An Early Arabidopsis Demonstration. Resolving a Few Issues Concerning Photorespiration

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One of the great discoveries of 20th century biology was the elucidation of the pathway of photosynthetic CO₂ fixation by Calvin, Benson, and colleagues (18). Among the many loose ends that remained after the photosynthetic carbon reduction cycle had been defined was a series of observations showing that when ¹⁴CO₂ was supplied to higher plants in the light, glycolate, Gly, Ser, and several other metabolites that could not be placed in the cycle were also rapidly labeled. By the late 1960s Ed Tolbert, Israel Zelitch, and others had identified the steps of a metabolic pathway in which two molecules of glycolate were converted in a series of enzymatic reactions through glyoxylate, Gly, Ser, and hydroxypropionate to one molecule each of CO₂ and phosphoglycerate (Fig. 1; 23, 24). It had also been established that the CO₂ released from glycolate metabolism was the source of at least some of the CO₂ released during a process that had become known as photorespiration (4, 25). However, there was no generally accepted explanation for the biosynthetic origin of glycolate or why it was rapidly labeled by ¹⁴CO₂.

Photorespiration was discovered shortly after the first infrared gas analyzers became available in the mid 20th century (6). The phenomenon was described as the light-dependent release of CO₂, a difficult process to measure against a background of concurrent photosynthetic CO₂ fixation and mitochondrial or “dark” respiration. To accurately measure the magnitude of photorespiration it was necessary to use elaborate pulse-chase isotope labeling methods that could distinguish recently fixed carbon from carbon fixed during an earlier time period (2). The best estimates suggested that under normal circumstances, a C₃ plant could photorespire as much as 25% of the carbon fixed by photosynthesis. Thus, photorespiration was considered a potentially wasteful process that was limiting plant productivity.

My interest in the problem was stimulated by a theory advanced by Bill Ogren and George Bowes that was the equivalent of the Grand Unified Theory of Photosynthesis and Photorespiration. In the late 1960s, Ogren had been intrigued by the observation that photosynthetic CO₂ fixation is strongly inhibited by oxygen. This is simply demonstrated: Plants grown in 350 μL L⁻¹ CO₂ and 2% (v/v) O₂ have much higher rates of CO₂ fixation than plants grown in 350 μL L⁻¹ CO₂ and 21% (v/v) O₂. Higher levels of CO₂, however, suppress the negative effect of O₂. These effects were exhaustively measured in a series of carefully executed experiments on photosynthetic gas exchange that became a scientific touchstone for Ogren (7). He resolved to try to find a mechanistic model of photosynthetic CO₂ fixation that would explain the inhibitory effect of O₂ and the salutary effect of CO₂. The recognition that O₂ and CO₂ had mutually competitive effects on photosynthesis led him invariably to the conclusion that O₂ must compete with CO₂ as a substrate for the enzyme responsible for photosynthetic CO₂ fixation, RuBP carboxylase. I consider this to be one of the most brilliant examples of deductive reasoning in 20th century plant biology.

On the basis of this theory, Ogren’s postdoc, George Bowes, carried out a protracted search for RuBP oxygenase activity. After more than a year of many failed attempts, RuBP oxygenase activity was at last detected and determined to be a property of RuBP carboxylase (1, 14). The enzyme was subsequently renamed RuBP carboxylase/oxygenase, or Rubisco. I think about this experiment frequently and that it is that nothing substitutes for a good theory (and tenacity). It was not just George Bowes who initially had trouble demonstrating RuBP oxygenase activity. About a year after the oxygenase paper was published, George Lorimer, a student of Ed Tolbert’s at the time, reportedly burst into Ogren’s office with an armful of O₂ electrode tracings from failed attempts to measure RuBP oxygenase activity and dumped them on Ogren’s desk with the words “It doesn’t work.” Lorimer was so inflamed with the idea that Bowes’ and Ogren’s paper was erroneous, and that he had wasted time testing their idea, that he had driven all the way from Lansing to Urbana to deliver the message in person. Of course it did work and Lorimer went on to show why with an elegant
series of papers on the mechanistic basis of catalysis by the enzyme (5).

The product of RuBP oxygenase activity is phosphoglycolate (14). Thus, the discovery of oxygenase activity provided a credible explanation for the origin of glycolate. Because CO₂ and O₂ are mutually competitive substrates of RuBP carboxylase/oxygenase, the discovery of oxygenase activity also explained the effects of CO₂ and O₂ concentration on photosynthesis and photorespiration. Ogren went one step further to suggest that photorespiration was not biologically necessary—that it had evolved only to recycle carbon from phosphoglycolate back into the Calvin cycle and that the CO₂ loss was the cost of recycling the other three carbons back into the Calvin cycle. The evidence for this was that plants grown in low levels of O₂ or high levels of CO₂ were more productive than plants grown in air despite strongly reduced levels of flux through the photorespiratory pathway. Thus, the implication was clear: Plant productivity could be strongly enhanced by identifying mutants with reduced amounts of photorespiration. However, in a precient analysis of Rubisco’s probable catalytic mechanism, Lorimer and John Andrews hypothesized that RuBP carboxylation and oxygenation could not be uncoupled because oxygenase activity is due to autoxidation of an obligatory intermediate in the carboxylation reaction (12).

Except for Tolbert and colleagues, who had verified for themselves the existence of RuBP oxygenase activity, the RuBP oxygenase theory of photorespiration gained acceptance rather slowly. I arrived in Ogren’s lab as a postdoc about 7 years after the first paper on RuBP oxygenase had been published and the topic was still a subject of heated debate; people on opposite sides of the issue were literally shouting at each other during long public arguments at scientific meetings. I have never witnessed any public arguments comparable with those that dominated the 1978 Gordon Conference on photosynthesis. The opposition was led by Israel Zelitch who was of the opinion that the RuBP oxygenase-based mechanism of glycolate synthesis was inconsistent with many miscellaneous observations that had been made during the long search for the source of photorespiratory glycolate (25). Zelitch and others also argued that phosphoglycolate could not be an important precursor of photorespiratory glycolate because measurements of flux through phosphoglycolate were much too low to account for the magnitude of photorespiratory CO₂ metabolism (25). Zelitch further claimed that he had been able to reduce glycolate synthesis and photorespiration by treating plant tissues with glycidate, that blocking glycolate oxidation inhibited photorespiration and increased photosynthesis, that Gly oxidation could not account for most photorespiratory CO₂ release, and that there was substantial genetic variation in the ratio of photosynthesis to photorespiration. These and many related observa-

Figure 1. An abbreviated scheme of the photorespiratory pathway. Phosphoglycolate produced by ribulose bisphosphate (RuBP) oxygenase activity is converted to glycolate by phosphoglycolate phosphatase in the chloroplast. Glycolate enters peroxisomes and is converted to glyoxylate by glycolate oxidase. Glyoxylate is transaminated to Gly by either Ser:glyoxylate aminotransferase or Glu:glyoxylate aminotransferase. In mitochondria, Gly is converted to CO₂, ammonia and the methylene group of methylene tetrahydrofolate (C₁-THF). Gly and C₁-THF condense to produce Ser. Peroxosomal Ser is deaminated to hydroxypyruvate, which is reduced to glycine by hydroxypyruvate reductase. Glycine enters the chloroplast and is phosphorylated to 3-phosphoglycerate, an intermediate of the Calvin cycle. Ammonia released during Gly decarboxylation is used by Gln synthetase to produce Gln. Gln synthase condenses 2-oxoglutarate (2-OG) and Gln to produce two molecules of Glu. A dicarboxylate transporter in the chloroplast envelope transfers oxoglutarate, Glu, and Gln across the chloroplast envelope. Overall, two molecules of phosphoglycolate are converted to one molecule of phosphoglycerate and one molecule of CO₂.


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tions seemed at variance not only with Ogren’s theory but also with some aspects of the scheme for glycolate metabolism developed by Tolbert and colleagues (23). In retrospect, it seems to me that Zelitch was misled by reliance on a number of technically flawed attempts to quantitatively measure photorespiration and metabolite flux.

Shauna Somerville and I arrived in Bill Ogren’s lab for graduate and postdoctoral work, respectively, in 1978 with three clear ideas. The first was that plant biology as a field needed a model organism with good experimental properties. The second was that Arabidopsis was that organism. The third was an idea about how to solve some of the problems that were generating heated debate among plant physiologists and biochemists at that time. We had formulated the idea of developing Arabidopsis as a model organism for plant biology during an extended visit to Paris where we spent our time reading in the library of the Institut Pierre et Marie Curie and doing gedanken experiments in the cafes. The groundbreaking work of Chilton et al. (3), showing that Agrobacterium tumefaciens transferred a fragment of DNA from the Ti plasmid into plant genomes, led us to conclude that the development of methods for plant transformation were imminent. We realized that this new technology would create a new opportunity to develop the use of genetics and molecular biology as a general approach to problems in plant biology. We had begun using the tools of molecular biology as graduate students and were certain that these tools would be rapidly adapted by plant biologists and that, when that happened, plant biologists would recognize the need for a facile genetic system. Although there was a long history of very sophisticated genetics in plant biology, our impression was that plant genetics was dissociated from mainstream plant biology and the average plant biologist did not understand the power of genetics as a tool for dissecting problems in general biology. I think this may have arisen, in part, because many plant geneticists had a tendency to work on problems that were of interest primarily in the context of genetics rather than using genetic methods to solve problems of interest to physiologists or biochemists. Therefore, we decided that to stimulate the interest of plant biologists in the use of genetic methods, we should focus on solving a problem that was of broad interest and was amenable to a genetic approach. In the cafes of Paris we formulated a “demonstration experiment” in which we envisioned solving a problem in plant biology using the kind of genetic approach that was used in Escherichia coli genetics. We spent our mornings reading the current issues of the major plant journals, and the rest of the day sitting in the cafes talking about the papers we had read that day and trying to envision a genetic approach to the problems they discussed.

In the course of our reading we had come upon an article by George Redei extolling the virtues of Arabidopsis as a model system for plant genetics (15). Redei pointed out that Arabidopsis was closely related to many important crop species, small, rapid cycling, diploid, self-fertilizing, easily mutagenized, had the smallest known plant genome, and already had the rudiments of a genetic map. These properties corresponded to those that we were looking for in a model plant and we determined to adopt Arabidopsis as our experimental system. We assumed that routine genetic transformation was imminent and that we should focus our efforts on genetically defining an interesting problem so that we could make use of the molecular tools that were being developed by others.

We were attracted to the problem of photorespiration because it seemed important and it was vividly controversial. One need only compare the views expressed in two contemporaneous reviews of the subject to get a clear impression of a major scientific controversy of the period (4, 25). The problem seemed important because, although there was a lot of disagreement about the mechanism of photorespiration, there was broad agreement that if it could be genetically reduced it would lead to a major increase in primary plant productivity.

One of the major challenges we faced from the outset was deciding which side of the controversy was most likely to be correct. I had done my graduate work in E. coli genetics and Shauna had done a masters degree in plant breeding so neither of us had any experience with plant physiology or biochemistry. We eventually decided that the proponents of the RuBP oxygenase theory were advocating the most convincing explanation for the biological phenomena. We realized that the competitive actions of O2 and CO2 on the outcomes of the RuBP carboxylase/oxygenase reactions could be used as a basis for mutant selection. We hypothesized that we could isolate plant mutants with defects in photorespiration by growing mutant populations in high concentrations of CO2, where the pathway was suppressed, then scoring for mutations in the pathway by placing them in air. We guessed that mutants with enzymatic defects in the pathway would be viable in high CO2 but would be inviable in air because of the drain of carbon from the Calvin cycle or other effects. After our return from Paris we joined the laboratory of Bill Ogren who agreed to let us test our ideas, and to help us learn plant physiology and biochemistry. Following methods that George Redei and others had developed for mutagenizing Arabidopsis with ethyl methane sulfonate we produced a mutagenized M2 population. We grew the plants in growth chambers in which the atmosphere was held at roughly 1% (v/v) CO2 by pumping air into the chambers at several liters per minute and bleeding inexpensive welding grade CO2 into the air stream. Once the plants became established, we removed any plants that were chlorotic, stunted, or morphologically abnor-
mal, then stopped supplementing the plants with CO₂. After several days of illumination in air, we scored the populations for plants that were chlorotic. To our delight, dozens of plants from the first screen turned chlorotic! As anyone who has done a mutant screen knows, the risk of investing a lot of effort for no result was behind us.

After verifying that the mutant phenotypes were heritable, we set about trying to determine the biochemical nature of the defects. A wealth of literature existed concerning the labeling of the products of photosynthesis with ¹⁴CO₂. By labeling the various mutants and then resolving the primary products by a combination of ion-exchange and thin-layer chromatography we were able to group the mutants into various classes based on what metabolites accumulated. We then performed enzymes assays on extracts of the various mutants. The first unambiguous result that we obtained was a mutant that was completely deficient in phosphoglycolate phosphatase (19). I finished the enzyme assays at about midnight and was so excited by the evidence that we had identified a mutant for the enzyme that I phoned Bill Ogren at home to share the news. Bill was characteristically calm but enthusiastic considering the late hour.

By similar approaches we identified mutants in Ser:glyoxylate aminotransferase, Gly decarboxylase, and Ser transhydroxymethyltransferase (13, 17) and Glu synthase (20). These mutants were very useful in resolving many of the problems in the area of photorespiration that had been intractable to conventional biochemical approaches (13). For instance, when illuminated in air, the phosphoglycolate phosphatase mutant rapidly accumulated large amounts of phosphoglycolate but failed to accumulate glycolate and essentially lacked photorespiration. This observation largely ended debate about the key issues of whether the amount of RuBP oxygenase activity in vitro was adequate to support photorespiration or whether there were alternate sources of glycolate. In a similar manner, when placed in air the Glu synthase mutants became rapidly depleted of Glu (20), confirming the recently proposed role for the enzyme in recycling photorespiratory nitrogen (9). Shauna also characterized a mutant deficient in the chloroplast dicarboxylate transporter and showed that the mutants were unable to recycle photorespiratory ammonia, demonstrating the operation of a Glu-oxoglutarate shuttle in the photorespiratory cycle (22). When provided with exogenous ammonium, the Ser transhydroxymethyltransferase mutant was found to be completely deficient in photorespiratory CO₂ release, providing unambiguous evidence that in plants with adequate nitrogen, Gly decarboxylation was normally the sole source of photorespiratory CO₂ (17). In short, the mutants provided novel and compelling tests of the various theories that had been proposed based on biochemical or physiological criteria. The results confirmed all of the predictions of the RuBP carboxylase/oxygenase-based theory of photorespiration and also confirmed the role of many of the steps in photorespiratory metabolism that had been proposed by Ed Tolbert and colleagues. The success of the approach generated some of the earliest converts to the utility of Arabidopsis genetics. In addition, Peter Lea, Ben Miflin, Alf Keys, and colleagues at Rothamstead used similar approaches to isolate a rich collection of photorespiratory mutants of barley that have been extensively utilized in continuing studies of photorespiration (8, 10).

In addition to the mutants in the photorespiratory pathway, we had isolated several mutants that we could not place in the pathway by isotopic labeling experiments. The mutants were clearly defective in photosynthetic CO₂ fixation at low concentrations of atmospheric CO₂ but had relatively normal levels of CO₂ fixation at high levels of CO₂ (21). In vitro assays with Rubisco showed that, in the mutants, the enzyme was present in an inactive form that could be converted to normal levels of activity by preincubation with high levels of sodium bicarbonate. Bill Lang and Ogren had discovered the activation of Rubisco by bicarbonate some years earlier. This effect had been shown by George Lormier to be due to the formation of a carbamyl group on a Lys group of the enzyme that was involved in binding a metal ion required for catalysis (11). This led to the idea that the mutants had a defect in RuBP carboxylase/oxygenase activation.

Shortly after isolating this mutant I left Ogren's lab to found my own lab at the University of Alberta in Edmonton. Mike Salvucci, a new postdoc in Ogren's lab, inherited the mutant and went on to show with Archie Portis that the mutant was deficient in an enzyme that was specifically required to activate Rubisco (16). This unique enzyme, now called Rubisco activase, is thought to activate Rubisco by removing an inhibitory isomer of RuBP from the active site of Rubisco. The discovery of Rubisco activase provides a satisfying example of the utility of the genetic approach to biological problems. The existence of the enzyme was not even hinted at in the hundreds of papers describing the properties of Rubisco prior to the isolation of the mutant.

In retrospect, the photorespiratory mutant work provided a timely example of the use of a directed genetic approach to dissect a complex problem in plant biology and helped pave the way for acceptance of Arabidopsis as a model organism. I think that the success of the project was due, in part at least, to having spent a lot of time thinking about it in a congenial setting before starting experimental work, a technique that seems as useful today as ever.

LITERATURE CITED


