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Impacts of genetically engineered alterations in carbon sink pathways on photosynthetic performance☆

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ABSTRACT

Genetic engineering of photosynthetic organisms typically redirects native metabolism towards desirable products, which thereby represent new metabolic sinks. There is limited information on how these modifications impact the evolved mechanisms of photosynthetic energy metabolism and cellular growth. Two engineered strains of *Synechocystis* sp. PCC 6803 with altered carbon sink capacity were assayed for their photosynthetic and CO_2 concentrating mechanism properties in conditions of high and low inorganic carbon (Ci) availability. In the Δ*glgC* mutant, glycogen cannot be synthesized and a carbon sink pathway has been effectively removed. The JU547 strain has been engineered by integration of the *Pseudomonas syringae* ethylene forming enzyme and provides a new sink. When cultured under high carbon conditions, Δ*glgC* displayed diminished photochemical efficiency, a more reduced NADPH pool, delayed initiation of the Calvin-Benson-Bassham cycle, and impairment of linear and cyclic electron flows. It also exhibited a large decrease in photochemical quenching indicative of the accumulation of Q_A-, normally associated with a reduced PQ pool, but appears instead to be the result of an undefined dissipative mechanism to spill excess energy. In the case of carbon sink integration, JU547 displayed slightly more oxidized PQ and NADPH pools and increased rates of cyclic electron flow and an enhanced demand for inorganic carbon as suggested by increase in the expression of the bicarbonate transporter, SbtA. Overall, the results highlight the importance of the native regulatory network of autotrophic metabolism in governing photosynthetic performance and provide cogent examples of both predicable and difficult to predict phenotypic consequences upon installation of new pathways in autotrophs.

1. Introduction

The cyanobacteria have long served as models for studying oxygenic photosynthesis and autotrophic carbon metabolism. This includes recent genetic engineering studies that investigate the feasibility of producing biofuels and economically valuable compounds that represent diversions of photosynthetic products to these alternative products (reviews, see references [1–3]). Presently there is limited information on how such engineered modifications will impact the evolved mechanism of photosynthetic energy metabolism and cellular growth. Conceivably, it is possible that the evolved regulatory mechanisms built into the native enzymes and cell regulatory circuits will be sufficient to accommodate the alterations in photosynthetic and metabolic flux imposed by the engineered pathways. That is, the introduction of genetic modifications leading to desired products or restricting flux to competing pathways may produce little in the way of deleterious or unexpected effects, apart from anticipated losses in cell growth and productivity due to partitioning of photosynthate away from growth substrate. Such introduced pathway alterations would be considered benign. On the other hand, it is also possible, in principle, that the introduced changes in metabolism will 'inappropriately' interact with the native regulatory mechanisms. In this case, native regulatory mechanisms and flux characteristics that normally give rise to evolutionary fitness and robustness in the natural system instead produce deleterious

Abbreviations: Chl, chlorophyll; C_i, inorganic carbon, primarily [$HCO_3^- + CO_2$]; 2PG, initial product of photorespiration due to the oxygenation reaction of RuBP by RubisCO; RuBP, ribulose bisphosphate; RubisCO, ribulose bisphosphate carboxylase/oxygenase; 2OG, 2-oxoglutarate, α-ketoglutarate; CBB, Calvin-Benson-Bassham; CEF, cyclic electron flow; LEF, linear electron flow; DCMU, diuron, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; PSI, photosystem I; PSII, photosystem II; DCBQ, 2,6-dichloro-1,4-benzoquinone, artificial electron acceptor of PSII; PQ/PQH_2, plastoquinone/plastoquinol; $NADP^+$/NADPH, oxidized/reduced nicotinamide adenine dinucleotide phosphate; Fd, ferredoxin; FNR, ferredoxin-$NADP^+$ oxidoreductase; CCM, CO_2-concentrating mechanism.

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misregulation of gene and metabolic activity. For example, it was recently observed that carbon fixation capacity was severely diminished upon large diversion of photosynthate towards, 2,3-butanediol, an engineered sink [4]. However, the physiological basis for that observation remained unresolved. Indeed, an understanding of the 'interaction' between introduced pathways and photosynthetic function generally remains poorly understood. An important approach to this question is to examine photosynthetic performance in the face of contrasting limiting nutrient conditions because nutrient or light availabilities may have a strong influence on whether or not these hypothesized metabolic impacts become significant [2–4].

The availability of inorganic carbon (C_i) is very often the limiting factor in the growth of algae and cyanobacteria in natural and engineered settings [5–10]. For cyanobacterial growth, the two main substrate forms are CO_2 and bicarbonate (HCO_3^-). Cyanobacteria have evolved mechanisms to acquire and concentrate both forms in order to saturate the active site of RubisCO with CO_2. The overall mechanism is termed the CO_2-concentrating mechanism (CCM) [11–13]. The CCM consists of a set of HCO_3^- transporters and a set of redox powered CO_2 uptake (Cup) proteins, which together function to supply HCO_3^- to the carboxysome. The latter contains RubisCO, as well as carbonic anhydrase [14]. The action of the HCO_3^- transporters and Cup proteins results in high cytoplasmic concentrations of HCO_3^-, driving its transport across the carboxysome boundary through selectively permeable pores in the surrounding proteinaceous shell. Carbonic anhydrase within the carboxysome converts incoming bicarbonate into CO_2 thereby supplying RubisCO. The CCM also exists in two forms: a constitutive low affinity, high flux uptake system and an inducible high affinity system that is controlled by C_i availability via changes in $NADP^+$ and 2-oxoglutarate levels, which are metabolic signals that respond to changes in C_i availability [45]. An additional metabolic interface between the CCM and metabolism relates to the power demands imposed by the operation of the CCM. The CCM requires both ATP and reductant (NADPH or reduced ferredoxin, Fd_{red}), and therefore, the CCM is intimately connected to photosynthetic electron transport. Accordingly, an important metabolic impact of the CCM involves its demands for ATP and Fd_{red}/NADPH production. A stoichiometric accounting of the NADPH and ATP requirements of the Calvin-Benson-Bassham (CBB) cycle shows a 2:3 ratio of NADPH to ATP required for carbon metabolism [15,16], although there remains debate whether linear electron transport can fulfil this ratio (briefly reviewed in [15]). Nevertheless, the demanded actual ratio, as tuned to metabolism, almost certainly changes in response to changes in the environment: limited inorganic carbon availability and extremes in pH and light intensity very likely modify this ratio. Furthermore, the introduction of new metabolic channels, and/or the closing of other channels, is predicted to impact these demands on the photosynthetic mechanism. To cope with changes in the cellular demand ratio for NADPH/ATP, oxygenic photosynthesizers have evolved mechanisms of returning electrons to the electron transport chain, thereby increasing the proton motive force (and therefore producing ATP) without the net production of Fd/NADPH. Collectively, these mechanisms are referred to as cyclic electron flow (CEF; see Fig. 1). There are several putative CEF pathways in cyanobacteria, involving SDH, NDH and FNR, but they remain poorly understood. The availability of CEF pathways, combined with energy dissipation pathways provides the photosynthetic electron transport system flexibility required to cope with fluctuations in light intensity and nutrient supply.

Here we perform a detailed analysis probing for possible changes in photosynthetic electron transport to address the question of how two types of engineered modification, one closing a metabolic sink, the other creating a new sink, are tolerated in the face of contrasting availability of the major photosynthetic macronutrient, inorganic carbon (Ci). Two engineered strains of *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*) were chosen as models for analysis of differentially altered carbon metabolism.

The first strain, Δ*glgC*, has a deletion of the gene encoding glucose-1-phosphate adenylyltransferase and cannot synthesize glycogen [17]. Consequently, this mutant lacks a carbon sink available to the wild-type strain. The second strain, JU547, has been engineered to express the ethylene forming enzyme (EFE) from the organism *Pseudomonas syringae* [18]. The ethylene-forming enzyme uses 2-oxoglutarate (2OG, aka α-ketoglutarate) as a cofactor for the synthesis of ethylene, a volatile compound, which escapes the medium as a gas. The JU547 strain therefore has an additional, engineered carbon and electron sink available. Furthermore, the ethylene-forming reaction also produces succinate in addition to ethylene, which can serve as an electron donor to the plastoquinone pool through the succinate dehydrogenase complex, which may serve as a major donor of electrons during respiration [19]. Appropriately, lower 2OG and higher succinate concentrations have been reported in JU547 [20].

2. Methods

2.1. Strains and culturing conditions

The glucose tolerant *Synechocystis* served as the background control strain and was maintained in standard BG-11 medium [21]. The Δ*glgC* and JU547 strains have been previously described [17,18]. Experimental

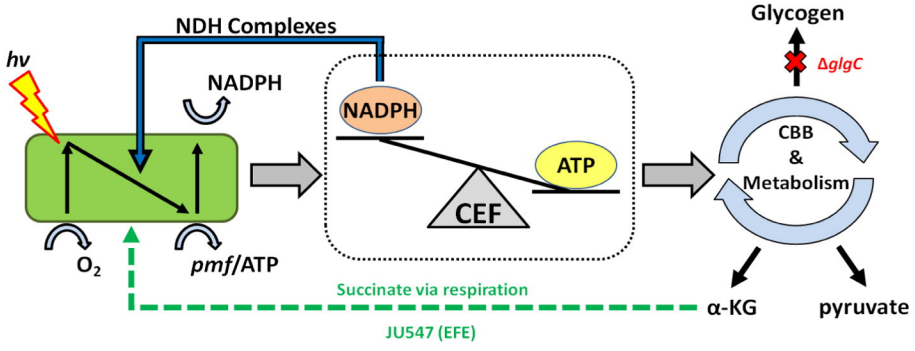

Fig. 1. Simplified model of electron flow and metabolism in *Synechocystis* sp. PCC 6803. The photosynthetic light reactions obtain electrons from water, producing gaseous oxygen as a result. Electrons are transferred through the photosynthetic electron transport chain ultimately reducing $NADP^+$, forming NADPH. During these reactions, a proton motive force (*pmf*) is established and used to drive the formation of ATP. NADPH and ATP are used to energize the Calvin-Benson-Bassham (CBB) cycle in order to integrate inorganic carbon into cellular metabolism. Cyclic electron flow (CEF) returns electrons back to the electron transport chain and regulates the NADPH/ATP ratio as needed, although the regulatory mechanisms are not fully elucidated. In nutrient replete conditions, glycogen serves as a primary storage molecule for excess electrons and carbon skeletons. In the Δ*glgC* strain (red), an enzyme responsible for glycogen synthesis has been deleted, and glycogen synthesis is removed. In the JU547 strain (green), the ethylene forming enzyme (EFE) gene has been integrated into the genome. As a result, ethylene and succinate are formed as products. Succinate itself may serve as a form of CEF through the succinate dehydrogenase complex (SDH) and return electrons to the electron transport chain. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)