Biochimica et Biophysica Acta 893 (1987) 219–224
Elsevier

BBA 42597

Inhibition of inorganic carbon transport by oxygen in a high CO_2-requiring mutant (E_1) of *Anacystis nidulans* R_2

Teruo Ogawa [a], Tatsuo Omata [a], Yehouda Marcus [b] and Aaron Kaplan [b]

[a] *Solar Energy Research Group, The Institute of Physical and Chemical Research (RIKEN), Wako (Japan) and* [b] *Department of Botany, The Hebrew University of Jerusalem, Jerusalem (Israel)*

(Received 26 August 1986)
(Revised manuscript received 13 May 1987)

Key words: Inorganic carbon transport; Carbon transport; Oxygen inhibition; Action spectrum; (*A. nidulans* mutant)

The effect of O_2 on inorganic carbon (C_i) transport was studied with a high CO_2-requiring mutant (E_1) of *Anacystis nidulans* R_2. Oxygen (above 2%) inhibited C_i transport by 15–35% at CO_2 concentrations above 200 μl/l, but had no apparent effect at low, limiting CO_2 concentration. The action spectra for C_i transport measured in the presence or absence of 20% O_2 showed two peaks around 684 and 625 nm, corresponding to chlorophyll *a* and phycocyanin absorption, respectively. The difference between these two spectra (anaerobic minus aerobic) showed one peak around 625 nm, which indicates that a linear electron transport from water to O_2 is involved in the O_2 inhibition of C_i transport. Dithiothreitol could overcome the inhibition by O_2. The results suggested that the O_2 inhibition is a result of inactivation of the C_i-transporting system.

Introduction

Cyanobacteria possess a CO_2-concentrating mechanism which involves active inorganic carbon (C_i) transport [1,2]. This transporting system is both activated and energized by light. The activation requires very low activity of PS II [3] and energization can occur by PS I [4]. Oxygen inhibits the activity of C_i transport [4]. The mechanism of the O_2 inhibition is not yet understood. Oxygen may draw electrons from ferredoxin, leading to the formation of free radicals and H_2O_2. The latter was shown to inactivate ferredoxin-dependent enzymes [5] as well as C_i transport [3]. Draining of electrons to O_2 will slow down the cyclic electron flow driven by PS I and hence the rate of ATP formation [6]. This may result in a suppression of the C_i transport.

In order to elucidate the mechanism of O_2 inhibition of C_i transport, we examined the effect of O_2 concentration on CO_2 uptake by the high CO_2-requiring mutant (E_1) isolated from *Anacystis nidulans* R_2 [7]. This mutant does not perform CO_2-dependent O_2 evolution at an atmospheric concentration of CO_2, although it has the ability to accumulate C_i internally. Thus, the mutant is unable to utilize the intracellular C_i pool for photosynthesis. When the suspension of the mutant cells is illuminated while bubbling with gas containing an atmospheric level of CO_2, there is an influx of CO_2 from the gas phase into the medium due to uptake of CO_2 by the cells from the medium, either as CO_2 or after being hydrated

Abbreviations: C_i, inorganic carbon; Chl, chlorophyll; PS, Photosystem; Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

Correspondence: T. Ogawa, Solar Energy Research Group, The Institute of Physical and Chemical Research (RIKEN), Wako-shi, Saitama 351-01, Japan.

to HCO_3^- [7,8]. Uptake of C_i by the mutant under these low CO_2 conditions is independent of photosynthetic CO_2 fixation and represents the activity of the C_i-transporting system. A portion of C_i taken up by the mutant is accumulated within the cells; the rest is extruded from the cells and partly stays in the medium as HCO_3^- producing nonequilibrium conditions between CO_2 and HCO_3^- in the medium, with the concentration of HCO_3^- being higher than that expected under equilibrium conditions [9]. It was inferred that the nonequilibrium conditions are produced as a result of CO_2 uptake and HCO_3^- efflux [9]. The mutant can adapt from high to low levels of CO_2 as exhibited by the increased ability to take up C_i and the large increase in the amount of plasmalemma located 42 kDa polypeptide [8]. We report here the effect of O_2 concentration on CO_2 uptake by low-CO_2-adapted E_1 cells under various CO_2 concentrations and light conditions and discuss possible mechanism of the O_2 inhibition on C_i transport.

Materials and Methods

Cells of E_1 were grown in BG 11 medium [10] supplemented with 10 mM Hepes-NaOH (pH 7.5) at 4% CO_2 in air, 34° C. Continuous illumination was provided by tungsten lamps (120 $\mu E/m^2$ per s, 400–700 nm). At a late logarithmic phase of growth the cultures were aerated with air for 10 h followed by 1–2 h of dark incubation under the same conditions. This period of dark incubation was required in order to obtain maximal capability of CO_2 uptake. The cells were then harvested (3000 × *g* for 5 min) and resuspended in 30 ml of 20 mM Hepes-NaOH (pH 7.0) at a chlorophyll concentration of 6–8 $\mu g/ml$ and then placed in a reaction vessel. Gas exchange measurements were carried out at 30° C using an open gas-analysis system previously described [9,11] which records the rate of CO_2 exchange directly. Unless otherwise stated, the CO_2 concentration in the gas led into the reaction vessel was 400 $\mu l/l$. The flow rate of the gas was kept at 1.0 l/min. The exchanged gas was dried and then measured with an infrared CO_2 analyzer (model ZAP; Fuji Electric CO., Tokyo).

Illumination was provided by a projector with 650-W halogen lamp (Procabin 667, Cabin Co., Tokyo). A water-bath (7 cm in thickness) was placed in front of the projector for heat absorption. Fig. 1 presents the transmission spectra of the filters used to obtain 684 and 625 nm monochromatic light and far-red light. Light energy was measured with a thermocouple (model E2, Kipp en Zonen, Delft, The Netherlands) or a quantum sensor (L1-190S, LiCor, Lincoln, NE).

Unlike previous studies, we did not determine the extent of the CO_2 burst, but rather the maximal rate of CO_2 uptake obtained (M, see Fig. 2) following illumination. The integrated (with time) rate of CO_2 uptake is equal to the sum of the intracellular C_i pool and the amount of HCO_3^- accumulated in the medium in excess of that expected under equilibrium conditions (presumably as a result of HCO_3^- efflux during C_i transport) [9]. The CO_2 burst technique does not enable kinetic studies of the response of initial rate of CO_2 uptake to the various treatments. On the other hand, M is affected by the time response of the system (due to the volume of the air between the cell suspension and the CO_2 detector), by exchange of CO_2 between the gas phase and the buffer (including rate of solubilization of CO_2) and by the activity of the cells. The first two

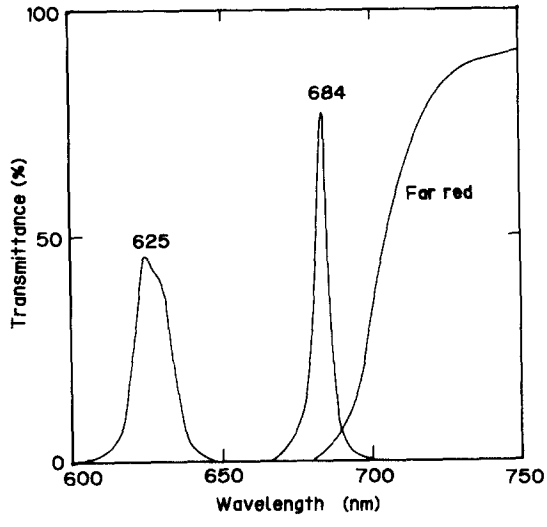

Fig. 1. The transmittion spectra of the 684 and 625 nm interference filters and the far-red filter used in the gas-exchange system.

factors will tend to slow the observed response, thus leading to an underestimation of the capacity of the cells to take up CO_2 upon illumination.

For ATP analysis, approx. 1 ml of cell suspension was drawn rapidly from the reaction vessel into a 1.5 ml syringe containing 125 μl of 70% perchloric acid. The extract was neutralized by adding 5 M KOH/1 M triethanolamine and ATP in the neutralized extract was determined by the firefly luciferase procedure as described in Ref. 12.

Results

Typical behavior of CO_2 uptake as a function of time in E_1 is shown in Fig. 2. The rate of CO_2 uptake increased upon illumination to a maximum and then slowly decreased to zero, due to filling up of the intracellular C_i pool and release of CO_2 from HCO_3^- accumulated in the medium. When the light was turned off, the C_i accumulated within the cells and in the medium during the light period was released into the gas phase as CO_2 burst and the rate of CO_2 evolution decreased to the initial dark respiration rate. Similar behavior was observed in *Anacystis* cells treated with iodoacetamide, which inhibited CO_2 fixation [4].

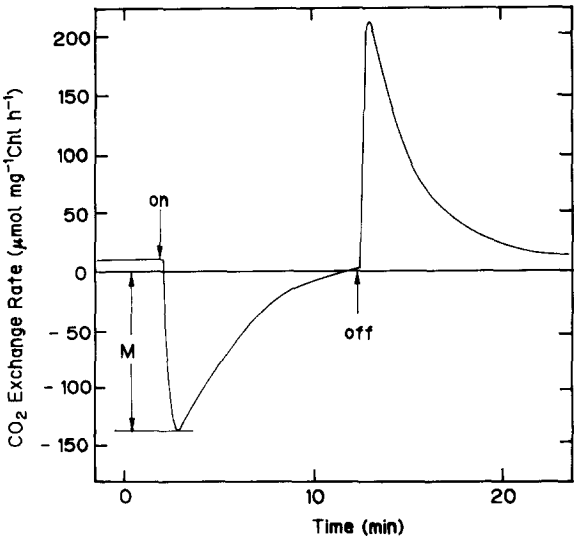

Fig. 2. Changes of CO_2 concentration in the gas phase of a cell suspension upon white light (26 mW/cm^2) on and off. The measurement was done under N_2 containing 20% O_2 and 400 μl CO_2/l. For details, see text.

The effect of O_2 concentration on the maximal rate of CO_2 uptake as affected by its concentration is shown in Fig. 3. At low, limiting CO_2 concentration, O_2 had no apparent effect on the rate of CO_2 uptake. At CO_2 concentrations higher than 200 μl/l, where the rate of CO_2 uptake was not limited by its concentration, O_2 inhibited CO_2 uptake (Fig. 3). There was some variations among the cultures in the extent of O_2 inhibition, which ranged between 15 and 35%. The effect of O_2 was already pronounced at 2% O_2.

Fig. 4 shows the effect of O_2 on the rate of CO_2 uptake measured as a function of light intensity. In these experiments cells were provided with 625 or 684 nm monochromatic light (see Fig. 1 for transmittance spectra of the filters used). When cells were illuminated with 684 nm light (lines A, B), there was hardly any effect of O_2 at low light intensity, whereas at high light intensity the maximal rate at 20% O_2 (line B) was only 70% that obtained under anaerobic conditions (line A). On the other hand, when cells were illuminated with 625 nm light (lines C, D), O_2 inhibition of CO_2 uptake was observed even at low light intensity.

The action spectra for CO_2 uptake by E_1 cells measured in the presence or absence of 20% O_2 are shown in Fig. 5, where the maximal rates of CO_2 uptake (M, see Fig. 2) normalized to a quantum flux of 10 μmol/m^2 per s are plotted as a function of wavelength. Both spectra show a peak

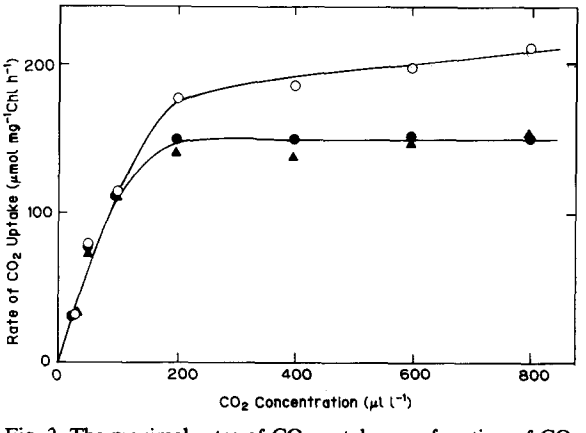

Fig. 3. The maximal rates of CO_2 uptake as a function of CO_2 concentration in the gas phase under different O_2 concentrations of 0 (○), 2 (●) and 20% (▲). The light conditions as in Fig. 2.

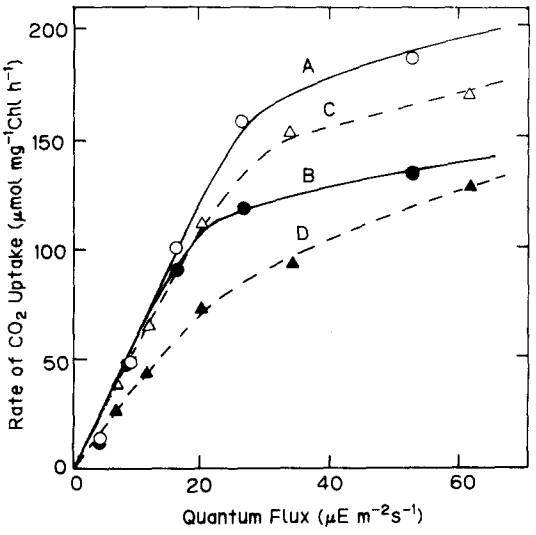

Fig. 4. The maximal rates of CO_2 uptake as a function of intensity of 684 or 625 nm monochromatic light under 0 or 20% O_2. (A), (B) 684 nm light; (C), (D) 625 nm light; (A), (C) 0% O_2; (B), (D) 20% O_2. The CO_2 concentration was 400 μl/l.

around 684 nm, corresponding to absorption by Chl *a*, with a broad band in the 625 nm region, corresponding to absorption by phycocyanin. These spectra are essentially the same as those reported previously for C_i accumulation in *Anabaena variabilis* [13] and for the generation of CO_2 burst in *A. nidulans* [4] and indicate that the energization of C_i transport occurs primarily by

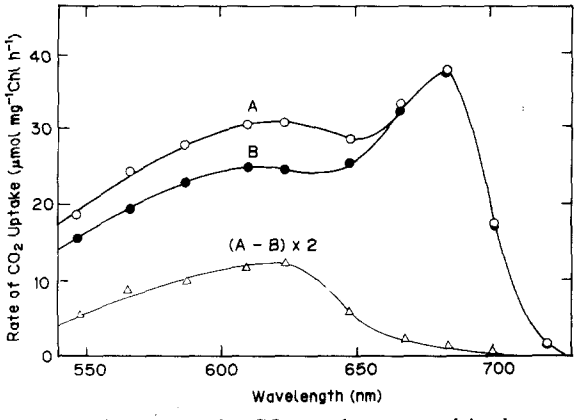

Fig. 5. Action spectra for CO_2 uptake measured in the presence (curve B) or absence (A) of 20% O_2. The maximal rates of CO_2 uptake were normalized to those at a quantum flux of 10 μE/m^2 per s. The incident fluxes of monochromatic light were 18.5–26.4 μE/m^2 per s. Curve (A – B) shows the difference between curves A and B.

TABLE I

THE ATP POOL SIZE OF E_1 CELLS BEFORE AND AFTER 1 MIN OF ILLUMINATION WITH THE FAR-RED LIGHT IN THE PRESENCE OR ABSENCE OF O_2 AND/OR 10 mM DITHIOTHREITOL

Conditions	ATP pool size (nmol/mg Chl)	
	dark	light
– Dithiothreitol		
0% O_2	91	195
20% O_2	97	177
+ Dithiothreitol		
0% O_2	53	143
20% O_2	68	252

PS I. The height of the phycocyanin peak in the action spectrum measured at 20% O_2 (curve B) was lower than that measured under anaerobic conditions (A), whereas the height of the Chl *a* peak was not affected by O_2 concentration. The difference between these two spectra (A–B in Fig. 5) shows a peak around 625 nm (PC) and was similar in shape to the action spectrum for O_2 evolution in *A. nidulans* [4]. These results suggest that a linear electron transport from water to O_2 via PS II and PS I is involved in the O_2 inhibition of C_i transport.

Dithiothreitol was shown to activate C_i transport even in the presence of DCMU [3]. When dark-adapted cells were exposed to the far-red light (see Fig. 1 for transmittance spectrum of the filter used) in the presence of 20% O_2, the rate of CO_2 uptake increased slowly to a maximal level (Fig. 6) due to slow activation of the system under the far-red light by the very low PS II light transmitted by the filter. In the presence of 20% O_2, the addition of dithiothreitol (10 mM) activated the system and led to a faster maximal rate. Further, dithiothreitol increased the maximal rate of CO_2 uptake to the level attained under anaerobic conditions. Thus, dithiothreitol could overcome the inhibition by O_2. The results suggested that the O_2 inhibition of C_i transport is a result of inactivation of the transporting system, which was released by dithiothreitol. In the absence of O_2, dithiothreitol strongly inhibited CO_2 uptake (Fig. 6). Table I presents the ATP pool size within the cells before and after 1 min of illumina-

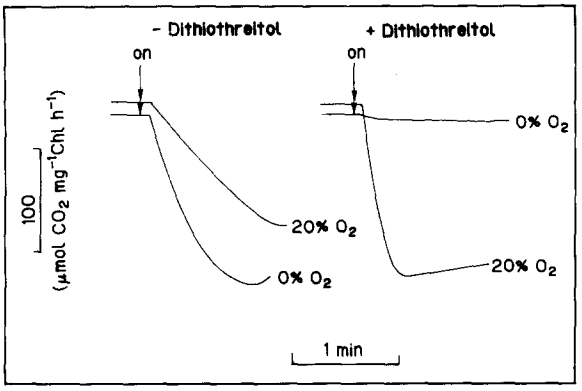

Fig. 6. The effect of dithiothreitol (10 mM) on CO_2 uptake in the presence or absence of 20% O_2. The cells were illuminated with the far-red light (6.5 mW/cm^2) under the gas containing 400 μl CO_2/l.

tion with the far-red light under the experimental conditions used to obtain the data in Fig. 6. The addition of dithiothreitol increased the ATP level in the light when O_2 was present, but decreased the level under anaerobic conditions. The presence of dithiothreitol under anaerobic conditions might cause overreduction of the electron transporting system, resulting in a suppression of ATP synthesis.

Discussion

The rate of C_i transport at any given set of conditions is affected by the activity of the transporting system and the amount of available energy. The activity of the system depends on the density of active sites in the membrane [1,14] and the state of activation of these sites [3]. The availability of light energy is a function of the intensity and spectra of the light. Energization of C_i uptake by light is most probably due to light-dependent ATP formation. Under anaerobic conditions, ATP must be produced by cyclic electron transport. The action spectrum for CO_2 uptake measured under anaerobic conditions (curve A in Fig. 5) clearly showed that energy derived from absorption by Chl *a* in PS I is used for CO_2 uptake. The action spectrum also showed a peak around 625 nm due to phycocyanin with the peak height being 80% of the Chl *a* peak at 684 nm. Thus, the light energy absorbed by phycocyanin is efficiently transferred to PS I.

It is presumed that, in the E_1 mutant in the presence of O_2, pseudocyclic electron transport also produces ATP to drive the C_i transport and the contribution of PS II reaction to the C_i transport becomes larger. However, the light energy absorbed by phycocyanin in PS II was rather inhibitory when O_2 was present (Figs. 4 and 5). Thus, it is clear that the linear electron transport from water to O_2 via PS II and PS I which produces free radicals and H_2O_2 is involved in the O_2 inhibition of C_i transport. We have shown that the C_i transport in E_1 cells was strongly inhibited by H_2O_2 [3]. These results suggested that the O_2 inhibition is a result of inactivation of the C_i-transporting system by free radicals of O_2 or H_2O_2 produced in the light.

Draining of electrons to O_2 may slow down the formation of ATP coupled to cyclic electron flow [6]. Under the 684 nm monochromatic light, which is mostly absorbed by PS I, O_2 had no effect on CO_2 uptake at low light intensity. Under high intensity of the 684 or far-red light, O_2 inhibited C_i transport (Figs. 4 and 6). Since dithiothreitol overcomes the inhibition by O_2, we may assume that the main effect of O_2 is inactivation of the C_i-transporting system by H_2O_2 and/or free radicals produced in the light. Probably, the production of these peroxide and free radicals will be too slow under low intensity of 684 nm light to inactivate the transporting system. However, at present we cannot resolve, based on the data presented here, the relative effect of O_2 on energization and on activation.

Acknowledgements

This study was supported by a grant for Solar Energy Conversion by Means of Photosynthesis from the Science and Technology Agency of Japan. A.K. wishes to thank RIKEN for support during these studies.

References

1 Kaplan, A., Badger, M.R. and Berry, J.A. (1980) Planta 149, 219–226
2 Miller, A.G. and Colman, B. (1980) J. Bacteriol. 143, 1253–1259
3 Kaplan, A., Zenvirth, D., Marcus, M., Omata, T. and Ogawa, T. (1987) Plant Physiol., in the press

4 Ogawa, T., Miyano, A. and Inoue, Y. (1985) Biochim. Biophys. Acta 808, 77–84
5 Heldt, H.W., Chon, C.J., Lilley, R.M. and Portis, A. (1979) in Proceedings of the 4th International Congress on Photosynthesis (Hall, D.O., Comb, J. and Goodwin, T.W., eds.), pp. 469–478, Biochemical Society, London
6 Heber, U., Egneus, H., Hanck, U., Jensen, M. and Koster, S. (1978) Planta 143, 41–49
7 Marcus, Y., Schwarz, R., Friedberg, D. and Kaplan, A. (1986) Plant Physiol. 82, 610–612
8 Omata, T., Ogawa, T., Marcus, Y., Friedberg, D. and Kaplan, A. (1987) Palnt Physiol. 83, 892–894
9 Ogawa, T. and Kaplan, A. (1987) Plant Physiol. 83, 888–891
10 Stanier, R.Y., Kunisawa, R., Mandel, M. and Cohen-Bazire, G. (1971) Bacteriol. Rev. 35, 171–205
11 Ogawa, T., Omata, T., Miyano, A. and Inoue, Y. (1985) in Inorganic Carbon Uptake by Aquatic Photosynthetic Organisms (Lucas, W.L. and Berry, J.A., eds.), pp. 287–304, American Society of Plant Physiologists, Rockville, MD.
12 Lilley, R.M., Stitt, M. and Heldth, H.W. (1982) Plant Physiol. 70, 965–970
13 Ogawa, T. and Ogren, W.L. (1985) Photochem. Photobiol. 41, 853–857
14 Omata, T. and Ogawa, T. (1986) Plant Physiol. 80, 525–530