CO_2 ACCUMULATION BY MICROALGAE

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Abstract

Microalgae and cyanobacteria grown under ordinary air show a high affinity for inorganic carbon (Ci) in photosynthesis, which cannot be seen in terrestrial higher plants. This may be achieved with the cooperation of Ci accumulating system, carbonic anhydrase and pyrenoid (or carboxysome). The mechanism, however, is diverse among species and still under investigation. Information on the photosynthetic characteristics is important with respect to the technological utilization of microalgae against the increase in atmospheric CO_2 concentration.

Introduction

Concentration of carbon dioxide in the atmosphere is now increasing year by year, which has been seriously concerned as one of factors causing greenhouse effect of the earth. It is

mainly due to the consumption of fuels by human activity. The total amount of CO_2 produced in the world is estimated at about 2 x 10^{10} tons per year and 1 x 10^9 tons from Japan. We should reduce the CO_2 production as soon as possible. Increasing the efficiency in various industrial processes to diminish CO_2 dispersion may be the first step which should be taken, but it is also important to remove CO_2 from the waste gases. There are several chemical and physical methods to fix CO_2, e.g. absorption of CO_2 into monoethanolamine and adsorption to lumps of clay. However, extra energy must be required to store CO_2 somewhere such as bottom of deep sea and to produce or recycle the chemicals and materials, which may cause another production of CO_2. In this respect, photosynthetic CO_2 fixation by plants and algae is an efficient biological phenomenon which is possibly applied to industry in future. As one of the possibilities, a "green smokestack" which takes up CO_2 as well as poisonous gases will be very pleasant.

However, we must solve several problems to realize it. The rate of photosynthesis per biomass is not so high as to fix all of the CO_2 produced from factories. Calculation reveals that at least 6-27 x 10^4 km^2 of leaves or 3-7 x 10^6 m^3 of Chlorella cells are required for the fixation of CO_2 produced from Japan, assuming that leaves of C_3 plants or Chlorella cells can proceed photosynthesis at their maximum rates for 10 hrs a day with following parameters: maximum rate of photosynthesis of C_3 plants, 10-50 mg $CO_2/dm^2 \cdot hr$[1]; the rate of photosynthesis of

Chlorella per biomass, 40-80 mg CO_2/ml packed cell volume·hr. We have, therefore, to find or manipulate plants and/or algae which can fix CO_2 much faster than those reported so far. For this purpose, it is important to investigate limiting factors in photosynthesis. Kinetic analyses of photosynthesis in terrestrial C_3 plants show that $K_{1/2}$ values for CO_2 (the concentration of CO_2 which gives a half of the maximum rate) are about the concentration of atmospheric CO_2 (300-400 ppm)[2], indicating that photosynthesis at atmospheric CO_2 concentration proceeds at only a half of the maximum rate. The $K_{1/2}$ values of C_4 plants are much lower than those of C_3 plants due to CO_2 accumulating system in mesophyll cells[2]. Microalgae growing under ordinary air (low-CO_2 cells) show still higher affinity for CO_2 and this characteristic changes depending on the CO_2 concentration during growth[3,4]. We have been investigating the mechanism why the microalgae show such a high affinity for CO_2 in photosynthesis.

Two parameters, accumulating system(s) of HCO_3^- and/or CO_2 in the cells and carbonic anhydrase, have been considered for the explanation of the photosynthetic characteristics[3,4]. Recently, attention became to be payed to pyrenoid and carboxysome. They are the compartments which contain ribulose 1,5-bisphosphate carboxylase (RuBisCO) in the chloroplast of eucaryotic algae and in cyanobacteria, respectively. The detailed discussion on these problem was made in the Second International Symposium on Carbon Utilization by Aquatic

Photosynthetic Organisms held in 1990 and the papers presented by the participants were published in vol. 69, No. 5 of Canadian Journal of Botany in 1991. We intend to summarize the current understanding on the high affinity for CO_2 in photosynthesis of eucaryotic microalgae and cyanobacteria for the researchers of technology and its related fields in this paper.

Chemical reaction of inorganic carbon in water

It is well known that, when CO_2 is dissolved in water, it is hydrated and ionized to bicarbonate ion and then to carbonate ion[5]. Percentage of the concentrations of these chemicals depends on pH, and bicarbonate ion is dominant at physiological pH (pH 7-8.5). All forms of compounds derived from CO_2 in water will be referred to inorganic carbon (Ci). Carbon dioxide can penetrate through lipid membrane easily, while it is not the case for HCO_3^- because of its (negative) charge.

Photosynthetic Ci fixation is a very fast reaction and its rate in normal condition is roughly 10 times higher than that of respiration in Chlorella. Therefore Ci transfer from outside of the cells to the reaction site of RuBisCO would be a possible limiting step in the algal photosynthesis if Ci moves by simple diffusion. RuBisCO which is a key enzyme of Calvin-Benson cycle reacts with CO_2, but not with HCO_3^-[6], whereas the substrate for phosphoenolpyruvate carboxylase (PEPCase) which is the initial carboxylation enzyme in C_4 photosynthesis is HCO_3^-[7]. Furthermore, the rate of conversion between CO_2 and HCO_3^- is

relatively low[5] and the reaction is catalyzed by carbonic anhydrase (CA).

Ci accumulating system in the cells

Accumulation of Ci can be observed with silicone oil layer filtering centrifugation[8,9,10,11] and with CO_2 burst after the illumination[12]. The level of Ci accumulation during photosynthesis at limiting Ci concentrations reaches the order of thousand in cyanobacteria, and about a hundred or less in eucaryotic algae such as green algae[3,13]. From kinetics, HCO_3^- transporter has been postulated in the cytoplasmic membrane of cyanobacteria[9,11]. However, in spite of many efforts, any peptide of the HCO_3^- transporter has not been illustrated yet. One of the genes which are responsible to the Ci accumulation was assumed to be NADH dehydrogenase based on the predicted amino acid sequence[14], suggesting that ATP is necessary for the Ci transport. Recently some also considered the active transport of free CO_2 in cyanobacteria[15], but its molecular mechanism is still not clear.

In Chlorella, CO_2 is the substrate which passes through plasma membrane in photosynthesis[10,16,17]. Some of HCO_3^- is also taken up into the cells when HCO_3^- is absent in the cells. However, HCO_3^- fixation does not continue after its addition to the cell suspension. Many researchers assume the existence of Ci transporter, because the Ci concentration in the cells reaches much higher than the level explained by the difference of pH

between inside the cells and medium assuming that only CO_2 penetrates through plasma membrane and that HCO_3^- is produced to equilibrium with CO_2 in the cells. At present none of the report has demonstrated the Ci transporter in eucaryotic algae at neither peptide nor gene level. We think that, Ci transporter should be localized in the plasma membrane, if it exists, from the data of kinetics in Chlorella[4,10,18], Chlamydomonas[19] and Euglena[20]. Nevertheless, the substrate (CO_2 or HCO_3^-) and the locality of the transporter (in plasma membrane or chloroplast envelope) wait further investigation. The existence of the Ci transporter itself is not yet clearly demonstrated.

Carbonic anhydrase

Carbonic anhydrase activity has been found in various plants and animals. Human CA II in erythrocyte has been well analyzed so far[21]. In plants and algae, CA is usually detectable in green tissues, and its activity is commonly higher in the terrestrial plants than in low-CO_2 cells of microalgae. CA activity in microalgae is usually suppressed under 2-5% CO_2.

Carbonic anhydrase in green algae is divided into two types with respect to its cellular locality: One is located inside the cells, probably in the chloroplast and the other on the outer surface of cells such as cell wall, periplasmic space (between cell wall and plasma membrane), or on plasma membrane. However, not all of the algal strains have CA on cell surface and conversely some of Chlorella strains have the cell wall-bound CA

even when the cells are grown under high CO_2 conditions (high-CO_2 cells). The activity of the cell-surface CA, if it exists, is commonly higher than the internal CA[22]. It prevents us from precise determination of the internal CA activity. By subtracting the value obtained with the suspension of intact cells from that obtained with the cell homogenate it has been shown that the internal CA is always higher in low-CO_2 cells than in high-CO_2 cells[22]. This suggests that the internal CA plays more important role for the high affinity for CO_2 in photosynthesis than the cell-surface one.

Nonetheless, cell-surface CA of Chlamydomonas reinhardtii has been a good target of research so far. Firstly because its activity is relatively high and stable, and secondly because it is soluble after homogenization of the cells or after digestion of cell wall. The structure of the protein and its gene have been identified[23,24].

What is the function of the cell-surface CA in photosynthesis? Analysis of Ci fixation showed that both Chlorella and Chlamydomonas take up CO_2, not HCO_3^-, through plasma membrane in photosynthesis and their cell-surface CA's catalyze the reaction from HCO_3^- to CO_2 before the absorption to the cell.

CA activity in cyanobacteria is relatively lower than that of green algae. CA activity in intact cells, spheroplasts of low-CO_2 cells of Anabaena variabilis were both 0.7 units/mg chlorophyll and that of broken spheroplasts was 0.8 units/mg

chlorophyll[25]. This result suggests that the most of CA is present on or in the cytoplasmic membrane. Since HCO_3^- is the substrate of the transporter in cytoplasmic membrane, the CA may play a role of conversion from CO_2 to HCO_3^- prior to the transport into cells. Thus, CA on cell surface may function in the opposite manner between green algae and cyanobacteria.

CA is also located in the chloroplast of eucaryotic algae. Because fractionation of organelles is very hard from microalgae, there are still a few data indicating its intracellular locality exactly. CA was found in stroma in Porphyridium cruentum R-1 with a technique of gold particle in electron microscopy[26]. Differential centrifugation and non-aqueous fractionation of Chlorella vulgaris 11h suggest the CA locality in chloroplast, probably about 80% being on thylakoid membrane[22], and the remains in stroma. Role of the CA in the stroma may be the promotion of CO_2 supply to RuBisCO which is shown by a mathematical model assuming that only CO_2 can penetrate through the chloroplast envelope and the CO_2 molecules diffuse in the stroma some of which is fixed by RuBisCO simultaneously. Since stromal pH is usually higher than 8, HCO_3^- concentration reaches the level much higher than that of CO_2 in the presence of CA. And since HCO_3^- is not trapped by RuBisCO, it spreads over the chloroplast. Consequently CO_2 can be supplied not only by diffusion of itself but also by dehydration of HCO_3^- by CA. CA thus catalyzes both directions of the reaction between CO_2 and HCO_3^- in photosynthetic CO_2 fixation. This mechanism had been

speculated kinetically [10,17,27]. The principle of this mechanism is the same as facilitated diffusion reported in animal cells[28,29,30].

Pyrenoid and carboxysome

Carboxysomes which had been identified as polyhedral bodies in electron microscopy may be important for the efficient CO_2 fixation in cyanobacteria. Firstly because number of carboxysome increases during the adaptation to low CO_2 concentration[31], and secondly because some of mutants which can grow only under high CO_2 conditions have abnormal carboxysome[32], in which the 5'-franking region of RuBisCO gene (rbc) are mutated[32].

In green microalgae, pyrenoid and starch plates surrounding the pyrenoid develop under low CO_2 conditions. So far, pyrenoid had been considered as a storage compartment of nitrogen in the form of RuBisCO[33]. However, the rate of photosynthetic CO_2 fixation can not be explained by only stromal RuBisCO without contribution of RuBisCO in the pyrenoid[34]. Since there are few reports which show the RuBisCO activity in isolated pyrenoid, the real function of the pyrenoid is not yet established.

Concluding remarks

Microalgae and cyanobacteria can adapt to various CO_2 concentrations and show almost the maximum rate of photosynthesis at the CO_2 concentration during growth. They are possibly applied to the CO_2 absorber from atmosphere and also from waste

gases in the future. Some of the algae and cyanobacteria have already been used for production of chemicals such as β-carotene and polysaccharides[35,36]. It must be a great progress for human being if we can apply the photosynthetic characteristics of microalgae to produce some chemicals and/or materials from solar energy and the waste gases from the industry.

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