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Rapid report

Bicarbonate-reversible formate inhibition at the donor side of Photosystem II

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Abstract

Flash-induced absorbance changes at 295 nm show that in the spinach Photosystem II preparation called DT-20 the four-step redox cycle of the oxygen evolving complex is blocked by very low formate concentrations, 50% at 10 μM, and fully restored by a 2-fold higher bicarbonate concentration. These concentrations are four orders of magnitude lower than those involved in the well-known acceptor-side inhibition by formate and did not affect 295 nm absorbance changes in thylakoids, 'BBY' PS II membrane fragments, or PS II core particles. The effect is not related to the chloride dependence of oxygen evolution and appears to be an all-or-none inhibition already present on the first flash.

Keywords: Photosystem II; Formate inhibition; Bicarbonate; Oxygen evolution

The requirement for bicarbonate in electron transport at the acceptor side of Photosystem II (PS II) [1] is attributed to a bicarbonate ion bound as a ligand of the non-heme iron [2]. Formate and other carboxylate anions can replace bicarbonate at this site, resulting in inhibition, but it takes prolonged incubation and millimolar concentrations [3].

Recently, Klimov et al. [4,5] have shown that in the preparation of PS II particles called DT-20 [6] partial depletion of bicarbonate or addition of a few micromolar formate led to a slower and smaller increase of the chlorophyll (Chl) fluorescence yield on illumination, while the addition of 1 mM bicarbonate reversed these effects. This is the opposite of the effects of formate inhibition at the acceptor side and, together with other indications, suggested an inhibition at the electron donor side, with a much higher affinity for formate than that at the acceptor side. In order to verify that suggestion, we have investigated the formate-sensitivity of PS II electron transport on illumination of dark-adapted DT-20 membrane fragments by single-turnover flashes, using UV absorbance difference spectroscopy.

Subchloroplast PS II membrane fragments (DT-20) were isolated according to [6] with some modifications. Spinach chloroplasts were suspended at 0.2 mg Chl/ml in 50 mM MES-NaOH buffer (pH 6.5)/0.5 M sucrose/35 mM NaCl. After addition of 0.4% digitonin (from a 1% stock solution) and 2% NaCl, the suspension was incubated for 60 min at 4°C with stirring, unbroken material was removed by centrifugation for 15 min at $4000 \times g$, 0.1% Triton X-100 was added (from a 10% stock solution) to the supernatant and after a further incubation on ice for 15 min the DT-20 membrane fragments were harvested as the sediment obtained by 50 min centrifugation at $20000 \times g$. The DT-20 fragments were stored at 77 K in 0.1 M Mes buffer (pH 6.2)/0.1 M KCl/10% glycerol at a Chl concentration of 2 mg/ml. Before measurement they were diluted 10-fold in the same medium without glycerol and incubated for 20 min on ice after addition of the indicated concentration of sodium formate. The high concentration of KCl was used only to avoid potential complications by chloride-depletion effects of formate. UV absorbance changes induced by a series of single-turnover flashes (532

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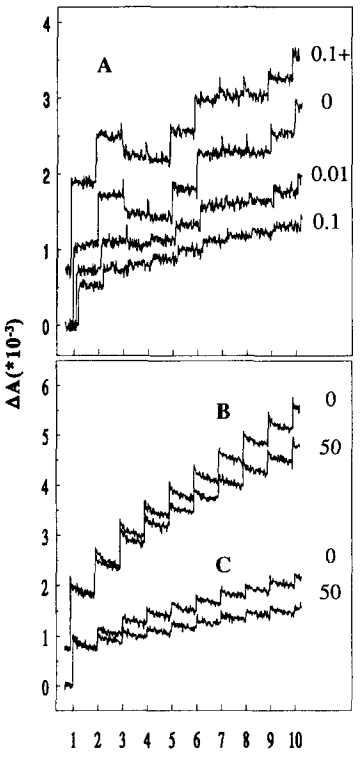

Fig. 1. Absorbance changes at 295 nm in DT-20 particles on illumination by a series of 10 saturating flashes, spaced at 100 ms, in the presence of 50 μM DCBQ (A), 50 μM TPB and 50 μM DCBQ (B), or 50 μM TPB and 50 μM DMBQ (C). Formate concentrations are indicated in mM for each trace; in the upper trace in (A), 0.2 mM bicarbonate was present in addition to 0.1 mM formate.

nm, 20 ns) in dark-adapted samples were measured as in [7].

Fig. 1A shows the absorbance changes at 295 nm in DT-20 particles upon illumination by a series of single-turnover flashes in the presence of the artificial electron acceptor 2,5-dichloro-*p*-benzoquinone (DCBQ). Electron transport from water to DCBQ caused the characteristic absorbance oscillations with a periodicity of four flashes attributed to the S-state cycle, superimposed on a steady increase of absorbance with flash number attributed DCBQ reduction (trace marked 0). The absorbance changes were very similar to those in BBY particles [7]. When only 0.01 mM formate was added (20 min. before measurement) the amplitude of the absorbance oscillation was halved (trace marked 0.01) and it virtually disappeared in the presence of 0.1 mM formate (trace marked 0.1). The inhibition affects only the amplitude and not the shape of the oscillation, indicating that the number of active centers is decreased and not their efficiency. The average absorbance increase per flash, disregarding the first flash on which an extra contribution by inactive centers occurs [7,8], is decreased by only 50% at 0.1 mM formate. Addition of bicarbonate in 2-fold excess over formate completely reversed the effects of formate: the upper trace (marked 0.1 +), measured in the presence of 0.1 mM formate and 0.2 mM bicarbonate, is essentially indistinguishable from the control.

Measurements as in Fig. 1A were also performed on thylakoid membranes, 'BBY' PS II membrane fragments [9], also with storage and measurement in MES/KCL with glycerol as described above, NaCl-washed BBY as in [10] and in Mes/KCl, and on PS II core particles as in [11]. In none of these preparations was the period-four oscillation of UV absorbance changes affected by submillimolar formate concentrations. The properties of the DT-20 preparation are otherwise comparable to those of 'BBY'-type PS II membrane fragments: it appears to have similar purity and antenna size of PS II, all of the extrinsic proteins, and oxygen evolution activity (although only 140–200 μmol O_2(mg Chl)$^{-1}$ h^{-1}) which does not depend on the addition of calcium or chloride.

Fig. 1B shows that DT-20 particles also exhibit the 'normal' sensitivity to high formate concentrations. In the presence of the artificial electron donor tetraphenylboron (TPB) water oxidation is effectively bypassed and the oscillation of the absorbance with flash number was completely suppressed (Fig. 1B). The average absorbance increase per flash (disregarding the first) is about twice that observed in the absence of TPB. Since TPB oxidation by PS II does not cause significant absorbance changes at 295 nm [12], DCBQ reduction was about doubled, indicating that in the absence of TPB about half of the PS II centers were inactive in oxygen evolution, consistent with the rate

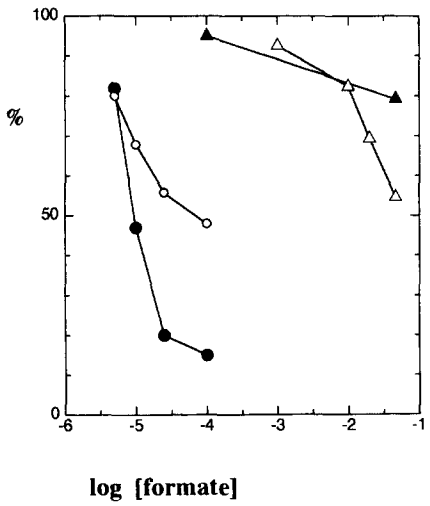

Fig. 2. Electron transport activities in DT-20 particles as a function of formate concentration, in % of the corresponding measurements without formate, measured by flash-induced 295 nm absorbance changes as in Fig. 1. Solid circles: water oxidation with DCBQ as electron acceptor, measured as the absorbance after two flashes plus that after 6 minus 2x that after 4. Open circles: DCBQ reduction in the same measurements, measured as the average absorbance increase on flashes 2 to 10. Solid triangles: DCBQ reduction with TPB as electron donor. Open triangles: DMBQ reduction with TPB as electron donor.

of oxygen evolution in saturating continuous light. Electron transport from TPB to DCBQ was largely insensitive even to 50 mM formate. When the acceptor 2,5-dimethyl-*p*-benzoquinone (DMBQ) was used instead of DCBQ, the normal acceptor-side inhibition by formate became visible at 50 mM, although even at this concentration the inhibition does not exceed 50% after 20 min incubation.

Fig. 2 shows the formate concentration dependence of flash-induced electron transport measured as in Fig. 1. Note that the concentration scale is logarithmic. The amplitude of the period 4 oscillation (solid circles, measured as $A_{\text{flash }2} + A_{\text{flash }6} - 2\,A_{\text{flash }4}$) was halved at 10 μM formate. The inactivation of the oscillating centers was not associated with complete inhibition of DCBQ reduction as indicated by the average absorbance increase on flash numbers 2 to 10 (open circles). Since the origin of the remaining absorbance changes is not clear, we cannot rule out a contribution by absorbance changes of the electron donor involved here. Electron transport from TPB to DCBQ (solid triangles) or to DMBQ (open triangles) was not affected by submillimolar concentrations of formate. The inhibition of DMBQ reduction at formate concentrations above 10 mM presumably reflects the replacement of bicarbonate by formate at the non-heme iron [3], which apparently does not prevent DCBQ reduction.

Our findings show conclusively that in DT-20 membrane fragments the 4-step redox cycle of the oxygen evolving complex is blocked by submillimolar formate concentrations and that this effect is bicarbonate-reversible. It has been proposed that the formate inhibition discussed here is due to replacement of a bicarbonate ion essential for the binding of manganese in the oxygen-evolving complex [4]. Recent EPR measurements indicate that it is associated with the reversible release of one Mn(II) per PS II from the particles (Hulsebosch et al., unpublished data). It remains unclear why the proposed bicarbonate binding site is exposed (or created) only in the DT-20 preparation.

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