The effects of elevated temperature on starch deposition in developing wheat grains

Paul J Harris[1], **Ian J Tetlow**[2] and Michael J Emes[2]

[1] School of Biological Sciences, University of Manchester, Manchester, M13 9PT, United Kingdom
[2] College of Biological Science, University of Guelph, Guelph, Ontario, N1G 2W1, Canada

Abstract

Rates of fresh and dry weight accumulation, *in vitro* assays of enzymes involved in the conversion of sucrose to starch and starch synthase (SS) zymograms and immunoblots were used to examine the effects of elevated temperature on the deposition of starch in developing wheat grains. Wheat plants were grown under two different temperature regimens after anthesis: 20/16?C and 27/16?C day/night, 16 hr photoperiod. Comparing plants subjected to the two post-anthesis temperature regimens, the high temperature effect on grain filling was shown to occur during a distinct period in grain development, between 200 and 300 degree days post-anthesis (?C DPA), and resulted in an approximately 25% reduction in mean mature grain weight. The *in vitro* activity of starch synthase, and the specific activity of granule bound starch synthase I (GBSSI) determined by activity zymograms and immunoblots appeared to be markedly reduced in endosperm extracts from wheat plants grown under high post-anthesis temperature conditions during this 200 and 300?C DPA period. A possible role for soluble GBSSI in initiation of B-type starch granules in wheat endosperm is suggested.

Media Summary

Elevated temperature affects grain filling during a distinct period of grain development. This coincides with a reduction in activity of a soluble form of GBSSI.

Keywords

Carbon partitioning, *Triticum aestivum*.

Introduction

Starch typically accounts for around 75% of wheat grain weight and so is a major determinant of yield in wheat crops. Elevated temperatures during grain filling decrease starch deposition and therefore adversely affect yield (Gibson and Paulsen, 1999). The reduction in starch accumulation resulting from exposure to high temperature is likely due to a decrease in the conversion of sucrose to starch because the supply of photosynthates to developing grains does not seem to be a major limitation to starch production (Wardlaw et al., 1980). Reduction in final starch content with increased temperatures is thought to be due either to an increase in starch synthesis being insufficient to compensate for a shortened growth period, or in more severe cases, a reduction in both rate of starch synthesis and the growth period (Denyer et al., 1994).
In this study, we show that the high temperature effect on grain filling occurs during a distinct period in grain development, which coincides with an apparent reduction in a soluble form of GBSSI activity in endosperm whole cell extracts.

Methods

Plant material

Spring wheat (*Triticum aestivum* L. cv. Axona) was grown under greenhouse conditions (20/15?C day/night, 16 hr photoperiod in daylight supplemented with sodium light, photosynthetically active radiation 160µmol m^{-2} s^{-1}) until anthesis. Plants at anthesis were transferred to two controlled environment chambers (20/16?C and 27/16?C day/night, 16 hr photoperiod). Developing grains were harvested between 3 and 46 days post-anthesis (DPA), and fresh and dry weights determined.

Preparation of endosperm whole cell extracts

Whole cell extracts were prepared from grains harvested from plants grown in the 20/16?C chamber (grown to 13 DPA) and the 27/16?C chamber (grown to 11 DPA). Endosperm tissue was ground in extraction buffer [50mM glycylglycine (pH7.5), 2mM $MgCl_2$, 1mM KCl, 1mM EDTA, 10% (v/v) ethanediol, 10% (v/v) glycerol, 5mM DTT] on ice. The extracts were centrifuged at 14000g in a refrigerated microfuge for 5 min and desalted on PD-10 columns (Amersham Biosciences) pre-equilibrated with extraction buffer. Protein contents were determined using Bio-Rad protein dye reagent (Bradford, 1976). Desalted whole cell extracts were used in the subsequent analyses.

Measurement of the activities of enzymes involved in the conversion of sucrose to starch

The following enzymes were assayed at 25?C as described previously: sucrose synthase (Huber and Akazawa, 1986), UDPglucose pyrophosphorylase (M?ller-R?ber et al., 1992), phosphoglucose isomerase (Simcox et al., 1977), phosphoglucomutase (Journet and Douce, 1985), ADPglucose pyrophosphorylase (Smith, 1990), SS (Smith, 1990).

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

Desalted extracts were mixed with gel sample buffer [60mM tris-HCl (pH 6.75), 2% (w/v) SDS, 10% (v/v) glycerol, 5% (v/v) 2-mercatoethanol, 0.001% (w/v) bromophenol blue] and heated to 100?C for 3 min prior to loading on 7.5% gels containing 0.3% rabbit liver glycogen following the method of Laemmli (1970).

Zymogram analysis

Zymogram analysis of SS activity was performed on gels following SDS-PAGE according to Bul?on et al. (1997).

Immunoblotting

Proteins separated by SDS-PAGE were transblotted onto nitrocellulose membranes, blocked with 1.5% bovine serum albumin and exposed to antibodies raised against starch synthase I (SSI) (1:5000 dilution) and GBSSI (1:1000 dilution) using the methods described by Harlow and Lane (1988). Bound antibodies were detected with alkaline phosphate-conjugated goat anti-rabbit IgG using a 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium liquid substrate.

Results

Grain weights through development

Grain weights were plotted against thermal time after anthesis (Figure 1) to account for differences in the rates of development of the plants grown under the two different temperature regimens. ?C DPA were calculated by multiplying the mean daily temperatures after anthesis by DPA. Grains from plants grown under the 20/16?C regimen reached greater maximum fresh weights and had greater dry weights at maturity than grains grown under the 27/16?C regimen. The rates of deposition of dry weight for the two regimens were the same until about 200?C DPA, when the rate increased rapidly in grains under the 20/16?C regimen but was not matched with a similar increase in rate in grains grown under the 27/16?C regimen. By 300?C DPA the rate of dry matter deposition in grains grown under the 27/16?C regimen was similar to that in grains grown under the 20/16?C regimen, suggesting that the high temperature effect on starch synthesis could be specific for the period of development between 200 and 300?C DPA. Subsequent analyses were therefore carried out on endosperm extracts prepared from grains during this period.

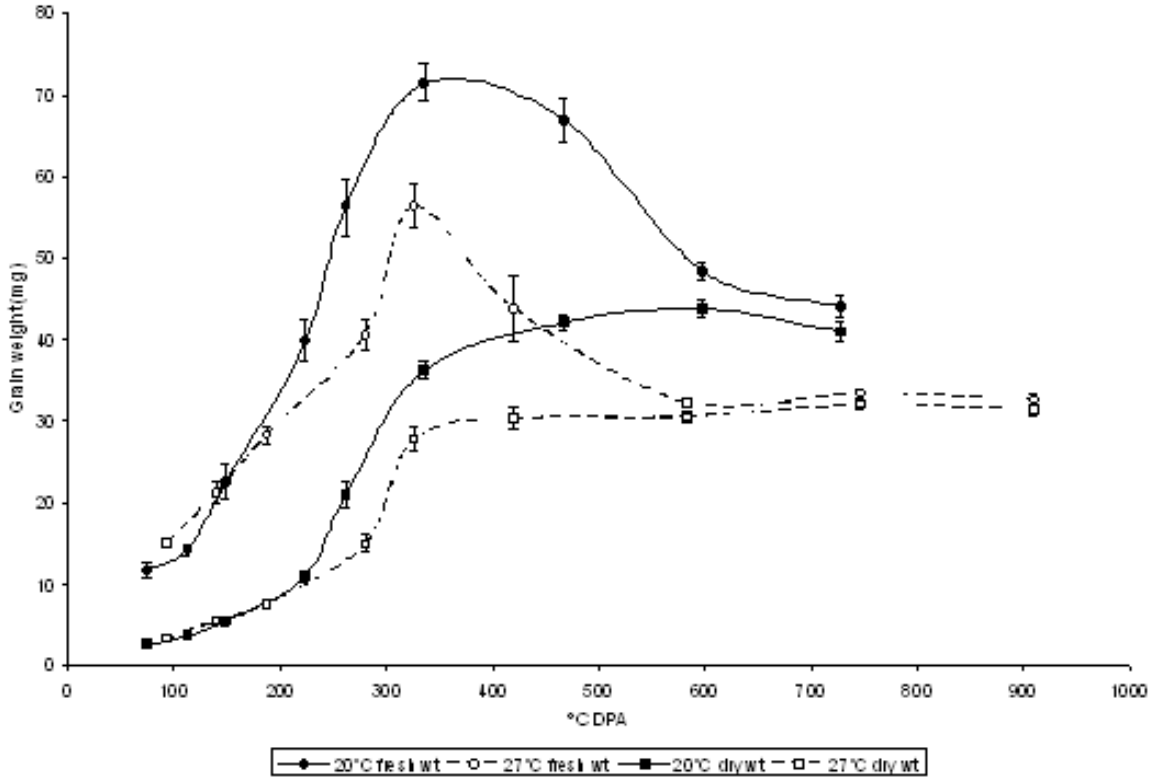

Figure 1. Fresh and dry weights of grains from wheat plants grown under 20/16?C and 27/16?C regimens post-anthesis. Values presented are means ? SEM (n ≥ 6).

Activities of enzymes involved in the conversion of sucrose to starch

All of the enzymes assayed had similar *in vitro* activities under the different temperature regimens with the exception of SS, which showed a 3-fold greater activity under the 20/16?C regimen (Table 1).

Table 1. Activities of enzymes involved in the conversion of sucrose to starch in desalted whole cell extracts of endosperm from wheat grown under 20/16?C and 27/16?C regimens after anthesis. 20/16?C extract: 243 ?C DPA (13 DPA), 27/16?C extract: 257 ?C DPA (11 DPA).

Enzyme	20/16?C activity (nmol min^{-1} mg $protein^{-1}$)	27/16?C activity (nmol min^{-1} mg $protein^{-1}$)
Sucrose synthase	44.1	37.6
UDPglucose pyrophosphorylase	1060	1170
Phosphoglucose isomerase	191	204
Phosphoglucomutase	385	419
ADPglucose pyrophosphorylase	49.2	43.2
Starch synthase	2.95	0.968

Zymogram and immunoblot analyses of SS activity

The SS activity of a 60kD protein that co-migrated with protein recognized by anti-GBSSI antibodies was higher in endosperm whole cell extracts from plants grown under the 20/16?C regimen than the same

amount of protein extracted from the endosperm of plants grown under the 27/16?C regimen (Figure 2). The 60kD bands in lanes 4 and 5 of Figure 2B (immunoblot) show similar intensities, suggesting that the reduced activity of GBSSI observed in Figure 2A (zymogram) is due to a difference in the specific activity of the GBSSI protein between the two regimens.
Endosperm whole cell extracts from plants grown under the 27/16?C regimen showed relatively more SS activity of a 75kD protein that co-migrated with protein recognized by anti-SSI antibodies than the same amount of protein extracted from plants grown under the 20/16?C regimen (Figure 2). However, the increased SSI activity in the 27/16?C extract over the 20/16?C extract could have been due to different amounts of SSI protein in the samples as the 75kD band in lane 3 of Figure 2B is more intense than the corresponding band in lane 2, suggesting the 27/16?C extract contained more SSI protein.

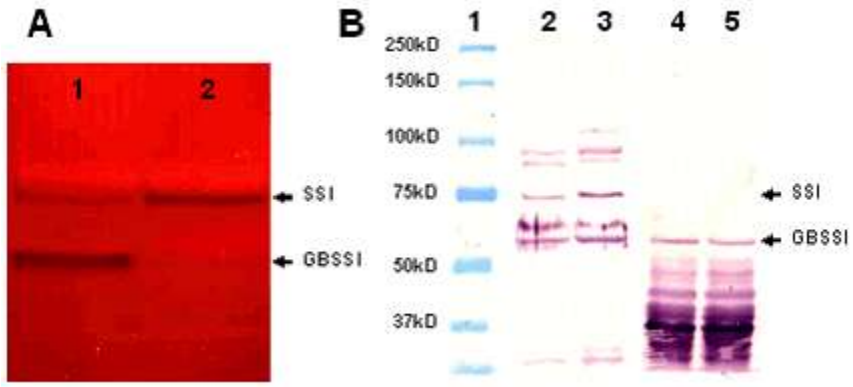

Figure 2. A: Zymogram of SS activity in protein extracts from the endosperm of wheat grown under 20/16?C and 27/16?C regimens after anthesis. Lane 1: 20/16?C, 243 ?C DPA (13 DPA). Lane 2: 27/16?C, 257 ?C DPA (11 DPA). 400µg of protein loaded per lane. B: Immunoblots of endosperm proteins probed with anti-SSI (lanes 2 and 3) and anti-GBSSI (lanes 4 and 5) antibodies. Lanes 2 and 4: whole cell extracts of wheat endosperm from plants grown at 20/16?C, 243 ?C DPA (13 DPA). Lanes 3 and 5: whole cell extracts of wheat endosperm from plants grown at 27/16?C, 257 ?C DPA (11 DPA). Lane 1: molecular mass markers.

Conclusions

Elevated temperature post-anthesis affected the rate of dry matter deposition most markedly between 200 and 300?C DPA and resulted in an approximately 25% lower mean mature grain weight compared with the lower temperature regimen (Figure 1). When the *in vitro* activities of several enzymes involved in the conversion of sucrose to starch were measured in extracts prepared from endosperm harvested during this period (243?C DPA for the 20/16?C regimen and 257?C DPA for the 27/16?C regimen), only the activity of SS was markedly different between the temperature regimens (Table 1). SS zymograms (Figure 2A) and immunoblots (Figure 2B) suggest that the reduced SS activity observed in the *in vitro* enzyme assay with increased temperature could at least in part be due to reduced activity of GBSSI. It has previously been proposed that GBSSI could have a role in the initiation of B-type starch crystals (Wattebled et al., 2002) and B-type granules are thought to be initiated around 200 to 300?C DPA in wheat endosperm (Peng et al., 2000). If this is the case, it is feasible that elevated temperatures could cause a reduction in the rate of dry matter deposition between 200 and 300?C DPA by an effect on soluble GBSSI, interfering with B-type granule initiation. Effects of elevated temperature on B-type granule formation in wheat endosperm will be the subject of future investigations.

References

Bradford M (1976) *Analytical Biochemistry* **72** 248-254

Bul?on A, Gallant DJ, Bouchet B, Mouille C, D'Hulst C, Kossmann J and Ball S (1997) *Plant Physiology* **115** 949-957

Denyer K, Hylton CM and Smith AM (1994) *Australian Journal of Plant Physiology* **21** 783-789

Gibson LR and Paulsen GM (1999) *Crop Science* **39** 1841-1846

Harlow E and Lane D (1988) *Antibodies: A Laboratory Manual* (Cold Spring Habor Laboratory Press, NY)

Huber SC and Akazawa T (1986) *Plant Physiology* **81** 1008-1013

Journet E and Douce R (1985) *Plant Physiology* **79** 458-467

Laemmli UK (1970) *Nature* **227** 680-685

M?ller-R?ber B, Sonnewald U and Willmitzer L (1992) *EMBO Journal* **11** 1229-1238

Peng MS, Gao M, Baga M, Hucl P and Chibbar RN (2000) *Plant Physiology* **124** 265-272

Simcox PD, Reid EE, Canvin DT and Dennis DT (1977) *Plant Physiology* **59** 1123-1132

Smith (1990) *Methods in Plant Biochemistry* **3** 93-102

Wardlaw IF, Sofield I and Cartwright PM (1980) *Australian Journal of Plant Physiology* **7** 387-400

Wattebled F, Bul?on A, Bouchet B, Ral JP, Li?nard L, Delvall? D, Binderup K, Dauvill?e D, Ball S and D'Hulst C (2002) *European Journal of Biochemistry* **269** 3810-3820