EFFECTS OF CARBONIC ANHYDRASE ON RIBULOSE 1,5-BISPHOSPHATE CARBOXYLASE AND OXYGENASE

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1. Introduction

The apparent K_m value for CO_2 in photosynthesis in algal cells grown with ordinary air (low-CO_2 cells) is much lower than that in those grown with CO_2-enriched air (high-CO_2 cells) [1–4]. Between the two types of cells there was practically no difference in activities as well as K_m (CO_2)-values of ribulose 1,5-bisphosphate carboxylase (RuBPCase, EC 4.1.1.39) [5,6]. In contrast, carbonic anhydrase (CA, EC 4.2.1.1) was almost exclusively confined to low-CO_2 cells of *Chlorella* and its activity in high-CO_2 cells was marginal, if any [5,6]. In the chloroplasts of high-CO_2 cells which show very low CA activity, CO_2 is supplied by simple diffusion, then directly used as the substrate for RuBPCase (direct use of CO_2) [6]. On the other hand, CO_2 which crossed the chloroplast envelope of low-CO_2 cells will be quickly converted to bicarbonate via CA, enhancing the transport of CO_2 from outside into the chloroplasts. There are two possible ways by which CO_2 may be supplied to RuBPCase in low-CO_2 cells.

(i) The direct use of CO_2 (as described above for high-CO_2 cells);
(ii) HCO_3^- formed from CO_2 in the stroma is converted again into CO_2 via CA and fixed by RuBPCase (indirect use of CO_2).

Under CO_2-limiting conditions, photosynthetic CO_2 fixation would be enhanced by the indirect use of CO_2, and CA is essential for this enhancement.

CO_2-fixation by RuBPCase is actually enhanced by CA [7]: When [$^{14}CO_2$] dissolved in the reaction medium containing RuBPCase was low (in equilibrium with 400 ppm $^{14}CO_2$ gas), the addition of CA greatly enhanced $^{14}CO_2$ fixation. On the other hand, no such enhancement was observed when [$^{14}CO_2$] dissolved in the reaction medium was 10-times higher.

The enzyme RuBPCase also catalyzes the oxygenation of RuBP (RuBP oxygenase (RuBPOase)) [8]. The RuBPCase and RuBPOase are inhibited by O_2 and CO_2, respectively, in a competitive manner [9]. Here we show that CA not only increases RuBPCase activity but also decreases RuBPOase activity. The effect of CA was more pronounced as [CO_2] dissolved in the reaction medium was decreased. As a result, K_m (CO_2) for RuBPCase was greatly decreased by CA.

2. Materials and methods

RuBPCase (partially purified from spinach leaves, purchased from Sigma, St Louis) was dissolved in 100 mM bicine–NaOH solution (pH 8.30) containing 20 mM $MgCl_2$ and 10 mM $NaH^{14}CO_3$ (1.13 μCi/μmol). The solution was kept in a 1.5 ml rubber stoppered vial at 30°C for ≥30 min to activate the enzyme. The activity of the activated enzyme remained constant during the incubation at 30°C for >5 h.

RuBPOase and RuBPCase activities were measured simultaneously in a Clark-type oxygen electrode vessel (Rank, London) [10]. The reaction was started by injection of 40 μl activated enzyme solution (260 μg enzyme) into CO_2-free reaction mixture with or without CA (from bovine erythrocytes, Boehringer-Mannheim) (final vol. 1500 μl). CA was added 2 min before the start of the reaction. Unless otherwise mentioned, the amount of CA added was 2 μg (4 units)/vessel. The reaction mixture contained 18.3 mM bicine–NaOH (pH 8.30), 7 mM $MgCl_2$, 0.3 mM RuBP and (unless otherwise indicated)

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0.27 mM $NaH^{14}CO_3$ (1.13 μCi/μmol). To prepare the reaction mixture free of CO_2, each reagent was dissolved in CO_2-free water (the pH value of the medium containing buffer was adjusted by adding CO_2-free concentrated NaOH solution under nitrogen flush). The reaction was started at 240 μM O_2 and the temperature was kept at 30°C. RuBPOase activity was monitored by RuBP-dependent O_2 uptake and recorded with a highly sensitive R-12 recorder (Rikadenki, Tokyo). Oxygen uptake was linear at least for the initial 90 s and the rate was determined from the slope during this period. After 90 s, the reaction was stopped by adding 250 μl 50% acetic acid, and RuBPCase activity was determined from the ^{14}C incorporated into acid-stable fraction as follows. Portions (200 μl) of the acidified mixture were taken into scintillation vials. Unfixed $^{14}CO_2$ was purged with N_2, then 2.5 ml liquid scintillator solution (Tritosol) [11] was added. The radioactivity was determined with a liquid scintillation spectrophotometer (Beckman LS 230).

$NaH^{14}CO_3$ and Diamox were purchased from Daiichi Chemical, Tokyo and Tokyo Kasei, Tokyo, respectively.

3. Results and discussion

In the presence of 240 μM O_2 and 0.27 mM $NaHCO_3$ (which contains 3 μM CO_2 under equilibrium state at pH 8.30) at 30°C, the addition of CA decreased RuBPOase activity, while RuBPCase activity was increased by the same treatment (fig.1). Both effects intensified with increasing [CA] reaching a maximum at ~2.0 μg CA/vessel. As a consequence, the ratio of RuBPOase to RuBPCase activity decreased with the increase in [CA].

Fig.2(a) shows that the effects of CA on RuBPOase and RuBPCase are dependent on [CO_2]: The most pronounced effects were observed at the lowest [CO_2] (3 μM). The effects of CA decreased with the increase in [CO_2] and practically disappeared at 20 μM CO_2. Likewise, the decrease in the ratio of RuBPOase to RuBPCase activity disappeared at 20 μM CO_2 (fig.2(b)). In the absence of CA, K_m (CO_2) for RuBPCase was ~18 μM while the addition of CA decreased the K_m (CO_2) to ~7 μM (determined from double reciprocal plots of fig.2(a)). The [CO_2] and [O_2] in water in equilibrium with atmospheric air at 25°C are 10 and 254 μM, respectively. Therefore, CA should greatly

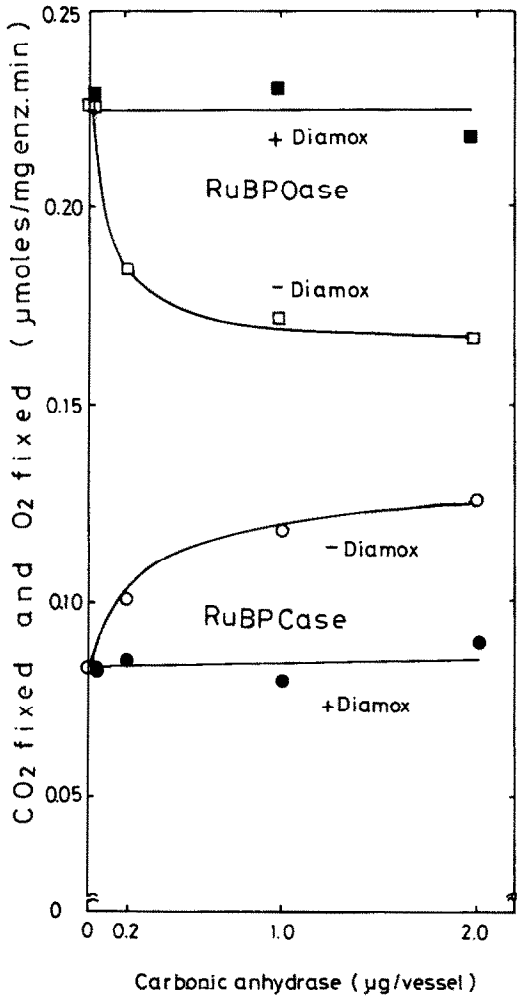

Fig.1. Effects of CA and Diamox on activities of RuBPOase and RuBPCase.

enhance RuBPCase and reduce RuBPOase reaction in the algal photosynthesis under atmospheric conditions and the presence or absence of CA in the algal chloroplasts should be very effective in regulating the photosynthetic carbon flow between the reductive pentose phosphate cycle and the glycolate pathway. It has been shown that glycolate excretion from algal cells was maximal when high-CO_2 cells were incubated at low [CO_2] [12,13] and the addition of Diamox to low-CO_2 cells of *Chlorella* increased the glycolate excretion to almost the same level as in high-CO_2 cells [13]. These findings support the present conclusion. The molecular weights of these sources of RuBPCase and CA have been reported as 557 000 [14] and

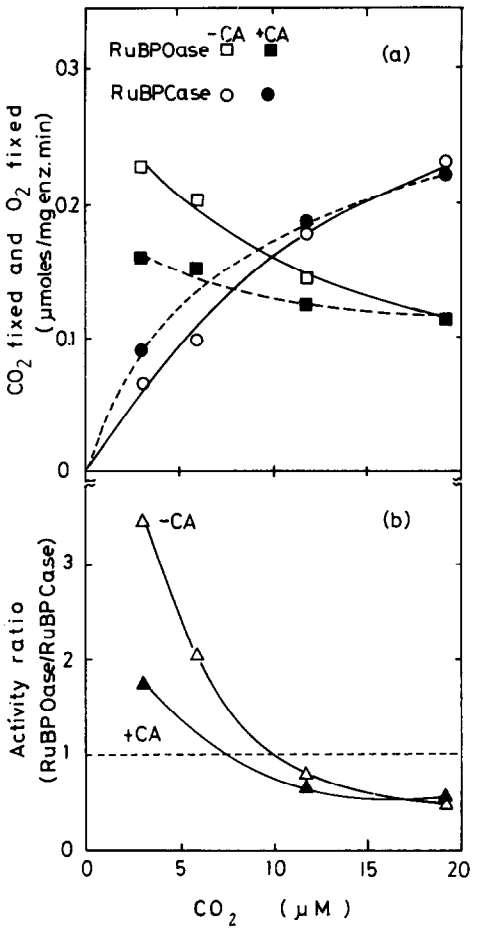

Fig.2. Effects of CA on activities of RuBPOase and RuBPCase in the presence of varied $[CO_2]$. The $[CO_2]$ was calculated from the $NaHCO_3$ added, assuming that CO_2 and HCO_3^- are in equilibrium.

31 000 [15], respectively. Therefore, from the data in fig.1, it is clear that a molar ratio of CA to RuBPCase/RuBPOase of ~1/7 (at 2 μg CA and 250 μg RuBPCase/RuBPOase) was already saturating for the CA-effects. This fact indicates that CA does not directly associate with RuBPCase/RuBPOase.

The determination of K_m (CO_2) for RuBPCase reaction has been generally carried out with $NaH^{14}CO_3$ as substrate. The substrate for RuBPCase is known to be free CO_2 [16]. As was done in this experiment, the free $[CO_2]$ in the medium was calculated with Henderson-Hasselbach equation [17] assuming that the equilibrium between CO_2 and HCO_3^- was established. These results indicate that, in the absence of CA, the rate of conversion of HCO_3^- to CO_2 is usually not fast enough to bring about the equilibrium during CO_2 fixation. As a result, the K_m (CO_2) obtained without CA is usually higher than the actual value. The detailed kinetic consideration of this matter will be published elsewhere.

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