BBA Report

INHIBITION OF THE REOXIDATION OF THE SECONDARY ELECTRON ACCEPTOR OF PHOTOSYSTEM II BY BICARBONATE DEPLETION

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Summary

In bicarbonate-depleted chloroplasts, the chlorophyll a fluorescence decayed with a halftime of about 150 ms after the third flash, and appreciably faster after the first and second flash of a series of flashes given after a dark period. After the fourth to twentieth flashes, the decay was also slow. After addition of bicarbonate, the decay was fast after all the flashes of the sequence This indicates that the bicarbonate depletion inhibits the reoxidation of the secondary acceptor R^{2-} by the plastoquinone pool; R is the secondary electron acceptor of pigment system II, as it accepts electrons from the reduced form of the primary electron acceptor (Q^-). This conclusion is consistent with the measurements of the DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea)-induced chlorophyll a fluorescence after a series of flashes in the presence and the absence of bicarbonate, if it is assumed that DCMU not only causes reduction of Q if added in the state QR^-, but also if added in the state QR^{2-}.

Recent experiments have shown that the site of inhibition of the Hill-reaction by bicarbonate depletion [1] is at the reducing side of Photosystem II [2, 3]. A two-fold slowing down of the Hill reaction due to bicarbonate-depletion at low light intensities and in light flashes was explained [3, 4] by a reversible inactivation of half the reaction centers of Photosystem II. In addition, Jursinic et al. [3] demonstrated that in the absence of bicarbonate the reoxidation time of the reduced primary acceptor, Q^-, as revealed by the dark decay of the chlorophyll a fluorescence yield after a short saturating flash [5],

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was increased from the normal 0.6 ms to 2.6 ms. Since in normal chloroplasts the rate-limiting step has a halftime of about 20 ms, this result could not explain the 5—10 times decreased rate of the Hill reaction in saturating light [4]. To explain a 5—10-fold decrease in the maximal rate of the Hill reaction in continuous light, one has to assume that one or more reactions are slowed down to 100—200 ms and now form the rate-limiting step [5].

We report experiments concerning the effect of bicarbonate-depletion on the electron transport from the primary acceptor, Q, to the plastoquinone pool, via the secondary electron acceptor, R [6—8]; R must accumulate two negative charges before it can be reoxidized by the plastoquinone pool. Our results show that the reoxidation of R^{2-} by the plastoquinone pool is drastically slowed down, thus providing an explanation of the effect of bicarbonate-depletion on the Hill reaction in saturating light.

Chloroplasts were isolated from market spinach (*Spinacea oleracea*) in phosphate buffer (sodium phosphate, 50 mM; NaCl, 200 mM, pH 8.0). They were given an osmotic shock by suspending them in a buffer containing 50 mM sodium phosphate and 10 mM NaCl at pH 8.0. Bicarbonate (HCO_3^-) was removed by suspending a concentrated sample of chloroplast fragments in a buffer containing 50 mM phosphate, 100 mM NaCl and 100 mM sodium formate at pH 5.0 at room temperature; nitrogen gas was passed over the suspension while it was shaken gently by hand for about 10—12 min. Then, the suspension was transferred, by a glass syringe, into closed test tubes previously flushed with nitrogen gas. After centrifugation, these tubes were stored on ice until use. Just before the assay, the supernatant was discarded and the chloroplast fragments were resuspended in a buffer containing 50 mM phosphate, 100 mM NaCl and 100 mM formate at pH 6.8. Addition of 10—100 mM sodium bicarbonate provided the plus bicarbonate samples. Where indicated, 20 mM ferricyanide was added as a Hill oxidant.

Chlorophyll *a* fluorescence was excited by a weak measuring beam (480 nm). Short (3 μs at half height) saturating flashes passed through a filter
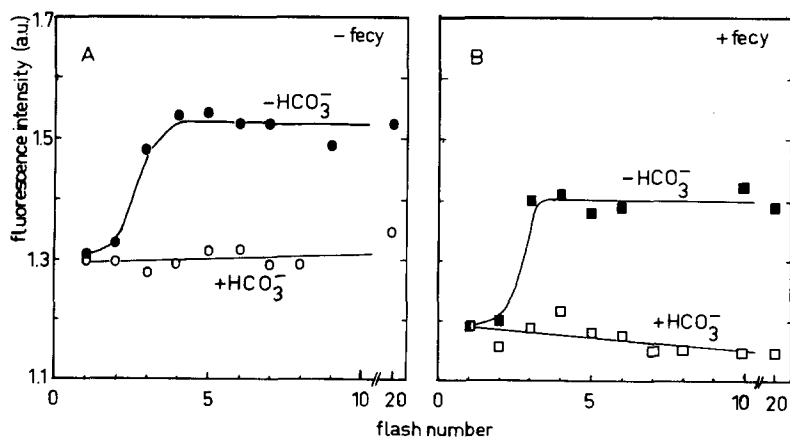

Fig. 1. Fluorescence intensity 160 ms after the last of a series of 3 μs saturating flashes, spaced at 30 ms, as a function of the number of flashes. Addition as indicated. Concentrations: bicarbonate, 20 mM. Chlorophyll, 20 μg·ml^{-1} of spinach chloroplast suspension. Ferricyanide (fecy), 20 mM.
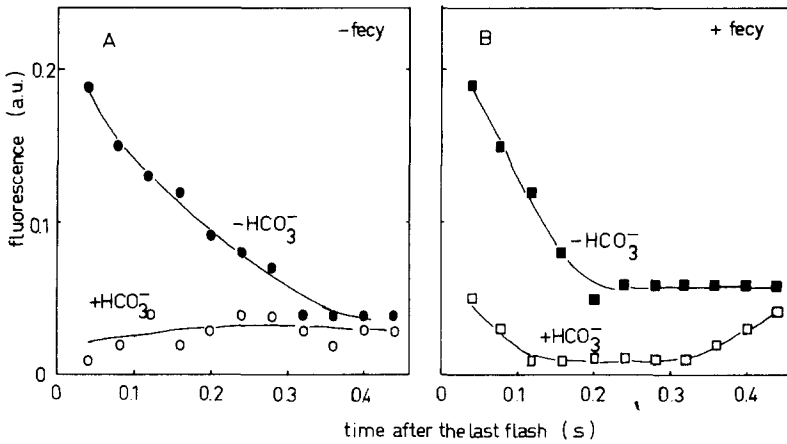
Fig. 2. The fluorescence intensity after the third flash minus that after the second flash as a function of time. Additions as indicated; see legend of Fig. 1.

transmitting wavelengths above 645 nm. Fluorescence was detected in a wavelength region around 680 nm with 20 nm halfwidth. During actinic illumination, the photomultiplier was shielded by a mechanical shutter and measurements started a few ms after cessation of the illumination.

Fig. 1 shows the fluorescence intensity 160 ms after a series of short saturating flashes spaced at 30 ms, as a function of the number of flashes. The fluorescence decay is slowed down from the third flash onwards in bicarbonate-depleted chloroplasts, both in the absence and the presence of a Hill acceptor (ferricyanide). Fig. 2 shows the kinetics of the slow decay, plotted as the difference between the decay after the third and the second flash. The half time of this decay is in the 120—160 ms range. This slow decay can explain the 5—10 times decreased rate of the Hill reaction in saturating continuous
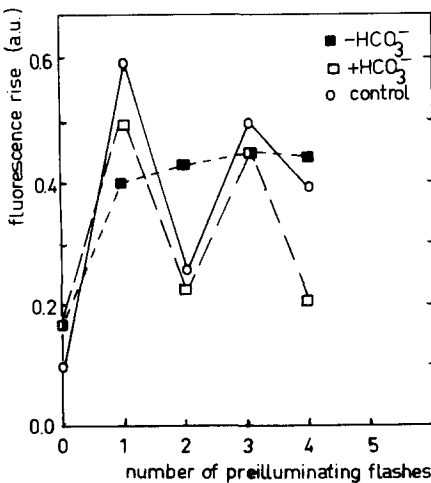

Fig. 3. DCMU-induced fluorescence increase as a function of the number of preilluminating flashes. Additions as indicated. Concentrations: DCMU, 5 μM; hydroxylamine, 1 mM; bicarbonate, 20 mM. Chlorophyll, 20 μg·ml^{-1} of spinach chloroplast suspension.

light. Since the slowly decaying state is reached after three turnovers, we suggest that in the absence of bicarbonate the reaction centers are blocked in the state Q^-R^{2-}. After the first and second flash, Q^- is reoxidized by R (halftime 2.6 ms, 3) or R^- respectively in the time between the last flash and the measurement. Since the presence or the absence of ferricyanide (see Figs. 1 and 2) did not greatly modify the kinetics of the decay, the slow reoxidation of R^{2-} reflects mainly electron transfer to the plastoquinone pool rather than to ferricyanide directly.

Velthuys and Amesz [7] have shown that the redox state of R can be monitored by measuring the fluorescence rise induced by the addition of DCMU. DCMU causes a shift of the equilibrium $QR^- \rightleftarrows Q^-R$ towards Q^-R resulting in a fluorescence increase dependent upon the concentration of R^- before the addition. The presence of an artificial electron donor is required to prevent reoxidation of Q^- by oxidized components on the donor side of the reaction center.

Fig. 3 shows the DCMU-induced chlorophyll a fluorescence rise both in the presence and absence of bicarbonate and in control chloroplasts as a function of the number of flashes given before the addition; 1 mM hydroxylamine was used as an artificial electron donor. In bicarbonate-depleted chloroplasts, the normal periodicity of two [7] disappeared; instead, one observes a rather large fluorescence increase after preilluminating flashes, irrespective of their number. Addition of 20 mM bicarbonate restored the normal flash number dependence. This result is consistent with our conclusion that the reoxidation of R^{2-} by the plastoquinone pool is drastically slowed down in the absence of bicarbonate, if it is assumed that the addition of DCMU to centers in the state QR^- and in the state QR^{2-} (ref. 7) results in reduction of Q, and that this reduction proceeds to an about equal concentration of Q^-.

Acknowledgements

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