Volume 226, number 2, 347-351 FEB 05463 January 1988

Involvement of bicarbonate in the protonation of the secondary quinone electron acceptor of photosystem II via the non-haem iron of the quinone-iron acceptor complex

Jack J.S. van Rensen, Willem J.M. Tonk and Steef M. de Bruijn

Laboratory of Plant Physiological Research, Agricultural University Wageningen, Gen. Foulkesweg 72, 6703 BW Wageningen, The Netherlands

Received 1 October 1987; revised version received 5 November 1987

The mechanism of the bicarbonate effect was investigated by monitoring flash-induced pH changes. In control chloroplasts the proton yields exhibit a binary oscillation with a period of four. In CO_2-depleted chloroplasts the binary oscillation disappears and only the period four pattern remains, which can be described by proton liberation in the water-oxidizing system. It is concluded that bicarbonate is involved in the protonation of Q_B^{2-}. The affinity of bicarbonate to its binding site is much lower in the presence of dithionite. It is suggested that bicarbonate exerts its influence through being a ligand for the non-haem iron between Q_A and Q_B.

Electron transport; Bicarbonate effect; Quinone protonation; Quinone-iron complex; (Pea chloroplast)

1. INTRODUCTION

Electron flow driven by PS II is affected by monovalent anions [1,2]. The rate of electron transport can be inhibited by several monovalent anions of which formate is the most active. In isolated broken chloroplasts which have been inhibited by monovalent anions, addition of bicarbonate produces a large stimulation of the Hill reaction rate. Formate has been shown to be a competitive inhibitor of the bicarbonate stimulation of electron flow [3]. It is suggested that bicarbonate, and not carbon dioxide, is the species required for the stimulation [4]. The antagonistic action of formate and bicarbonate on electron flow is termed the bicarbonate effect. Details of the bicarbonate effect can be found in several reviews [5–7].

The site of the bicarbonate effect has been a matter of debate for a long time. While Stemler [8] has advocated localization at the water-splitting side of PS II, most evidence indicates that the major effect is located at the reducing side of PS II [5–7]. Formate and bicarbonate have an antagonistic action on electron flow from Q_A to the PQ pool. Vermaas and Rutherford [9] showed that formate and bicarbonate affect the Q_A^--Fe^{2+} acceptor complex of photosystem II.

Whereas the site of the bicarbonate effect has been established, nothing is known about its mechanism of action. It has been suggested several times that formate and bicarbonate interfere with protonation reactions near Q_B [2,3,5,9–11].

Correspondence address: J.J.S. van Rensen, Laboratory of Plant Physiological Research, Agricultural University Wageningen, Gen. Foulkesweg 72, 6703 BW Wageningen, The Netherlands

Abbreviations: Chl, chlorophyll; FeCy, ferricyanide; PS, photosystem; PQ, plastoquinone; Q_A/Q_B, primary/secondary quinone electron acceptor of PS II; Q-400, redox group at acceptor side of PS II (high-spin Fe^{2+})

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

However, evidence for such an action is very scarce. Here, we describe the effects of formate and bicarbonate on flash-induced proton translocation and those of oxidation and reduction of the quinone-iron complex on the time course of CO_2 depletion of chloroplasts. Our results indicate that the bicarbonate effect is related to the protonation of Q_B^{2-} via the quinone-iron complex.

A preliminary report on the proton translocations has been presented earlier [2].

2. MATERIALS AND METHODS

Broken pea chloroplasts were isolated as in [12]. Thylakoids were depleted of C_2 [3]. The depletion medium contained 0.3 M sorbitol, 25 mM sodium formate, 20 mM sodium phosphate (pH 5.8), 10 mM NaCl and 5 mM $MgCl_2$. For measurement of the rate of electron flow a sample of 200 μl chloroplasts was transferred to 2 ml of the reaction medium. This had the same composition as the depletion medium, except sodium phosphate was at 50 mM (pH 6.5); Chl concentration was 25 μg/ml. The Hill reaction was measured as O_2 evolution with 0.5 mM FeCy as electron acceptor and 5 mM NH_4Cl as uncoupler at 25°C as reported [13]. For determination of the Hill reaction rate, we used the initial slope of the electrode response. This was of particular importance under the CO_2-free condition, since in this case the rate levels off very rapidly (fig.1).

Flash-induced proton translocation was measured using a pH electrode assembly as described by Fowler and Kok [14]. The reaction medium contained 0.4 M sorbitol and 50 mM NaCl, the volume being 1 ml and the final Chl concentration 25 μg/ml. Measurements were performed in the presence of uncoupler (30 mM methylamine or 5 μM gramicidin D) and 1 mM FeCy or 100 μM methyl viologen as electron acceptor. In the presence of FeCy, on average one proton is released per flash (figs 2–4). In the presence of methyl viologen the oscillations are the same, but the steady-state translocations average at zero. In the control experiment (fig.2), a 12.5 μl sample was taken from a chloroplast suspension which contained 20 mM Tricine-NaOH (pH 7.8). CO_2-depleted chloroplasts were suspended in a CO_2-free medium of 0.3 M sorbitol, 50 mM sodium formate, 10 mM NaCl and 20 mM Tricine-NaOH (pH 7.8) before introducing a 50 μl sample into the pH electrode chamber (fig.3). In the experiment of fig.4, CO_2-depleted chloroplasts were suspended in a medium containing 0.3 M sorbitol, 50 mM sodium formate, 10 mM $NaHCO_3$ and 20 mM Tricine-NaOH (pH 7.8) before introducing the 50 μl sample into the pH electrode. Chloroplasts were incubated in the dark for 10 min before commencing illumination with short saturating flashes at a frequency of 0.33 Hz.

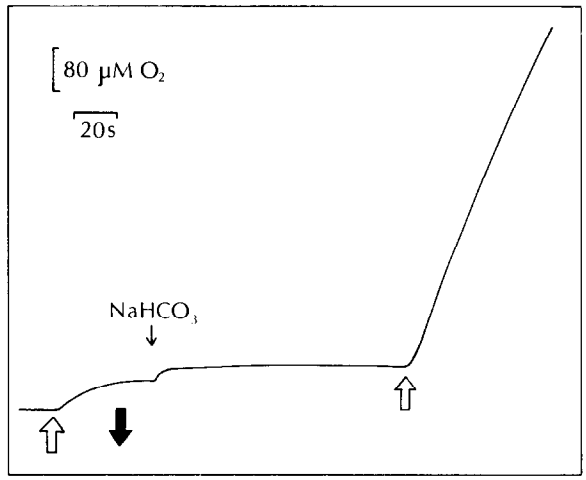

Fig.1. Recording of the Hill reaction of chloroplasts deplted of CO_2 over 30 min, before and after addition of 10 mM $NaHCO_3$. Arrow pointing upwards, light on; arrow downwards, light off.

The amplitude of the pH changes is influenced by the quality of the pH-glass membrane and the buffering capacity of the reaction medium. The glass membrane is very fragile and must be exchanged regularly. The buffering capacity of the reaction medium is determined by the amount and composition of the sample of chloroplasts which is introduced into the reaction chamber of the pH electrode assembly. For this reason the amplitudes in figs 2–4 are difficult to compare. In this paper only the oscillation patterns will be discussed.

3. RESULTS AND DISCUSSION

Fig.2 shows pH changes induced by short saturating flashes in control chloroplasts. The oscillations are the same as those reported by Fowler [15]. The proton flash yields display binary oscillation with a period of 4. The yields at the

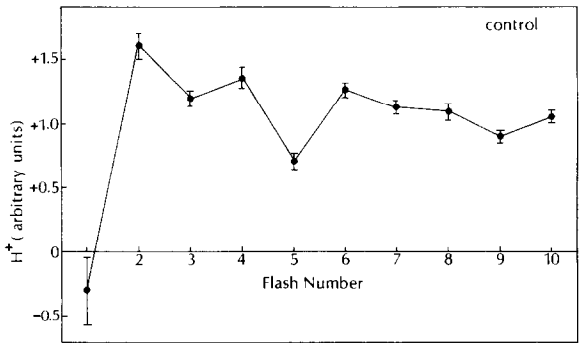

Fig.2. Flash-induced pH changes in control chloroplasts; average of 12 experiments.

even flashes are high, those at the uneven flashes are lower and there is a damping similar to that with oxygen flash yield. The proton translocations at the cytochrome *b/f* complex are the same for each turnover of the Q cycle [16]. Therefore, the oscillations must be due to a combination of proton liberation in the water-splitting reaction and the protonation of Q_B^{2-}.

The oscillation pattern of proton release by the water-oxidizing enzyme system can be described by a 1,0,1,2, stoichiometry for the redox transitions $S_i \longrightarrow S_{i+1}$ ($i = 0$–3) [17]. After 10 min in the dark most of the reaction centres are in the S_1 state. It is clear that the oscillations in fig.2 do not follow proton liberations at the water-splitting site only. In order to explain the binary oscillations, and especially the dip at the third flash, we must conclude that there is proton uptake at Q_B^{2-} at odd flashes.

The high-spin Fe^{2+} located between Q_A and Q_B [18], which was recently shown to be identical with the component Q-400 [19], may be involved in proton uptake [20,21]. Recently, Renger (personal communication) proposed a reaction sequence based on reductant-induced oxidation of Fe^{2+} to Fe^{3+} [22,23]. This mechanism implies that negatively charged Q_A^- reduces the redox potential of the Fe^{2+}/Fe^{3+} couple so that bound quinone is able to oxidize the Fe^{2+}. The reaction sequence proposed by Renger may lead from $Q_AFe^{2+}Q_B$ after dark incubation, to $Q_AFe^{3+}Q_B^{2-}$ after the first and further odd flashes. This leads to proton uptake by Q_B^{2-} at uneven flashes as observed in fig.2. After protonation of Q_B^{2-} and exchange with the plastoquinone pool, $Q_AFe^{3+}Q_B$ results. The second and further even flashes reduce Q_A, which forwards its electron to the Fe, so that after these flashes the complex is in the state $Q_AFe^{2+}Q_B$. A comparable series of reactions was recently proposed by Petrouleas and Diner [23].

In CO_2-depleted chloroplasts the binary oscillations are absent (fig.3). The pattern can now be better described by a period 4 oscillation following the 1,0,1,2 stoichiometry of proton liberations at the water-splitting site. The restoration of the binary oscillations by addition of bicarbonate to CO_2-depleted chloroplasts (fig.4) shows that the observation in fig.3 is indeed due to the absence of CO_2. We conclude that the absence of CO_2 prevents protonations at Q_B^{2-}.

The stimulation of the Hill reaction of CO_2-depleted chloroplasts used in the experiment of fig.3 by bicarbonate was about 3-fold. This means that about 30% of the reaction centres remained active. In CO_2-depleted chloroplasts electron flow through the Q_B site remains possible and only becomes slower as was demonstrated by Robinson et al. [24]: the overall half-time for the oxidation of Q_A^- after one flash was 1.2 ms and became 13 ms after the third of three flashes. In flash experiments with sufficiently long dark times between flashes, electron flow can proceed although at a slower rate. In our experiments the dark time between flashes was 3 s. Thus, electron flow at the Q_B site remained possible, as did water splitting. The proton release pattern of the water-splitting reaction should not be influenced greatly during the 10 flashes of our experiment. However, due to the absence of CO_2, electron flow appears to be unaccompanied by proton uptake at the Q_B site.

In addition to four histidines, glutamic acid is a ligand for the non-haem iron between Q_A and Q_B in the reaction center of *R. viridis*. Glutamic acid is not present as such as a ligand in the reaction centre proteins of PS II. It was recently proposed that in PS II bicarbonate should be a ligand for the non-haem iron (Michel, H., personal communication). We suggest that bicarbonate may be the protonatable group associated with the quinone-iron complex [20,21] and is involved in the protonation of Q_B^{2-} via the non-haem iron. This protonation may occur via the protein matrix.

If bicarbonate is a ligand for this non-haem iron, the affinity of bicarbonate for its binding site

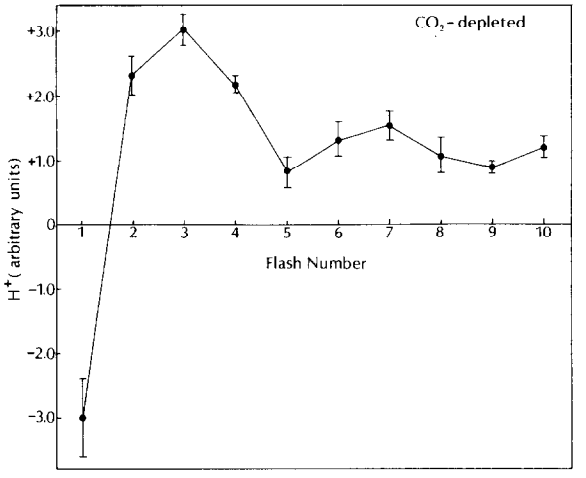

Fig.3. Flash-induced pH changes in CO_2-depleted chloroplasts; average of 4 experiments.

may depend on the redox state of the quinone-iron complex. We studied the kinetics of CO_2 depletion, while the quinone-iron complex was either oxidized or reduced. Chemical oxidation was achieved by adding 2 mM FeCy [19], reduction by adding 2 mM dithionite. It is highly probable that under the latter conditions (darkness, anaerobiosis, pH 5.8, 2 mM dithionite) the quinone-iron complex is reduced. Fig.5 shows that the half-time of CO_2 depletion was about 15 min when FeCy was added to the reaction medium. When the quinone-iron complex was reduced by addition of dithionite, the half-time was reduced to about 5 min (fig.6). It appears that the affinity of bicarbonate to its binding site is much lower when the quinone-iron complex is in the reduced state. This finding explains several

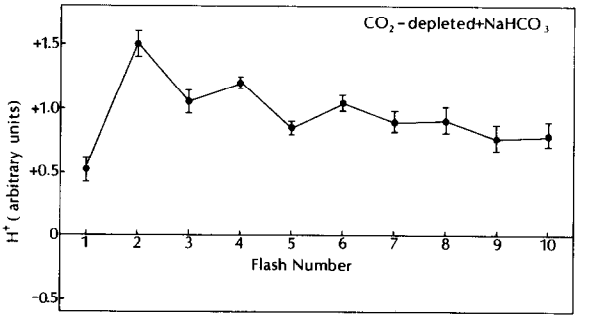

Fig.4. Flash-induced pH changes in CO_2-depleted chloroplasts to which 10 mM bicarbonate was added; average of 4 experiments.

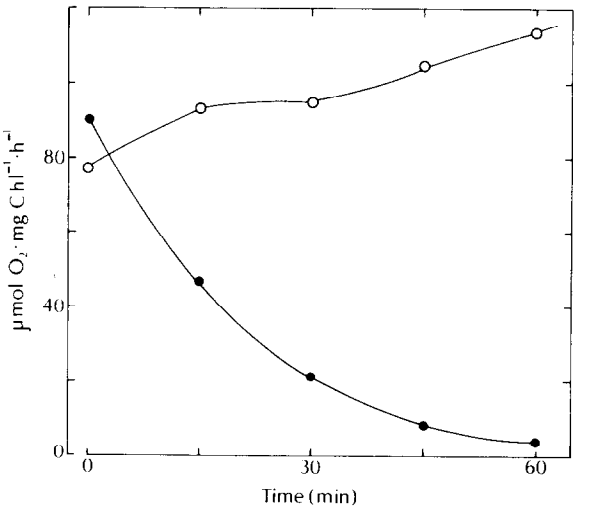

Fig.5. Rate of the Hill reaction after various duration times of CO_2 depletion while 2 mM FeCy was added to the depletion medium. At each time a sample was measured before (●) and after addition of 10 mM bicarbonate (○). Average of 3 experiments.

earlier observations of the antagonistic action of formate and bicarbonate on electron flow, of which only a few can be mentioned here. When chloroplasts are illuminated in the presence of formate, the Hill reaction rate decreases very rapidly (fig.1 and [25,26]). Reactivation of CO_2-depleted chloroplasts by bicarbonate requires a dark in-

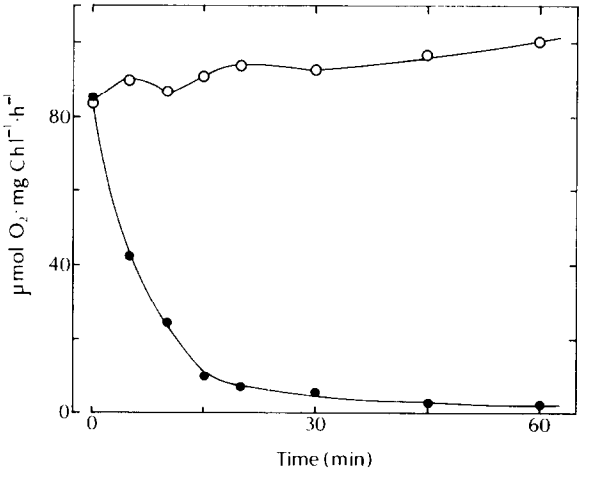

Fig.6. Same experiment as in fig.5, however instead of FeCy, 2 mM sodium dithionite was added to the depletion medium. Average of 3 experiments.

cubation time (fig.1 and [27]). Light decreases the binding constant for bicarbonate in the presence of formate [28]. These results can now be explained by the redox state of the quinone-iron complex, it being more reduced in the light than in the dark. In addition, it may be mentioned that a bicarbonate effect has never been found in photosynthetic bacteria. Possibly glutamic acid has the same function in photosynthetic bacteria as bicarbonate in the higher plants.

Our data can be interpreted as indicating that bicarbonate is a ligand for the non-haem iron between Q_A and Q_B; it is involved in the protonation of Q_B^{2-}. Formate is an inhibitor of bicarbonate binding and prevents the protonation. While CO_2 and H_2O may be diffusing species, bicarbonate may bind to the iron. $CO_2/HCO_3^-/CO_3^{2-}$ can serve as a proton shuttle, since the pK_a of (CO_2 + H_2O) is 6.4 at 25°C. Formate is not able to function in such a way, because its pK_a is 3.8.

REFERENCES

[1] Stemler, A. and Jursinic, P. (1983) Arch. Biochem. Biophys. 221, 227–237.

[2] Govindjee and Eaton-Rye, J.J. (1986) Photosynthesis Res. 10, 365–379.

[3] Snel, J.F.H. and Van Rensen, J.J.S. (1984) Plant Physiol. 75, 146–150.

[4] Blubaugh, D.J. and Govindjee (1986) Biochim. Biophys. Acta 848, 147–151.

[5] Govindjee and Van Rensen, J.J.S. (1978) Biochim. Biophys. Acta 505, 183–213.

[6] Vermaas, W.F.J. and Govindjee (1981) Proc. Indian Natl. Sci. Acad. B47, 581–605.

[7] Van Rensen, J.J.S. and Snel, J.F.H. (1985) Photosynthesis Res. 6, 231–246.

[8] Stemler, A. (1982) in: Photosynthesis (Govindjee, ed.) vol.II, pp.513–539, Academic Press, New York.

[9] Vermaas, W.F.J. and Rutherford, A.W. (1984) FEBS Lett. 175, 243–248.

[10] Hope, A.B. and Matthews, D.B. (1983) Aust. J. Plant Physiol. 10, 363–372.

[11] Vermaas, W.F.J. (1984) PhD Thesis, Agricultural University, Wageningen, The Netherlands.

[12] Van Rensen, J.J.S., Wong, D. and Govindjee (1978) Z. Naturforsch. 33c, 413–420.

[13] Van Rensen, J.J.S., Van der Vet, W. and Van Vliet, W.P.A. (1977) Photochem. Photobiol. 25, 579–583.

[14] Fowler, C.F. and Kok, B. (1976) Biochim. Biophys. Acta 423, 510–523.

[15] Fowler, C.F. (1977) Biochim. Biophys. Acta 459, 351–363.

[16] Ort, D.R. (1986) in: Encyclopedia of Plant Physiology (Staehelin, L.A. and Arntzen, C.J. eds) New Series vol.19, Photosynthesis 3, pp.143–196, Springer, New York.

[17] Förster, V. and Junge, W. (1985) Photochem. Photobiol. 41, 183–190.

[18] Petrouleas, V. and Diner, B.A. (1982) FEBS Lett. 147, 111–114.

[19] Petrouleas, V. and Diner, B.A. (1986) Biochim. Biophys. Acta 849, 264–275.

[20] Rutherford, A.W. and Zimmerman, J.-L. (1984) Biochim. Biophys. Acta 767, 168–175.

[21] Renger, G., Wacker, U. and Völker, M. (1987) Photosynthesis Res. 13, 167–189.

[22] Zimmermann, J.-L. and Rutherford, A.W. (1986) Biochim. Biophys. Acta 851, 416–423.

[23] Petrouleas, V. and Diner, B.A. (1987) Biochim. Biophys. Acta 893, 126–137.

[24] Robinson, H.H., Eaton-Rye, J.J., Van Rensen, J.J.S. and Govindjee (1984) Z. Naturforsch. 39c, 382–385.

[25] Stemler, A. (1979) Biochim. Biophys. Acta 545, 36–45.

[26] Snel, J.F.H., Naber, J.D. and Van Rensen, J.J.S. (1984) Z. Naturforsch. 39c, 386–388.

[27] Snel, J.F.H. and Van Rensen, J.J.S. (1983) Physiol. Plant. 57, 422–427.

[28] Stemler, A. and Murphy, J. (1983) Photochem. Photobiol. 38, 701–707.