ref. 11). Because the increase in V_{max} activity is paralleled by increases in SPS subunit abundance and synthesis, it is clear that the low-temperature response is not simply an alteration in the activation state of the enzyme. Instead, the change in SPS activity stems from increased production of the SPS subunit. Whether the increase in SPS synthesis results from increased mRNA abundance, as seems to be the case for wheat SS (7, 19, 20) or by another mechanism remains to be determined. The increased SPS enzyme and activity associated with the accumulation of sucrose suggests that the response is not simply an adjustment of this metabolic process to overcome the slower enzyme kinetics at low temperature. Rather, it is probably an adaptive process to maximize synthesis of a compound with important cryoprotectant properties for the purpose of increasing tolerance to freezing stress.

NOTE ADDED IN PROOF

It has come to our attention that the work of Koster and Lynch (Plant Physiol [1992] **98:** 108–113) shows most of the sucrose present in cold accumulated rye leaf protoplasts to be localized in extravacuolar compartments.

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