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Minireview

C_4 photosynthesis: discovery and resolution

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Abstract

This Minireview provides a brief account of the scene and interesting turn of events surrounding the discovery and resolution of the mechanism of C_4 photosynthesis, as well as the recognition of the process by the wider plant science community.

Abbreviations: PCR – photosynthetic carbon reduction; 3-PGA – 3-phosphoglyceric acid; PEP – phosphoenolpyruvate

Introduction

The events surrounding the discovery and resolution of the mechanism of C_4 photosynthesis provide many of the elements of a good mystery thriller: good and bad luck, people, politics, wisdom of hindsight, serendipity, and being in the right place at the right time. This story is briefly outlined here. A more detailed account of these events is provided in earlier chapters and articles (Hatch 1992; Hatch and Slack 1998; Hatch 1999), to which I will frequently refer.

Prehistory

A retrospective search of the plant science literature of the early 1960s shows that it abounded with hints of a different form of photosynthesis operating in a particular group of tropical grasses. Of course, at that time it was reasonably assumed that the then recently defined Photosynthetic Carbon Reduction (PCR) cycle (or Calvin–Benson–Bassham cycle) accounted for CO_2 assimilation in all photosynthetic organisms (Calvin 1989; Fuller 1999). However, a keen and insightful reader of that literature should notice that a small group of tropical grasses share an array of unique or unusual anatomical and physiological features, all related in one way or the other to the photosynthetic process. Included amongst these (see Hatch and Slack 1998; and Hatch 1999 for details and references) were:

- a specialized leaf anatomy termed Kranz anatomy,
- dimorphic and bifunctional chloroplasts,
- high efficiency of water use,
- a very low CO_2 compensation point,
- high growth rates at higher light and temperature,
- high leaf photosynthesis rates associated with high light and temperature optima for photosynthesis.

However, this coincidence of unusual features went largely unnoticed, or at least it did not stimulate any search for a biochemical explanation. The links between these features and the operation of the C_4 pathway gradually emerged after the discovery of the process.

Discovery

The C_4 story begins with a brief unreferenced note in the 1954 Annual Report of the Hawaiian Sugar Planters Association Experiment Station. It noted that some compounds other than 3-phosphoglyceric acid (3-PGA) were rapidly labeled during $^{14}CO_2$ assimilation

by sugarcane leaves. In subsequent annual reports over the next six years, there were brief accounts of further studies identifying malate and aspartate amongst the early labeled products. A third labeled compound initially identified as phosphomalate was later shown to be 3-PGA. These results were briefly mentioned in the abstracts of a meeting of the Hawaiian Academy of Science (Kortschak et al. 1957) and again by George Burr in a paper presented at the 1961 Pacific Science Congress on the use of radioisotopes in the Hawaiian sugar industry. The proceedings of this meeting were published later in the International Journal of Applied Radiation and Isotopes (Burr 1962).

It is of historical interest to note that about this time a young Russian worker, Yuri Karpilov, reported similar early labeling of malate and aspartate during $^{14}CO_2$ assimilation by maize leaves. 3-PGA and sugars phosphates were labeled after longer times. These results appeared in a publication from the Kazan Agricultural Institute (Karpilov 1960). According to the translation, Karpilov concluded that the results were 'not characteristic of other plant species.' Soon after, he published a short paper with a more senior Russian colleague in which they considered artifactual effects of different killing and extraction procedures on the pattern of labeled products. However, Karpilov did not pursue this work further for another eight or nine years, at which time scientists in the West first became aware of this earlier report. I met Yuri Karpilov several years later, at the 1975 International Botanical Congress in Leningrad. Over dinner and a great deal of vodka, we reminisced about fate and good fortune in science and life in general. I was saddened to learn that he died soon after in a bicycling accident.

In the early 1960s, both Roger Slack and I were working in the laboratory of the Colonial Sugar Refining Company in Brisbane, Australia, on the biochemistry of sugar production and storage in sugarcane. This laboratory maintained contacts with the Hawaiian group and exchanged Annual Reports. Hence, we were aware of their work on photosynthesis and had often pondered on its significance. However, it was not until 1965 that they published their results in a detailed and accessible form (Kortschak et al. 1965). By this time, George Burr had retired and Constance Hartt was about to retire. This paper clearly showed the predominant early labeling of malate and aspartate when sugarcane leaves assimilated $^{14}CO_2$ in the light. This was followed by a phase of more rapid labeling of 3-PGA and then PCR-cycle intermediates and sucrose after longer periods. They concluded that 'in sugarcane carbon assimilation proceeds by a path qualitatively different from many other plants.'

I have often been asked why the Hawaiian group delayed publication of their work for so long, and then apparently only after prompting by the new head of the laboratory, Lou Nickell (see Nickell 1993). Andy Benson has suggested to me that this may have been due to a 'discouraging' reaction that they got during contact with Melvin Calvin's laboratory. It is interesting to note in this regard that C_4 acids were always amongst the early-labeled products of $^{14}CO_2$ assimilation in *Chlorella* and most higher plants. In fact, C_4 acids were even proposed as a critical intermediate in one of the earliest models of algal photosynthesis (Bassham et al. 1950). I also refer the reader to Andrew Benson (this issue) for his recollections on this topic.

It would be appropriate and relevant at this point to set the record straight by responding briefly to some comments made in the article referred to above (Nickell 1993). In this otherwise timely and well-deserved tribute to Hugo Kortschak, Lou Nickell made some unfounded inferences about the earlier involvement of Roger Slack and myself with respect to the work on photosynthesis by the Hawaiian group. Briefly, my responses are that: (1) prior to the publication of the Hawaiian work in 1965 (Kortschak et al. 1965), Roger Slack and I knew little more than the very brief comments appearing in the annual reports from that laboratory, (2) we did not start our work on sugarcane photosynthesis until more than six months after publication of this 1965 paper, (3) we did not visit the Hawaiian laboratory to discuss the photosynthesis work until 1969, (4) we did not name the process the Hatch–Slack pathway or ever use this term; we introduced the term 'C_4 dicarboxylic acid pathway' which was later abbreviated to C_4 pathway, (5) we clearly acknowledged the Hawaiian work in our early publications, and (6) we have no recollection of being 'admonished' by the head of our laboratory, Ken Glasziou.

I should add that somewhere around the time of the publication of the Hawaiian work on photosynthesis (Kortschak et al. 1965), we did receive word from Hugo Kortschak that he would be happy for us to further pursue this line of investigation. As I recall, Kortschak felt that they lacked the necessary biochemical–enzymological know-how to effectively follow up their work. In our subsequent contacts with Hugo Kortschak, we never had any indication that he was unhappy with how things had transpired.

So, with that aside, I should return to the main game. Towards the latter part of 1965, both Roger Slack and I were concluding current research projects on aspects of sugar storage in sugarcane. Over a glass of beer or two, we decided to confirm and extend the observations of the Hawaiian group; as I have said before (Hatch 1999), 'to see if we could understand what it all meant.' These studies confirmed the observations of the Hawaiian group that, during the assimilation of $^{14}CO_2$ by sugarcane leaves, malate and aspartate were strongly labeled before 3-PGA and other PCR cycle intermediates (Hatch and Slack, 1966). Our results were also very similar to those reported by Karpilov (1960) for $^{14}CO_2$ assimilation by maize leaves. But it was another three years before we were to learn about that work.

In that first paper, we extended knowledge about the process in several important ways. For instance: (1) the unstable dicarboxylic acid oxaloacetate was labeled at the same time as malate and aspartate, (2) the dicarboxylic acids were labeled initially in the C-4 carboxyl, and critically, (3) in a 'chase' experiment this C-4 carboxyl gave rise to the C-1 carboxyl of 3-PGA and then to the carbons of sugar phosphates in a manner consistent with the operation of the PCR cycle.

A model based on these observations (Hatch and Slack 1966) proposed that a 3-carbon compound, either pyruvate or phospho-enol pyruvate (PEP), is carboxylated to give a C_4 dicarboxylic acid. The carbon 4 of this acid (or a related dicarboxylic acid) is then transferred to an acceptor yielding 3-PGA and leaving the remaining 3-carbon compound as a source of the primary CO_2 acceptor.

In the following paper (Hatch et al. 1967), this unusual labeling pattern was shown to be similar in leaves of different age, and when CO_2 and light were varied. Significantly, in a survey of different plant species, several other grasses from different tribes, including maize and sorghum, showed similar C_4 acid-dominated earlier labeling, together with a sedge from the family Cyperaceae. By this time, we were reasonably confident that we were looking at a distinctly different mechanism for CO_2 assimilation. General recognition of this was to take a few more years.

Mechanism and function

The model referred to above provided the basis for making predictions about the possible enzymes involved. As a result, phospho-enol-pyruvate (PEP) carboxylase was identified as the likely primary carboxylating enzyme; its activity in the leaves of plants showing the unusual ^{14}C- labeling was about 20-fold higher than in other species (Slack and Hatch 1967). In the same study, we identified normal levels of key PCR cycle enzymes, except that ribulose bisphosphate carboxylase-oxygenase (Rubisco) activity was apparently much lower. The fallout from the latter erroneous observation, and the ultimate solution to the problem, are related elsewhere (Hatch 1997, 1999).

There followed a cycle of predictions and discoveries of other key enzymes. The need for an enzyme to convert pyruvate to PEP led to the discovery of pyruvate, P_i dikinase. An account of the discovery of this enzyme, and also the resolution of the many remarkable features of this reaction and its regulation, are provided elsewhere (see Hatch 1997). Also, a search for an enzyme capable of using photoreduced NADP to convert oxaloacetate to malate led to the discovery of the then novel NADP-specific malate dehydrogenase located in chloroplasts (Hatch and Slack 1969).

The Rubisco dilemma was then resolved (see Hatch 1997), thanks to the observations of Björkman and Gaul (1969), followed by various studies on the inter- and intracellular location of key enzymes in plants such as sugarcane and maize (see Hatch 1999). It clearly emerged from these results that, in this group of species, malate was decarboxylated in the bundle sheath chloroplasts via an NADP-specific malic enzyme and the released CO_2 refixed by the PCR cycle. By the time of the watershed international meeting on photosynthesis held in Canberra (Australia) towards the end of 1970 (see proceedings, Hatch et al. 1971), it was possible to formulate a detailed scheme (Figure 1) to account for CO_2 assimilation in sugarcane and related species (Hatch 1971); this process was later termed 'NADP-malic enzyme-type' C_4photosynthesis. Also at this meeting, it was suggested for the first time that the special reactions of the C_4 pathway might serve to concentrate CO_2 in bundle sheath cells (Björkman 1971; Hatch 1971).

Amongst the participants at that meeting in 1970 were most of the key contributors to the early development of the C_4 pathway story and the related special features of C_4 plants (see Hatch et al. 1971). They included Hugo Kortschak, Olle Björkman, Clanton Black, John Downton, Hilary Johnson, Mac Laetsch, Barry Osmond, as well as Roger Slack, and me. Amongst those missing were John Andrews, Bruce Tregunna, John Hesketh, and, in particular, Gerry

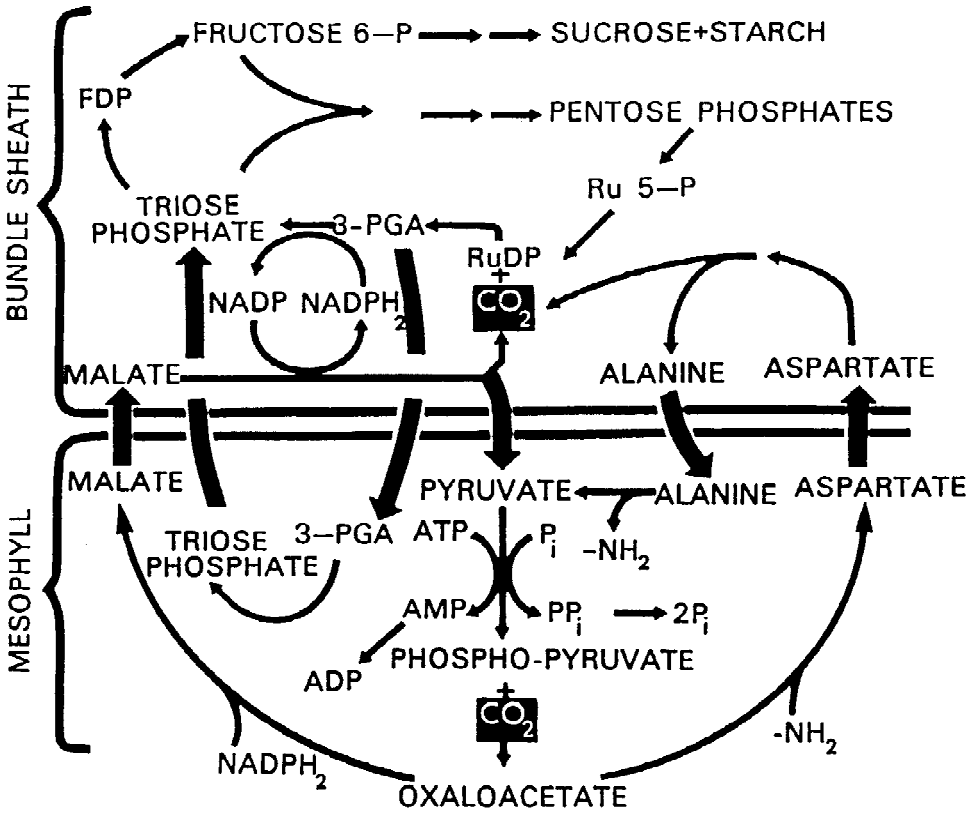

Figure 1. A scheme for C_4 photosynthesis as viewed in 1970 (Hatch 1971). The left hand side depicts the path of carbon in species like sugarcane and maize that transport malate to bundle sheath cells. The right-hand side shows an option involving aspartate, only elucidated in detail some years later.

Edwards. Edwards was a post-doc in Clanton Black's laboratory at the time and was later to contribute so strongly in a wide variety of C_4 related areas. It was probably not until about this time that it could be said that the C_4 pathway was truly 'discovered' and generally accepted by the wider plant research community.

It is interesting to note that for most of this early research we worked very largely in isolation from the mainstream plant science community. I believe this was no disadvantage, since it allowed us to concentrate on the problems at hand free of peripheral distractions and pressures. Compared with this most exciting period up to 1970, what was to follow seemed like relatively hard work.

Of course, it turned out that this was just the start of the final story. Highlights of the events to follow are summarized in other publications (Hatch 1987, 1999; Hatch and Slack 1998) and in a recent comprehensive book on the biology of C_4 plants (Sage and Monson, 1999). They include:

- the resolution of two other variants for C_4 photosynthesis, NAD-malic enzyme-type and PEP carboxykinase-type in different C_4 species, with the key difference revolving around the mechanism of C_4 acid decarboxylation,
- the recognition of the CO_2 concentrating function of the C_4 acid cycle and its role in reducing photorespiration,
- the complex mechanisms of light–dark regulation of pyruvate, P_i dikinase, and PEP carboxylase,
- the critical role of mitochondria in two of the three different C_4 mechanisms,
- the specialized transport systems to move particular metabolites through boundary membranes of chloroplasts and mitochondria,
- the proliferation of plasmodesmata to allow the adequate flux of metabolites between mesophyll and bundle sheath cells,
- identification of the order of 10 000 C_4 species occurring in 2 monocotyledonous and 14 dicotyledonous families,

Figure 2. Participants at the 1970 meeting on photosynthesis and photorespiration held in Canberra, Australia. As indicated in the text, this was a critical meeting for the general acceptance of the C_4 process. In the front row, from the left: Barry Osmond, Irwin Ting, Clanton Black, Eric Waygood, and Martin Gibbs. In the second row, from the left: Ed Tolbert, Ralph Slatyer, John Lyttleton, Roger Slack, John Downton, Harry Beevers, and Hal Hatch. Hugo Kortschak is immediately behind Hal Hatch on the right of the third row. You may also recognize Ulrich Heber and Kozi Asada on the left of the third row.

Figure 3. Some of the original and more recent of the contributors to the C_4 photosynthesis story gathered after a small C_4 meeting held in Canberra in 1996. From left to right: Hediaki Usuda, Jim Berry, Roger Slack, Hal Hatch, Gerry Edwards, and Ryuzi Kanai.

- the recognition of a few species showing features intermediate between C_3 and C_4 species,
- last but not least, the gradual recognition of the causative links between the operation of the C_4 pathway and the various anatomical, physiological, and performance features mentioned at the start of this article.

Figures 2 and 3 show two group photographs that include scientists involved in aspects of C4 photosynthesis research.

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