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Optimization of CO_2 fixation in photosynthetic cells via thermodynamic buffering

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ABSTRACT

Stable operation of photosynthesis is based on the establishment of local equilibria of metabolites in the Calvin cycle. This concerns especially equilibration of stromal contents of adenylates and pyridine nucleotides and buffering of CO_2 concentration to prevent its depletion at the sites of Rubisco. Thermodynamic buffering that controls the homeostatic flux in the Calvin cycle is achieved by equilibrium enzymes such as glyceraldehyde phosphate dehydrogenase, transaldolase and transketolase. Their role is to prevent depletion of ribulose-1,5-bisphosphate, even at high [CO_2], and to maintain conditions where the only control is exerted by the CO_2 supply. Buffering of adenylates is achieved mainly by chloroplastic adenylate kinase, whereas NADPH level is maintained by mechanisms involving alternative sinks for electrons both within the chloroplast (cyclic phosphorylation, chlororespiration, etc.) and shuttling of reductants outside chloroplast (malate valve). This results in optimization of carbon fixation in chloroplasts, illustrating the principle that the energy of light is used to support stable non-equilibrium which drives all living processes in plants.

1. Introduction

Although living systems operate far from thermodynamic equilibrium, non-equilibrium fluxes that support their metabolism should be steady (Igamberdiev and Kleczkowski, 2009). The external energy is used primarily to support stable non-equilibrium state (Bauer, 1982), which becomes an internal source of work conducted by a biosystem. This can be reached, in particular, via a "fitting function" of special thermodynamic buffering enzymes that equilibrate fluxes of load and fluxes of consumption of major metabolic components, e.g., ATP and pyridine nucleotides (Igamberdiev and Kleczkowski, 2003). Thermodynamic buffering generates steady metabolic fluxes via local equilibrations and regulated uncoupling or "slippage" of the oxidation processes from ATP synthesis (Igamberdiev and Kleczkowski, 2009). Understanding mechanisms of such equilibration will help in developing a computational approach for calculating major parameters of metabolism. This may include metabolic flux analyses which provide tools to measure and model metabolism and its functions (Allen et al., 2009). These approaches consider a co-existence of both equilibrium and non-equilibrium reactions in complex metabolic networks (Galimov, 2004). Equilibration of metabolites by the equilibrium (thermodynamic buffer) enzymes (Stucki, 1980) leads to a steady non-equilibrium flux through irreversible reactions and prevents depletion of substrates for non-equilibrium enzymatic reactions. This general idea of a homeostatic flux control (Fridlyand and Scheibe, 2000) provides a metabolic substantiation for the concept of homeorhesis (Waddington, 1968), i.e. of temporal steady trajectories in the development of biological systems. The key properties of biochemical networks should be robust in order to ensure their proper functioning (Barkai and Leibler, 1997) and to maintain stable non-equilibrium state (Bauer, 1982).

Photosynthetic metabolism is an example of optimized homeostatic flux where carbon is efficiently fixed using the energy (ATP) and the reducing power (NADPH) formed in light reactions. For optimization of the flux, the following conditions should be satisfied: (1) the homeostatic flux control of metabolic pathways, including the Calvin cycle and photorespiration; (2) the buffering of adenylates both within their own pool and in relation to the pool of pyridine nucleotides, thus providing the maintenance of a stable ATP/NADPH ratio; (3) the buffering of reductants including pyridine nucleotides and ascorbate; (4) the buffering of the substrate of photosynthesis (CO_2). In this paper, we discuss possible mechanisms providing the thermodynamic buffering and maintaining stable non-equilibrium during photosynthesis.

2. Homeostatic Flux Control in the Calvin Cycle

Metabolic cycles are organized in a way that they maintain stable non-equilibrium flux by providing a steady turnover of substrates through the cycle (Igamberdiev, 1999; Qian et al.,

Abbreviations: AK, adenylate kinase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; PFK, phosphofructokinase; PGA, 3-phosphoglycerate; RuBP, ribulose bisphosphate; Rubisco, RuBP carboxylase/oxygenase.

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2003). Every metabolic cycle contains both equilibrium and non-equilibrium reactions. For instance, in the citric acid cycle, the equilibrium reactions are catalyzed by malate dehydrogenase, fumarase, aconitase (MacDougall and ApRees, 1991; Hagedorn et al., 2004) and NADP-isocitrate dehydrogenase (Igamberdiev and Gardeström, 2003). As earlier suggested (Igamberdiev, 1999), the simplest metabolic substrate cycle includes one irreversible (non-equilibrium) and one reversible (equilibrium) reactions. Examples include the cycle composed of irreversible NAD- and reversible NADP-isocitrate dehydrogenase reactions (Igamberdiev and Gardeström, 2003), the glycolate oxidase/NAD(P)H-glyoxylate reductase cycle (Kleczkowski and Givan, 1988; Kleczkowski and Randall, 1988; Igamberdiev and Lea, 2002), and ATP- and PPi-dependent phosphofructokinase (PFK) cycle (Huang et al., 2008). Most metabolic cycles/pathways evolved via transformations of these simple substrate cycles (Igamberdiev, 1999).

The commonly accepted idea that irreversible reactions in cycles limit their turnover has been shown to be generally incorrect (Fridlyand and Scheibe, 1999a, 2000; Fridlyand et al., 1999; Morandini, 2009). There are strong theoretical arguments against the idea that highly regulated enzymes catalyzing reactions far from equilibrium must be considered a priori rate limiting. Conversely, contrary to accepted wisdom, the reactions close to equilibrium frequently limit flux when the amount of the enzyme is reduced (Morandini, 2009). Changes in provision of a substrate to the cycle or pathway are controlled by thermodynamic buffering reactions that can reverse a product back to the substrate. Also, when levels of a metabolite provided by the equilibrium enzyme are insufficient for maximum flux, the cycle flux could still be increased by involving separate depots of stored intermediates. For the Krebs cycle, this is achieved by the involvement of large pools of malate, citrate and other stored acids (including fumarate, transaconitate, isocitrate in some species). For the Calvin cycle, the pool of triose phosphates (e.g. from the cytosol), and probably ribose-5-phosphate and erythrose-4-phosphate, may play a similar role. Another example is photorespiration which eventually supplies 3-phosphoglycerate (PGA) back to the Calvin cycle (Kleczkowski and Givan, 1988; Igamberdiev and Lea, 2002).

Fridlyand and Scheibe (1999a, 2000) considered the importance of turnover times (or pool sizes) of the Calvin cycle intermediates to optimize metabolism. Presumably, there are mechanisms for adjusting uniformly the rates of individual reactions inside the cycle (Fridlyand and Scheibe, 1999a, 2000). Indeed, the carboxylation rate can completely limit the rate of photosynthesis, at least at low [CO_2] when the rate of RuBP carboxylation is strongly limited by Rubisco (Farquhar et al., 1980; Woodrow and Berry, 1988). Even at high [CO_2], it has been proposed that RuBP is not limiting, but rather it serves as a gatekeeper and, thus, the paramount controller of CO_2 fixation under any condition (Farazdaghi and Edwards, 1988; Farazdaghi, 2009). Such a model substantiates the robustness of the Calvin cycle and its relative insensitivity to precise values of biochemical parameters.

Steady operation of the Calvin cycle assumes that the contribution of every individual step to the turnover period (τ_i/τ) is determined by the relative concentration of the metabolite at a given step. In these conditions, $\tau_i/\tau = S_i/\sigma$, where S_i is the concentration of the metabolite at a given step, τ_i is the transient time for this step, τ is the sum of the lifetimes of all metabolite pools (it can be regarded as the turnover period of the cycle or the mean time of the turnover of one acceptor molecule in the cycle), and σ is the total concentration of metabolites in the cycle (Fridlyand and Scheibe, 1999a).

Main equilibria in the Calvin cycle are achieved by glyceraldehyde dehydrogenase (GAPDH), transketolase, and aldolase. GAPDH catalyses the reversible reduction of 1,3-bisphosphoglycerate. Its rate depends on the ATP/ADP ratio (really MgATP/MgADP) and

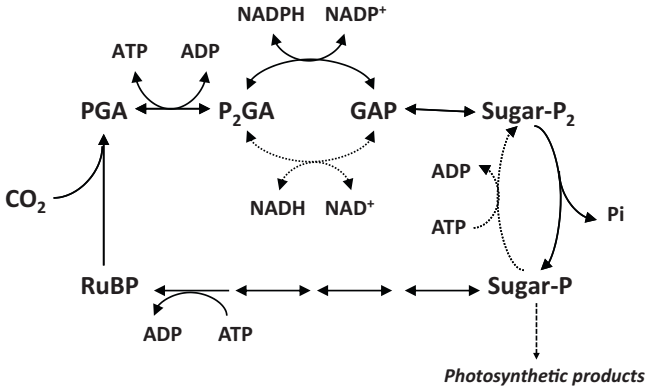

Fig. 1. A generalized structure of the Calvin cycle. The bypasses forming substrate cycles and catalyzed by chloroplastic glycolytic enzymes are shown by dotted lines. Abbreviations: RuBP – ribulose-1,5-bisphosphate, PGA – 3-phosphoglycerate, P_2GA – 1,3-bisphosphoglycerate, GAP – glyceraldehyde-3-phosphate.

levels of 1,3-bisphosphoglycerate, but considering an unlimited formation of the latter in the kinase reaction, it depends on PGA formed in the Rubisco reaction. Phosphoglycerate kinase and triose-phosphate isomerase exhibit very high activities and do not exert any limitation on the Calvin cycle turnover (Fridlyand, 1992). The equilibrium transketolase and transaldolase reactions, together with phosphopentose isomerase result in the production of ribulose-5-P which, after entering the irreversible phosphoribulokinase reaction, forms RuBP, the substrate for Rubisco.

The net result of the coupling of equilibrium and non-equilibrium reactions in the Calvin cycle is to produce a sufficient amount of RuBP for Rubisco to adjust rates of CO_2 assimilation to turnover rates of the cycle intermediates (Fridlyand and Scheibe, 1999a). In conditions of homeostatic operation of the Calvin cycle, Rubisco becomes its main controlling point through CO_2 concentration (and also the O_2 level). This would not be possible in case of a direct reduction of CO_2 via formate and formaldehyde, the mechanism largely abandoned by living matter, although there are some indications that it may have a minor contribution to photosynthetic metabolism (reviewed in Igamberdiev et al., 1999).

From the analysis of operation of the Calvin cycle (Fig. 1), we see the basic principle of the structure of the metabolic cycle. Any cycle consists of both equilibrium and non-equilibrium reactions. The equilibrium enzymes in cycles buffer them, while the non-equilibrium enzymes drive them. The equilibrium reactions are generally limiting because they create equilibrium concentrations to supply substrates for fast non-equilibrium fluxes. These equilibria set turnover times for cycles and provide conditions when the cycle operation is limited only by the availability of the entry substrate (in the Calvin cycle – CO_2) and supply of the energy (ATP) and the reductant (NADPH) at optimum rate and stoichiometry. This can be achieved via mechanisms that buffer supply of ATP, NADPH, and CO_2. We discuss them below.

3. Buffering of Adenylates in the Chloroplast by Adenylate Kinase

To provide the stable and sustainable rate of CO_2 fixation, it is important that the ATP/ADP ratio in chloroplasts is maintained at certain optimal level. Too low level will result in the suppression of Calvin cycle turnover at the levels of PGA phosphorylation/reduction and ribulose-5-P phosphorylation (Fridlyand and Scheibe, 1999a), while too high level will result in the ADP depletion for ATP synthesis and in the depletion of Mg^{2+} in the stroma (Igamberdiev and Kleczkowski, 2006). Under non-photorespiratory conditions, the values of ATP/ADP in leaf chloroplasts are relatively