Crassulacean Acid Metabolism

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Crassulacean acid metabolism (CAM) is a CO₂ acquisition, CO₂ transient storage and CO₂ concentrating mechanism based on organic acid synthesis. In this variant of photosynthesis CO₂ can be fixed nocturnally in the dark and is used during the day for assimilation in the light.

CAM Pathway Characteristics and Metabolic Carbon Flux

Dark period (phase I) of CAM

Crassulacean acid metabolism (CAM) is a CO₂ acquisition, CO₂ transient storage and CO₂ concentrating mechanism based on organic acid synthesis. CO₂ acquisition occurs in the dark. CO₂ is fixed and stored in the form of organic acids. Remobilization of CO₂ from the organic acids occurs in the light and leads to massive CO₂ concentration inside photosynthesizing plant organs (green leaves or stems, in some cases also aerial roots of epiphytes). No particular biochemical reactions or enzymes participate in CAM. Although special isoforms of enzymes may be involved, basically all reactions have well-known housekeeping functions in all green plants. It is the special linkage of metabolic elements that makes up the CAM network (Figure 1; Cushman and Bohnert, 1997; Lütğe, 1998).

The key enzyme of CO₂ dark fixation is phosphoenolpyruvate carboxylase (PEPC, (1) in Figure 1) in the cytosol. It forms oxaloacetate, which is then reduced to malate. This is the only, or at least the dominating, form of nocturnal CO₂ storage in most CAM plants. In some CAM plants, however, citrate may also play a considerable role additionally or alternatively to malate. It can be formed in the mitochondria.

Protons are generated together with the organic acid anions. To maintain cytosolic pH homeostasis, these are pumped into the cell sap vacuole by tonoplast H⁺ pumps, mainly the vacuolar H⁺ ATPase (V-ATPase, (2) in Figure 1). Proton pumping energizes vacuolar organic acid accumulation as it establishes an H⁺ electrochemical gradient (ΔµH⁺) driving organic acid anions across the tonoplast, the vacuolar membrane. Malate must be removed from the cytosol because it effects a feedback inhibition of its own synthesis via PEPC.

The central aqueous cell sap vacuole is a suitable storage compartment occupying often 98% or even more of the volume of cells in succulent CAM tissues (leaf or stem succulence). Nocturnally accumulated organic acids typically amount to 100–300 mmol L⁻¹ malate but may be as high as 1.4 mol L⁻¹ titratable proton equivalents in species of Clusia, which accumulate both malate and citrate with a stoichiometry of 2 and 3 titratable protons, respectively, per malate and citrate (Lütğe, 1998).

Light period (phase III) of CAM

During the light period, organic acids are remobilized from the vacuole by passive efflux and are decarboxylated. Malate is decarboxylated by NAD(P)-dependent malic enzymes in the cytosol (NADP-ME) and mitochondria (NAD-ME), respectively ((3) in Figure 1) or via PEP-carboxykinase (PEPCK). Citrate could be partially decarboxylated by cytosolic isocitrate dehydrogenase ((4), in Figure 1) and/or metabolized via the tricarboxylic acid cycle (TCA-C), where it can be broken down totally to CO₂. The CO₂ regenerated is fixed and reduced in the Calvin cycle (PPC_red) to carbohydrate, part of which can be used for energy metabolism, maintenance and growth ([CH₂O]ₙ in Figure 1). Another part is stored in the form of starch in the plastids or as free sugars (hexoses but also sucrose) in the vacuole to serve as precursor for the glycolytic synthesis of PEP as CO₂-acceptor in the subsequent dark period. Gluconeogenesis with the key enzyme pyruvate P₁ dikinase (PPDK, (5) in Figure 1) may also contribute to this carbohydrate pool.

Organic acid influx and efflux at the tonoplast are mediated by carboxylate carriers and/or anion channels in the membrane. (Details still await clarification.)

Ecophysiological Advantages of CAM

CAM-phases

Nocturnal CO₂ acquisition and storage has been named phase I of CAM, during which stomata are normally open and CO₂ is taken up from the atmosphere. Daytime CO₂ remobilization and refixation via the Calvin cycle in the light, during which stomata are closed and large internal
CO₂ concentrations of up to a few per cent build up in the intercellular airspaces, has been called phase III of CAM. These two phases are separated by a phase II when stomata open widely in the morning and a peak of uptake of atmospheric CO₂ is explained by concomitant operation of PEPC and ribulose-bisphosphate carboxylase/oxygenase (Rubisco), the CO₂-fixing enzyme of the Calvin cycle, and by a phase IV, when stomata open in the afternoon after organic acid remobilization is completed and again CO₂ is taken up from the atmosphere (Figure 2; Osmond, 1978).

**Ecophysiological roles of diurnal malate rhythms**

Examination of the CAM phases offers a clue to the evaluation of the ecophysiological significance of CAM (Lütge, 1998). Stomatal rhythms are evidently associated with this, but they are not the *sine qua non* of CAM, because CAM may also occur in stomata-less leaves of water plants and in green aerial roots of orchids. In leaves of submerged freshwater plants, nocturnal malate synthesis of CAM facilitates acquisition of CO₂ when it is more readily available by nocturnal respiratory activities in the aqueous ecosystem than during the day when other photosynthesizing organisms are competing.
Crassulacean Acid Metabolism

Ecophysiological roles of diurnal citrate rhythms

In contrast to malate, nocturnal citrate accumulation does not provide a net gain of carbon, because for each hexose unit used as precursor (C₆) only one citrate (C₆) is formed as one CO₂ is fixed but also one CO₂ is lost in the pathway; with malate accumulation, two malates (2 × C₆) are formed for each hexose unit used, because two CO₂ are fixed (Figure 1). However, citrate can contribute significantly to night/day carbon cycling, which becomes important under severe water stress. Citrate is also known to be an effective buffer substance. Its vacuolar accumulation therefore allows considerable total acid accumulation (titratable acidity) while control of vacuolar pH is maintained. Indeed, the highest nocturnal accumulation of titratable protons (~1.4 mol L⁻¹) with vacuolar pH still close to 3) was observed in the genus Clusia, where citrate accumulation is very important (Lüttge, 1998).

Productivity of CAM Plants

Metabolic schemes such as that of Figure 1 can be used to work out stoichiometric energy budgets. While this may be a gratifying exercise on paper, the fact that in ecophysiological reality various meshes of the metabolic network may be used simultaneously and switches may also occur makes this a more bewildering task. What becomes clear at a glance, though, is that CAM is energetically more costly than straightforward C₃-photosynthesis (Calvin cycle). In fact, productivity of CAM plants in natural ecosystems is normally much lower than that of C₃ plants. CAM appears as an adaptation for ecological survival but not for maximum productivity. Conversely, in agroecosystems with the appropriate maintenance, CAM plants can achieve a productivity close to or even better than that of C₃ or also C₄ plants. This is always based on a considerable contribution of phase IV to total carbon gain with direct CO₂ fixation via Rubisco (Nobel, 1996). Major CAM crops are pineapple (Ananas, Bromeliaceae), Opuntia (Cactaceae) and Agave (Agavaceae).

Figure 2 Diurnal course of net CO₂ exchange (JCO₂), leaf-conductance for water vapour (gH₂O) and malate levels (per gram fresh weight, gFW) in a leaf of the CAM-plant Kalanchoe daigremontiana, with nocturnal (dark bar on top of the graph) stomatal opening, CO₂ uptake and malate accumulation (phase I); an early morning peak of CO₂ uptake (phase II); daytime malate remobilization and stomatal closure (phase III); and afternoon stomatal opening, CO₂ uptake and assimilation in the Calvin cycle (phase IV).
Evolution of the CAM Pathway

Since enzymologically there is nothing particular in CAM (Figure 1), it is no surprise that CAM evolved polyphyletically. CAM plants emerged in very many taxa dispersed over all three classes of the subdivision Angiospermae in the division of Spermatophyta, namely the Magnoliopsida (primitive dicots), the Rosopsida (‘eudicots’) and Liliopsida (monocots). Polyphyletic evolution of CAM has also been traced within a given family, i.e. the Bromeliaceae (Smith, 1989). The occurrence of CAM plants scattered over very different phylogenetically advanced taxa of the angiosperms has supported the contention that emergence of CAM is a relatively recent event in plant evolution. It ought to be noticed, however, that CAM plants also occur in the division of the Pteridophyta, i.e. in the classes of the Lycopodiopsida (order Isoëtales: Isoëtes and Stylites) and the Pteridopsida (order Polypodiales: Pyrrosia and Drymoglossum), which emerged much earlier in evolution (∼3 × 10⁸ years ago) than the Angiospermae (∼1.5 × 10⁸ years ago).

Constitutive and Inducible CAM: Developmental and Environmental Regulation

While looking for the traces of CAM plants in the evolutionary tree of the Spermatophyta gives us some ideas about phylogeny, we can also observe development of CAM expression in ontogeny. In the genus of so called obligate or constitutive CAM plants Kalanchoë (Crassulaceae) the youngest leaves have a more C₃-like metabolism and CAM is only fully expressed in mature leaves. In Kalanchoë blossfeldiana cv. Tom Thumb, CAM expression is dependent on developmental regulation elicited by short-day photoperiod conditions that also elicit flowering and this, hence, is considered a C₃/CAM intermediate species. Another extensively studied C₃/CAM intermediate species is the annual facultative halophyte Mesembryanthemum crystallinum (Aizoaceae) (Cushman and Bohnert, 1997). With increasing age this plant exhibits some degree of CAM. However, this is considerably amplified by stress, i.e. salinity, osmotic or water stress, and is reversible depending on plant age. Hence, in this case both developmental and environmental regulation determine the degree of CAM expression, where phytohormones, such as abscissic acid and cytokinins, and secondary messengers, such as Ca²⁺, may be involved in signal transduction. Other C₃/CAM intermediate taxa are the bromeliad Guzmania monostachia and in the genera Sedum, Peperomia and Clusia (Lüttge, 1998). In the latter, a genus of neotropical woody plants, C₃–CAM switches are very rapid, occurring within days or even several hours, and are governed by environmental rather than developmental signals, such as availability of water, light intensity and day–night temperature regimes. In perennial tropical plants using their leaves for more than one season, it appears sensible that environmental control should dominate developmental regulation. It is also noted that such phenotypic plasticity allows Clusia to cover a wide ecological amplitude rather than being a straightforward adaptation to a given type of stress (Lüttge, 1999).

CAM Enzymes

More readily than analysis of evolutionary relations of enzymes and their genes, C₃/CAM intermediates draw our attention to CAM enzymes. It has been noted above that there are no really typical CAM enzymes in the CAM pathway (Figure 1). Nevertheless, PEPC ((1) in Figure 1) certainly has a key role. Its phylogenetic analysis does not give any evidence that it falls into a class of its own in CAM plants. However, study of steady-state mRNA levels shows that during the C₃–CAM transition in M. crystallinum there is transcriptional activation of a CAM-specific isogene of PEPC. Similarly PPDK ((5) in Figure 1), the glycolytic enzyme NAD-glyceraldehyde-3-phosphate dehydrogenase ((6) in Figure 1), and an NADP-dependent malic enzyme ((3) in Figure 1) are products of transcriptionally activated specific CAM genes (Cushman and Bohnert, 1997). The H⁺-pumping V-ATPase, a multisubunit enzyme of the tonoplast with a membrane integral proteolipid (Vₒ-domain) and a head and stalk structure (V₁-domain) is also affected transcriptionally (subunit mRNA levels) and posttranslationally (protein processing), leading to changes in activity and ATP/H⁺ coupling during C₃–CAM transitions (Lüttge and Ratajczak, 1997).

Regulation of CAM: Diurnal and Circadian Rhythmicity

The key enzyme of carbon acquisition in CAM, PEPC, must be regulated during the normal night/day (diurnal) rhythm of CAM. In its active state it has an ∼6-fold higher affinity for CO₂ than does Rubisco, and this would imply futile cycling of CO₂ via PEPC in the light (phase III). Activity is regulated posttranslationally by phosphorylation (PEPC-kinase) and dephosphorylation (phosphorylase) (Carter et al., 1991; Cushman and Bohnert, 1997). The active phosphorylated night-form of PEPC has high CO₂ affinity and low malate sensitivity (malate feedback inhibition). Dephosphorylation downregulates CO₂ affinity and increases malate sensitivity during the light period, and thus, minimizes futile CO₂ refixation.
CAM also shows free running (circadian) rhythmicity under constant external conditions in continuous light. PEPC activity follows these endogenous oscillations due to phosphorylation/dephosphorylation oscillations associated with endogenous oscillations of PEPC-kinase (Carter et al., 1991). The endogenous rhythmicity of CAM has been studied theoretically and by establishing ‘skeleton models’ for computer simulations, where the degrees of freedom were reduced as much as possible. It turns out that the PEPC-phosphorylation rhythm makes the model more stable against perturbations but can be eliminated without destroying rhythmicity of simulations. It is also confirmed experimentally that CAM can oscillate without PEPC-kinase oscillations. Conversely, simulations break down if a tonoplast-located beat oscillator (hysteresis switch) is removed from the model. The decisive regulation of CAM must therefore lie in malate influx/efflux at the tonoplast (Grams et al., 1997).

References


Further Reading