CARBOHYDRATE METABOLISM DURING SPROUTING

Dr. Howard V. Davies[1]

Introduction

The polymeric carbohydrate starch constitutes approximately 70% to 85% of tuber dry weight. It represents not only an important source of energy for man but also a reserve which developing shoots and roots rely on to supply their structural and functional requirements. This dependency can continue for some time after emergence, allowing sustained growth until plants become autotrophic. Even then the phloem network of the mother tuber continues to transfer reserves to the haulm, probably until reserves have been completely exhausted or the tuber has succumbed to disease (25). Of the dry weight lost by tubers during sprouting the majority (ca. 80 to 90%) appears to be translocated to shoots and roots and only approximately 10% lost by respiration (20). The tuber is therefore efficient in its function as a storage organ. Respiratory losses of transported carbon by rapidly growing shoots is extensive, however. Carbohydrate metabolism in sprouting tubers also has an important bearing on the quality of tubers destined for processing into chips, French fries and reconstituted products. The high reducing sugar content of sprouting tubers causes discoloration during high-temperature frying following a typical Maillard reaction with amino groups (40). Chemical sprout suppressants are used to alleviate the problem but the growing environmental lobby may preclude their usage in the future. It is for this reason that many laboratories are researching the low temperature sweetening phenomenon. An understanding of the biochemical processes which control carbohydrate metabolism in sprouting tubers will also benefit these programs.

Reserve Mobilization and Sprout Growth

Several investigations have suggested that sprout growth can be limited by the availability of mother tuber reserves. For example, an increase in the number of sprouting eyes per tuber reduces dry weight accumulation per eye. The onset and degree of this "competition" is delayed and reduced, respectively, by increasing tuber size and lowering ambient temperatures (20, 28). Furthermore, the use of tuber pieces or cores of differing sizes has indicated that the time to emergence is inversely proportional to the amount of substrate available (24, 39). Headford (21), on the other hand, was of the opinion that the supply of carbohydrates is unlikely to limit sprout growth

[1]Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, Scotland, United Kingdom.
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severely because the reserves of starch are appreciable, sprout dry weight is positively associated with tuber size in a linear manner, whereas tuber carbohydrate content departs from a linear relation due to relatively greater respiratory losses with a decrease in tuber size, and sprouts from smaller tuber pieces contain a higher percentage of soluble sugars on a dry weight basis compared with larger pieces. Mikitzel and Knowles (23) showed that with advanced tuber age apical dominance decreases together with the strength of individual shoots as sinks for tuber carbohydrates. In their experiments the rate of starch breakdown was unaffected by tuber age but evidence for more extensive sucrose breakdown in older tubers was presented. This was offered as one possible explanation for the poor growth response of aged tissue, *i.e.* reduced availability of transportable carbohydrate. It is unlikely that the rate of sucrose transport presents a major limitation as Edelman *et al.* (15), have shown that an efficient transport system exists within the tuber. They restricted the cross-sectional area of the tuber available for transport to 5% of the control. This did not interfere appreciably with movement of reserves from basal to apical regions of the tuber. It is also apparent that the rate of starch breakdown is similar in all parts of the tuber during sprout growth (10, 14). It seems unlikely, then, that the proximity of growing shoots to regions of more rapid starch breakdown confers any advantage in terms of carbon availability.

It would appear that if sprout growth is limited by the supply of carbohydrate, then the rate of starch breakdown and sucrose turnover are more likely to regulate growth than either the quantity of reserves or the rate of sucrose transport. Sucrose does not accumulate appreciably during sprouting, again a good indication that transport is not limiting. Hexoses may increase substantially, if only transiently (10, 11, 14). The accumulation of hexoses within the mother tuber is relatively small compared with the quantity of starch lost. Since respiratory losses are also small compared with the quantity of carbohydrate translocated it appears unlikely that sucrose breakdown and hexose accumulation/metabolism limit the availability of sucrose for transport extensively.

By comparison with our knowledge of reserve mobilization in germinating seeds, little is known about the biochemical control of starch breakdown in potato tubers. At this point, therefore, it is worth outlining the pathways of starch metabolism as elucidated from other species. Those wishing to obtain more detailed information on starch structure and the biochemistry of starch breakdown should consult recent reviews by Steup (46) and Beck and Ziegler (2). A scheme indicating the principle enzymes and reactions involved is shown in Fig. 1. The major controversy with the potato continues to be the relative involvement of amylase and phosphorylase in catalyzing starch breakdown. This is discussed later. Even less is known about the other enzymes, *e.g.* debranching enzyme, maltose phosphorylase. The functions of the major enzymes are:

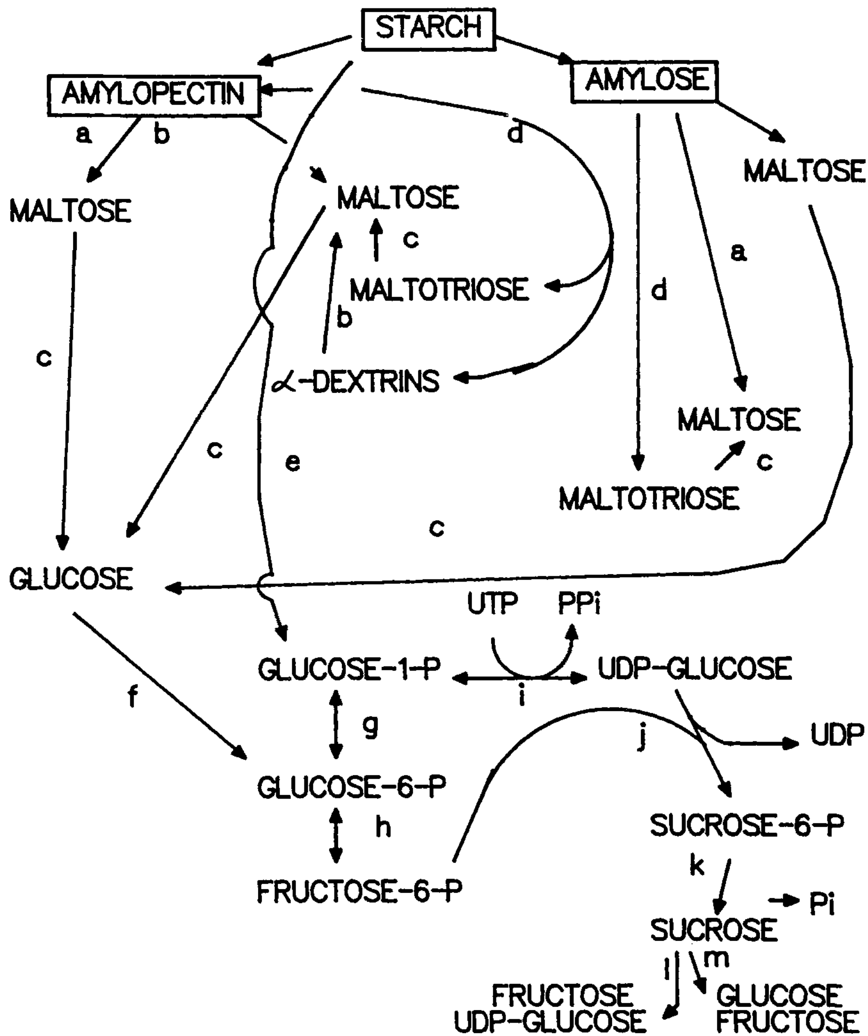

FIG. 1. Schematic representation of pathways of starch breakdown sucrose synthesis and sucrose breakdown in higher plants. a = β- amylase, b = debranching enzyme, c = α-glucosidase, d = α- amylase, e = phosphorylase, f = hexokinase, g = phosphoglucomutase, h = phosphohexoseisomerase, i = UDP-glucose pyrophosphorylase, j = sucrose phosphate synthase, k = sucrose phosphatase, l = sucrose synthase, m = invertase.

α-amylase (endoamylase): hydrolysis of α-1,4-linked oligo- or polyglucans by transferring a glucosyl residue to water. Internal α-1,4-bonds of linear or branched glucans are cleaved randomly to yield products with a new reducing group in the α-configuration. Links adjacent to non-

reducing ends or ∝-1,6- branch points are not hydrolyzed. Amylose degradation yields maltose, glucose and oligoglucans with a degree of polymerization of up to seven (46). Amylopectin breakdown yields the same sugars together with branched oligosaccharides (∝-limit dextrin).

β-amylase (exoamylase): hydrolyzes ∝-1,4- links in both amylose and amylopectin releasing maltose units from the non-reducing end of the chain. With amylopectin hydrolysis again stops at ∝-1,6- links. The products in this case are maltose and β-limit dextrin.

∝-1,4- glucan phosphorylase: catalyzes the formation of glucose-1-phosphate from amylose and orthophosphate (46). The reaction is potentially reversible but is generally believed to function in the direction of starch breakdown *in vivo.*

debranching enzyme (limit dextrinase): ∝-1,6- interchain glucosidic links are hydrolyzed by specific ∝-1,6- glucosidases.

maltose phosphorylase: converts maltose and orthophosphate to glucose and glucose-1-phosphate.

D-enzyme (glucosyl transferase): transfers a portion of an ∝-1,4- glucan chain (donor) to a new 4 position. The smallest donor is maltotriose, *e.g.* maltotriose (donor) + maltotriose (acceptor) = glucose + maltapentose.

∝-glucosidase: cleaves maltose and related oligosaccharides to glucose.

Amylase, Phosphorylase and Starch Breakdown

As stated previously, there is some controversy over the relative roles of amylase and phosphorylase in catalyzing starch breakdown in tubers. Morrell and ap Rees (27) did not detect either ∝- or β- amylase in mature tubers but demonstrated high phosphorylase activity. It was concluded that, in stored tubers at least, starch breakdown was phosphorylytic. Similarly, Sowokinos *et al.* (45), showed that starch depletion in tubers which develop a translucent pith in storage was associated with a 5-fold increase in phosphorylase whereas ∝- amylase activity was not affected significantly. Doehlert and Duke (13) employing a starch azure assay, indicated that tubers were low in β- amylase compared with other species tested but that ∝- amylase accounted for approximately 50% of total activity. Ross and Davies (36), on the other hand, found that the inclusion of Ca^{2+} ions in the assay medium had no effect on amylase activity extracted from sprouted tubers. A spectral analysis of the starch-iodine complex before and after incubating soluble starch with tuber extracts showed a shift in the absorbance which was almost identical to that obtained with pure β- amylase. These findings point to the predominance of β- amylase over ∝- amylase in sprouting tubers. Recent work using chromogenic substrates for amylase and exoamylase determinations (p-nitrophenyl maltoheptaoside and p-

nitrophenyl maltopentaoside, respectively) has confirmed that β- amylase is 5- to 10-fold more active in stored tubers than ∝ - amylase (Cochrane *et al.*, personal communication). The contradictory reports that appear in the literature on amylase activities in potato tubers may, at least in part, be due to the various extraction and assay protocols used. Potato ∝ - amylase, unlike that from barley, is heat labile (16). Thus the traditional use of a 70°C treatment to eliminate activity could result in considerable errors. It is also interesting that Singh (44) reported that amylase activity was principally confined to the particulate fraction following centrifugation of buffered extracts. This clearly requires further investigation. There are reports which state that complete starch breakdown is only accomplished if phosphorylase accompanies the action of amylases (41). It would appear difficult not to ascribe an important role to amylase in the complete dissolution of the starch granules in potato. Maltose, a product of amylolytic cleavage of starch, does not accumulate to any extent in sprouting tubers, however (Davies, unpublished data). Maltase (∝ - glucosidase) activity is present in tubers and the enzyme has been partially purified (22). Rapid turnover of any maltose released would explain the absence of appreciable quantities of this disaccharide.

There are several reports of increases in amylolytic and phosphorylitic acitivities during dormancy break in tubers (1). Porter (31) reported that starch degradation during the dormancy period was mainly phosphorylytic. Bruinsma (5) observed a decrease in phosphorylase activity during the period of dormancy but a striking rise in amylase at the beginning of sprouting. Bailey *et al.* (1), found no correlation between amylase and phosphorylase activities and reducing sugar levels in tubers. Although there were general increases in enzyme activities at about the time of sprouting, activities declined thereafter. Unfortunately, as in many other cases, enzyme determinations were not continued into the rapid phase of starch breakdown that accompanies extensive sprout growth. This does not allow the establishment of any correlations between enzyme activity and rates of starch mobilization.

The work of Singh (44) suggested that phosphorylase activity increased only slightly during the period of rapid starch depletion. Amylase activity actually decreased during sprout growth. More recent studies have shown that starch phosphorylase activity declines during the period of rapid starch breakdown and sprout growth (10, 11). However, the maximum extractable activity of the enzyme was found to be sufficient to account for the rate of starch breakdown at all stages of sprouting. Amylase activity, measured by reducing sugar release from starch, also declined with sprouting and was apparently insufficient to account for observed rates of starch mobilization. Use of the iodine binding method confirmed that β- amylase activity predominated in unsprouted tubers and that β- amylase activity declined as starch breakdown progressed. This particular assay also indi-

cated that β- amylase activity was always present in excess. α- amylase (β-limit dextrin substrate) was not detected in unsprouted tubers but an increase in activity accompanied starch breakdown. Although the changes in absorbance during the assay of α - amylase were small, sufficient activity accumulated to account for the observed rate of breakdown.

When all the evidence is closely scrutinized it can only be concluded that there is, as yet, little convincing evidence that substantial increases in phosphoryltic and hydrolytic enzyme activities accompany starch breakdown in potato tubers. The fact that there have been several reports of amylase and phosphorylase activities in dormant tissues casts some doubt upon the involvement of coarse control in regulating starch breakdown. Further clarification of the role of α - amylase is required, including a detailed examination of isozymes and of transcript levels, exploiting the possibility of using cDNA probes for cereal amylases. Potato tuber phosphorylase has received considerably more attention, Brisson *et al.* (4), reporting the isolation of a cDNA clone. The protein is nuclear encoded and synthesized as a precursor containing a transit peptide. Phosphorylase antibody and immunogold localization was employed to show that the enzyme is located inside the amyloplast stroma in young, developing tubers, but in the cytosol in the immediate vicinity of the plastid in mature tubers. Similar results were obtained by Schneider *et al.* (42). These authors also suggested that the presence of phosphorylase in the cytosol of aged tubers coincided with lesions in the amyloplast membrane, offering the possibility of protein diffusion. However, Brisson *et al.* (4), point out that an inhibition of phosphorylase import into amyloplasts of mature tubers cannot be discounted. The fact that the enzyme is spatially separated from its substrate in mature tubers implicates compartmental control of any starch breakdown via phosphorylase. This would certainly agree with the poor correlation between activity and rates of starch breakdown. Again it is possible that specific isoforms of phosphorylase increase during starch breakdown. Gerbrandy and Doorgeest (17) reported the presence of several isoenzymes in tubers, whereas Shivaram (43) detected only two. Schneider *et al.* (42), were of the opinion that proteolytic cleavage *in vitro* of one major native enzyme was responsible for such discrepancies [see also Brisson *et al.* (4)]. However, Moreno and Tandecarz (26) demonstrated quite clearly the presence of two forms of phosphorylase with different acceptor requirements. The role of each enzyme in tuber carbohydrate metabolism is not known.

Hormonal or Sink Control of Starch Breakdown?

The classical case of hormonally regulated reserve breakdown in storage tissue is the breakdown of barley endosperm starch through processes initiated by gibberellins transported to the aleurone layer from the embryo (49). It is not surprising, therefore, that researchers have sought a similar mechanism in potato tubers. As with embryo removal in the cereal grain,

excision of growing sprouts from tubers severely inhibits further starch breakdown (14). This, together with reports of increases in endogenous gibberellins during sprouting (34), began to favor the hypothesis that hormones transported from the growing sprout were responsible for initiating/accelerating reserve mobilization in the same way as in the cereal system. Weight was added to this argument by observations that gibberellic acid increases reducing sugar levels in tuber tissue (1, 6). However, several sets of data cast doubt upon the hormone hypothesis. First, while gibberellic acid stimulates sprout extension it can reduce the accumulation of dry matter in sprouts (18). Second, the measurement of reducing sugar content is not a good determinant of rates of starch mobilization (similar concentrations can be found in tubers actively degrading starch and in tubers where breakdown has been inhibited (14). Coleman and King (7) also reported that tuber reducing sugar content, whilst responding to storage temperature, was not necessarily related to dormancy and sprouting. Third, removal of buds or growing sprouts (a procedure which inhibits starch breakdown) or treatment of tubers with GA_3, has no effect on amylase and phosphorylase activities (1, 11).

Davies and Ross (9) reported that there was no relation between the number of potential hormone-producing sites, *i.e.* the number of shoots per tuber, and the rate of starch breakdown. Thus, if hormones are involved, their sites of action must be easily saturated. They also observed that starch breakdown was initiated in the region of the phloem bundles and argued that the inherent capacity of sprouts for growth (sink strength) was an important determinant of the rate of reserve breakdown. It was hypothesized that phloem loading of sugars at the earliest stages of sprout growth would interrupt steady state conditions within the tuber and allow hydrolytic enzyme activity to be expressed in full. Fig. 2 is an autoradiograph showing the distribution of ^{14}C between several sprouts growing from a single apical bud. The ^{14}C sucrose was originally supplied to wells bored into the phloem — rich perimedulla of unsprouted tubers. It is clear that the accumulation of ^{14}C varies between sprouts of similar size, again suggesting that the sink strength of individual sprouts is an important determinant of carbohydrate allocation. The same experiment showed no relation between the extent of ^{14}C accumulation by sprouts and their distance from the point of ^{14}C application. When Oparka *et al.* (29), supplied ^{14}C sucrose to wells bored in non-sprouting tubers, sprouts that developed in storage contained up to 6% of the total ^{14}C supplied to the tuber but less than 0.2% of the tuber dry matter. Autoradiographs of tubers showed that areas around the phloem bundles contained high levels of ^{14}C, raising the possibility that substrates utilized during the initial stages of sprout growth were located in storage cells close to the sieve elements. In this case they would be used on a "last in - first out" basis. More recently, Wright and Oparka (48) examined the ability of excised tuber tissues from source (sprouting) and sink

FIG. 2. Autoradiograph showing the distribution of ^{14}C between sprouts growing from a single eye position following the supply of ^{14}C sucrose to the mother tuber.

(growing) potato tubers to accumulate and convert exogenously supplied sucrose. They found that sucrose entered storage cells of sprouting tubers passively with no conversion to starch. By contrast, in growing potato tubers sucrose was taken up by an active, carrier-mediated process, which was acutely sensitive to cell turgor pressure. This turgor-sensitive uptake mechanism declined as the tubers aged and was absent from stored or sprouting tubers (48). Autoradiographs of source and sink tuber slices have shown intense localizations of ^{14}C in the phloem of the former, but not the latter, when the slices are incubated with ^{14}C sucrose, indicating the onset of phloem loading in the sprouting state. This process is inhibited by compounds known to block the sucrose carrier (Wright and Oparka, unpublished data).

Davies and Viola (11) proved that immersing the cut surface of half tubers in GA_3 can stimulate both starch breakdown and reducing sugar accumulation when tubers are allowed to sprout in a moist medium in darkness. Shoot dry weight accumulation was also stimulated. Enhanced rates of starch breakdown again occurred without increases in enzyme activities (phosphorylase, amylase, invertase). Similar results were obtained with intact tubers during the initial period of sprout growth (up to 30 days after planting) but eventually shoot dry weight increased more rapidly in water-treated controls. This was associated with a substantial development of daughter tubers in the former treatment. Application of GA_3 to aged tubers of Maris Bard also prevented "little tuber" formation and again the rate of starch breakdown was reduced compared with water-treated control. GA_3 evidently increases the sink strength of sprouts, probably by stimulating elongation growth and cell expansion. This stimulates starch breakdown. By inhibiting tuber formation, however, GA_3 prevents the development of more active sinks for the products of starch breakdown. Once tuber formation occurs the control treatment develops more active sinks and the rate of starch breakdown is accelerated.

Sugar Metabolism in Tubers

Little is known about the products of starch breakdown in sprouting tubers and nothing about the nature of compounds transported from amyloplast to cytosol. This assumes, of course, that the transport properties of the amyloplast are maintained during rapid starch degradation and that breakdown products do not simply "diffuse" through damaged membranes. Recent work (19, 47) has shown that six-carbon compounds (*e.g.*, hexose or hexose phosphate) are transported into the amyloplast to support starch synthesis. If six-carbon compounds are also transported out of the amyloplast then the likely candidates are glucose (derived from the action of amylase and α - glucosidase) and/or hexose phosphates derived from the glucose-1-phosphate produced through the action of phosphorylase. The transport of maltose also cannot be precluded. Once in the cytosol hexoses are phosphorylated by hexokinase and the resulting hexose phosphates further metabolized. A considerable proportion will be used to synthesize sucrose, either through the action of sucrose synthase or sucrose phosphate synthase. Pressey (33) showed that both enzymes were present in stored tubers. However, recent work (37) has shown that on detachment of the tuber from the mother plant the activity of sucrose synthase decreases to barely detectable levels. Furthermore, studies with ^{13}C - labelled glucose in combination with Nuclear Magnetic Resonance have shown that the glucosyl and fructosyl moieties of sucrose become equally labelled with ^{13}C (Viola and Davies, unpublished data). This indicates that the sucrose is synthesized by sucrose phosphate synthase. Once formed the sucrose is either transported to the growing sprout or stored in the vacuole. There is

no information available on the control of sucrose and hexose transport into and out of the vacuole in potato tubers. Control of the compartmentation of sugars is again an important issue and the problem needs to be addressed.

In many potato cultivars the ratio of glucose to fructose in storage approximates unity (12) and sucrose hydrolysis often accompanies reducing sugar accumulation in storage (30, 35). The evidence therefore favors sucrose hydrolysis via the action of invertase rather than sucrose synthase in stored and sprouting tubers. Upon detaching immature tubers from the mother plant there is a considerable increase in acid invertase activity which accompanies an accumulation of reducing sugars (35, 37). Again this implicates acid invertase in regulating sucrose breakdown during storage. Sasaki *et al.* (38), demonstrated five forms of invertase in cold-stored tubers, three forms showing lower activity when tubers were stored at 20°C. Bracho and Whitaker (3) reported that the major tuber acid invertase was a 60kDa protein with 30kDa subunits. Davies and co-workers (unpublished) have purified a 58kDa protein showing acid invertase activity but the protein is apparently a monomer. There is still some controversy over the existence of an alkaline invertase in potato tubers. Morrell and ap Rees (27) have reported that both acid and alkaline invertases are present in tubers while Sowokinos (personal communication) also observed an alkaline invertase in cell suspension cultures. However, Richardson *et al.* (35), could only detect significant activity at acid pH. Since alkaline invertase is reported to be a cytosolic enzyme and acid invertase vacuolar (8), it is important that this issue is resolved. Significant alkaline invertase in sprouting tubers would not only compound the sweetening of stored tubers but would also limit the availability of sucrose for transport to growing sprouts. The activity of acid invertase *in vitro* is affected by the presence of a proteinaceous invertase inhibitor which forms a normally undissociable complex with the enzyme (3, 32). Whether or not the inhibitor plays a role *in vivo* remains to be established. Acid invertase activity is considerably higher in potato sprouts than in tubers (Burch, personal communication). This is rapidly expanding tissue where hexose formation will assist in maintaining the turgor required for elongation growth. It is tempting to speculate that if the sink strength of sprouts drives starch breakdown then invertase may have an important role to play in the process.

Conclusions

With the data presently available one might suggest, albeit arguably, that improving the rate of starch breakdown and sucrose biosynthesis in sprouting tubers would have little influence on the rate of sprout growth. One way to resolve many of the issues raised in this review is to manipulate, in a targeted or non-targeted fashion, the expression of genes encoding the proteins which catalyze starch breakdown and sucrose turnover.

Such techniques could include transposon tagging, the generation of mutants by chemical or physical means and over - or under expressing the genes of interest using Agrobacterium armed with sense or antisense constructs. Answers to such questions may be available within the next three to five years.

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