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SOLUBLE-STARCH-SYNTHESIZING AND SUCROSE-DEGRADING ENZYMES IN *ONOCLEA* SPORES

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Key Word Index—*Onoclea sensibilis*; Onocleaceae; fern spores; light effects; soluble starch anabolic enzymes; soluble sucrose catabolic enzymes.

Abstract—Sucrose degradation and starch accumulation in imbibed spores of *Onoclea sensibilis* occur slowly in the dark and are accelerated two- to four-fold when irradiated with low fluence of red light. The accelerated rates of starch accumulation and sucrose degradation begin 4–6 hr after irradiation and precede the completion of germination. The effect of 5 min of red irradiation on the extractable activities of the enzymes involved in starch and sucrose metabolism was investigated as a possible means by which light accelerates the flux of metabolites through these pathways. The accumulation of starch from exogenous sucrose in unirradiated spores and the specific radioactivity of newly synthesized starch in irradiated and unirradiated spores were determined to see if the lack of sucrose degradation and starch synthesis in unirradiated spores is due to limited availability of substrates. Irradiation did not increase the maximum catalytic activities of the soluble starch synthesizing or soluble sucrose degrading enzymes during the 24 hr period following irradiation when compared with those in the unirradiated spores. Incubation of unirradiated spores on exogenous sucrose increased starch accumulation to a level comparable to that in irradiated spores incubated on distilled water suggesting that enzymes for sucrose catabolism and starch anabolism are active *in situ* in unirradiated spores. Irradiation caused a decrease in the specific radioactivity of starch without decreasing the incorporation of label. Therefore, light regulation of starch synthesis and sucrose degradation in *Onoclea* spores is not a result of enhanced activity of the soluble enzymes investigated. With respect to starch synthesis, irradiation causes an increase in availability of substrates that can be utilized for starch synthesis.

INTRODUCTION

Dehydrated spores of *Onoclea sensibilis* L. contain large amounts of sucrose, proteins and lipids, and minute amounts of free reducing sugars and starch [1]. Hydrated spores maintained in the dark slowly mobilize sucrose and accumulate starch without any measurable changes in the storage proteins or lipids [1]. When hydrated spores are irradiated with low fluences of light, sucrose degradation and starch accumulation are accelerated. Red light is the most efficient in causing these effects but shorter and longer wavelengths are also effective if high enough fluences are given [2]. The same light regime is responsible for induction of germination of *Onoclea* spores [2].

Changes in the rates of starch synthesis and sucrose degradation in other systems [3] have been attributed to changes in the activities of the enzymes involved in sucrose degradation and in starch synthesis. Sucrose can be mobilized by sucrose synthetase (EC 2.4.1.13) or invertase (EC 3.2.1.26). Starch is synthesized from G-1-P via ADPG pyrophosphorylase (EC 2.7.7.29) and starch synthetase [4]. Starch phosphorylase (EC 2.4.1.1) in certain situations [3] may also be important in starch biosynthesis. Although the predominant role of UDPG pyrophosphorylase (EC 2.7.7.9) is to supply UDPG to the nucleotide–sugar pools [5], in some systems exhibiting starch–sucrose interconversions this enzyme may function in supplying G-1-P needed for starch synthesis [3, 5].

In this paper we report the maximal *in vitro* activities of the aforementioned soluble enzymes in irradiated and unirradiated spores over a 24 hr period. We also report the effects of both tracer amounts of [U-^{14}C]sucrose and exogenous sucrose concentration ranging from 30 to 140 mM on the specific radioactivity of starch and on the synthesis of starch in unirradiated spores, respectively.

RESULTS

Starch accumulation and sucrose degradation

Accelerations of both sucrose degradation and starch synthesis occur as a result of irradiation (Fig. 1). Statistical analysis indicates that the increase in starch content 24 hr after irradiation and the decrease in sucrose levels 12 hr after irradiation were highly significant ($F_{1,24} = 6.75$; $p < 0.025$ and $F_{1,12} = 60.24$; $p < 0.001$, respectively). It should be noted that the accelerations begin 3–4 hr after the termination of irradiation and precede by 14–18 hr the onset of cell division, the first sign of germination [1]. Thus, sucrose degradation and starch synthesis are not affected directly by light and are not associated with the growth process. These time courses were consistent with those of previous investigations [1].

Activities of soluble-sucrose-degrading and starch-synthesizing enzymes

The effects of the inductive light regime on the maximum extractable catalytic activities of the soluble cata-

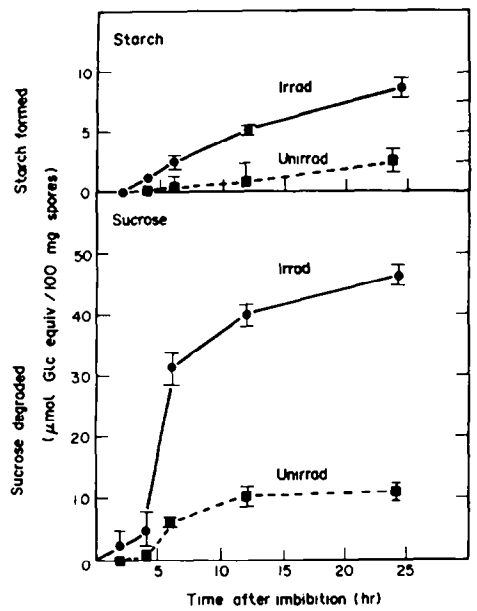

Fig. 1. Amount of starch accumulated and sucrose degraded in unirradiated and irradiated *Onoclea* spores. Spores were imbibed in distilled water in the dark for 24 hr. One set of spores was then irradiated with 5 min of red light (38 mW/m^2) at 0 hr and placed back in the dark until collection. Unirradiated spores were maintained in the dark for comparable time periods.

bolic and soluble anabolic enzymes were investigated. Extraction buffers were designed to optimize enzyme activities in the subsequent assays. Buffers used to extract neutral invertase, sucrose synthetase, ADPG pyrophosphorylase and starch phosphorylase contained dithiothreitol (DTT). DTT has been shown to activate certain enzymes which are activated *in situ* by light [6]. To see if DTT affected the activity of the above enzymes, unirradiated spores were extracted with buffers containing no reducing agent, 0.5 mM reduced glutathione, 0.5 mM mercaptoethanol or 0.5 mM DTT and the activities of the enzymes compared. Extraction and assays were done under dim green safelight. The results indicated that DTT did not enhance the activities of the enzymes compared with those of the other reducing agents but did enhance the activities to 5–10% when compared to the extraction buffer without the reducing agent. Similarly, the presence of the reducing agents enhanced the activities of enzymes from irradiated spores to a comparable extent. DTT did not enhance the level of *in vitro* incorporation of ^{14}C from sucrose into starch.

The enzyme assays were optimized in extracts from irradiated spores which were exhibiting maximal rates of sucrose degradation and starch synthesis (6 and 12 hr after irradiation, respectively) and from unirradiated spores incubated in the dark for time periods comparable to that of irradiated spores. The assays were optimized for pH and for the concentration of substrates and effector molecules. The activity of each enzyme was linear with respect to time and to the amount of extract utilized.

The amount of protein in extracts from unirradiated spores were comparable to that in extracts from irradiated spores at every time point. Therefore, any differences in the specific activities (μmol of product/mg protein/min) of the enzymes between irradiated and unirradiated spores reflect changes in the extractable activities rather than changes in total protein.

The activities of sucrose synthetase and soluble acid invertase in extracts from irradiated and unirradiated spores over a 24 hr period after imbibition are given in Table 1. There are no statistically significant differences in the specific activities of either of these sucrose-degrading enzymes as a result of irradiation. The activity of soluble acid invertase remains high over the time course compared with that of sucrose synthetase. Extraction of invertase with either citrate phosphate buffer at pH 5.0 or Tris–HCl buffer at pH 6.9 and subsequent assays at varying pH revealed a major peak of activity at pH 4.5 and a minor peak of activity *ca* pH 6.5. The activity of the invertase at the neutral pH was comparable to that of sucrose synthetase and was similar in extracts of irradiated and unirradiated spores.

The specific activities of UDPG and ADPG pyrophosphorylases and ADPG starch synthetase over the 24 hr incubation period are given in Table 2. At any given time during the 24 hr period, there were no statistically significant differences in the activities of any of these enzymes in extracts from irradiated and unirradiated spores. The activities of the pyrophosphorylases are similar and are less than the activity of ADPG starch synthetase. The activity of starch phosphorylase was found to be negligible throughout the time course of the experiment.

Utilization of exogenous sucrose for starch synthesis

The lack of differences in the *in vitro* activities of the starch-synthesizing and sucrose-degrading enzymes between irradiated and unirradiated spores suggests that photoenhancement of sucrose degradation and starch accumulation is not a result of irradiation induced changes in enzyme activities. However, another possibility is that the activities of one or more enzymes are inhibited *in situ* in unirradiated spores and that extraction releases them from inhibition.

Table 1. Specific activities of soluble acid invertase and sucrose synthetase in extracts from unirradiated and irradiated *Onoclea* spores

Time after imbibition (hr)	Specific activity (μmol Glc eq/mg protein/min)			
	Soluble acid invertase		Sucrose synthetase	
	Unirrad	Irrad	Unirrad	Irrad
0	109 (8)	99 (16)	3.6 (0.5)	0.9 (0.4)
4	115 (30)	131 (36)	3.5 (0.7)	3.8 (0.4)
6	115 (30)	113 (26)	2.1 (0.2)	3.9 (0.7)
24	117 (19)	120 (13)	2.0 (0.3)	2.1 (0.5)

Spores were imbibed on distilled water in the dark for 24 hr. One aliquot of spores was then irradiated with 5 min of red light (38 mW/m^2) and placed back in the dark until they were collected. Unirradiated spores were maintained in the dark for comparable period of time. Standard errors given in parentheses. $n = 4$.

Table 2. Specific activities of soluble forms of ADPG pyrophosphorylase, UDPG pyrophosphorylase and ADPG-starch synthetase in extracts from unirradiated and irradiated *Onoclea* spores

Time after imbibition (hr)	Specific activity (nmol product formed/mg protein/min)					
	UDPG PPiase		ADPG PPiase		ADPG-starch synthetase	
	Unirrad	Irrad	Unirrad	Irrad	Unirrad	Irrad
0	46.0 (12.9)	37.1 (3.4)	64.9 (10.3)	29.7 (10.3)	211 (50)	293 (51)
2	31.8 (7.5)	25.7 (9.2)	80.1 (20.1)	56.4 (15.1)	311 (30)	515 (52)
4	34.3 (10.3)	29.2 (9.5)	70.9 (25.0)	70.9 (23.5)	307 (65)	366 (63)
6	42.8 (10.8)	44.6 (1.1)	67.5 (21.5)	67.6 (17.1)	222 (43)	403 (82)
12	20.4 (4.4)	27.7 (7.6)	32.2 (15.5)	47.7 (13.5)	—	—
24	25.7 (—)	43.5 (—)	59.6 (—)	90.0 (25.0)	268 (62)	228 (54)

Experimental protocol as described in Table 1.

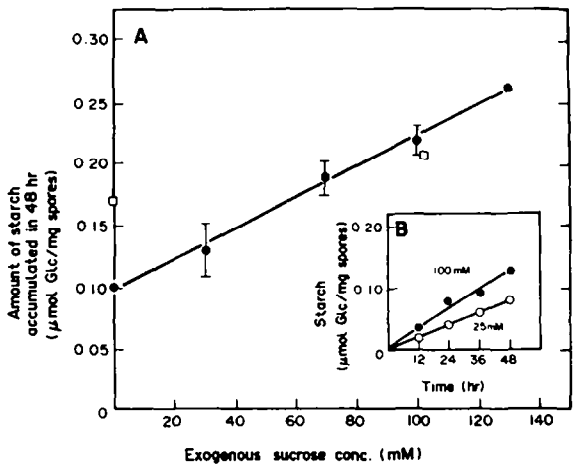

Fig. 2. Amount of starch accumulated in unirradiated *Onoclea* spores incubated on exogenous sucrose. (a) Spores were sown on sucrose concentrations ranging from 30 to 140 mM and incubated in the dark for 48 hr. Open squares represent aliquot of spores that received 5 min of red light after 24 hr of incubation. (b) Spores were sown on either 25 or 100 mM sucrose and incubated in the dark for varying periods of time.

To see if unirradiated spores were able to catabolize sucrose and utilize the breakdown products for starch synthesis, spores were incubated on concentrations of sucrose ranging from 30–140 mM for a 48 hr period in the dark. Two aliquots of spores, one maintained on distilled water and the other on 100 mM sucrose, were irradiated with 5 min of red light in the middle of the dark period.

Figure 2a shows that there is a linear increase in starch content with increasing concentrations of sucrose and that the amount of starch that can accumulate in unirradiated spores incubated on high concentrations of sucrose is comparable to that which accumulates in irradiated spores incubated on distilled water. The increase in starch is linear for at least 48 hr of incubation on exogenous sucrose (Fig. 2b). Spores which are first incubated on distilled water to activate the enzymes and to develop photosensitivity and then either irradiated or transferred to 120 mM sucrose accumulated during a 16 hr period comparable amounts of starch (0.089 and 0.112 μmol Glc/mg spores, respectively). These results indicate that there are functional sucrose-degrading and starch-synthesizing enzymes present in unirradiated spores which are operating at rates comparable to those in irradiated spores.

Specific radioactivity of newly synthesized starch

If the starch synthesizing enzymes are functional in unirradiated spores, then the lack of starch accumulation in spores maintained in the dark may be due to a limited availability of precursors for starch synthesis. Irradiation then would increase the availability of starch precursors.

The specific radioactivity of newly synthesized starch was compared in irradiated and unirradiated spores to see if irradiation increased the availability of starch precursors. Spores were incubated in tracer amounts of [U-^{14}C]sucrose for 8 or 12 hr after imbibition and the amount of starch that accumulated and the amount of label in the starch during those periods were determined. Tracer amounts were employed so that endogenous pools would not be perturbed. Red irradiation was given at the time of label addition. The results shown in Table 3 indicate a 70–75% decrease in the specific radioactivity of starch as a result of irradiation. Since the amount of label incorporated into starch is the same for both irradiated and unirradiated spores, the decrease in starch specific radioactivity is due to a greater increase in starch content in the irradiated spores. Similar decreases in the specific radioactivity of starch as a result of irradiation have been observed using tracer amounts of [2-^{14}C]acetate, [U-^{14}C]glucose and [U-^{14}C]alanine. Thus, irradiation causes an increase in the availability of an unlabelled pool utilized for starch synthesis.

DISCUSSION

Irradiation stimulates an enhancement in the rate of sucrose degradation in *Onoclea* spores relative to that of the unirradiated controls. Our data indicated that this

Table 3. The amount of label incorporated into, and the specific radioactivity of, starch in unirradiated and irradiated *Onoclea* spores

Tracer	Time of incubation (hr)	Unirrad (−) or irrad. (+)*	Newly synthesized starch† (μmol Glc)	(dpm/μmol Glc × 10^{-3})	% Reduction of spec. radioactivity due to irrad
[U-^{14}C]Sucrose	8	−	0.50	3.24	70%
		+	1.60	0.96	
[U-^{14}C]Sucrose	12	−	0.55	15.6	75%
		+	1.98	3.9	
[2-^{14}C]Acetate	8	−	0.77	3.39	74%
		+	2.06	0.87	
[U-^{14}C]Alanine	20	−	0.19	23.8	79%
		+	1.02	5.0	
[U-^{14}C]Glucose	12	−	0.26	2.29	58%
		+	2.02	0.96	

*15 min red irradiation given at time of label addition.

†Amount of starch present at time of label addition is subtracted from the total amount of starch accumulated after label addition.

enhancement could not be accounted for by an increase in the activities of soluble acid invertase or sucrose synthetase as the activities of these enzymes were similar in extracts from both irradiated and unirradiated spores during the 24 hr period after imbibition. This is in contrast to the situation in buds of etiolated peas [7] and in hypocotyls and cotyledons of radish seedlings [8] in which light via phytochrome has been shown to increase the activities of both acid and neutral invertases. Additionally, the transfer of invertase from the cytoplasm to cell wall has been shown to be under phytochrome control [9, 10]. Preliminary experiments with the pellet fraction from the crude extract of irradiated and unirradiated spores revealed the presence of invertase activity which could be solubilized with Tween 20 or Triton X 100. However, there was no difference in the activity of released enzyme nor in the residual activity retained in the pellet between irradiated and unirradiated spores. Therefore, the extent of bound acid invertase is probably not the explanation for the enhanced rate of sucrose degradation in irradiated spores.

Results of this investigation revealed two features of sucrose catabolism in *Onoclea* spores which are unusual. Firstly, in many systems high acid invertase activity is usually associated with cell expansion or other processes in which tissues require hexoses for maintenance of rapid metabolism [11]. Unirradiated spores and irradiated spores prior to 24 hr after irradiation are not expanding. Vogelmann and Miller [12] have shown that the first cell division and subsequent expansion of the cells does not occur until 36 hr after the spores have been sown. Secondly, in most systems investigated [13] high acid invertase activity is correlated with low levels of storage sucrose. In unirradiated *Onoclea* spores, a co-existence of high levels of sucrose (6–12% of the dry weight) and apparent active acid invertase is observed.

One possible explanation for the latter observation is that the activity of acid invertase in unirradiated spores is inhibited *in situ* and that extraction releases the enzyme from that inhibition. Invertase inhibitors have been described for several plant systems [14, 15] and the inhibitor can be inactivated upon extraction [16]. However, extraction of unirradiated spores by techniques which inactivate the invertase inhibitor [16] did not reduce the activity of the enzyme. Further evidence for active sucrose catabolic enzymes in unirradiated spores is the observation that exogenous sucrose provides carbon skeletons for starch synthesis (Fig. 2). Since exogenous sucrose provides the carbon skeletons for not only starch synthesis but also lipid synthesis and respiration [17], the rate of degradation of exogenous sucrose in unirradiated spores is most likely comparable to the rate of degradation of endogenous sucrose in irradiated spores. Therefore, the more plausible explanation for the lack of catabolism of sucrose in unirradiated spores is sequestration of sucrose and the sucrose-degrading enzymes in different compartments.

The pathway by which starch is formed in *Onoclea* spores is via ADPG pyrophosphorylase and starch synthetase as starch phosphorylase activity was negligible throughout the 24 hr period. The activity of ADPG pyrophosphorylase in *Onoclea* spores corresponds to levels found in leaf tissues [18] and maize pollen [19] but is lower than that reported for starch storing tissues [20].

The activities of the aforementioned enzymes are similar in irradiated and unirradiated spores (Table 2). Further, the accumulation of starch from exogenous sucrose in unirradiated spores is comparable to that accumulated in irradiated spores in the same time period. This indicates that the starch-synthesizing enzymes are as active *in situ* in unirradiated spores as in the irradiated spores. In contrast, there is a decrease in specific radioactivity of starch extracted from irradiated spores relative to that extracted from unirradiated spores (Table 3). The decrease in specific radioactivity is not due to a decrease in ^{14}C incorporated into starch but rather from an increase in incorporation of unlabelled carbons. These results indicate that irradiation stimulates starch synthesis by increasing the availability of substrates used for starch synthesis and not by enhancing the activities of either

ADPG pyrophosphorylase or starch synthetase.

EXPERIMENTAL

Plant material and experimental protocol. Onoclea sporophylls were collected in December 1980, from the location described in Towill and Ikuma [21], and the spores harvested and stored as described therein. For the enzyme extractions 250 mg of spores were sown under dim green safelight on 500 ml of glass distilled water in a 20 cm crystallizing dish and placed in light-tight drawers at room temp. for 24 hr to develop maximum photosensitivity. The spores were irradiated with 5 min of broad band red light (38 mW/m^2) and then placed in the dark until collection under dim green safelight at various times over a 24 hr period following light induction. Control spores were maintained in continuous darkness for comparable periods of time. After collection, the spores were immediately frozen and maintained at −4° until lyophilization.

For experiments employing exogenous sucrose, 10 mg spores were sown on 10 ml sucrose at concentrations ranging from 30 to 140 mM in a 5 cm Petri dish. Spores were maintained in the dark on these concentrations for varying time periods until collection. Spores from 3 Petri dishes were pooled for each treatment. Collected spores were air dried, weighed and then immediately utilized for carbohydrate determinations.

For experiments measuring the incorporation of ^{14}C into starch, multiple 5 cm Petri dishes each containing 10 mg spores on 10 ml distilled H_2O were maintained in the dark for 24 hr. At that time, one aliquot of spores (3 Petri dishes) was collected in order to determine the starch content present at the time of label addition. From the remaining Petri dishes, the water was withdrawn by aspiration and repaced by 10 ml tracer amounts of [U-^{14}C]sucrose (552 mCi/mmol), [U-^{14}C]glucose (250 mCi/mmol), [2-^{14}C]acetate (58.7 mCi/mmol) or [U-^{14}C]alanine (174.0 mCi/mmol). One aliquot of spores (3 Petri dishes) for each tracer was irradiated with 5 min of red light at the time of label application. A second aliquot of spores (3 Petri dishes) for each tracer was maintained in the dark for a comparable period of time. After collection, spores were air dried, weighed and immediately utilized for starch determination.

Viability of the light-treated and control spores was checked by retaining a small aliquot of the spores for the determination of the extent of germination. Germination was scored if the rhizoid or protonema protruded through the spore coat. Throughout this study, the extent of germination as a result of irradiation ranged between 60 and 80%; for any given time course, germination values varied less than 5%. Dark germination ranged from 5 to 15%. Light sources and spectral emission characteristics have been described previously [21].

Carbohydrate determination. The starch extraction procedure followed the method of Towill and Ikuma [1], and reducing sugars and sucrose were extracted from the spores by homogenization in hot 70% EtOH. The EtOH extract was evaporated to dryness and the residue dissolved in 1.0 ml H_2O. Reducing sugars and sucrose content in the solubilized residue was analysed as previously described [1].

To determine the incorporation of ^{14}C into starch, the starch purification and solubilization procedures were modified. Starch precipitated according to the procedures described in [1] was washed successively with 3.5 ml ethanolic NaCl (70% EtOH v/v and 2% NaCl w/v) and 5 ml of ethanolic NH_4OH (70% EtOH v/v and 2.4 N NH_4OH) without disrupting the pellet. The purified starch pellet was dried at 95° and then hydrolysed by refluxing in 1 M HCl in a boiling H_2O bath for 4 hr. The HCl soln was neutralized with 1 M NaOH. One aliquot of the neutralized soln was assayed for radioactivity using a liquid scintillation procedure to determine the incorporation of ^{14}C into starch. A second aliquot was analysed for glucose content by the Somogyi–Nelson procedure using glucose as the standard [22].

Enzyme extraction and analysis. Lyophilized spores were ground in 7 ml, Ten-Broeck tissue grinders with appropriate buffer as listed below. Polyclar AT was added to minimize effects of phenolic compounds [23] and DTT or GSH were used as reducing agents. Preliminary experiments indicated that 2 extractions were required to completely homogenize the spores and to maximally release soluble enzymes. After the final centrifugation, the supernatant was assayed after desalting by CC on Sephadex G-25 in the cold using the extraction buffer as the eluant. Initial experiments determined the amount of enzyme required for maximal catalytic activity relative to substrate, the effector molecule concentrations and the optimal pH under the assay conditions. For each enzyme assay, a reaction mixture containing the boiled extract was included to accommodate for nonspecific binding in the case of the radioactive assays or for the presence of endogenous products in the extract. Assays were run at 37° in duplicate, and the data presented represent the mean of at least 4 experiments.

Results are presented as μmol of product formed/mg protein/min. Protein content was determined by the method of Lowry *et al.* [23], using BSA as a standard. Radioactive products were counted by liquid scintillation spectrometry.

ADP-glucose pyrophosphorylase and UDP-glucose pyrophosphorylase. The extraction procedure followed the method of Hannah and Nelson [24]. 100 mg of lyophilized spores were ground for 5 min in a total vol. of 3.0 ml of 30 mM Pi buffer (pH 7.0) with 0.5 mM EDTA, 5 mM $MgCl_2$ and 0.1 mM DTT. The supernatant obtained from centrifugation of the extract at 39 000 *g* for 20 min was passed over a G-25 Sephadex column and then used for analysis of the activities of ADPG and UDPG pyrophosphorylases. Minimal activities of these enzymes were found in the pellet fraction. ADPG pyrophosphorylase was measured following the method of Hannah and Nelson [25] using 5 μl of the G-25 eluate and 2 μmol of 3-PGA in a final vol. of 0.1 ml. UDPG pyrophosphorylase was assayed by the above method with the following differences: UTP replaced ATP; 10 μl of G-25 eluate was used in the reaction mixture; and 3-PGA was eliminated.

Starch synthetase. Soluble ADPG-starch synthetase was extracted with 0.1 M Pi buffer (pH 7.5) containing 0.1 M EDTA and 0.005 M GSH. The enzyme activity was assayed according to the method of Ozbun *et al.* [26] which measured the incorporation of the radioactive glucosyl moiety of ADP-[^{14}C]glucose into soluble potato starch primer. A modification of a method developed by Thomas [27] for measuring the extent of incorporation of glucose into glycogen was used to analyse the radioactive product formed. The reaction mixture is adsorbed onto a 2 cm square of Whatman 31 ET paper. The papers are air dried and then washed twice in 75% MeOH (10 ml/paper) followed by a single rinse in 100% Me_2CO. Papers were again air dried and counted.

Starch phosphorylase. Starch phosphorylase was extracted using a modification of a procedure described by Downton and Hawker [18]. 100 mg of spores were homogenized in 1.5 ml 30 mM Tris–HCl (pH 6.5) containing 2 mM DTT and 50 mM EDTA; the homogenate was centrifuged at 300 g for 10 min, and the pellet resuspended in 1.5 ml extraction buffer and treated as above. The supernatants from these two low speed spins were combined and centrifuged at 30 000 *g* for 10 min. Activity in the supernatant was assayed in the direction of synthesis by measuring the incorporation of the [^{14}C]glucosyl moiety of G-1-P into a soluble potato starch primer. The reaction mixture consisted of 25 μl G-25 eluate, 5 μmol NaF, 1 μmol [^{14}C]G-1-P

(0.2 μCi/μmol) and 10 μmol HEPES buffer (pH 6.7) containing 1.0 mg heat treated soluble potato starch/ml in a total vol. of 0.1 ml. After incubation for 15 min, the radioactive product was analysed as described above for the starch synthetase.

Acid invertase. To extract soluble acid invertase 100 mg of spores were homogenized in 1.0 ml 50 mM citrate phosphate buffer (pH 5.0), and the homogenate centrifuged at 900 *g* for 15 min. The pellet was resuspended in 1.0 ml of the extraction buffer, re-homogenized, and centrifuged as above. The combined supernatants were centrifuged at 30 000 *g* for 15 min. After G-25 Sephadex de-salting, a 0.1 ml aliquot of the 30 000 *g* supernatant was incubated for 1 hr with 0.9 ml of 50 mM citrate–phosphate buffer (pH 5.0) containing 0.1 M sucrose. Reducing sugars released were assayed using the Somogyi–Nelson method [22].

Sucrose synthetase. Spores were homogenized with 3.0 ml 30 mM Tris–HCl buffer (pH 6.9) containing 0.1 mM DTT and 10 mM EDTA. The extraction procedure followed that for the soluble acid invertase except the final centrifugation was at 30 000 *g* for 30 min. In each of 4 test tubes, 0.3 ml of the G-25 eluate was combined with 0.25 μmol sucrose, 10 μmol NaF, and 50 μmol Tris–Pi buffer (pH 7.5) in a total volume of 1.0 ml. In two of the tubes 5 μmol of UDP were added to the reaction mixture. The reactions were allowed to proceed for 1 hr at 37° and were stopped by boiling for 1 min. Reducing sugars released from sucrose were determined by the Somogyi–Nelson method [22]. Invertase activity was estimated from the reducing sugar content in the reaction mixtures lacking UDP, whereas sucrose synthetase activity was determined from the difference in concentration of reducing sugars between the reaction mixture with and without UDP.

Statistical analysis. Data were analysed statistically using the two factor analysis of variance [27]. This test allows the simultaneous testing of the effects of the two experimental variables, time of incubation and light treatment, and of their interaction. Only statistically significant ($p < 0.05$) F ratios are given.

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