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Review article

Mechanisms of carbon fixation and engineering for increased carbon fixation in cyanobacteria

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ABSTRACT

Cyanobacteria, gram-negative prokaryotic microorganisms, perform oxygenic photosynthesis with a photosynthetic machinery similar to higher plants which includes ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO) as the main CO_2-fixing enzyme. Currently, there is a growing interest to use cyanobacteria as photosynthetic microbial cell factories for the direct production of solar fuels or other compounds of human interest. However, rates and efficiencies to produce e.g. biofuels are still very low. The amount of available fixed carbon for the synthesis of desired product(s) may be one of the limiting steps. This contribution reviews CO_2-fixation in cyanobacteria with focus on CO_2-concentrating mechanisms, RuBisCO, phosphoenolpyruvate carboxylase and other carboxylases, engineering approaches for increased carbon fixation, and finally the synthetic malonyl-CoA-oxaloacetate-glyoxylate pathways.

Contents

1. Introduction

Cyanobacteria are among the oldest organisms on earth [1]. These microorganisms are gram-negative prokaryotes and perform oxygenic photosynthesis, with a photosynthetic machinery similar to higher plants [2]. It is thought that they raised the O_2 levels in the atmosphere when the earth was anoxic, 2.3 billion years ago [3]. Presently, cyanobacteria are broadly distributed in different natural habitats such as marine and freshwater, different terrestrial and extreme environments [4]. Structurally they range from unicellular to colony forming and filamentous forms. Regardless of form, certain cyanobacteria have the capacity to fix atmospheric nitrogen (N_2) into ammonia through the action of the enzyme complex nitrogenase. This makes cyanobacteria important contributors of nitrogen into nutrient poor environments [3].

There is a rapidly growing interest to use cyanobacteria as photosynthetic microbial cell factories for the direct production of solar fuels [5–8] and [9] or other compounds of human interest. However, rates and efficiencies are still very low [10]. The amount of available carbon for the synthesis of desired product(s) may be one of the limiting

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steps. In addition, an increased production level of purpose designed, carbon-containing molecules that are excreted from the cells and harvested for further use highlights the need for additional carbon fixation in the cells. Thus, possibilities to make the process of carbon fixation more efficient with higher rates and yields attract new as well as increased attention.

Six different carbon fixation mechanisms have been identified in autotrophic organisms; (i) the Calvin–Benson–Bassham (Calvin) cycle, (ii) the reductive citric acid cycle, (iii) the reductive acetyl-coenzyme A pathway, (iv) the 3-hydroxypropionate bicycle, (v) the hydroxypropionate–hydroxybutyrate cycle, and (vi) the dicarboxylate–hydroxybutyrate cycle [11]. The main carbon fixation cycle used for primary biomass production (including in cyanobacteria) is the Calvin cycle [12], and cyanobacteria contribute to almost a quarter of the global carbon fixation [13].

2. CO_2 fixation and subsequent carbon metabolism

2.1. Carbon concentrating mechanisms (CCM)

One main issue in aquatic systems, where most cyanobacteria live, is the low availability of inorganic carbon (Ci), which is controlled by different external parameters like pH, temperature, and gas-exchange. [14]. The CO_2 diffusion in air is faster than in water and consequently the equilibrium between CO_2 and HCO_3^-, in water, is relatively slow for pHs between 7 and 8.5. Therefore, cyanobacteria have developed a CO_2-concentrating mechanism (CCM) in order to ensure that RuBisCO is surrounded by CO_2 and thereby avoid the oxygenase reaction [15]. Since the cyanobacterial RuBisCO has low affinity for CO_2, these microorganisms may not have survived in the aquatic habitat without a CCM [16].

Cyanobacteria have developed up to five different transporters to actively acquire and transport inorganic carbon into the cells. Some transporters are expressed constitutively while others are induced when only limited levels of carbon are present [15] and [17]. In addition, light is a pre-requisite for the expression of the genes encoding Ci uptake transporters, and their transcription is dependent on cyclic photosynthetic electron flow around photosystem I [18]. Cyanobacteria may incorporate two different forms of Ci, CO_2 and HCO_3^-. In addition, there are specific uptake systems; three for the import of HCO_3^- and two for the import of CO_2 (Fig. 1) [15];

(i) BCT1 is an ATP-binding cassette transporter located in the plasma membrane and encoded by the *cmpABCD* operon. Its transcription is induced under low levels of Ci and enhanced by high light conditions. This transporter shows high affinity for HCO_3^- and it is common in β-cyanobacteria while it is present only in some α-cyanobacteria [15] and [19];
(ii) SbtA is a Na^+-dependent transporter positioned in the plasma membrane. It is highly induced under carbon limited conditions and has a relatively high affinity to HCO_3^- [15]; and
(iii) BicA is the second Na^+-dependent HCO_3^- transporter. However, this transporter shows low affinity to HCO_3^- and the genes encoding BicA are primarily constitutively expressed [15].

The two CO_2 transporters, NDH-I_3 and NDH-I_4, are based on plastoquinine oxidoreductase NADPH dehydrogenase respiratory complexes. Both CO_2 transporters are present in β-cyanobacteria while α-cyanobacteria possess only one of them or even lack them completely. Since CO_2 can cross the plasma membrane in a passive way, the main activity of these transporters is hydration of CO_2 by the CpX or CpY proteins which perform carbonic anhydrase (CA)-like reactions [17] and [18];

(i) NDH-I_3, located in the thylakoid membrane, is inducible under low levels of Ci with CpY being the CA-like reaction protein [19].
(ii) NDH-I_4 is constitutively expressed and contains the CpX protein. The location of this complex is still unclear; either in the plasma or thylakoid membrane [17] and [21].

Once inside the cyanobacterial cell, the HCO_3^- is further transported into the carboxysomes where both RuBisCO and CA are located. CA catalyzes the dehydration of HCO_3^- into CO_2. The action of this enzyme results in an increased level of CO_2 around RuBisCO leading to significantly higher carbon fixation efficiencies [20]. The carboxysomes are polyhedral cytosolic inclusion bodies composed by several proteins (Table 1). Experiments where genes encoding carboxysome proteins were deleted resulted in cells able to grow under high concentration of CO_2 only due to a non-functional CCM [22]. Therefore, carboxysomes are essential for an active CCM. In cyanobacteria, two different forms of carboxysomes are distinguished (α and β) depending on both the type of RuBisCO (form 1A or 1B) and the presence of other structural and functional carboxysomal proteins (Table 1) [20,23] and [24].

2.2. Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO)

RuBisCO, the carbon-fixing enzyme in the Calvin cycle, has the capacity to catalyze two different reactions. In the carboxylation reaction, it uses CO_2 and ribulose-1,5-bisphosphate (RuBP) to produce two molecules of 3-phosphoglycerate (3PGA). 3PGA is used in the regeneration of RuBP in the Calvin cycle as the precursor for further cyanobacterial carbon metabolism. In cyanobacteria, the regulation of 3PGA is performed by sedoheptulose 1,7-bisphosphate proteins (SBPase proteins) [25]. Besides the carboxylase activity, RuBisCO can also act as an oxygenase resulting in photorespiration. In this case, RuBisCO uses O_2 and RuBP in order to form 3-phosphoglycerate and 2-phosphoglycolate. The 2-phosphoglycolate is further converted to glycolate. The latter can be metabolized by the C2 cycle, the glycerate pathway or through decarboxylation.

RuBisCO may exist in up to four different forms in nature. The most abundant is form I found in all higher plants, red and brown algae, chemoautotrophic bacteria, purple bacteria and cyanobacteria [12] and [26]. The enzyme is composed of 8 large and 8 small subunits forming a hexadecameric structure. Single large subunits (LSU, about 55 kDa) form dimers and a tetramer of dimers. The small subunits (SSU, about 15 kDa) form tetramers which are placed on the top and the bottom of the tetramer of the LSU. Each SSU makes contact with three LSU and two SSU [26] and [27]. In the cyanobacterium *Synechococcus* ACMM 323 it has been shown that the SSU is not essential for catalysis but it stabilizes the complex structure of the enzyme, and contributes to the maximal activity, efficiency and specificity [28]. Further subdivisions of RuBisCO form I have been made based on amino acid sequences; cyanobacteria possess forms IA and IB [23]. Form II of RuBisCO is present in purple non-sulfur bacteria, several chemoautotrophic bacteria and eukaryotic dinoflagellates. This form consists of only LSU forming dimers [12]. Form III is found in archaea and it is relatively diverse, with different structures such as LSU associated in dimers, octamers or decamers. The final form IV is composed by enzymes that are similar to RuBisCO sequences, but they neither use CO_2 as substrate nor perform the carboxylation reaction. Form IV is e.g. involved in sulfur metabolism in the green sulfur bacterium *Chlorobium tepidum* [29], the thermophilic bacterium *Geobacillus kautophilus* [30] as well as in other bacteria [12].

Even though the origin of RuBisCO can be diverse, the secondary structure is well conserved among all types. The large subunits have mainly two domains; the N-terminal and the C-terminal domain, respectively. The N-terminal domain is shorter than the C-terminal and composed of 4–5 mixed β-sheets and α-helices (reviewed in [12]) while the C-terminus is formed by eight-stranded parallel α/β barrels. Each subunit has an active site in the C-terminus of the respective β-strands. The entrance of the active site is facing out of the enzyme [12] and [31] and the loops that connect the α/β barrels are involved in