

# Plant ADP-Glucose Pyrophosphorylase – Recent Advances and Biotechnological Perspectives (*A Review*)

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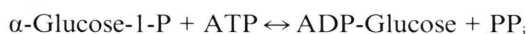
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ADP-Glucose Pyrophosphorylase, Gene Expression, Metabolic Regulation, Starch Biosynthesis

Recent advances in studies on plant ADP-glucose pyrophosphorylase (AGP), the key enzyme of starch biosynthesis, are presented. AGP constitutes the first committed and highly regulated step of starch synthesis in all plant tissues. The importance of AGP in carbohydrate metabolism and several of its features, such as potent regulation by cellular effectors (3-phosphoglycerate and  $P_i$ ), an unusual two subunit-types structure, tissue-specific and developmentally-regulated expression, and presence of the AGP-deficient mutants, make it an attractive, but complex, target for biotechnological manipulations. Some strategies for future research on AGP are discussed.

## Introduction

ADP-glucose pyrophosphorylase (ATP:  $\alpha$ -glucose-1-P adenylyl-transferase, EC 2.7.7.27) (AGP) is an important regulatory enzyme in the biosynthesis of starch and glycogen in plants and bacteria, respectively. The enzyme catalyzes the following reaction:



ADP-glucose, the product of AGP, is the major, if not the sole, glucosyl donor for starch biosynthesis in plants [1]. Starch is a predominant carbon sink of storage organs and leaves of many species, and has an obvious importance as a component of human diet, foodstock for husbandry, as well as in brewing and fermentation industries, biodegradables, paper and textile production – to mention only major applications [2].

Considerable evidence indicates that plant AGP is the rate-limiting step in starch biosynthesis for both photosynthetic and non-photosynthetic tissues [1]. A close relationship between the activity of AGP and starch content has been demonstrated for numerous mutants with impaired levels of starch [1, 3–8]. The lack of, or low, activity in

these mutants was correlated with the deficiency of AGP protein [4, 5, 9]. A close relationship was also found between levels of starch, the AGP protein, and the mRNA coding for AGP [10].

The last few years have brought a number of important reports on the structure and regulation of AGP, and a significant progress in the application of molecular biology techniques toward studying the plant enzyme. In the present review, we intend to focus only on these latest developments in the research on plant AGP, and to underline some tasks facing prospective researchers in the future. More detailed and comprehensive information on AGP and other enzymes involved in starch metabolism can be found in recent excellent reviews by Preiss [1] and Beck and Ziegler [6].

## Metabolic Control

AGP has been found in all plant tissues containing starch granules, confirming its role as the committed step in starch biosynthesis. In leaves, AGP activity is localized exclusively in the chloroplasts, *i.e.* plastids specialized in photosynthesis [1]. A study by Kim *et al.* [11], employing immunocytochemical techniques, has provided an unequivocal evidence that, in potato tubers, AGP protein is confined exclusively to the amyloplasts, *i.e.* plastids specialized in starch storage. In the amyloplasts, AGP appears to be localized within specific regions of the starch grain, which may reflect sites of active starch synthesis [11]. Thus, for both pho-

**Abbreviations:** AGP, ADP-glucose pyrophosphorylase; dap, days after pollination; kb, kilobases;  $M_r$ , molecular mass; PGA, 3-phosphoglycerate;  $P_i$ , inorganic phosphate;  $PP_i$ , pyrophosphate.

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tosynthetic and non-photosynthetic tissues, plastids are probably the only intracellular compartments containing AGP.

Pioneering studies by Preiss and co-workers (see [1]) have established that plant AGP is potentially activated by PGA and inhibited by  $P_i$ . A significant stimulatory effect on AGP has also been found with some other phosphoesters, *e.g.* fructose-6-P, glucose-6-P [12] and pyridoxal-P [13, 14]. The activation (PGA) and inhibition ( $P_i$ ) constants for plant AGP are generally of the order of micromoles.  $P_i$  has been found to inhibit the activating effect of PGA, but higher concentrations of PGA usually overcome the inhibition caused by  $P_i$ , suggesting that both effectors bind to the same site on the enzyme. These data have also suggested that a ratio of PGA/ $P_i$  may play a key regulatory role for AGP *in vivo* [1]. In leaves, a high ratio of PGA/ $P_i$  is observed during photosynthetic carbon fixation, which produces PGA as the first stable product in many plants, while  $P_i$  levels are low because of photophosphorylation. These processes coincide with starch formation, which occurs predominantly during light conditions. Studies using intact leaves and isolated chloroplasts (see [1, 15]) directly support the role of the PGA/ $P_i$  ratio in regulating starch biosynthesis *via* the modulation of AGP activity.

The regulation by PGA and  $P_i$  has also been found for AGP from non-photosynthetic tissues. Partially purified AGPs from maize endosperm [12] and potato tubers [16] showed similar mode of regulation as the leaf enzyme. Olive *et al.* [17], on the other hand, have argued that wheat endosperm AGP might not be activated by PGA and that it is less sensitive to  $P_i$  inhibition, but the actual data are yet to be published. It seems important to note that the effector sensitivity of AGP may depend on the conditions of isolation and purification of the enzyme. For instance, the regulatory properties of maize endosperm AGP were substantially affected by an endogenous proteolytic degradation; the proteolysis decreased sensitivity of the enzyme to PGA and  $P_i$ . On the other hand, the enzyme isolated in the presence of protease inhibitors exhibited regulatory properties similar to leaf AGP [12]. The proteolytic effect may perhaps explain some earlier reports, which suggested that AGP from non-photosynthetic tissues was insensitive to the PGA/ $P_i$  regulation (see [1]).

The problem of the mode of regulation of AGP from the non-photosynthetic (sink) tissues is emphasized by the fact that precursors for starch synthesis there are different from precursors found in the photosynthetic (source) tissues. In the latter, both glucose-1-P and ATP, as well as PGA, are derived from photosynthetic carbon metabolism and photophosphorylation. In the sink, however, precursors for starch biosynthesis need to be imported to the cells as sucrose. Studies with cultured sycamore cells [18] and wheat endosperm [19, 20] have suggested that glucose-1-P, the substrate of AGP, might be derived directly from the imported carbohydrate. Furthermore, the same studies have indicated that glucose-1-P rather than triose-P is transported into the amyloplasts (see also [21]). Although the limited role of triose-P in starch formation in the amyloplasts raises the question of whether PGA serves as the *in vivo* activator of AGP in this tissue (triose-P is a direct precursor to PGA through glycolysis), the recent enzymatic evidence [12, 16] suggests that there are no major differences in effector-sensitivity between AGP from the chloroplasts and amyloplasts. This suggests, in turn, that there are no functional differences for the enzyme with regard to various starch allocation and storage strategies found in plants [1].

In contrast to plant AGP, the regulation of the enzyme from *Escherichia coli* is primarily mediated by fructose-1,6-bisP (activator) and AMP (inhibitor) [22, 23]. This may reflect a different type of metabolism in *E. coli* when compared to higher plants. The enzyme from green algae and cyanobacteria, *i.e.* lower photosynthetic organisms, was found to be sensitive to the PGA/ $P_i$  control [1], suggesting an evolutionary link between photosynthesis and the mode of AGP regulation.

### Subunit structure

Despite the key position of AGP in starch biosynthesis in plants, numerous attempts to purify the enzyme either from leaves or storage tissues have failed to yield pure AGP. To our knowledge, only the enzyme from spinach leaves has been purified to homogeneity, as attested to by electrophoretic evidence and by the production of specific polyclonal antibodies [9, 13]. In fact, most of our knowledge about the structure of plant AGP comes from examination of the spinach AGP and

from the use of antibodies directed against this protein.

Plant AGP exists as a tetramer (*ca.* 210–240 kDa) composed of two small (*ca.* 50–55 kDa) and two large (*ca.* 51–60 kDa) subunits. This is in contrast to the AGP enzyme from bacteria, which is composed of four equally-sized subunits [9]. In the case of a potato tuber AGP, the two subunit types share almost identical  $M_r$  values, but their structure appears different, based on immunological properties and pI values [16]. It is believed that there are no exceptions to this two subunit type structural organization of plant AGP, even though some earlier studies, based either on electrophoretic or immunological evidence, have suggested a single type subunit for the enzyme, similarly to the bacterial AGP (for discussion see [9, 24]). A possible explanation for this could be that one of the two subunit types of plant AGP is very unstable during preparation of protein extracts or during purification [12], or that their  $M_r$  values are similar, as found for the potato tuber enzyme [16]. It seems also possible that, in certain cases, one of the subunits is expressed in excess over the other, which may perhaps lead to a preferential formation of homotetramers. As demonstrated by Muller-Rober *et al.* [25], under some metabolic conditions, mRNA coding for the large subunit of potato tuber AGP is overexpressed *in vivo*, when compared to the small subunit transcript, which has led to increased starch production.

The controversy regarding the question of heterogeneity of the subunit structure of plant AGP was at least partly due to the fact that the antibodies against the native spinach AGP, used for immunodetection, reacted preferentially with only one of the subunit types of the enzyme [9] (see also [12, 26]). Thus, western blots of crude or partially purified AGPs frequently yielded only one band, indicating a homotetrameric structure of AGP. More recent studies, however, using antibodies prepared against each of the subunit types of spinach AGP [9, 16, 24], have provided an unequivocal evidence in favour of the two subunit type structure of plant AGP.

For plant AGP, the residues responsible for substrate binding are probably lysine and phenylalanine, while a lysine residue at the carboxyl end of the protein is responsible for the activator binding. These conclusions were drawn from studies on a

irreversible binding of labelled pyridoxal-P (analog of PGA) and 8-azido-ADP-glucose (analog of ADP-glucose) to the purified spinach enzyme [13, 14, 27], and from the comparison of conserved regions of AGP from several species [17, 28, 29]. Based on studies with spinach AGP, the activator-binding site was postulated to be located both on the small and large subunit [13, 27], while the substrate-binding site is present almost exclusively on the large subunit [27]. However, an *Arabidopsis* mutant entirely lacking the large subunit of AGP and having only about 4% of the small subunit protein has been reported [5] to have about 5% of the AGP activity, when compared to wild type plants, suggesting that the small subunit of AGP in this species does contain the substrate-binding domain. Unfortunately, the state of oligomerization of the small subunits of native AGP in this *Arabidopsis* mutant is unknown at present. The homotetrameric AGP from *E. coli* differs from plant AGP in the structure of the activator-binding site, but not the substrate-binding domain [14, 27–29], probably reflecting the distinct nature of activators of the bacterial and plant AGP.

Both subunit types of plant AGP are synthesized as precursor peptides, having  $M_r$  values larger than the mature proteins [17, 26, 28]. These additional sequences are attached to the amino-termini of the mature proteins, and probably represent transit peptides which direct the proteins into plastids. Following a cleavage of the transit peptide of the immature AGP upon sequestration into plastids, the enzyme apparently is not subjected to any other post-translational modification process *in vivo*. An observation that plant AGP is phosphorylated *in vitro* by a calcium-dependent protein kinase [1] needs yet to be proved in a more physiological system.

### Molecular Biology

The last few years have brought very significant progress in the molecular biology research on AGP. Tissue-specific transcripts for AGP have been identified and sequenced from several species (Table I). The identification of cDNAs coding for AGP was accomplished by screening expression libraries with the antibodies against either spinach [17, 27, 28] or potato AGP [24], by hybridization to cDNA probes [17, 29, 30] and, most recently, by

Table I. A survey of plant species and tissues from which cDNA sequences encoding the small and large subunit of AGP are available.

Species and tissue	Literature ref.
Large subunit ( <i>Shrunken-2</i> )	
Barley endosperm <sup>1</sup>	[P. Villand, unpublished]
Maize endosperm <sup>1</sup>	[29]
Potato tubers <sup>1</sup>	[25]
Wheat endosperm <sup>1,2</sup>	[17]
Wheat leaves <sup>1,2</sup>	[17]
Small subunit ( <i>Brittle-2</i> )	
Barley endosperm <sup>1</sup>	[P. Villand, unpublished]
Maize endosperm <sup>1</sup>	[30]
Potato tubers <sup>1</sup>	[24, 25]
Rice endosperm	[28]
Spinach leaves <sup>1</sup>	[27]
Wheat endosperm	[C. C. Ainsworth, personal communication]

<sup>1</sup> Partial sequence.

<sup>2</sup> Tentatively identified as coding for the large subunit, based on its high homology to cDNA for the large subunit of potato AGP or low homology to cDNA for the small subunit of the potato enzyme [25], and based on secondary structure predictions [17].

polymerase chain reaction technique, using oligonucleotide primers based on conserved regions of the cDNAs (P. Villand, unpublished). The latter technique seems especially promising, since it allows a direct amplification of transcripts and genomic sequences, opening new opportunities for the characterization of the structure and expression of different homologous genes of AGP.

It is clear at present that plant AGP is encoded by two structural genes, which are responsible for the synthesis of the small and large subunit of the enzyme. Early studies by Tsai and Nelson [3] have established that, in maize, genes for AGP are unlinked, and mutations at either locus abolish most of the AGP activity. The loci have been called *Brittle-2* and *Shrunken-2*, respectively, which referred to distinct phenotypes of seeds of the mutant plants [3]. Partial sequences of cDNAs coding for both subunits of maize endosperm AGP have already been published [29, 30]. Based on biochemical and genetic studies, as well as analysis of isolated cDNA clones, the large and small subunits of spinach, rice and potato AGP have also been proposed to be the products of two separate genes [14, 25, 28]. Biochemical and genetic studies on the AGP-deficient mutants from *Arabidopsis* have indicated that synthesis of AGP is under the

control of at least two genes, one of which has perhaps a regulatory function [4, 5]. In contrast to plant AGP, the bacterial enzyme, which is composed of four equal subunits, is encoded by a single gene [1].

The expression of genes for AGP has been found to be developmentally regulated and tissue-specific. In rice endosperm, mRNA for AGP was detected at 3 days after pollination (dap), reaching its maximum at 14 dap. The mRNA levels were correlated with the appearance of the AGP protein, which was first detected at 6 dap, and reached its maximum at 14–18 dap [10]. The small subunit-encoding gene from potato tubers was found to be expressed in the tuber and leaves, but only low (or none) expression was in the stolon tissue [24, 25]. Based on transcript sizes or sequence analysis, studies on rice and wheat have indicated presence of different genes encoding the same subunit type of AGP in leaves and endosperm [17, 26], while in potato the same gene might be responsible for the expression of the small subunit in tubers and leaves [24]. In addition, the gene coding for the large subunit of potato AGP was found to be strongly inducible by sucrose, and its expression led to an increase in starch levels [25]. The data suggest a link between expression of the gene encoding the large subunit and the status of a tissue as either a sink or as a source of carbohydrates [25].

Based on cDNA sequences, genes coding for the small and large subunits of plant AGP share a relatively high degree of identity at the protein level. Even though the subunits clearly differ with respect to their N-terminal sequences, tryptic peptide patterns and immunological reactivity [13, 14], there is at least 52% identity between the respective derived amino acid sequences for the two subunits from potato tuber and maize endosperm [25, 30]. In our studies on cDNA fragments (about 500 bases each) encoding putative small and large subunits of barley endosperm AGP, there was about 50% identity in the derived amino acid sequences [P. Villand, unpublished]. It seems likely, thus, that genes encoding the small and large subunit of AGP may have common origin, and that they diverged to the point where the expression of each locus is necessary for AGP function [30].

There is usually a higher degree of identity, both at the cDNA and protein levels, between the same subunit types of AGP from different species or or-

gans than between the small and large subunits from the same source [29, 30]. For instance, the derived amino acid sequence of the large subunit from wheat endosperm [17] shows 83 and 94% identity to the large subunit from potato tuber [25] and barley endosperm [P. Villand, unpublished], respectively. The degree of identity of the rice endosperm small subunit of AGP [28, 29] to the corresponding proteins from potato tubers [25], maize endosperm [30], spinach leaves [27] and barley endosperm [Villand, unpublished] is 88, 89, 76 and 91%, respectively. The nucleotide sequences are less conserved (*e.g.* 85% identity at the nucleotide level between cDNAs for the small subunit of the rice and maize AGP [30], and most of the changes are at the third base of the codons [30]).

Still very little is known about the structure and identity of genomic loci encoding plant AGP. The AGP genes are probably entirely confined to the nuclei, as demonstrated by an exclusive hybridization of cDNA for AGP to the nuclei fraction of potato tubers [24]. Preliminary analyses of genomic clones, using cDNA probes from rice endosperm and potato tubers, have indicated that the rice AGP gene spans nearly 6.5 kb and contains 9 introns, while the AGP gene from potato spans up to 6.7 kb [24]. Compared to an average AGP transcript size of about 2 kb [10, 25, 26, 28, 29], it appears that genes for plant AGP have a complex intron-intervened structure, perhaps indicating a role for introns in regulating gene expression of AGP [24].

### Perspectives

As mentioned above, there is a need for more information on the structure and regulation of purified plant AGP, especially from non-photosynthetic tissues. The purification procedure, usually consisting of heat treatment, ion-exchange and hydrophobic chromatography, has proved to yield homogeneous enzyme from spinach leaves [13], but was not as effective for the AGP from maize endosperm [12] and potato tuber [16]. Furthermore, to study the structure and function of plant AGP, it is desirable to obtain large quantities of pure enzyme – a difficult task because of low abundance of the AGP protein in plants [13]. Other approaches are required, perhaps involving novel affinity chromatography matrixes, “tagging” the protein with labelled 8-azido-ADP-glu-

cose, or overproducing the plant AGP in *E. coli*, yeast or other suitable organisms. The overproduction of plant AGP could provide an efficient system not only to yield sufficient amount of the enzyme, but also to conduct site-specific mutagenesis studies. It is unknown, however, whether a given expression system would be able to correctly assemble the two subunit types of plant AGP into the active tetrameric protein.

The information about amino acid sequences of AGP subunits from different species and tissues is crucial for biotechnological manipulations of the regulatory functions of the enzyme. For instance, when considering the nature of the binding of PGA and  $P_i$  to AGP, it is assumed that  $P_i$  inhibits PGA binding by interacting electrostatically with the same amino acid residue that interacts with the phosphate group of PGA (lysine, for the small subunit of spinach AGP [14]). At the same time, the carboxyl group of PGA may react electrostatically with an arginyl or another lysyl residue. A site-directed mutagenesis approach, leading to a replacement of the phosphate group-binding amino acid with one that would still allow anchoring of the PGA molecule within a polar pocket, may decrease the affinity of  $P_i$  for the site. Similar studies with AGP from *E. coli*, leading to a replacement of a lysine residue responsible for the binding of fructose-1,6-bisP (activator) with glutamic acid, have resulted in the enzyme which was less dependent on the activator for the expression of maximal activity [23] (see also [22]).

A model developed by Neuhaus and Stitt [8] has predicted that altering the sensitivity of plant AGP to PGA and  $P_i$  could be a useful strategy to control partitioning between starch and sucrose. As the first committed step in starch synthesis, AGP constitutes an obvious target for biotechnological manipulations leading to changes in starch quantity. Under physiological conditions, levels of AGP are well in excess of those required for starch production [4, 5], suggesting that the enzyme operates in a strongly inhibited mode *in vivo*. An effort directed at the increase of sensitivity of the enzyme to PGA activation could, thus, result in enhanced starch production. In *E. coli*, mutants with increased sensitivity to fructose-1,6-bisP (activator) and decreased sensitivity to AMP (inhibitor) showed up to three-fold higher rates of glycogen synthesis than the wild type [22]. An analogous effect with

respect to starch production would also be expected if a given plant species were transformed with AGP gene(s) encoding the enzyme which is insensitive to the  $P_i$  inhibition (*e.g.* AGP from *E. coli*). An opposite strategy, involving manipulations leading to an increased sensitivity of AGP to  $P_i$  and a decreased PGA effect, could result in a redirection of carbon usually stored in starch for synthesis of quality proteins [1].

The alteration in carbon partitioning could also be studied by the so-called antisense strategy (*e.g.* [31]), involving the use of antisense transcripts of AGP in transgenic plants. This may allow the study of the effect of a reduced AGP level on starch quantity and on levels of other enzymes involved in starch production. The antisense approach may also be used in studies on the relative importance and stoichiometry of the small and large subunits in the native AGP protein. In relation to starch production, the antisense strategy has already proved useful when applied to the starch synthase gene from potato [32] (see also [33]). Antisense DNA for this enzyme was introduced to potato roots, resulting in plants which had reduced, albeit varying, starch levels. Modification of starch quantity by the antisense technique appears to yield results more rapidly than the traditional mutant induction and selection methods, and may be especially useful in polyploid species (*e.g.* potato) because the introduced DNA acts as a dominant suppressor gene [32].

Mutants having low, or none, AGP activity frequently contain altered levels of starch synthase and branching enzyme as well as other enzymes involved in starch metabolism [4–7, 34], which suggests a common regulatory mechanism for the expression of genes coding for these proteins. Whether an increased activity of AGP will also correspond to altered levels of the other enzymes remains to be determined. The mutant of *Arabidopsis*, lacking the large subunit of AGP [5], is of particular biotechnological significance, because it offers a model system for mutant complementation studies and for studies on the *native* small subunit AGP protein. The mutant may serve as a genetic background into which foreign and chemically mutated genes coding for the large subunit can be introduced and studied in physiological conditions. Changes in starch quantity in such a system can, then, be unequivocally related to the

characteristics of the introduced gene [5]. Potato plants with varying levels of amylose starch have already been created by complementation of a starch synthase-deficient mutant with the starch synthase gene (see [33]).

There is clearly a need for a comprehensive model of gene expression of AGP and for identification of DNA regions that confer tissue-specific and developmentally regulated expression of the enzyme. The prerequisite for such a study is cloning and characterization of *all* genes coding for AGP in a given plant species, and preparation of specific probes for each locus. All the data on gene expression of plant AGP have been obtained using probes of rather poorly defined specificity with regard to any particular gene coding for AGP, so it is still premature to definitively assign a given AGP expression pattern to a specific locus. At present, it appears that potato plants offer the most promising system to study gene expression of AGP, given the availability of cDNA clones for both the small and large subunit of AGP from potato tubers (see Table I), and the relatively advanced status of work on the physical and enzymatic properties of the potato AGP protein [1, 16]. Furthermore, transformation of potato with *Agrobacterium* is well established (*e.g.* [32]). Concerning cereal species, the key problem is the lack of a reliable stable transformation method for biotechnological studies. Before such a method is developed, the major thrust of the cereal AGP research will probably be directed at studying gene expression with specific probes, and using other systems for transformation with the cereal AGP gene(s) (*e.g.* *Arabidopsis*, tobacco).

In our studies on barley AGP, clones containing genes of AGP have been isolated by screening genomic library with cDNAs coding for the small and large subunit of the enzyme. Preliminary results have indicated that barley AGP is encoded by two distinct gene families, each corresponding to genes coding for the small and large subunits, similarly to the AGP genes in maize [30] and potato [25]. Each family can be further divided into at least two sub-groups (based on hybridization studies under different stringency conditions), possibly representing genes that code for the endosperm and leaf-specific AGPs [P. Villand, unpublished]. Studies involving cloning and sequencing of these genes, including promotor regions, are actively

pursued in our lab. In studies of upstream sequences of the AGP genes, the lack of a suitable method for stable transformation of barley may be partly overcome by the use of the biolistic procedure [35] for transient gene expression. The method has already found application using whole grains and transverse sections of grains of barley [36]. Characterization of specific loci coding for barley AGP, together with studies on the promoter strength of a given AGP gene (using fusion constructs of the promoter and a reporter gene for transient gene expression) should allow a detailed dissection of the promoter regions to identify elements specifying the tissue-specific and temporal patterns of expression. Oligonucleotide probes for each locus may prove useful in screening of our collection of barley monogenic mutants with altered starch levels [37] to isolate possible mutants of AGP.

Another revealing strategy should be to screen several species of different evolutionary past (*e.g.* C<sub>3</sub>, C<sub>4</sub> and CAM plants) and different organs (*i.e.* seeds, leaves, roots, *etc.*), using antibodies and cDNA probes. This type of a study may resolve the dilemmas, mentioned earlier, of whether all plant AGPs are composed of two-types of subunits, what are the exact  $M_r$  values of the subunits in different species and organs, what are the evolu-

tionary patterns in AGP expression, *etc.* The side-by-side comparison of the subunit size of a given enzyme from several species and organs may tentatively lead to the identification of isozyme(s) with altered regulation and/or catalysis characteristics (*e.g.* see [38]). It seems worth mentioning that, in carrot embryo cells, the antibodies against the native spinach AGP were reported to recognize two "unusual" peptides of 60 and 100 kDa [39], suggesting presence of distinct isozyme(s) of AGP in the embryonic tissue. It remains to be further tested whether these apparently embryo-specific proteins [39] are indeed functionally related to AGP.

Further progress is needed in order to fully understand the protein/gene structure and regulation of plant AGP. The current knowledge, however, appears sufficient to initiate efforts to gain biotechnological control of some known properties of the enzyme. The approaches discussed above, involving alterations of the allosteric site of AGP and of the AGP gene(s) expression, together with biotechnological manipulations of the enzymes directly involved in starch production (starch synthase and branching enzyme), could facilitate the control of photosynthetic carbon partitioning, hopefully leading to improvements in starch quantity and structure.

- [1] J. Preiss, in: *The Biochemistry of Plants*, pp. 181–254, **vol. 14**, Academic Press, New York 1988.
- [2] F. Rexen and L. Munck, *Cereal Crops for Industrial Use in Europe*, The Royal Veterinary and Agricultural University, Copenhagen 1984.
- [3] C. Y. Tsai and O. E. Nelson, *Science* **151**, 341–343 (1966).
- [4] T.-P. Lin, T. Caspar, C. Somerville, and J. Preiss, *Plant Physiol.* **86**, 1131–1135 (1988a).
- [5] T.-P. Lin, T. Caspar, C. Somerville, and J. Preiss, *Plant Physiol.* **88**, 1175–1181 (1988b).
- [6] E. Beck and P. Ziegler, *Annu. Rev. Plant Physiol.* **40**, 95–117 (1989).
- [7] A. M. Smith, M. Bettey, and I. D. Bedford, *Plant Physiol.* **89**, 1279–1284 (1989).
- [8] H. E. Neuhaus and M. Stitt, *Planta* **182**, 445–454 (1990).
- [9] J. Preiss, S. Danner, P. S. Summers, M. Morell, C. R. Barton, L. Yang, and M. Nieder, *Plant Physiol.* **92**, 881–885 (1990).
- [10] C. D. Reeves, H. B. Krishnan, and T. W. Okita, *Plant Physiol.* **82**, 34–40 (1986).
- [11] W. T. Kim, V. R. Franceschi, T. W. Okita, N. Robinson, M. Morell, and J. Preiss, *Plant Physiol.* **91**, 217–220 (1989).
- [12] W. Plaxton and J. Preiss, *Plant Physiol.* **83**, 105–112 (1987).
- [13] M. K. Morell, M. Bloom, V. Knowles, and J. Preiss, *Plant Physiol.* **85**, 182–187 (1987).
- [14] M. K. Morell, M. Bloom, and J. Preiss, *J. Biol. Chem.* **263**, 633–637 (1988).
- [15] M. Stitt and W. P. Quick, *Physiol. Plant* **77**, 633–641 (1989).
- [16] T. W. Okita, P. A. Nakato, J. M. Anderson, J. Sowokinos, M. Morell, and J. Preiss, *Plant Physiol.* **93**, 785–790 (1990).
- [17] M. R. Olive, R. J. Ellis, and W. W. Schuch, *Plant Mol. Biol.* **12**, 525–538 (1989).
- [18] J. Ngernprasirtsiri, P. Harinasut, D. Macherel, K. Strzalka, T. Takabe, T. Akazawa, and K. Kojima, *Plant Physiol.* **87**, 371–378 (1988).
- [19] P. L. Keeling, R. J. Wood, R. H. Tyson, and I. G. Bridges, *Plant Physiol.* **87**, 311–319 (1987).
- [20] R. H. Tyson and T. ap Rees, *Planta* **175**, 33–38 (1988).
- [21] G. Entwistle and T. ap Rees, *Biochem. J.* **271**, 467–472 (1990).
- [22] J. Preiss, in: *Bacteria in Nature*, pp. 189–258, **vol. 3**, Plenum Press, New York 1989.
- [23] A. Gardiol and J. Preiss, *Arch. Biochem. Biophys.* **280**, 175–180 (1990).
- [24] J. M. Anderson, T. W. Okita, and J. Preiss, in: *The Molecular and Cellular Biology of the Potato* (M. E. Vayda, W. D. Park, eds.), pp. 159–180, CAB International, Wallingford 1990.
- [25] B. T. Muller-Rober, J. Kossman, L. C. Hannah, L. Willmitzer, and U. Sonnewald, *Mol. Gen. Genet.* **224**, 136–146 (1990).
- [26] H. Krishnan, C. D. Reeves, and T. W. Okita, *Plant Physiol.* **81**, 642–645 (1986).
- [27] J. Preiss, D. Cress, J. Hutny, M. Morell, M. Bloom, T. Okita, and J. Anderson, in: *Biocatalysis in Agricultural Biotechnology* (J. R. Whitaker, P. E. Sonnet, eds.), pp. 84–92, American Chemical Society, Washington 1989.
- [28] J. M. Anderson, J. Hnilo, R. Larson, T. W. Okita, M. Morell, and J. Preiss, *J. Biol. Chem.* **264**, 12238–12242 (1989).
- [29] M. R. Bhawe, S. Lawrence, C. Barton, L. C. Hannah, *Plant Cell* **2**, 581–588 (1990).
- [30] J. M. Bae, M. Giroux, and L. Hannah, *Maydica* **35**, 317–322 (1990).
- [31] M. Cannon, J. Platz, M. O'Leary, C. Sookdeo, and F. Cannon, *Plant Mol. Biol.* **15**, 39–47 (1990).
- [32] R. G. F. Visser, W. J. Feenstra, and E. Jacobsen, *J. Cell. Biochem.* **14E** (Suppl.), 271 (1990).
- [33] R. B. Flavell, *AgBiotech. News* **2**, 629–630 (1990).
- [34] A. H. Schulman and H. Ahokas, *Physiol. Plant.* **78**, 583–589 (1990).
- [35] T. M. Klein, E. D. Wolf, R. Wu, and J. C. Sanford, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 8502–8505 (1989).
- [36] S. S. Klemsdal, W. Hughes, A. Lönneborg, R. B. Aalen, and O.-A. Olsen, *Mol. Gen. Genet.* (in press) (1991).
- [37] M. Bosnes, E. Harris, L. Aigeltinger, and O.-A. Olsen, *Theor. Appl. Genet.* **74**, 177–187 (1987).
- [38] L. A. Kleczkowski and D. D. Randall, *Planta* **173**, 221–229 (1988).
- [39] G. L. Keller, B. J. Nikolau, T. H. Ulrich, and E. S. Wurtele, *Plant Physiol.* **86**, 451–456 (1989).