

The Path from C3 to C4 Photosynthesis - A Study

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Abstract

This paper attempts to study how **C4 photosynthetic** carbon cycle is an elaborated addition to the **C3** photosynthetic pathway. The C4 photosynthetic carbon cycle is an elaborated addition to the C3 photosynthetic pathway. It evolved as an adaptation to high light intensities, high temperatures, and dryness. Therefore, C4 plants dominate grassland floras and biomass production in the warmer climates of the tropical and subtropical regions.

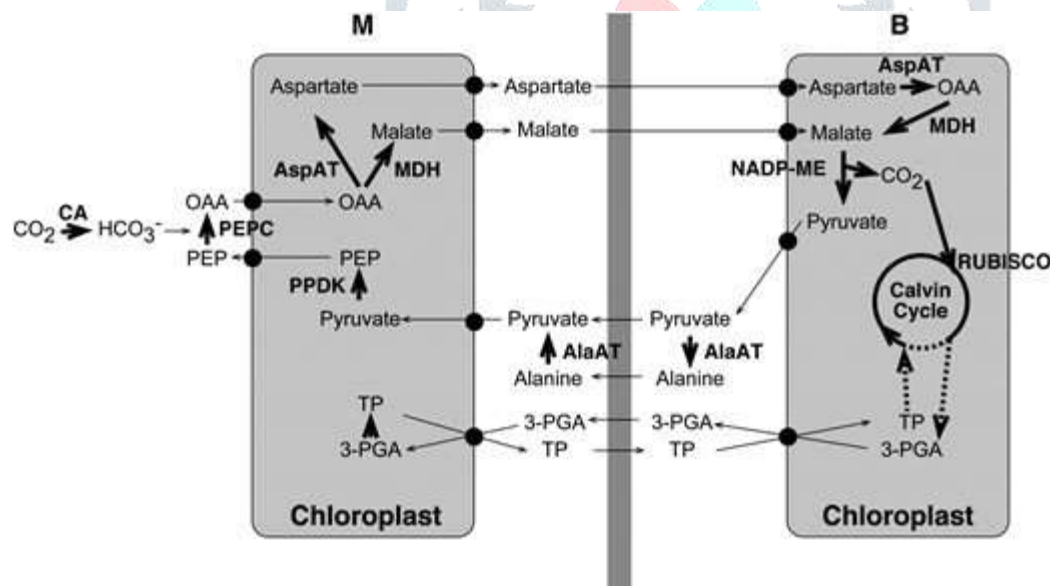
In all plants CO₂ is fixed by the enzyme Rubisco. It catalyzes the carboxylation of ribulose-1,5-bisphosphate, leading to two molecules of 3-phosphoglycerate. Instead of CO₂, Rubisco can also add oxygen to ribulose-1,5-bisphosphate, resulting in one molecule each of 3-phosphoglycerate and 2-phosphoglycolate. Phosphoglycolate has no known metabolic purpose and in higher concentrations it is toxic for the plant (Anderson, 1971). It therefore has to be processed in a metabolic pathway called photorespiration. Photorespiration is not only energy demanding, but furthermore leads to a net loss of CO₂. Thus the efficiency of photosynthesis can be decreased by 40% under unfavorable conditions including high temperatures and dryness (Ehleringer et al., 1991). The unfavorable oxygenase reaction of Rubisco can be explained as a relict of the evolutionary history of this enzyme, which evolved more than 3 billion years ago when atmospheric CO₂ concentrations were high and oxygen concentrations low. Apparently, later on, it was impossible to alter the enzyme's properties or to exchange Rubisco by another carboxylase. Nevertheless, plants developed different ways to cope with this problem. Perhaps the most successful solution was C4 photosynthesis. The establishment of C4 photosynthesis includes several biochemical and anatomical modifications that allow plants with this photosynthetic pathway to concentrate CO₂ at the site of Rubisco. Thereby its oxygenase reaction and the following photorespiratory pathway are largely repressed in C4 plants. In most C4 plants the CO₂ concentration mechanism is achieved by a division of labor between two distinct, specialized leaf cell types, the mesophyll and the bundle sheath cells, although in some species C4 photosynthesis functions within individual cells (Edwards et al., 2004). Since Rubisco can operate under high CO₂ concentrations in the bundle sheath cells, it works more efficiently than in C3 plants. Consequently C4 plants need less of this enzyme, which is by far the most abundant protein in leaves of C3 plants.

Key words: C4 photosynthetic, CO₂, C4 plants, Phosphoglycolate, oxygenase.

Introduction

In the mesophyll cells of C_4 plants CO_2 is converted to bicarbonate by carbonic anhydrase and initially fixed by phosphoenolpyruvate (PEP) carboxylase (PEPC) using PEP as CO_2 acceptor. The resulting oxaloacetate is composed of four carbon atoms, which is the basis for the name of this metabolic pathway. Oxaloacetate is rapidly converted to the more stable C_4 acids malate or Asp that diffuse to the bundle sheath cells. Here, CO_2 is released by one of three different decarboxylating enzymes, which define the three basic biochemical subtypes of C_4 photosynthesis, NADP-dependent malic enzyme (NADP-ME), NAD-dependent ME (NAD-ME), and PEP carboxykinase (PEPCK). The released CO_2 is refixed by Rubisco, which exclusively operates in the bundle sheath cells in C_4 plants. The three-carbon compound resulting from CO_2 release diffuses back to the mesophyll cells where the primary CO_2 acceptor PEP is regenerated by pyruvate orthophosphate dikinase by the consumption of, at the end, two molecules of ATP (Hatch, 1987).

Figure 1 shows a scheme of the NADP-ME subtype of C_4 photosynthesis. Here malate is the dominant transport metabolite while Asp can be used in parallel. The synthesis of malate occurs in the mesophyll chloroplasts, the decarboxylation by NADP-ME in the bundle sheath chloroplasts.



NADP-ME type of C_4 photosynthesis. 3-PGA, 3-Phosphoglyceric acid; AspAT, Asp aminotransferase; AlaAT, Ala aminotransferase; CA, carbonic anhydrase; MDH, malate dehydrogenase; OAA, oxaloacetate; PPDK, pyruvate orthophosphate dikinase; TP, triosephosphate.

This leads to a better nitrogen-use efficiency of C_4 compared to C_3 plants, since the rate of photosynthesis per unit nitrogen in the leaf is increased (Oaks, 1994). Additionally C_4 plants exhibit better water-use efficiency than C_3 plants. Because of the CO_2 concentration mechanism they can acquire enough CO_2 even when keeping their stomata more closed. Thus water loss by transpiration is reduced (Long, 1999).

Objective:

This paper intends to explore and analyze C₃ to C₄ Photosynthesis

- **C₃ plants**, referring to the fact that the first carbon compound produced during photosynthesis contains three carbon atoms. Under high temperature and light, however, oxygen has a high affinity for the photosynthetic enzyme Rubisco.
- **C₄ photosynthesis**, where a four-carbon compound is produced, unique leaf anatomy allows carbon dioxide to concentrate in 'bundle sheath'

POLYPHYLETIC EVOLUTION OF C₄ PHOTOSYNTHESIS

C₃ angiosperms evolved more than 50 times independently into C₄ plants (Muhaidat et al., 2007). Most of the C₄ species occur in the grasses (approximately 4,600) and sedges (approximately 1,600). Only a total of about 1,600 C₄ species are found in the dicots where they are spread over 16 families with 75% of them clustering in the four families Chenopodiaceae, Amaranthaceae, Euphorbiaceae, and Asteraceae (Muhaidat et al., 2007). C₄ grasses probably evolved in the early Oligocene about 30 million years ago, while C₄ dicots appeared later, less than 20 million years ago (Sage, 2004).

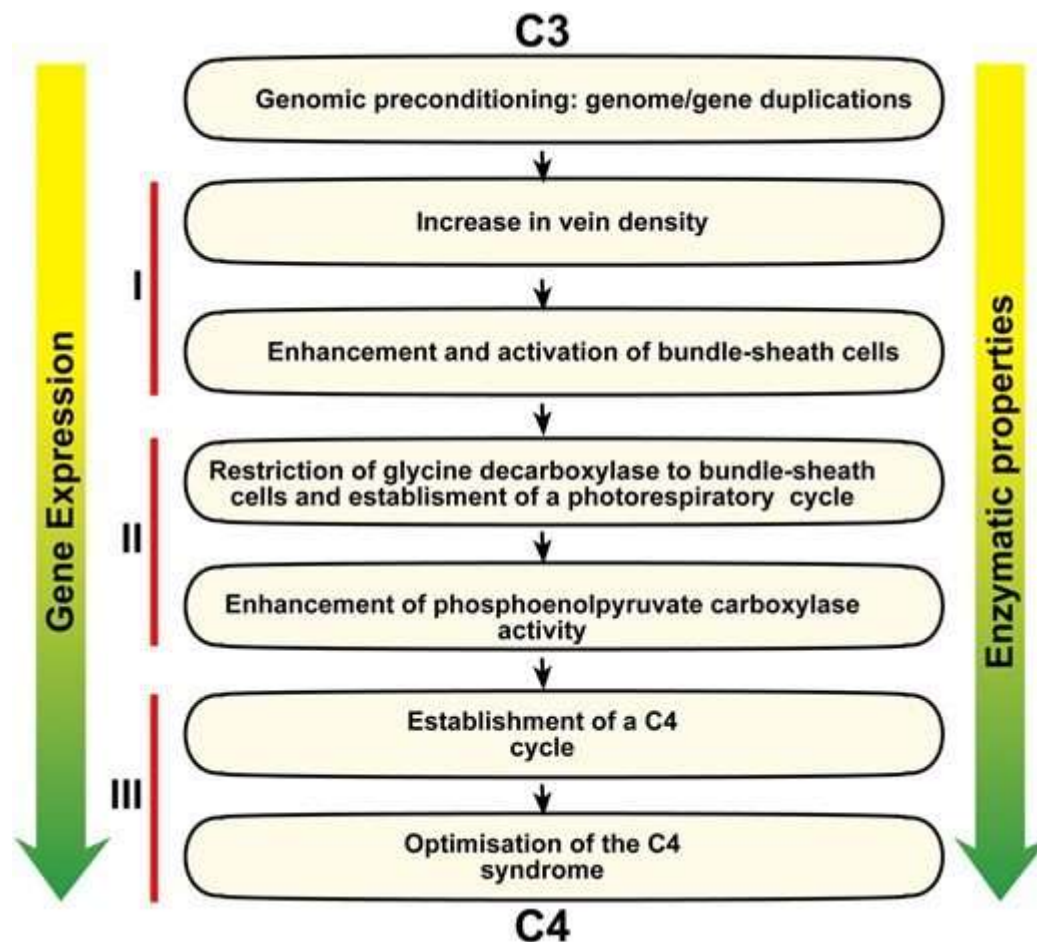
The polyphyletic origin of C₄ photosynthesis indicates that only relatively small evolutionary changes were required for the establishment of this photosynthetic pathway. It can be assumed that C₄ evolution must have been easy in genetic terms. This raises the question of whether we can use the information about the genetic architecture and evolution of this pathway and introduce modules of C₄-ness into present C₃ plant and thereby transform them into C₃-C₄ intermediate or even C₄-like plants (Sheehy et al., 2007).

The two other biochemical subtypes differ from the NADP-ME type by the transport metabolites used and the subcellular localization of the decarboxylation reaction. In NAD-ME plants Asp, which is synthesized in the mesophyll cytosol, is used as transport metabolite. After deamination and reduction, the resulting malate is decarboxylated by NAD-ME in the bundle sheath mitochondria. Plants of the PEPCK type use Asp as well as malate as transport metabolites. Asp is synthesized in the cytosol of mesophyll cells and decarboxylated in the cytosol of bundle sheath cells by the combined action of Asp amino transferase and PEPCK. As in NADP-ME-type C₄ species, malate is synthesized in the mesophyll chloroplasts but decarboxylated by NAD-ME in the mitochondria of bundle sheath cells. This reaction produces NADH that is used in the mitochondria to produce the ATP needed to drive the PEPCK reaction (Hatch, 1987). If Asp is used as transport metabolite, usually the three-carbon decarboxylation product, pyruvate, is partially transported back to the mesophyll cells in the form of Ala to maintain the ammonia balance between the two cell types (Hatch, 1987).

Compared to C₃ plants the bundle sheath cells of C₄ plants have expanded physiological functions. This is reflected by the enlargement and a higher organelle content of these cells in most C₄ species. For the efficient function of the C₄ pathway a close contact between mesophyll and bundle sheath cells is indispensable and they are tightly interconnected to each other by high numbers of plasmodesmata (Dengler and Nelson, 1999). To ensure a direct contact between bundle sheath and mesophyll cells, C₄ plants possess a characteristic leaf anatomy. The bundle sheath cells enclose the vascular bundles and are themselves surrounded by the mesophyll cells. The high vein density in the leaves of C₄ plants leads to a nearly one-to-one ratio of the volumes of mesophyll and bundle sheath tissues. The internal anatomy of a C₄ leaf is often composed of a repeating pattern of vein-bundle sheath-mesophyll-mesophyll-bundle sheath-vein. Because of its wreath-like structure this type of leaf anatomy was termed Kranz anatomy by the German botanist G. Haberlandt (1904). Kranz anatomy is found with more or less considerable variations in nearly all monocotyledonous and dicotyledonous lineages that use the two-cell mode of C₄ photosynthesis.

THE PATH TO C₄ PHOTOSYNTHESIS

The currently most widely accepted model of C₄ evolution proposes a stepwise sequence of changes leading from C₃ to C₄ plants (Fig. 2). Each of these changes on its own is leading to a distinct evolutionary benefit for the resulting species independent of whether it will progress toward the full expression of the C₄ syndrome. This scenario explains why the evolution of this complex trait could occur so many times independently. The model is mainly based on comparative analyses of extant C₃, C₄, and especially C₃-C₄ intermediate species, and a detailed elaboration can be found in Sage (2004). Here, we only present a short summary and elucidate how the evolutionary changes might have been realized through modifications at the molecular/genetic level.



Stepwise evolution of C₄ photosynthesis.

It is thought that the existence of many redundant genes in the genomes of the relevant organisms and species was a general prerequisite for C₄ evolution (Monson, 2003). These gene redundancies have been acquired by duplications of whole genomes, genome segments, or only single genes. Multiple copies of a gene allow evolutionary modifications of one copy without losing the original function of the gene itself. Thus redundant gene copies prevent deleterious consequences of evolutionary changes that alter or switch off the specific function of a certain gene. In further steps, leaves have been altered toward Kranz anatomy, a photorespiratory CO₂ pump was established, and finally a C₄ cycle was created. All these steps were accompanied by massive changes in gene regulation. Also the kinetic properties of enzymes, involved in metabolic pathways that were affected by these evolutionary changes, were adjusted to the new requirements

While the above differences are directly related to the CO₂ concentration mechanism, there are many further modifications known that evolved to integrate the C₄ pathway optimally into the plant's

metabolism. For instance, C₄ species of the NADP-ME subtype are depleted in PSII in their bundle sheath cells to lower oxygen production in these cells. Accordingly, the production of reduction equivalents in the bundle sheath cells is reduced and the reduction phase of the Calvin-Benson cycle, i.e. the conversion of 3-phosphoglycerate to triose phosphate, has been at least partially shifted to the mesophyll cells (Fig. 1). There is another adaptation in C₄ plants that affects the light reactions of photosynthesis. Compared to C₃ photosynthesis the C₄ pathway consumes one (PEPCK type) or two (NADP-ME and NAD-ME type) additional molecules of ATP per fixed CO₂ without the need of additional reduction equivalents. This increase in ATP-to-NADPH ratio is compensated for in some C₄ plants by enhancing cyclic electron flow around PSI, which provides additional ATP without concomitantly producing NADPH. Large-scale transcriptomic and proteomic approaches also revealed that other metabolic pathways such as amino acid synthesis, nitrogen or sulfur assimilation, and lipid metabolism are compartmentalized between mesophyll and bundle sheath cells in at least some C₄ plants

Development of Kranz Anatomy

The first step toward C₄ evolution was the development of the Kranz anatomy. To establish a mechanism that efficiently concentrates CO₂ in bundle sheath cells the mean distance of a mesophyll cell to the next bundle sheath cell must be as short as possible. Ideally each mesophyll cell should be directly adjacent to at least one bundle sheath cell. Therefore, in planar leaves the vein density had to be enhanced. A higher vein density increased also the mechanical integrity of the leaves, which could be beneficial in windy habitats, or improved the water supply of leaves in dry and hot biotopes (Sage, 2004). In succulent terete or semiterete leaves, evolution of C₄ occurred in some dicots with development of a single Kranz unit surrounding the vascular and water storage tissue (Edwards et al., 2004).

A comparative analysis of the leaf development in both monocot and dicot C₃ and C₄ species revealed that the close vein spacing in leaves of C₄ plants is due to changes in the initiation frequency and patterning of the minor and not the major veins (Ueno et al., 2006; McKown and Dengler, 2009).

In *Arabidopsis thaliana* the formation of veins from ground tissue is triggered by polar auxin flow mediated by auxin efflux carriers. Cell files along the auxin transport route convert to procambial cells and later on develop into vascular bundles (Scarpella et al., 2006). Either modifications of auxin production and allocation and/or modifications of the competency of ground tissue cell to become procambial cells are responsible for the greater vein density observed in C₄ compared to C₃ leaves (McKown and Dengler, 2009). Since the molecular events causing the initiation of veins are not even completely understood in C₃ model plants, it is presently challenging to predict the changes that led to the C₄ typical leaf anatomy.

The activation of bundle sheath cells—the enlargement of these cells and the increase in the number of organelles in this tissue might be a secondary effect of the higher vein density. Typically, the bundle

sheath cells of C_3 plants possess only a few chloroplasts, and the photosynthetic activity is low. With higher vein densities also the ratio of bundle sheath to mesophyll cells increases. Since only the mesophyll cells show high photosynthetic activity, this would imply that the overall photosynthetic activity of a leaf with a given size decreases. The evolutionary pressure to maintain the overall photosynthetic activity could have led to an increase of the number of chloroplasts in the bundle sheath cells. Due to the necessity to metabolize the photorespiratory Gly in bundle sheath cells the increase of chloroplast numbers would also require an increase in the numbers of mitochondria and peroxisomes in these cells.

The Photorespiratory CO_2 Pump: C_3 - C_4 Intermediate Photosynthesis

Extant C_3 - C_4 intermediate species possess a photorespiratory Gly shuttle that pumps CO_2 into the bundle sheath cells (Bauwe, 2010). This is achieved by restricting the Gly decarboxylation reaction to the bundle sheath mitochondria, thus all Gly produced by photorespiration in the mesophyll has to be transferred to the bundle sheath cells for further processing. The Gly shuttle affects photosynthetic CO_2 fixation in two ways. All photorespiratory CO_2 is set free inside the leaf far apart from the outer surface. Therefore it has to diffuse through several cell layers, before it could escape from the leaf. This enhances the plant's chances of refixing the photorespired CO_2 and minimizes the loss of carbon due to photorespiration. In some C_3 - C_4 intermediate species this refixation capacity is supported by the spatial distribution of the organelles within the bundle sheath cell, since the mitochondria concentrate adjacent to the vascular bundles (Rawsthorne et al., 1998). Additionally, the Gly shuttle enhances the CO_2 concentration within the bundle sheath cells. As a consequence, the carboxylation activity of Rubisco in the bundle sheath cells increases, while its oxygenase reaction is outcompeted (Bauwe, 2010).

It is assumed that the establishment of such a photorespiratory CO_2 pump is an important intermediate step on the way toward C_4 photosynthesis. A photorespiratory CO_2 pump can easily be accomplished at the molecular level. The expression of only one gene, encoding a subunit of the Gly decarboxylase multienzyme complex, had to be restricted to the bundle sheath cells. This might have been achieved through relatively subtle changes in the cis-regulatory elements that control the expression of these genes (compare with Akyildiz et al., 2007). In cases where several isogenes with different leaf expression specificities existed already in the respective C_3 ancestral species this process might also have included the pseudogenization of those isogenes that are not bundle sheath specific.

Conclusion

The world of the 21st century will face massive problems in feeding the growing human population. Green energy from plant biomass is being developed to help cover energy demands, and might compete with food production for terrain and resources in the future. It will be a challenge to increase crop production

adequately in a sustainable manner both in terms of harvestable yield and total biomass. The C₄ NADP-ME also acquired unique kinetic and regulatory properties during their evolution from nonphotosynthetic isoforms. Distinct enzyme regions could be identified that are involved in an altered pH-dependent inhibition by malate and differences in tetramerization of the enzyme. Interestingly, this ancestral carbonic anhydrase gene was already highly expressed in leaves, suggesting that the intracellular localization of the protein was of minor importance and altered during evolution. It is not clear so far to which extent other enzymes, which are not directly related to the C₄ pathway, were modified during C₄ evolution.

C₄ plants exhibit high photosynthetic capacity and efficient use of nitrogen and water resources. They have received an increasing interest in recent years and the transfer of C₄ photosynthesis into current C₃ crops is being considered (Sheehy et al., 2007). Currently there are attempts under way to implement a C₄-CO₂ concentration pathway into rice, perhaps the most important crop for human nourishment to date.

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