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Heat Inactivation of Starch Synthase in Wheat Endosperm Tissue

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ABSTRACT

The effect of temperature on accumulation of starch was studied in grain slices of wheat (*Triticum aestivum* cv SUN9E), taken 15 days after anthesis. As compared with pretreatment of such slices at 25°C, pretreatment at 30 or 35°C reduced the subsequent conversion of sucrose to starch. In contrast to rice (*Oryza sativa* cv Calrose), pretreatment of wheat soluble starch synthase *in vitro* at 30°C or higher temperatures reduced its activity. In zymograms using nondenaturing polyacrylamide gel electrophoresis followed by activity staining, the slowest migrating band represented the most temperature sensitive isozyme. Although preincubation of a soluble enzyme sample *in vitro* at 25°C did not result in loss of starch synthase activity, it did result in a gradual shift of zymogram banding pattern toward faster migrating species. Pretreatment of isolated starch granules at 40°C increased their bound starch synthase activity. Both soluble and bound enzymes in the grains of whole wheat plants lost activity when the plants were held above 30°C for 30 minutes or longer. Both activities lost from the grains after a 1 hour treatment at 37°C were restored in 1 to 2 days by a return to 21°C. In slices, inactivation of the soluble starch synthase was increased by incubation with 2,4-dinitrophenol. It is tentatively suggested that *in vivo* heat inactivation of soluble starch synthase may be a direct effect of heat on the enzyme protein and that of bound enzyme an indirect effect involving metabolic factors.

In wheat the optimum temperature, of plant culture for dry weight per grain at maturity occurs at a day/night temperature of 15°C/10°C (4). Increasing the temperature of the ear independent of the rest of the plant from 15 to 20°C and 25°C has resulted in an increased growth rate of the ear but this was accompanied by earlier senescence and a shorter duration of growth (6). Ear warming at 33°C/25°C (day/night) in comparison with 21°C/16°C, resulted in a reduction of grain growth rate after the 6 d warming period (1). From these data, it would be expected that at a suitably high temperature such a reduction in rate could be observed earlier and analyzed more directly. Also it has been suggested that for maize kernels grown *in vitro*, the adverse effects of high temperature on starch synthesis during the grain-fill period were due to deficiencies in the synthetic process rather than in the supply of substrate (8). In the following experiments grain slices, incubated in sugar solutions, have been used to investigate the temperature sensitivity of starch accumulation in developing wheat grains. Further, since starch synthase is the final specific link in the conversion of sugar to starch, effects of temperature on its stability *in vivo* and *in vitro* have also been investigated.

MATERIALS AND METHODS

The present procedures are based on those described elsewhere (8, 9).

Plant Material. Plants of *Triticum aestivum* cv SUN9E (and of *Oryza sativa* cv Calrose) were grown at 21°C/16°C and 27°C/22°C, respectively (8 h day/16 h night) in natural daylight in the CSIRO Phytotron (CERES) in Canberra. Ears of wheat were taken approximately 15 d after anthesis to provide grains in the 40 to 60 mg fresh weight range and ears of rice at a green stage with grains of approximately 18 mg fresh weight. In some experiments, plants were transferred to temperature controlled growth cabinets, lit artificially and continuously at 550 μmol m^{-2} s^{-1}.

Preparation and Processing of Wheat Endosperm Slices. The procedure was as described in Rijven and Gifford (14). The 0.9 mm slices, 10 per replicate, were incubated in scintillation vials with 500 μl of fresh aqueous medium including [U-^{14}C]sucrose, in a water-bath shaker and thereafter extracted exhaustively (14).

Estimation of Starch Production. Starch was estimated essentially as described before (14), incubating each replicate with 0.25 mg porcine pancreatic α-amylase and with 1 mg *Rhizopus niveus* glucoamylase (7) (instead of β-amylase) for 0.5 h at 40°C and 2.5 h at 50°C in an acetate buffer at pH 4.8. The 70% ethanol-soluble fraction formed was deemed to represent starch. As a control, label in starch granules, incorporated in a starch synthase reaction as described below, was 96 to 100% solubilized by the above incubation. Label in the insoluble residue was solubilized by acid hydrolysis (14).

Starch Granule Isolation and Enzyme Preparation. The grains were cut across at the basal end to remove the embryo. The starchy endosperm, uncontaminated by other tissue, was squeezed out by pressure exerted judiciously by the tip of the index finger and collected in PEG-1000 buffer (pH 7.4) that consisted of 300 mM (30%, w/v) polyethyleneglycol mol wt 1000 (PEG-1000), 25 mM Tes-KOH, pH 7.4, 50 mM KCl, 1 mM DTT. Starch granules were freed from the endosperm cells by chopping in an ice-cooled container for 1 to 1.5 min with a modified electric knife. The brei was filtered on ice through one layer of Miracloth with enough PEG-1000 buffer to give 5 endosperm equivalents of granules per ml. Triton X-100 was added to make 1.25% (v/v). The suspension was centrifuged at 4000*g* for 10 min at 2°C and the pellet twice washed, thereby removing an inhibitor and enzyme activity involved in cell wall synthesis (13). The pellet was resuspended in buffer without PEG (designated 'No PEG') to give a suspension equivalent to 4 endosperms (or 20 slices) ml^{-1}. Subsequent centrifugation separated the bound enzyme in the pellet from the soluble enzyme in the supernatant (13). The pellet was washed in No PEG buffer, and a gluten agglomerate was removed and discarded. The presence of an inhibitor in the crude extract prevented the estimation of enzyme recovery from the tissue (13).

In the case of rice, whole grains (husks removed) were chopped

in PEG buffer and the filtrate through Miracloth processed as above to the No PEG stage at 15 grains ml^{-1}.

Partial Enzyme Purification. Further purification of the soluble starch synthase was achieved by high speed centrifugation, (1 h at 160,000*g*) and $(NH_4)_2SO_4$ precipitation (0–30% saturation). The dialyzed 0 to 30% $(NH_4)_2SO_4$ fraction was mixed with 0.5 g of washed DEAE-cellulose equilibrated with 10 mM Tris-acetate buffer, pH 7.8, and the enzyme eluted between 80 and 200 mM KCl by centrifugation.

Assay for Enzyme Activities. The standard starch synthase assay of 30 min at 25°C contained PEG-1000 (180 mM), Tes-KOH (pH 7.4, 16 mM), KCl (50 mM), 1 mg glycogen (rabbit liver) plus 75 to 250 μl enzyme and 100 μl of the labeled substrate containing 1000 nmol [^{14}C]adenosine diphosphoglucose (0.5–1.0 Bq/nmol), in 1 ml total volume. In assays of the bound enzyme, the mixture contained no glycogen or PEG. The reaction was stopped by adding 10 ml 70% (v/v) ethanol and, processed as in (13).

Amylase assays were carried out according to (5).

Zymograms. Enzyme samples (20 μl) of No PEG supernatant or DEAE-cellulose eluate, made 30% w/v sucrose and containing the equivalent of 100 endosperms ml^{-1} were applied in PAGE (15). After 3.5 h at 2 mamp $tube^{-1}$ the gels containing 0.6 mg ml^{-1} glucogen were incubated with 1 mM adenosine diphosphoglucose and 600 mM trisodium citrate, 30 mM Tes-KOH, pH 7.5, 1 mM DTT overnight. After staining with iodine the gels were scanned at 550 nm with a Gilford 240 spectrophotometer and 2410-5 Linear Transport attachment. Pretreatments of enzyme samples were performed before the addition of 30% sucrose.

Chemicals. Adenosine diphospho-D-[U-^{14}C]glucose (9 GBq/mmol) and [U-^{14}C]sucrose (9 GBq/mmol) were obtained from Amersham Australia Pty Ltd., Sydney. Glycogen (rabbit liver), adenosine diphosphoglucose, α-amylase (rabbit liver) and PEG-1000 were from Sigma. *R. niveus* glucoamylase was supplied by Miles, Elkart, IN, and DEAE-cellulose (DE52) was from Whatman Ltd., Maidstone, Kent, U.K.

RESULTS

Uptake and Conversion of Sucrose by Grain Slices. As previously reported (14) in this system, uptake and conversion of sucrose to 70% ethanol insoluble matter are linear for several hours after the 1st h of incubation. Table I shows the results of an experiment in which grain slices (14 d after anthesis) were incubated with radiolabeled sucrose (150 mM) at 15, 25, and 35°C. At 35°C over the period 1 to 3 h, the conversion to starch was less than at 25°C. This contrasts with the conversion to the 70% ethanol-insoluble nonstarch residue and indeed with total uptake. The effect is not seen during the 1st h, perhaps because it resulted from gradual damage to the synthetic process.

To reduce the confusing effects of temperature on the uptake of sucrose and to observe those on its conversion more clearly, slices were preincubated at different temperatures for 2 h in the absence of sucrose, but in the presence of (100 mM) mannitol before being incubated at 25°C for 2 h with 100 mM radiolabeled sucrose (Table II). Total sucrose uptake was unaffected by temperature of preincubation but preincubation at 30°C reduced subsequent starch accumulation. At 35°C it was nearly halved and as in three other experiments (not shown) reduced more than the accumulation of nonstarch-containing residue. It seemed therefore of interest to study the thermostability of starch synthase, the final enzyme in the pathway for conversion of sucrose to starch.

Notes on Starch Synthase. Starch synthases, both soluble and bound, stay with the starch granule fraction if isolated in the presence of PEG (9). After repeated washing in PEG buffer the subsequent No PEG wash contains the soluble starch synthase but no measureable amylase activity (results not shown). Reports in the literature distinguish two seed soluble starch synthases by DEAE-cellulose chromatography (3, 11, 12); such differences between these two fractions as were found on the basis of citrate stimulated activity reflect differences in conditions for primer saturation (3). In this study, to provide for overall saturation of primer requirements glycogen plus PEG-1000 was added to the assays of the soluble starch synthase (13). As an additional point, it has now become apparent that the optimum PEG concentrations is a function of temperature (Fig. 1A). At 25°C, the same optimum concentration (180 mM) was found for wheat and rice but there were differences in the relative stimulations (Fig. 1, A and B). Linearity of activity with enzyme quantity over the range of the present estimates was observed (data not shown).

Thermostability of Starch Synthase *in Vitro*. Figure 2A shows two notable contrasts in the effects of heat on starch synthase *in vitro*: for wheat, pretreatment at 40°C of starch granules increased the bound activity, but pretreatment of No PEG supernatant at 35°C decreased the activity of the soluble enzyme; for rice, pretreatment of an identical supernatant at 35°C did not change the activity of the soluble enzyme. Activity in wheat No PEG supernatant was stable for 2 h at 25°C and when stored at 8°C for 12 and 24 h after a 30 min preincubation at 35°C did not recover any of the lost activity (data not shown). One way to provide protection against heat inactivation of the enzyme in the No PEG buffer was to add sucrose to it (Fig. 2B).

Figure 3 shows the effect of temperature on the stability of a DEAE purified starch synthase preparation (specific activity 0.21 μmol min^{-1} mg^{-1} protein). The enzyme was stable at 25°C, but activity decreased after a preincubation at 30°C and higher. At 35 and 45°C, an initial phase of fast decrease in activity followed by a phase of slow decrease may be distinguished suggesting isozymes with different thermostability.

Zymograms of No PEG supernatant showed various banding patterns depending on pretreatment (Fig. 4A). In the zymograms of control samples two close darkly stained bands, '2' and '3', predominated, but the slower migrating band '1' was also sub-

Table I. *Effect of Temperature on Uptake and Conversion of Sucrose by Wheat Grain Slices*

Incubation of 10 slices per replicate in 0.5 ml, 150 mM sucrose, 50 mM potassium chloride, 10 mM K-phosphate at pH 5.8. Soluble is complete 70% ethanol (70°C) soluble fraction of slices before grinding. Each value is the mean of four replicates; values for SE are in parentheses.

Fraction	Labeled Sucrose Equivalents								
	15°C			25°C			35°C		
	1 h	3 h	Δ	1 h	3 h	Δ	1 h	3 h	Δ
					nmol				
Soluble	1927 (30.0)	3106 (38.1)	1179	2275 (22.5)	3765 (51.8)	1490	2801 (36.5)	4490 (124.5)	1689
Starch	45 (1.7)	260 (15.5)	215	85 (1.7)	393 (29.1)	308	100 (1.8)	332 (20.8)	232
Residue	32 (2.0)	121 (5.8)	89	56 (0.8)	179 (8.8)	123	76 (2.3)	221 (9.1)	145
Total			1483			1921			2066

Table II. *Effect of Temperature during Preincubation for 2 Hours on Subsequent Uptake and Conversion of Sucrose by Wheat Grain Slices at 25°C for 2 Hours*

Preincubation of 10 slices per replicate in 0.25 ml 100 mM mannitol, 50 mM potassium chloride, 10 mM K-phosphate at pH 5.8. Incubation in 0.5 ml 100 mM radiolabeled sucrose, 50 mM mannitol, 50 mM potasium chloride, 10 mM K-phosphate at pH 5.8. Soluble (a) is complete 70% ethanol (70°C) soluble fraction of slices before grinding and soluble (b) is the 70% ethanol soluble fraction after grinding. Each value is the mean of five replicates; values for SE are in parentheses.

Fraction	Labeled Sucrose Equivalents with Preincubation at								
	25°C			30°C			35°C		
		nmol	%		*nmol*	%		*nmol*	%
Soluble (a)	2706	(34.8)	100	2805*	(13.6)	104	2908	(39.5)	107
Soluble (b)	53	(6.1)	100	46	(5.2)	87	34*	(8.2)	64
Starch	285	(6.6)	100	227**	(11.1)	80	154**	(5.0)	54
Residue	166	(4.4)	100	152**	(1.5)	92	113**	(5.7)	68
Total	3210		100	3230		101	3209		100

* Difference with 25°C, $P < 0.05$. ** Difference with 25°C, $P < 0.01$.

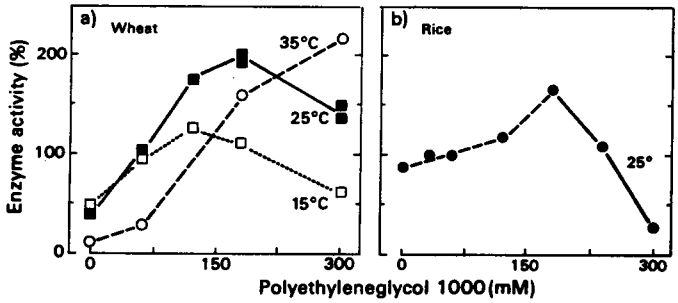

FIG. 1. Effect of PEG-1000 concentration in the assay system on the activity of soluble starch synthase from wheat at 15, 25, and 35°C, with glycogen (1 mg ml^{-1}) as primer and adenosine diphosphoglucose (1 mM) as substrate (a) and similarly from rice at 25°C (b). Activity observed at 25°C with 60 mM PEG was assigned the value of 100%.

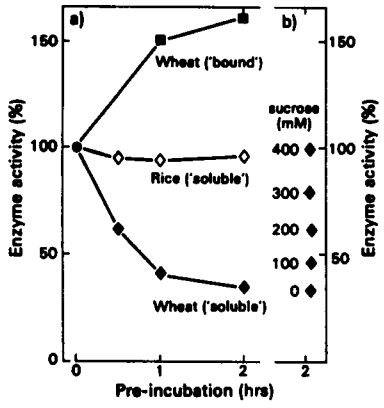

FIG. 2. The effect of preincubation at 35°C on the activity of wheat and rice soluble starch synthase and of preincubation at 40°C on the bound activity of washed wheat starch granules. The enzymes were prepared as described in "Materials and Methods" and were preincubated in No PEG buffer (a); in one experiment the No PEG buffer contained from 0 to 400 mM sucrose (b). Following preincubation the enzymes were assayed at 25°C as described in "Materials and Methods."

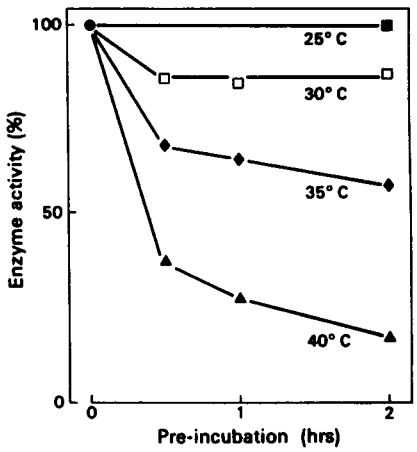

FIG. 3. Effect of temperature on the stability of partially purified soluble starch synthase in No PEG buffer. Following pretreatment the enzymes were assayed at 25°C as described in "Materials and Methods."

stantial (Figs. 4A and 5). Bands '2' and '3' were decreased markedly after preincubation of the enzyme sample of 25°C for 30 and 60 min while faster migrating bands became apparent (band '4', '5', '6', and '7' in Fig. 4, B and C). Higher heat resistance of these isozymes is suggested by their persistence after pretreatment at 35°C for 30 min, after which treatment band '1' had disappeared (Fig. 4D). The appearance of bands '4' to '7' after pretreatment at 25°C was a gradual process (Fig. 5). Zymograms of DEAE-cellulose fractions eluted with 80 to 140 mM KCl and 140 to 200 mM KCl, corresponding to respectively starch synthase I and II (3, 11, 12), both showed similar multiplicity. The higher salt fraction alone, however, contained band '1' and exhibited distinct sensitivity to pretreatment at 35°C by the disappearance of that band (Fig. 6). In the enzyme assay, this fraction lost 57% of its activity by such pretreatment and the lower salt fraction only 33% (no details shown).

Thermostability of Starch Synthase *in Vivo*. Rapid loss of the soluble starch synthase activity from the grains was observed after a transfer of plants from a growth cabinet at 21°C to one at 37°C (Fig. 7A). The critical temperature above which substantial loss of enzyme activity occurred was close to 30°C (Fig. 7B). This agrees with the inactivation *in vitro* (Fig. 3). But for a decrease in band '1' the Zymogram pattern resulting from a 1 h 35°C treatment of whole plants was the same as that of a control receiving no such treatment (Fig. 8).

The RH in the 37°C cabinet was close to 60%. This represents a vapor pressure deficit of 19 mbar for wheat plants (*i.e.* equivalent to zero RH at 22°C). However, an experiment with detached grains suggests that the contribution to enzyme inactivation by dry conditions is minor. Soluble starch synthase activity did not decrease for 2 h when batches of grains were kept in a dry beaker within a moist desiccator at room temperature (23–26°C). Approximately 13% of enzyme activity was lost after the following 4 h. The loss of activity of grains kept for 6 h in a

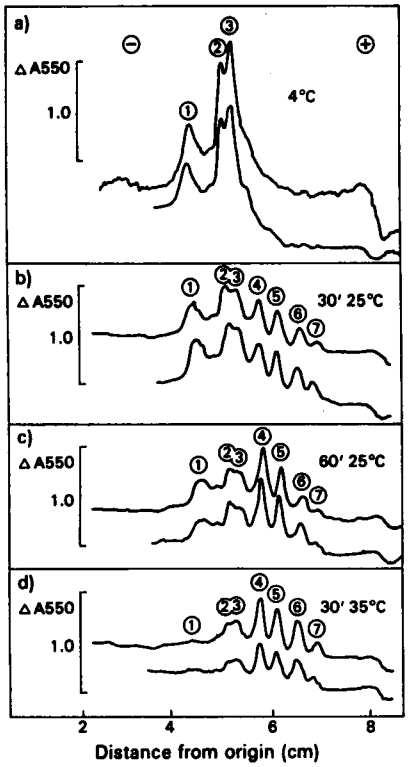

FIG. 4. Absorption scans of iodine-stained zymograms of wheat starch synthase (No PEG supernatant) held at various temperatures prior to PAGE: in (a) on ice, in (b) for 30 min at 25°C, (c) for 60 min at 25°C, and (d) for 30 min at 35°C. Duplicate PAGE columns shown for each treatment.

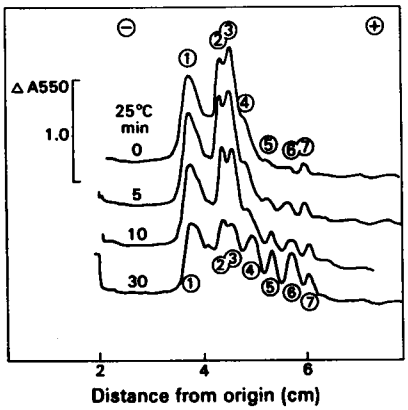

FIG. 5. Absorption scans of iodine-stained zymograms of wheat starch synthase, No PEG supernatants, treated for 0, 5, 10, and 30 min at 25°C.

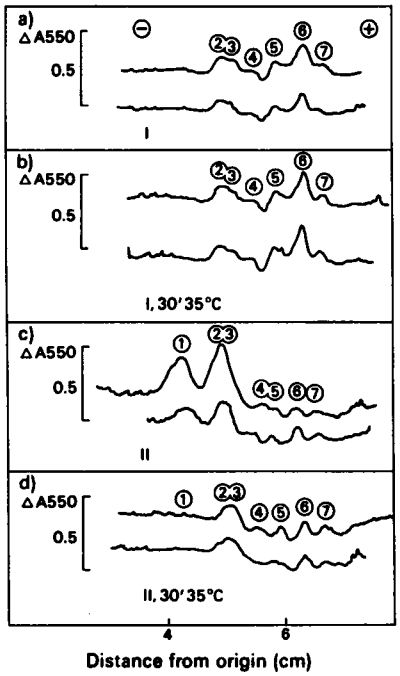

FIG. 6. Absorption scans of iodine-stained zymograms of wheat starch synthase, DEAE-cellulose fraction 80 to 140 mM KCl (a) and 140 to 200 mM KCl (c), and of the same fractions after treatment for 30 min at 35°C (b and d). Duplicate PAGE columns shown for each treatment.

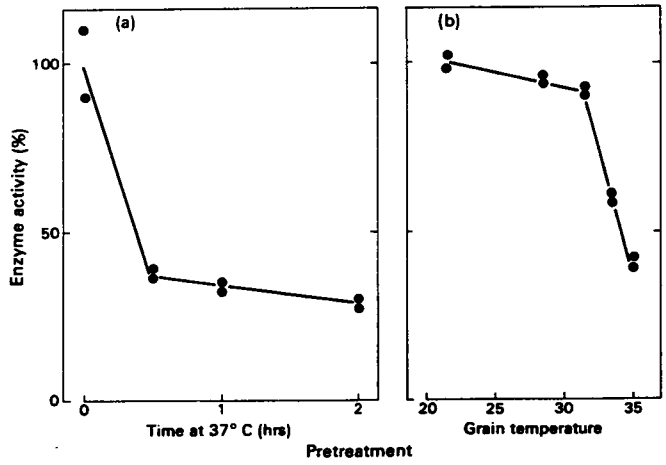

FIG. 7. a, Effect of transferring plants from 21°C to 37°C on the activity of the soluble starch synthase in the standard assay at 25°C. Each point represents a grain sample of 35 grains from 7 ears. (100% activity = 192 nmol 30 min^{-1} $grain^{-1}$). b, Effect of holding plants for 2 h at various temperatures on the activity of the soluble starch synthase in the standard assay at 25°C. Temperature in grains was checked by inserting a thermocouple. Each point represents an independent grain sample (100 = 197 nmol 30 min^{-1} $grain^{-1}$).

desiccator provided with oven-dry silica gel was 25%; the accompanying 7.5% fresh weight loss from the grain represents severe stress but its effect on enzyme activity appeared relatively mild (data not shown).

Recovery of Starch Synthase Activity. Enzyme activity lost from attached grains during a 1 h treatment of the plants at 37°C could be restored in 1 or 2 d at 21°C (Table III). In experiment 1, ears were harvested in triplicate allowing the assessment of variability. Immediately after the heat shock, the activities of both the soluble and bound fraction were significantly decreased although this decrease was relatively less for the bound fraction. This loss diminished and was not statistically significant after 24 h at 21°C and had certainly disappeared after 48 h at 21°C (Table III, experiments 1 and 2). No such recovery was observed when the plants were kept at 37°C for 2 d giving a final activity per grain of 35% of the initial value (no details shown).

Effects of Inhibitors. The loss of soluble enzyme activity in slices incubated in 100 mM sucrose was greater than in intact grains (Figs. 7 and 9). In contrast to observations on the purified enzyme or on grains, loss was observed at 25°C (Fig. 9) and even at 20°C (data not shown). At 20 and 30°C, the inactivation was increased by the presence of 0.5 mM 2,4-dinitrophenol (Fig. 9 and unpublished data). In contrast, in slices incubated for 2 h at 20°C no inactivation was found due to inhibitors of protein

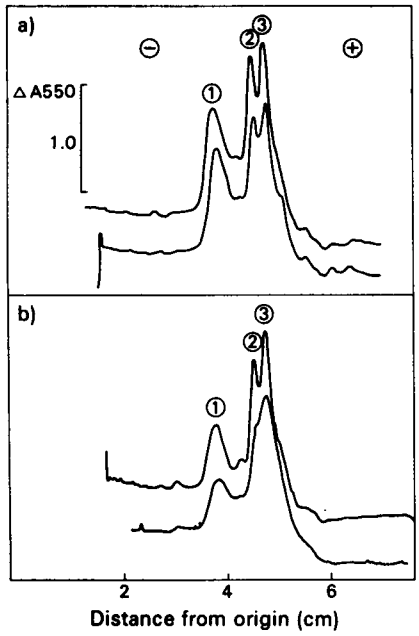

FIG. 8. Absorption scans of iodine-stained zymograms of wheat starch synthase, No PEG supernatant, prepared before (a) and after (b) plants had been held for 1 h at 35°C. Duplicate PAGE columns shown for each treatment.

synthesis: 0.1 mM cycloheximide, 0.1 mM chloramphenicol, 5 mM 1-azetidinecarboxylic acid (data not shown).

DISCUSSION

The data in Tables I and II show that in wheat endosperm slices the conversion of sucrose to starch was reduced by temperatures in the 30 to 35°C range. *In vivo* (Fig. 7) or *in vitro* (Fig. 3) starch synthase activity was shown to be reduced in that same temperature range. In contrast, in rice in which grain development has a notably higher temperature optimum (4), the enzyme appeared to be considerably less thermolabile (Fig. 2). Not all wheat starch synthase isozymes were equally temperature sensitive. Since *in vitro* the activity of the starch synthase complex is stable at 25°C (Fig. 3), the comparison of the enzyme patterns between 25 and 35°C must indicate the change that accompanies enzyme inactivation. This comparison points to the slowest migrating band '1' being most heat sensitive (Fig. 4, B and D). Also it is this band which disappears on heating to 35°C the DEAE-cellulose fraction corresponding to starch synthase II (Fig. 6, C and D). In waxy maize, starch synthase II showed a lower temperature optimum than starch synthase I (11). Here, this fraction lost relatively more activity on heating at 35°C. *In vivo*, the slower band '1' appeared to be reduced by a stay of whole plants for 1 h at 35°C (Fig. 8). It is to be considered that the relative peak areas, in the scan of the zymogram, after incubation overnight, do not represent applied activities quantitatively; the reduction in peak area due to heat shock probably underestimates the reduction in activity. It is suggested that the reduction of soluble activity *in vivo* may be a direct effect of heat on the enzyme protein.

A further noteworthy finding was the shift in isozyme banding pattern that followed incubation of No PEG supernatant at 25°C and featured a decrease in bands '2' and '3' and an increase in bands '4', '5', '6', and '7' (Fig. 4, A and B). An increase in multiplicity also attended the partial purification process (Fig. 6) and may also be noted in the purification of starch synthase of sweet corn reported by Schiefer *et al.* (16) (their Fig. 1, band 9a, b, and c) and of sorghum (12). Boyer and Preiss (3) reported that after thorough purification of maize starch synthase I 'the fraction contained many bands of near equal intensity.' Here the appearance of band '4' to '7' was noticed after 5 min at 25°C and proceeded gradually (Fig. 5). This process may well be an artifact of isolation and has not been identified. It was not accelerated by the inclusion of ATP during preincubation or slowed down by omitting DTT (data not shown).

No strict proportionality seems to exist between the relative heat-induced reductions of soluble starch synthase and starch accumulation: in slices starch accumulation proceeded undiminished for several hours at 25°C (14) but at that temperature enzyme inactivation was observed (Fig. 9); after 2 h at 35°C about 80% of enzyme activity was lost (Fig. 9) but the capacity to accumulate starch was reduced by 46% (Table II). The discrepancy may be accounted for by the observation that *in vivo* the bound activity is less heat labile than the soluble activity (Table III).

In relation to crop yield, it is of interest that the loss of starch synthase activity due to a short, high temperature treatment was readily restored after 24 h or longer at a lower temperature (Table III). This need not be due to resynthesis. It seems doubtful that the bound enzyme, encaged in the starch granule, could be replenished by introduction of new protein. The tight association between bound enzyme and starch granule is demonstrated by the fact that so far the enzyme's liberation from the granules has been successful to any extent only after amylase digestion (9).

Heat inactivation of bound starch synthase occurred *in vivo* but not *in vitro*. (Table III, Fig. 2). Pretreatment of granules for 2 h at 40°C resulted in an increase of activity but such pretreatment of the soluble enzyme resulted in a drastic decrease of activity (Figs. 2 and 3). The stimulation of the bound activity by heat *in vitro* may be due to changes in structure of starch rather than of protein. Loosening of the starch framework by heat

Table III. *Recovery of Starch Synthase Activity from Heat Shock in the Attached Grains of Wheat Plants at 21°C following Treatment for 1 h at 37°C, on Day 1*

Values for SE are in parentheses.

Day	Soluble Enzyme						Bound Enzyme					
	Control			Heat shock			Control			Heat shock		
	nmol/grain		%	*nmol/grain*		%	*nmol/grain*		%	*nmol/grain*		%
Experiment 1												
1	185.6	(6.50)	100	89.1**	(5.11)	48	14.9	(0.17)	100	10.3**	(0.44)	69
2	194.0	(6.36)	100	168.9	(8.29)	87	14.9	(0.17)	100	14.7	(0.73)	98
3	179.2	(17.38)	100	180.1	(4.17)	101	15.3	(1.25)	100	16.4	(0.08)	107
Experiment 2												
1	191.5		100	103.5		54	12.5		100	9.8		78
3	200.0		100	204.0		102	15.3		100	16.2		106

** $P < 0.01$.

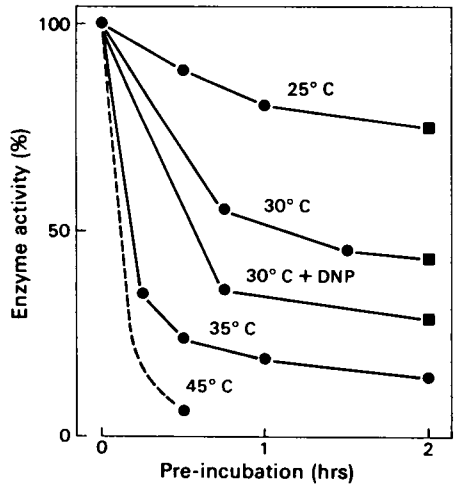

FIG. 9. Effect of preincubating grain slices in 100 mM sucrose (10 per vessel) at various temperatures on the activity of the soluble starch synthase in the standard assay at 25°C. Some of the 30°C samples contained 0.5 mM 2,4-dinitrophenol (DNP). Several experiments are combined in this figure. Each point represents the mean of duplicate samples of 40 slices. Square symbols represent values interpolated between 1 and 3 h of preincubation.

would permit easier access of substrate. Inactivation of the bound activity by heat *in vivo* may be indirect and due to metabolic factors. *In vivo*, in slices, inactivation of the soluble enzyme occurred at 25°C at which temperature *in vitro* that enzyme was stable (Figs. 3 and 9). Also the effect of 2,4-dinitrophenol furthering the inactivation *in vivo* indicates a possible role for metabolic factors (Fig. 9).

There are indications that heat sensitivity of dry weight accumulation may vary between some wheat varieties (17). It is now suggested that this could be due to differences in heat sensitivity of the soluble starch synthases *per se* as observed by differences between wheat and rice (Fig. 2A) and by differences between isozymes (Fig. 4) or to other factors affecting the 'molecular ecology' of the starch synthases such as suggested by effects due to sucrose concentration (Fig. 2B).

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