ANION EFFECTS ON THE ELECTRON ACCEPTOR SIDE OF PHOTOSYSTEM II IN A TRANSFORMABLE CYANOBACTERIUM SYNECHOCYSTIS 6803

JIANCHENG CAO AND GOVINDJEE, Univ. of Illinois, 289 Morrill Hall, 505 South Goodwin Ave., Urbana, IL 61801 USA

Abstract. Treatment with formate (1), nitrite or azide of Synechocystis 6803 thylakoids caused a slowing down of the oxidation of Q_A^-, as calculated form Chl a fluorescence decay after saturating flashes. Addition of 2.5 to 5 mM HCO_3^- fully reversed this inhibition in formate- and nitrite-treated samples; however, in 100 mM azide-treated samples only 50% inhibition was reversed at 2 ms after the actinic flash. The anion treatment (bicarbonate depletion) affects the electron acceptor side of PSII between Q_A^- and the PQ pool. Hill reaction in bicarbonate-depleted Synechocystis cells was stimulated more than 4 fold by 5 mM bicarbonate. The pH range for the optimum stimulatory effect was around 6.7.

1. INTRODUCTION. HCO_3^- causes a significant and reversible stimulation of anion-inhibited electron flow in chloroplasts (2). A working model of two HCO_3^- sites on the electron acceptor side of Photosystem II (PSII) was proposed (3): (a) as a ligand to Fe^{2+} in Q_A-Fe-Q_B complex where Q_A and Q_B are bound plastoquinone molecules; it is assumed that HCO_3^-, through this binding, keeps the reaction center in its proper functional conformation ; and (b) as a participant in the protonation of Q_B^- and/or Q_B^{2-}. Arginine was proposed to be responsible for the binding of the latter site. Information on specific binding site(s) of HCO_3^- can be obtained through the molecular genetic approach. In higher plants, however, no transformable system exists that could enable native genes to be removed and replaced with modified genes. On the other hand, in transformable photosynthetic bacteria, no "bicarbonate effect" has been observed (4). We have studied the "bicarbonate effect" in a cyanobacterium, since they evolutionarily link the gap between photosynthetic bacteria and plants. They are prokaryotic, but are fundamentally similar to oxygenic chloroplasts. We chose Synechocystis 6803 because site-directed mutagenesis has been applied in this transformable cyanobacterium for pinpointing the structure/function relationships in the D1 and D2 proteins (5).

2. MATERIALS AND METHODS. Synechocystis 6803 was grown in BG-11 medium at 28°C under continuous illumination (70 µmole photons m^{-2} s^{-1}). Cells in logarithmic phase were used. The formate treatment (interpreted by us as bicarbonate depletion) medium contained 0.3 M sorbitol, 25 mM $NaHCO_2$, 10 mM NaCl, 5 mM $MgCl_2$, and 10 mM $Na_2H_2PO_4$ (pH 5.8). The sample was incubated for 4 h at 20 °C in this medium over which N_2 gas was passed. The reaction medium contained 0.1 M sorbitol, 20 mM $NaHCO_2$, 10

M. Baltscheffsky (ed.), Current Research in Photosynthesis, Vol. I, 515–518.
© 1990 *Kluwer Academic Publishers. Printed in the Netherlands.*

mM NaCl, 5 mM $MgCl_2$, 0.1 µM gramicidin D and 20 mM NaH_2PO_4 (pH 6.5) or 20 mM HEPES (pH 7.5). [Q_A^-] was calculated from variable Chl a fluorescence, as in (1). The kinetics of the decay of this fluorescence was measured at 685 nm (10nm bandwidth) by a weak measuring flash. This flash was fired at variable times after each actinic flash. O_2 evolution was determined polarographically using a YSI Clark-type electrode. We used dimethylbenzoquinone as an electron acceptor.

3. RESULTS AND DISCUSSION. Fig.1 shows Q_A^- decay in *Synechocystis* thylakoids at pH 6.5 (A) and pH 7.5 (B). At both the pH, 25 mM formate caused a large slowing down of Q_A^- decay. By adding 2.5 mM HCO_3^- the inhibition of Q_A^- oxidation was relieved and the decay curve fully restored. Similarly, in chloroplasts of higher plants, a very large slowing down of Q_A^- decay after 3 or more flashes has been observed (6). The Q_A^- decay is composed of, at least, three exponential components (J. Cao and Govindjee, 1989, in preparation). In our present analysis, however, the slowest component (1-2s) was ignored and the data up to several ms were analyzed into only two components. At pH 6.5, halftimes for those components were 400 ± 5 µs and 26 ± 1 ms; at pH 7.5, halftimes were 330 ± 3 µs and 20 ± 1 ms. Upon formate treatment, no significant change in these halftimes were found. However, the amplitude of the fast component decreased by about 70% (pH 6.5) or 50% (pH 7.5); this was accompanied by a concomittant increase in the slow component.

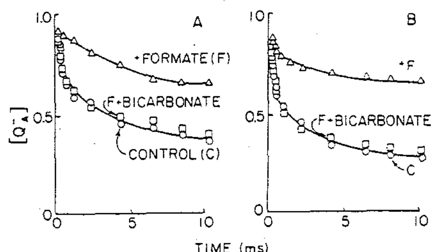

Figure 1. Decay of Q_A^- after the third flash, given at 1 Hz, in thylakoids of *Synechocystis* 6803 treated with 25 mM formate (▲) and recovered with 2.5 mM HCO_3^- (□) at pH 6.5 (A) and pH 7.5 (B). Controls (○) are also shown for comparison.

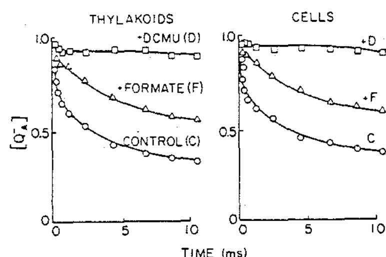

Figure 2. Decay of Q_A^- after the third actinic flash in thylakoids and cells of *Synechocystis* 6803 treated with formate (40 mM) (▲) and DCMU (5 µM) (□) at pH 6.5. Controls: ○ . The flash frequency was 1 Hz.

Fig.2 shows a replot of Q_A^- decay (1) in 5 µM DCMU and 40 mM formate-treated *Synechocystis* cells and thylakoids. DCMU is known to block reoxidation of Q_A^- by displacing Q_B from its binding site (7). Thus, a qualitatively similar inhibition of Q_A^- oxidation in DCMU-treated with those in formate-treated samples indicates that the inhibition of electron transport by bicarbonate depletion is between Q_A and the plastoquinone pool.

The inhibition of electron flow was also observed when, instead of formate (Fig. 3A), nitrite (Fig. 3B) or azide (Fig. 3C) anion was used. The inhibitory effect of nitrite was fully reversed by bicarbonate ions. However, addition of 5 mM HCO_3^- to 100 mM azide-treated samples only partially restored Q_A^- oxidation; at 2 ms, only 50% of the inhibition of Q_A^- oxidation was reversed.

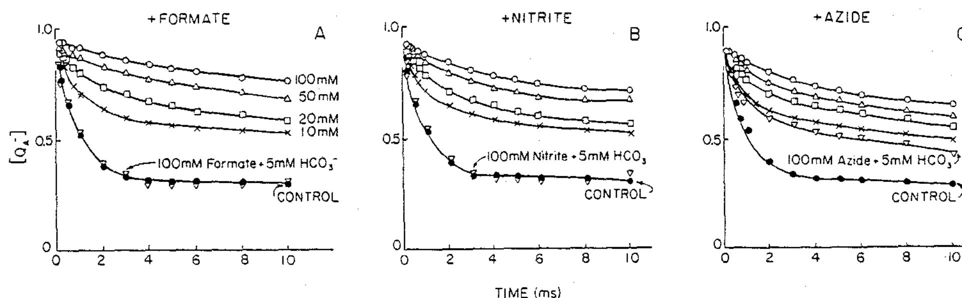

Figure 3. Recovery by 5 mM bicarbonate and the inhibitory effects of different concentrations (10-100 mM) of formate, nitrite and azide on Q_A^- decay in Synechocystis cells.

The stimulation of O_2 evolution by HCO_3^- addition in the HCO_2^--treated ($+HCO_2^-$) Synechocystis cells containing DMQ is shown in Fig.4. O_2 evolution rate in formate-treated cells was 40 µmol O_2 (mg Chla) $^{-1}h^{-1}$ (pH 6.5). By adding 10 mM HCO_3^- (pH adjusted to 6.5), it was stimulated to 260 µmol(mg Chla)$^{-1}h^{-1}$. In Synechocystis cells, HCO_3^- was also a source for carbon reduction since an O_2 evolution rate of 100 µmol(mg Chla)$^{-1}h^{-1}$ was observed in HCO_3^- recovered samples in the absence of DMQ. Thus, the net stimulation in electron transport from water to DMQ by HCO_3^- was 4 fold. In order to eliminate the effect of

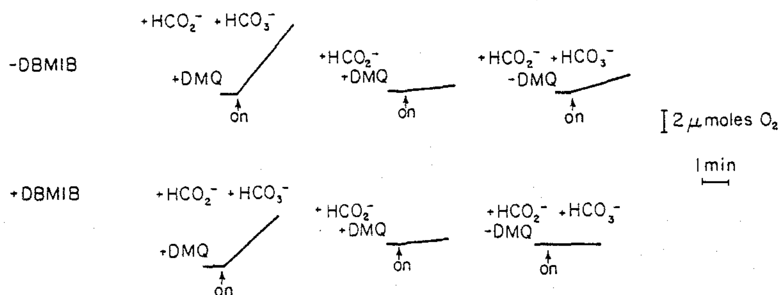

Figure 4. Effect of HCO_3^- on O_2 evolution in formate-treated Synechocystis cells. Cells containing 20 µg Chl a ml^{-1} were used. Reaction medium was adjusted to pH 6.5 after the addition of 5 mM HCO_3^-. A combination of two electron acceptors (DMQ, 0.5 mM; $K_3Fe(CN)_6$, 1 mM) was used. Twenty µM DBMIB was used to block electron flow beyond PQ pool, but before PS I.

CO_2 fixation, 20 μM DBMIB (8) was used. In the presence of both DBMIB and DMQ, addition of 10 mM HCO_3^- to the depleted sample also stimulated the electron transport rate by a factor of 4. A maximum HCO_3^- effect was found at approximately pH 6.7 (Fig. 5). This result is consistent with the conclusion that both CO_2 and HCO_3^-, not CO_2 or CO_3^- alone, may be the active species in the stimulation of Hill reaction. Blubaugh and Govindjee (9) have shown that HCO_3^- is the active species involved in spinach thylakoids.

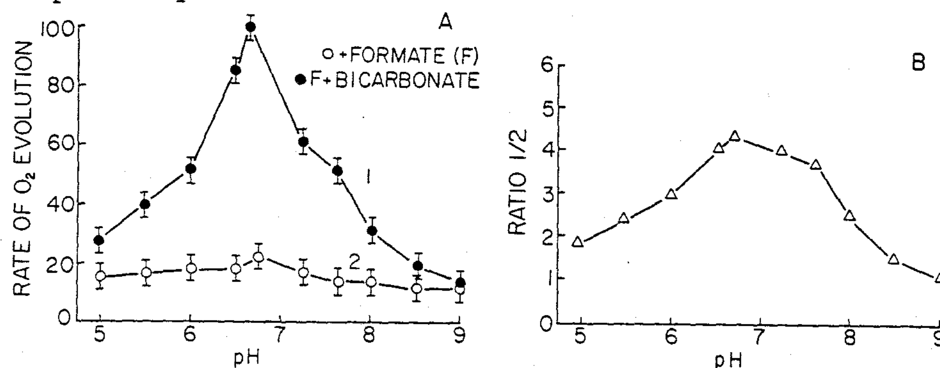

Figure 5. (A) Rate of oxygen evolution as a function of pH in the formate-treated (trace 1) and bicarbonate recovered (trace 2) cells. 100 arbitrary units = 150 μmol O_2(mgChl$\underline{a}$)$^{-1}$h^{-1}. (B) The ratio of O_2 evolution in HCO_3^--recovered cells and the formate-treated cells as a function of pH.

In **conclusion**, our data here and elsewhere (1) clearly show that a reversible "bicarbonate effect" exists in the transformable cyanobacterium Synechocystis 6803 and we believe that this applies to other cyanobacteria.

We thank the McKnight foundation for financial support.

References

1 Cao, J. and Govindjee (1988) Photosyn. Res.,19, 277-285
2 Govindjee and van Rensen, J.J.S. (1978) Biochim. Biophys. Acta 505, 183-213
3 Blubaugh, D.J. and Govindjee (1988) Photosyn. Res. 19, 85-128
4 Shopes, R.J., Blubaugh, D.J., Wraight, C. and Govindjee (1989) Biochim. Biophys Acta, 974, 114-118
5 Vermaas, W.F.J., Carpenter, S. and Bunch, C. (1989) in Photosynthesis: Molecular Biology and Bioenergetics (G. Singhal, J.Barber, R. Dilley, Govindjee, R. Haselkorn and P. Mohanty eds), Narosa Publishing House, New Delhi, pp. 21-35
6 Robinson, H.H., Eaton-Rye, J.J., van Rensen, J.J.S. and Govindjee (1984) Z. Naturforsch 30c, 225-274
7 Velthuys, B.R. (1981) FEBS Lett. 126, 277-281
8 Trebst, A. (1980) Methods in Enzymol. 69, 675-715
9 Blubaugh, D.J. and Govindjee (1986) Biochim. Biophys. Acta 848, 147-151