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

JTAN CHENG 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 QA, as calculated form Chl a fluorescence decay after saturating flashes. Addition of 2.5 to 5 mM HCO₃⁻ 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 QA 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. HCO₃⁻ causes a significant and reversible stimulation of anion-inhibited electron flow in chloroplasts (2). A working model of two HCO₃⁻ sites on the electron acceptor side of Photosystem II (PSII) was proposed (3): (a) as a ligand to Fe²⁺ in QA-Fe-QB complex where QA and QB are bound plastoquinone molecules; it is assumed that HCO₃⁻, through this binding, keeps the reaction center in its proper functional conformation; and (b) as a participant in the protonation of QB and/or QB²⁻. Arginine was proposed to be responsible for the binding of the latter site. Information on specific binding site(s) of HCO₃⁻ 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 NaHCO₃, 10 mM NaCl, 5 mM MgCl₂, and 10 mM Na₂HPO₄ (pH 5.8). The sample was incubated for 4 h at 20°C in this medium over which N₂ gas was passed. The reaction medium contained 0.1 M sorbitol, 20 mM NaHCO₃, 10
mM NaCl, 5 mM MgCl₂, 0.1 μM gramicidin D and 20 mM NaH₂PO₄ (pH 6.5) or 20 mM Hepes (pH 7.5). [QA⁻] was calculated from variable Chl a fluorescence, as in (1). The kinetics of the decay of this fluorescence was measured at 685 nm (10 nm bandwidth) by a weak measuring flash. This flash was fired at variable times after each actinic flash. O₂ evolution was determined polarographically using a YSI Clark-type electrode. We used dimethylbenzoquinone as an electron acceptor.

3. RESULTS AND DISCUSSION. Fig.1 shows QA⁻ 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 QA⁻ decay. By adding 2.5 mM HCO₃⁻ the inhibition of QA⁻ oxidation was relieved and the decay curve fully restored. Similarly, in chloroplasts of higher plants, a very large slowing down of QA⁻ decay after 3 or more flashes has been observed (6). The QA⁻ 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, half-times for those components were 400 ± 5 μs and 26 ± 1 ms; at pH 7.5, half-times were 330 ± 3 μs and 20 ± 1 ms. Upon formate treatment, no significant change in these half-times 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 concomitant increase in the slow component.

![Figure 1. Decay of QA⁻ after the third flash, given at 1 Hz, in thylakoids of Synechocystis 6803 treated with 25 mM formate (△) and recovered with 2.5 mM HCO₃⁻ (○) at pH 6.5 (A) and pH 7.5 (B). Controls (○) are also shown for comparison.](image1)

![Figure 2. Decay of QA⁻ 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.](image2)

Fig.2 shows a replot of QA⁻ decay (1) in 5 μM DCMU and 40 mM formate-treated Synechocystis cells and thylakoids. DCMU is known to block reoxidation of QA⁻ by displacing Qb from its binding site (7). Thus, a qualitatively similar inhibition of QA⁻ oxidation in DCMU-treated with those in formate-treated samples indicates that the inhibition of electron transport by bicarbonate depletion is between QA 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 HCO₃⁻ to 100 mM azide-treated samples only partially restored QA⁻ oxidation; at 2 ms, only 50% of the inhibition of QA⁻ oxidation was reversed.

![Graph showing recovery by 5 mM bicarbonate and the inhibitory effects of different concentrations (10-100 mM) of formate, nitrite and azide on QA⁻ decay in Synechocystis cells.](image)

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

The stimulation of O₂ evolution by HCO₃⁻ addition in the HCO₃⁻-treated (+HCO₃⁻) Synechocystis cells containing DMQ is shown in Fig. 4. O₂ evolution rate in formate-treated cells was 40 μmol O₂ (mg Chl)⁻¹ h⁻¹ (pH 6.5). By adding 10 mM HCO₃⁻ (pH adjusted to 6.5), it was stimulated to 260 μmol (mg Chl)⁻¹ h⁻¹. In Synechocystis cells, HCO₃⁻ was also a source for carbon reduction since an O₂ evolution rate of 100 μmol (mg Chl)⁻¹ h⁻¹ was observed in HCO₃⁻ recovered samples in the absence of DMQ. Thus, the net stimulation in electron transport from water to DMQ by HCO₃⁻ was 4 fold. In order to eliminate the effect of

![Diagram showing effect of HCO₃⁻ on O₂ evolution in formate-treated Synechocystis cells.](image)

Figure 4. Effect of HCO₃⁻ on O₂ 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 HCO₃⁻. A combination of two electron acceptors (DMQ, 0.5 mM; K₃Fe(CN)₆, 1 mM) was used. Twenty μM DBMIB was used to block electron flow beyond PQ pool, but before PS I.
CO₂ fixation, 20 µM DMIB (8) was used. In the presence of both DMIB and DMO, addition of 10 mM HCO₃⁻ to the depleted sample also stimulated the electron transport rate by a factor of 4. A maximum HCO₃⁻ effect was found at approximately pH 6.7 (Fig. 5). This result is consistent with the conclusion that both CO₂ and HCO₃⁻, not O₂ or CO₃⁻ alone, may be the active species in the stimulation of Hill reaction. Blubaugh and Govindjee (9) have shown that HCO₃⁻ is the active species involved in spinach thylakoids.

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 McKeen foundation for financial support.

References