Enhancing photosynthesis with sugar signals

Matthew Paul, Till Pellny and Oscar Goddijn

Photosynthesis has long been a target in the quest to maximize crop productivity to feed burgeoning populations. Recent evidence suggests that improved photosynthetic performance can be most easily achieved by modifying sugar-signalling mechanisms that control the expression of genes for whole pathways and processes that determine photosynthetic capacity and source-sink balance, rather than by directly targeting individual ‘key’ enzymes. Here, we highlight recent progress and support for the hypothesis that genetic modification of trehalose metabolism through its interaction with sugar-signalling pathways can enhance photosynthetic capacity.

For a long time, one of the aims of plant scientists has been to improve photosynthesis to maximize crop productivity. The logic of the argument is clear: the yield of crops broadly relates to the amount of light intercepted and the cumulative photosynthesis over a growing season. If photosynthesis could be increased then it would be possible to capitalize on and extend this relationship. Breeding and agronomic practice have effectively achieved this over the past century by creating the conditions under which photosynthesis can flourish. New varieties have been bred with bigger assimilation sinks and with better coordination of leaf production with the amount of light available during the growing season. High fertilizer use has further facilitated a high photosynthetic rate and, in the horticulture industry, ‘fertilization’ of tomato crops with CO₂ is used routinely to maximize carbon fixation.

As our knowledge of plant biochemistry, metabolism and, latterly, molecular biology has increased, our sights have shifted towards more targeted manipulation of metabolism itself. If we could find out which are the ‘key’ enzymes responsible for controlling photosynthetic rate or those that lead to photosynthetic losses of the CO₂, it would be possible to target these enzymes to improve photosynthesis. Isolation of the genes and genetic transformation would enable photosynthetic carbon acquisition to be increased directly. The reasoning behind this was good, as far as we knew at that time.

The last will be first and the first last in metabolic regulation

Genetic modification of plant metabolism proceeded apace during the 1990s and several strong messages have come from this. One is that our concept of ‘key’ enzymes has changed drastically. It was thought that enzymes that were essentially irreversible and subject to sophisticated fine control mechanisms represented the best targets because they exerted the most control on flux. In the Calvin cycle, through which carbon enters the reactions of photosynthesis and metabolism, there are four key irreversible enzymes: Rubisco, in the carboxylation phase of the cycle, and fructose-1,6-bisphosphatase (FBPase), sedoheptulose-1,7-bisphosphatase (SBPase) and phosphoribulokinase (PRK), in the regenerative phase (Fig. 1). In experiments on transgenic tobacco and potato expressing antisense cDNA to each of these enzymes, a decrease of 50% maximum catalytic enzyme activity had minimal impact on the rate of photosynthesis under standard growth-room conditions. The most extreme of these is PRK, 85% of whose maximum catalytic activity can be removed before there is any effect on photosynthesis. This is because sophisticated fine control of PRK can overcome a large genetic decrease in PRK protein.

The full story of control of Calvin cycle flux comes from analysis under a range of conditions that plants are likely to encounter in the natural environment. This emphasizes that control exerted on irreversible Calvin cycle enzymes occurs through allosteric and post-translational control mechanisms, and that targeting these enzymes for genetic modification is problematic because of the counteracting fine control mechanisms.

Another way in which control of enzyme activity is exerted is through expression level. The catalytic activity of reversible enzymes not subject to fine control is regulated in this way, and research using transgenic plants has shown that reversible enzymes have the potential to exert a high degree of control. This is true for aldolase, for example. However, it is not just the catalytic activity of reversible enzymes that is controlled by gene regulation. A second important conclusion of plant science over the 1990s, which developed from several approaches and has been confirmed by work on the response of plants to elevated CO₂, is that the control of photosynthesis at the molecular level by sugar and nitrogen signals through changes in whole plant carbon-nitrogen balance overrides the control of photosynthesis by other mechanisms. This gives us due to how to proceed with the genetic modification of photosynthesis. Direct genetic manipulation of photosynthesis by targeting key enzymes, even if it were possible to overcome post-translational control mechanisms and the flexibility of metabolism and to increase the photosynthetic rate, could be frustrated by the overproduction of a sugar signal that repressed expression of photosynthetic genes. To achieve significant positive changes in photosynthesis and resource allocation, it will be necessary to understand the signals and molecular mechanisms that control the expression not just of aldolase, PRK or Rubisco, but of whole pathways and suites of genes that determine photosynthetic capacity. These mechanisms underpin source-sink interactions and productivity in plants.
Signals from trehalose metabolism

In addition to the change in thinking about the control of metabolic fluxes that we did know about, a further surprise of plant metabolism over the past two or three years has been the finding that the genes for trehalose metabolism are universal in plants. Trehalose (α-D-glucopyranosyl–β-D-glucopyranoside), a non-reducing disaccharide consisting of two molecules of glucose, is the major disaccharide of microorganisms, fungi and insects, but has been largely superseded by sucrose in plants (although trehalose does accumulate in some resurrection species). Trehalose is synthesized from glucose-6-phosphate (G6P) and uridine-5-diphosphoglucose (UDPG) in a reaction catalysed by trehalose phosphate synthase (TPS) (Fig. 2). The phosphate group is removed from the trehalose-6-phosphate (T6P) formed in this reaction sequence by trehalose phosphate phosphatase (TPP). It is now clear that the genes are widespread if not universal in higher plants. At least five distinct sequences with homologies to microbial TPS genes can be found in the Arabidopsis genome, one of which has been doned and shown to synthesize T6P (Ref. 12). This raises the question of what the function of these genes and of trehalose metabolism in plants are.

In yeast, trehalose metabolism performs a signalling role13, although the precise mechanistic details of this are still being unravelled, as are interspecific differences between yeast species and other microorganisms14. Evidence from our current research suggests that a similar role has persisted in plants15. In experiments in which E. coli otsA and otsB genes, which encode TPS and TPP, respectively, were expressed in transgenic tobacco (Nicotiana tabacum), there were effects consistent with an effect on sugar signalling. In plants expressing E. coli TPS, rates of photosynthesis per unit leaf area are increased by up to a third under saturating irradiance; in plants expressing TPP, photosynthesis is decreased by a similar amount15. This shows that it is possible to increase the photosynthetic capacity of plants. Measurements are continuing to determine the biochemical and molecular basis of these results but the data are consistent with the hypothesis that genetic modification of trehalose metabolism has affected photosynthetic capacity through sugar signalling.

Our model to explain the findings centres on T6P. In yeast, T6P has a role in controlling glycolysis and sugar signalling through its interaction with hexokinase, a putative sensor in yeast and plants13. T6P has not been previously found in plant tissue, although this might be because researchers have not been looking and because methods have not been sensitive enough to detect it. We have developed a sensitive assay for T6P in plants, using a hexokinase isoform from Yarrowia lipolytica. The hexokinaseI from this yeast is particularly sensitive to inhibition by T6P and forms the basis of an accurate method to quantify T6P (Ref. 16). Our data using this assay technique have also been confirmed by HPLC measurements. In TPS lines with enhanced rates of photosynthesis, the T6P content is fivefold higher. This shows that it is possible to increase the photosynthetic capacity of plants.

In this model, T6P interacts with plant hexokinase as it does in yeast13 (Fig. 2). Plants have several isoforms of hexokinase and T6P might interact with just one of these. We suggest that T6P interacts with hexokinase, modifying perception of glucose content. In plants expressing E. coli TPS and hence with higher T6P content, a carbon deficit is perceived through interaction with hexokinase and photosynthetic capacity is enhanced. Plants expressing E. coli TPP, by contrast, have levels of T6P that are below detection. Low T6P content might mean that these plants sense a surfeit of glucose and hence that the expression of genes for photosynthesis is decreased. Clearly, more work is required to substantiate this hypothesis. In fact, the functioning
Acknowledgements

IACR receives grant-aided support from the Biotechnology and Biological Sciences Research Council of the UK. We are indebted to Klaus-Peter Krause for his part in setting up the collaboration between the laboratories. Our gratitude extends to Carlos Gancedo (Madrid, Spain) for providing us with Yarrowia lipolytica hexokinase.

Fig. 2. Interaction of trehalose-6-phosphate (T6P) with hexokinase (HXK) and sugar signalling pathways. T6P is synthesized from uridine-5-diphosphoglucone (UDPG) and G6P, catalysed by trehalose phosphate synthase (TPS). The phosphate group is removed from T6P by trehalose phosphate phosphatase (TPP). The reaction sequence is similar to that of sucrose, except that fructose-6-phosphate (F6P) substitutes for G6P in sucrose synthesis. HXK, sucrose-nonfermenting-1-related protein kinase1 (SnRK1) and 14-3-3 proteins are components of a sugar-sensing complex in plants. This putative complex underpins source–sink interactions and enables plants to redistribute resources between processes that produce carbon compounds in photosynthesis and those that store, mobilize and consume carbon in response to changing source–sink relationships caused by environment and development. Broken arrows indicate the link between HXK, sugar sensing and photosynthesis; dotted green line indicates putative trehalose-sensing complex.

of plant hexokinases and their role in sugar sensing requires more detailed characterization. What is clear, however, is that the function of hexokinase in plants is subtly different to that in heterotrophs. Plants can obtain hexose phosphate directly from photosynthesis without the need for hexokinase. Plant hexokinase functions in the re-entry of hexose into the hexose phosphate pool, mainly from sucrose and starch breakdown. Hexokinase might be part of cycles of sucrose and starch synthesis and breakdown that are part of the mechanism by which plants sense carbohydrate status and adjust resource allocation accordingly.

There are strong parallels between trehalose and sucrose metabolism that might help in interpreting the findings. Sucrose phosphate synthase (SPS) and sucrose phosphate phosphatase (SPP) exist as a complex. This might ensure efficient substrate channelling and that, under most conditions, sucrose-6-phosphate (S6P) is only seen by SPP. A similar enzyme complex has been shown for trehalose synthesis in yeast and again, under most conditions, T6P remains within the complex. In E. coli, TPS and TPP do not form a complex and, in plants expressing E. coli TPS, T6P synthesized by the transgene is free to interact with other enzymes and proteins within the cell. Thus, cells are exposed to a signal that is normally within the enzyme complex. ‘Normally’ might be the key word here: plant TPS, like SPS, interacts with 14-3-3 proteins. These are thought to be involved in the coordination of metabolism in response to carbon supply and affect protein–protein interactions. Loss of 14-3-3 binding under conditions of low carbon supply might affect the integrity of SPS–SPP and TPS–TPP complexes. Dissociation of the complexes under carbon starvation would liberate T6P to interact with enzymes and proteins such as hexokinase. A similar scenario for S6P can be envisaged. The effects of S6P on metabolism as for T6P in plants are largely unknown.

When interpreting the results, it is also important to bear in mind that any effect on hexokinase will have an effect on metabolite flux and metabolite content downstream of hexokinase, which might in turn affect sugar signalling. G6P, for example, the product of hexokinase, does not exert feedback control on hexokinase in plants and fungi as it does in animals, but it does affect the activity of SnRK1 (SNF1-related protein kinase1), a plant protein kinase related to SNF1 (sucrose nonfermenting1) from yeast. SnRK1 has been implicated in changes in the activities and expression of enzymes involved in sucrose and starch metabolism in response to carbon supply.

The plant material offers an unprecedented opportunity to study the interactions between metabolism, sugar signalling and resource allocation at the molecular, biochemical and physiological levels. In particular, it might be possible to isolate transcription factors that coordinate expression of the many genes that determine photosynthetic capacity and leaf development. Indeed, isolation of transcription factors might be a more readily attainable goal than understanding the complex mechanistic details of how signals arising from trehalose metabolism can enhance photosynthetic rate.

Conclusions

We have shown that it is possible to enhance photosynthetic capacity and photosynthetic rate in transgenic plants through the insertion of the E. coli TPS gene into A. There are many questions still to be resolved, but the main purpose of this article is to highlight the potential significance of the research. Evidence is accumulating that an ancient and once obscure metabolic pathway in plants has a role in sugar signalling. Full understanding of the mechanistic basis of enhanced photosynthesis in these plants will come from a detailed analysis of the interaction of metabolism with sugar signalling pathways. Of particular interest will be the characterization of plant hexokinase isoforms, plant TPS and TPP, and how
they interact with each other and with metabolism, and sugar signalling and its effect on resource allocation in plants and crops.

It is particularly exciting that the transgenic plant material gives us the opportunity to learn more about sugar signals and molecular mechanisms that underpin source–sink interactions and crop productivity. An opportunity now presents itself to enhance the photosynthesis of crops by manipulating sugar signals and signalling pathways.

References


3 Harrison, E.P. et al. (1998) Reduced sedoheptulose-1,7-bisphosphatase levels in transgenic tobacco lead to decreased photosynthetic capacity and altered carbohydrate accumulation. Planta 204, 27–36


7 Haake, V. et al. (1999) Changes in aldolase activity in wild-type potato plants are important for acclimation to growth irradiance and carbon dioxide concentration, because plastid aldolase exerts control over the ambient rate of photosynthesis across a range of growth conditions. Plant J. 17, 479–489


15 Paul, M.J. et al. (2000) A role for trehalose metabolism in resource allocation in plants. In Signals, Sensing and Plant Primary Metabolism, p. 57, Collaborative Research Centre, Potsdam, Germany


21 Cotelle, V. et al. (2000) 14-3-3s regulate global cleavage of their diverse binding partners in sugar-starved Arabidopsis cells. EMBO J. 19, 2869–2876


The apicoplast: a new member of the plastid family

Eric Maréchal and Marie-France Cesbron-Delauw

Protozoan parasites of the phylum Apicomplexa include pathogens such as Plasmodium, Toxoplasma and Cryptosporidium. They have been shown to contain a vestigial nonphotosynthetic plastid, the apicoplast, which might have arisen by secondary endosymbiosis. Little is known about the function of the apicoplast but the parasites exhibit delayed cell death when their apicoplast is impaired. The discovery of the apicoplast opens an unexpected opportunity to link current fundamental research on plant and algal plastids to the physiology of apicomplexans. For example, the apicoplast might provide new targets for innovative drugs that act as herbicides and do not affect the mammalian host.

Most closely related to ciliates and dinoflagellates, the phylum Apicomplexa constitutes a particularly diverse and ancient group of parasitic protists. They are classified as protozoa (>4000 species) that mostly have an obligately intracellular lifestyle. Some are important causative agents of human and animal diseases, the most potenct of which is Plasmodium, the agent of malaria, recognized by the World Health Organization as being in the top three killers in the world. Toxoplasma gondii and Cryptosporidium are major opportunistic pathogens in immunocompromised patients. Others cause economically important animal diseases such as babesiosis (Texas water fever), coccidiosis and theileriosis (East Coast fever). In spite of sustained efforts, no efficient vaccine is available and chemotherapy is facing the emergence of drug resistance, which is becoming crucial in malaria treatment.

Apicomplexan parasites have complex life cycles that involve both asexual and sexual reproductions, with many variations in the different groups. Apicomplexans are transmitted.