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Investigation into the CO_2 concentrating step rates within the carbon concentrating mechanism of *Synechocystis* sp. PCC6803 at various pH and light intensities reveal novel mechanistic properties

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ABSTRACT

Synechocystis sp. PCC6803 actively uptake inorganic carbon to elevate the carbon dioxide concentration around their Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) enzyme. The mechanism is comprised of a set of enzymatic complexes which act in an orchestrated manner to transfer extracellular dissolved inorganic carbon into the cell and concentrate it within a specialized compartment. NADPH dehydrogenase-1 is the first operational super-complex in light conditions and, therefore, allows a direct estimation of carbon flux into the cell, albeit before photosynthesis and the concentrating mechanism reach a chemical steady-state condition. We calculated the uptake rate during this period by fitting a nonlinear function to the sharp decline in the CO_2 gas exchange profile recorded with membrane inlet mass spectrometry, considering the constant change in rate during this step. We estimate the carbon dioxide uptake rates and pool sizes, showing that these values correspond to previously reported steady state rates in the literature. During the investigation, we reveal new properties of the uptake mechanism. At pH lower than six, the enzymatic complex responsible for the CO_2 uptake becomes inactive. Its activity recovers within minutes when attenuating the pH within the cell or elevating it back at or above pH 6.0 outside the cell, a characteristic which seems to be conserved in activity in *Symbiodinium microadriaticum*, *Isochrysis galbana*, *Phaeodactylum tricornutum* and *Emiliania huxleyi*. We, therefore, suggest that within the carbon concentrating mechanism, CO_2 insertion processes can be deactivated when the organism faces pH 5.7.

1. Introduction

Photosynthetic organisms can fixate carbon dioxide into organic molecules by using the enzyme Rubisco (Ribulose-1,5-BISphosphate Carboxylase/Oxygenase) [1]. As its name suggests, Rubisco can either carboxylate or oxygenate the precursor molecule, such that when it comes to oxygenation, there is a loss of 25% of photosynthetic efficiency and the accumulation of toxic products in the cells (termed photorespiration) [2, 3]. At the current atmospheric pressure of oxygen (O_2) and carbon dioxide (CO_2), photorespiration is inevitable [4]. This fact forced terrestrial plants to concentrate CO_2 near the Rubisco carboxylating site as C4 and CAM plant carboxylation events are separated by space and time, respectively [5, 6]. In water bodies, dissolved O_2 concentration exceeds that of dissolved CO_2 concentration, which is a situation similar to that present in the atmosphere, due to equilibrium between air and water [7]. In addition, the concentration of dissolved CO_2 decreases even further due to CO_2 interaction with water, whereby it is transformed into two additional forms: bicarbonate (HCO_3^-) and carbonate (CO_3^{-2}) ions; all three forms are found in equilibrium at any given pH of the water [8]. Thus, it was inevitable that photosynthetic microorganisms also develop active means in order to concentrate CO_2 in the vicinity of their Rubisco enzyme [9].

Synechocystis sp. progenitors first appeared 2.7 Ga [10] and have been subjected to a period of rapid evolutionary changes [11]. In particular, The Great Oxygenation Event (GOE) that occurred 350 Ma [12] forced the *Synechocystis* sp. to increase its carbon gain efficiency by elevating the CO_2 concentration around the Rubisco, *via* active means [13]. The Carbon Concentrating Mechanism (CCM) in *Synechocystis* sp. PCC6803 is comprised from active uptake of HCO_3^-, CO_2 hydrating super-complexes that lock CO_2 in the cell and prevent it from escaping out of it by transforming it to HCO_3^-, and a carboxysome compartment, into which HCO_3^- is concentrated and is dehydrated back to CO_2 in presence of Carbonic Anhydrase enzymes (Fig. 1). Three types of HCO_3^- transporters are situated on the plasma membrane: Na^+/HCO_3^- symporters SbtA, BicA, and the traffic ATPase Bct1 [14–16]. These vary in their expression profile and flux rates together with their

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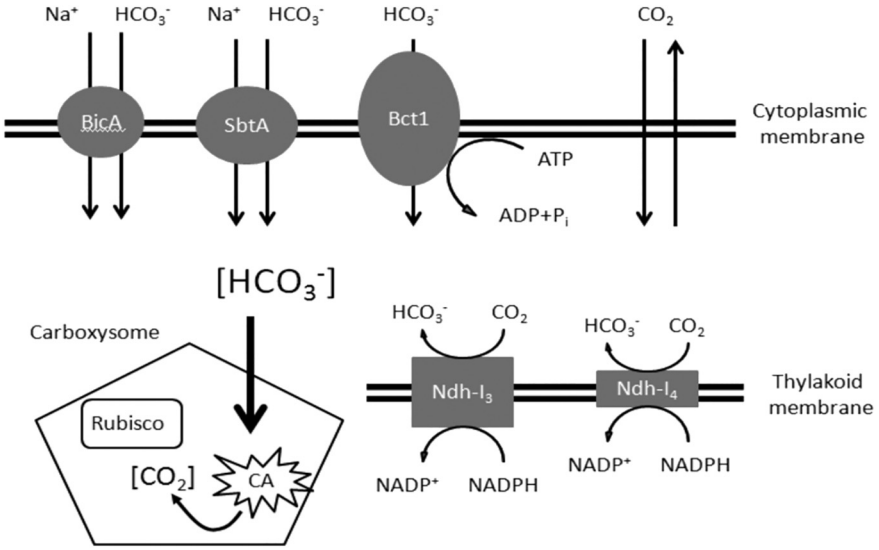

Fig. 1. Key players in the carbon concentrating mechanism of *Synechocystis* sp. PCC6803 (Pasteur Culture Collection). Simplified diagram of the enzymes involved in the cyanobacterial carbon concentrating mechanism (CCM), where clockwise represents directions of outside of the cell and into the carboxysome, respectively. CCM locks dissolved carbon dioxide (CO_2) within the cell by hydrating it to a charged form of bicarbonate ion (HCO_3^-). It also transports HCO_3^- directly into the cell by transporters located on the cytoplasmic membrane or indirectly by hydrating CO_2 which is free to enter and exit the cell. The HCO_3^- will penetrate the carboxysome where units of Carbonic Anhydrase enzyme will dehydrate it back to CO_2. This will, in turn be fixated by the Rubisco.
This diagram is copied with slight changes from [85] available at: https://doi.org/10.3390/life5010348.

Table 1
Mathematical formulation for analysis of dissolved carbon dioxide (CO_2) and bicarbonate ion (HCO_3^-) uptake rate fluxes into and out of the cell during light.

t_1 = CO_2 uptake; E_c = CO_2 evolution; k_2 = rate constant for de-hydration of HCO_3^-; [HCO_3^-] = concentration of HCO_3^- in the dark prior to light period; [CO_2] = concentration of CO_2 in the dark prior to light period; t_2 = bicarbonate uptake; E_b = bicarbonate evolution; net-fixation = fixation of CO_2 by Rubisco while estimating 1:1 ratio with oxygen (O_2) evolution; [C_i pool] = inorganic carbon (C_i) pool build-up; A^{max} = maximum asymptote; A^{min} = minimum asymptote; $t_{0.5}$ = time at the inflection point of the sigmoid; S = steepness/slope factor; t = time.

#	Equation	Citation
1[a]	$(t_1 - E_c) = k_2[HCO_3^-] - k_1[CO_2] - {}^{d[CO_2]}/_{dt}$	Badger [41]
2	$(t_2 - E_b) = net\,fixation - (t_1 - E_c)$	
3	$[HCO_3^-]_{dark} = \left\{\left({}^{d[CO_2]}/_{dt}\right)_{dark} + k_1[CO_2]_{dark} - \left({}^{d[O_2]}/_{dt}\right)_{dark}\right\}/_{k_2}$	
4	$(t_2 - E_b) = net\,fixation + E_c - {}^{d[Ci\,pool(t)]}/_{dt} - t_1$	Sültemeyer [36]
5	$(t_2 - E_b) = net\,fixation + E_c - {}^{d}/_{dt}\left\{\left[{}^{A^{max} - A^{min}}/_{1 + \exp\left(\frac{t_{0.5} - t}{s}\right)}\right] + A^{min}\right\} - t_1$	This work

[a] During the fitting procedure to zone I in the CO_2 traces, the uptake rate was corrected for the contribution of the inorganic carbonate system as in Eq. (1).

affinity to substrates, where BicA is active in any C_i concentration and SbtA and BCT-1 are expressed during inorganic carbon (C_i) limitation in much larger quantities [14, 15]. The two CO_2 hydroxylating complexes: NDH-I_3 (ndhD3/ndhF3/cupA/cupS) and NDH-I_4 (ndhD4/ndhF4/cupB), which are part of the NADPH dehydrogenase (NDH-IM) super-complex, belong to the energy-converting NADPH quinone:oxidoreductases family that is found in various microorganisms [17–20]; they are inducible under C_i limitation and constitutive in expression, respectively. Within the cell, the C_i penetrates into the carboxysome micro-compartment which holds most of the Rubisco units in a packed formation and encased in a proteinaceous shell of 200 nm width [21, 22]. The C_i will reach equilibrium between the three forms of ions at this point [9], where units of Carbonic Anhydrase enzyme (CA) obtain a zinc-bound water molecule which lowers the active site pKa from 15.7 to 7.0 [23], and transform the concentrated HCO_3^- back to CO_2 at the vicinity of the inactive Rubisco units [24, 25]. The Rubisco, in turn, will be transformed into the active mode of carboxylating enzyme [26].

The regulation of the CCM process occurs on multiple levels. During the transcription stage, the BCT-1 gene was found to be activated by a LysR transcription factor and coordinated to the gene promoter along with Ribulose-1,5-bisphosphate and 2-phosphoglycerate metabolites [27]. When the concentrations of the ccmR repressor coordinators $NADP^+$ and α-ketoglutarate decrease in the cell [28, 29], NDH-I_3 transcription repression is removed. Additional post-translational regulation on the complex arrangement level of NDH-IM complex was recently unraveled as the complex subunits ndh-Q and ndh-P stabilize and ndh-O destabilizes them, thus serving as means for yet unknown feedback regulation [30–32]. Despite the information regarding the regulation of the process, some questions remain open regarding the general activity of the NDH-I complexes. First, carbon uptake is active only in light conditions [33] and is controlled by redox reactions [34]. Secondly, upon illumination, the NDH-I complexes are activated immediately and the HCO_3^- transporters experience an activity lag of about 30 s [35, 36].

The quantity of concentrated inorganic carbon into the cell during a light period can be tracked directly by the amount of radioactive-labeled CO_2 (^{14}C) accumulated in the cell over time [37]. This can also be tracked indirectly by estimating the uptake rate by the amount of inorganic carbon expelled to the medium at the end of the light period, and then corrected to include the contribution of the inorganic carbon system which is operating outside of the cell [38]. In both cases, the cell volume into which the inorganic carbon is concentrated is estimated either directly by the Sorbitol Intermembrane Space (SIS) method [39] or indirectly by the cell volume calculation (with regard to its chlorophyll content or cell density) [40]. Another indirect method to estimate the size of the inorganic carbon pool within the cell can be carried out during the CO_2 insertion step at the beginning of the light period (Table 1). Badger [41] claims that during a light period there is a