EFFECTS OF CO_2 CONCENTRATION DURING GROWTH AND OF ETHOXYZOLAMIDE ON CO_2 COMPENSATION POINT IN *CHLORELLA*

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1. Introduction

Chlorella vulgaris 11h cells grown with ordinary air (containing 400 ppm CO_2) (low-CO_2 cells) were found [1] to have a much higher photosynthetic CO_2 affinity than those grown with air enriched with ~2% CO_2 (high-CO_2 cells). Similar changes in the affinity for CO_2 resulting from the difference in the CO_2 concentration during growth have been reported for *Scenedesmus obliquus* [2], *Chlamydomonas reinhardtii* [3] and *Anabaena variabilis* [4].

CO_2 concentration during growth has been shown not to affect either ribulose 1,5-bisphosphate (Ru-P_2) carboxylase (EC 4.1.1.39) activity or its K_m for CO_2 [3,5]. In contrast, carbonic anydrase (EC 4.2.1.1) (CA) activity was almost exclusively located in low-CO_2 cells of *Chlorella* and its activity in high-CO_2 cells was very small, if any [5,6]. These results indicate the possibility that CO_2 is accumulated within the algal cells via CA. This accumulation may lead to a CO_2 concentration available for fixation by Ru-P_2 carboxylase that is far higher in low-CO_2 cells than in high-CO_2 cells. Thus, the app. K_m (CO_2) in the former cells would become significantly lower than that in the latter cells. The finding that the app. K_m (CO_2) in low-CO_2 cells was greatly increased by Diamox, a potent inhibitor of CA [3,5] supported the above inference.

Photorespiration is generally assumed to be caused by Ru-P_2 oxygenase reaction which is active at high O_2 and low CO_2 concentration [7]. One may therefore assume that the photorespiration is more active in high-CO_2 cells than in low-CO_2 cells. With *Scenedesmus obliquus*, the CO_2 compensation point (Γ) which was adopted as an index of photorespiration was reported [8] greater in high-CO_2 cells than in low-CO_2 cells. However, they could not find any difference in Γ between high- and low-CO_2 cells of *Chlorella fusca*. The results described below showed that Γ in high-CO_2 cells of *Chlorella vulgaris* 11h was far greater than that in low-CO_2 cells. We further found that Γ in low-CO_2 cells which was 0–10 ppm increased to 50 ppm by the addition of ethoxyzolamide, another inhibitor of CA.

2. Materials and methods

Chlorella vulgaris 11h (Algensammulung des Pflanzenphysiologischen Institut der Universität Göttingen) was grown photoautotrophically by the method in [9]. Air enriched with ~2% CO_2 by volume was constantly bubbled through the algal suspension at 21–22°C. After ~1 week, the algal suspension was divided into 2 parts and the same CO_2-enriched air was bubbled through one part to obtain high-CO_2 cells, while ordinary air was supplied to the other part to obtain low-CO_2 cells. After 1 day, cells were harvested, washed twice with deionized water and suspended in 10 mM MES–NaOH buffer (pH 5.0). The cell density was 150 ml p.c.v./l. The vessel containing algal suspension was kept in ice.

Photosynthetic CO_2 fixation was carried out in a water-jacketed glass vessel 'lollipop'. Air containing predetermined concentration of CO_2 was kept in a 20 l reservoir [10]. The gas reservoir and 'lollipop' were connected with a vinyl tube. MES–NaOH buffer solution, 68 ml (pH 5.0, 10 ml) was placed in the

lollipop and the flow of gas mixture was started by means of water pressure. The concentration of CO_2 in the gas which had passed through the buffer solution was determined with an infrared gas analyzer (URA-2S, Shimazu Co. Ltd). After 5–20 min, the concentration reached a constant level, equal to that in the gas reservoir. Then, the algal suspension (2.3 ml) was injected into the lollipop under illumination with a reflector lamp (35 000 lux). Concentration of CO_2 in the gas which had passed through the algal suspension attained a new stationary level after another 5–10 min. The flow rate of the bubbling gas was carefully kept at 100 ml/min. The rate of CO_2 exchange by the algal cells was calculated taking account of the flow rate of the bubbling gas as well as the change in the stationary levels of CO_2 in the gas which was caused by the addition of algal cells. Temperature of the algal suspension was kept at 20°C or 32°C by means of water running through the water-jacket [10].

To study the effect of ethoxyzolamide, the algal suspension was preincubated with this inhibitor on ice under room light. After ~5 h, CO_2 fixation was determined by the above procedures. The final cell density and concentration of the inhibitor were 5 ml p.c.v./l and 0.5 mM, respectively. Ethoxyzolamide was purchased from Sigma Chemical Co., St Louis.

3. Results and discussion

Figure 1 shows that, at $CO_2 < 300$ ppm, the rates of net photosynthesis in low- and high-CO_2 cells were proportional to the CO_2-concentration given during the experiment. The rates of net photosynthesis in low-CO_2 cells were higher than those in high-CO_2 cells (cf. (A) and (B); (C) and (D)). In high-CO_2 cells, the temperature rise caused the lowering of net photosynthesis (cf. (B) and (D)). Such lowering was not observed in low-CO_2 cells (cf. (A) and (C)). When CO_2-free air was bubbled through the suspension of high-CO_2 cells, a significant CO_2 evolution was observed. This CO_2 evolution was enhanced when the temperature was raised from 20–32°C. In low-CO_2 cells, no such significant CO_2-evolution was observed even at 32°C. As a consequence, Γ in low-CO_2 cells was close to 0, while that in high-CO_2 cells was ~40 ppm at 20°C. At 32°C, the same value in low-CO_2 cells was 0–10, while that in high-CO_2 cells increased to 80–100 ppm. It was claimed [11] that various fresh water algae did not exhibit photorespiration. Since only low-CO_2 cells for the experiment were used, the conclusion is consistent with the present results. In this connection, it should be mentioned Γ was determined in a closed system [8]. It took several hours until the CO_2 concentration in this system reached Γ. During that long interval, high-CO_2 cells of *Chlorella* would have been changed to low-CO_2 cells. This would be the reason why they could not find any difference in Γ-values between two forms of the algal cells. Mostly low-CO_2 cells of various algae also showed lack of photorespiration [12]. However, these results with high-CO_2 cells should be re-examined from the same view point.

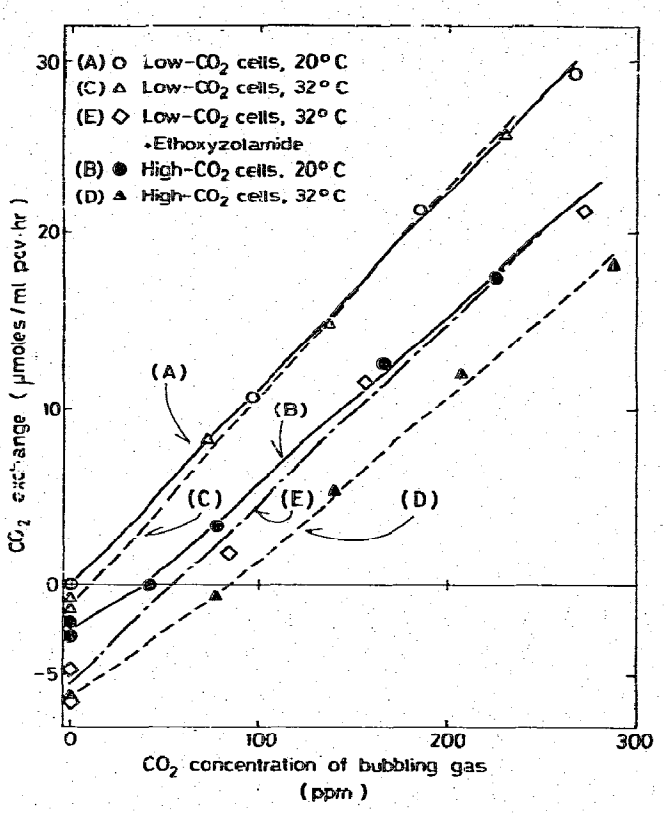

Fig.1. CO_2-exchange versus CO_2-concentration in low- and high-CO_2 cells of *Chlorella vulgaris* 11h. (○) Low-CO_2 cells at 20°C; (△) Low-CO_2 cells at 32°C; (●) High-CO_2 cells at 20°C; (▲) High-CO_2 cells at 32°C; (◇) Low-CO_2 cells in the presence of ethoxyzolamide (32°C).

We therefore concluded that photorespiration in green algae is active only in high-CO_2 cells, and that its activity is greater at higher temperature. Metabolically, photorespiration has generally been explained by the glycolate pathway [7]. With *Chlorella* cells ([13], Hogetsu and S. M. in preparation) evidence indicating that high-CO_2 cells produce and metabolize considerably more glycolate than low-CO_2 cells was provided. With high-CO_2 cells of *Chlorella vulgaris* 11h metabolism of glycolate was greatly enhanced by raising the temperature from 20–32°C (Nakamure and S. M. submitted). The elevation in Γ in high-CO_2 cells induced by the rise of the temperature, therefore, is in accord with the inference that the glycolate pathway is more active at higher temperatures.

Figure 1 also shows that Γ in low-CO_2 cells was shifted to 50 ppm by the addition of ethoxyzolamide (cf. (C) and (E)). We have shown that the inhibitory effect of ethoxyzolamide on photosynthetic O_2 evolution in high-CO_2 cells (in the presence of 1 mM $NaHCO_3$) is only 4% (data not shown, see also [5]), indicating that this inhibitor (0.5 mM) does not inhibit photosynthesis of the algal cells which do not contain CA. We therefore concluded that CA plays a role in concentrating CO_2 from outside to the site of the carboxylation reaction, and hence lowers the Γ-value in low-CO_2 cells.

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