Bicarbonate protects the donor side of photosystem II against photoinhibition and thermoinactivation

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Abstract The rate of photoinhibition of photosystem II (PSII) activities (photoinduced change of chlorophyll fluorescence yield, ΔF, and photoreduction of 2,6-dichlorophenol-indophenol) in O_2-evolving pea subchloroplast membrane fragments in medium depleted of CO_2 was considerably decreased upon addition of 5 mM $NaHCO_3$ before the light treatment. A similar effect was observed when 100 μM $MnCl_2$ was added before the photoinhibition. In PSII membrane fragments depleted of Mn, the pre-illumination led to irreversible loss of the capability of PSII to be reactivated by Mn^{2+}, and the rate of the photoinhibition was decreased by a factor of 2 or 5 if the pre-illumination was done in the presence of 0.2 μM $MnCl_2$ (≈4 Mn per PSII reaction center) added alone or in combination with 5 mM $NaHCO_3$, respectively. A similar protective effect of bicarbonate was also revealed in the dark, during thermoinactivation of O_2-evolving PSII at 40°C: the rate of thermoinactivation of ΔF was decreased by a factor of 3 if 5 mM $NaHCO_3$ was added to the medium. The results are consistent with the idea that bicarbonate is an essential component of the Mn-containing water-oxidizing complex of PSII, which decreases its susceptibility to photoinhibition and thermoinactivation.

Key words: Photosystem II; Bicarbonate; Photoinhibition; Thermoinactivation

1. Introduction

It is well established that bicarbonate (BC) is required for maximal activity of photosystem II (PSII) (reviewed in [1]). The interpretation of the stimulating effect of BC on PSII activities remains controversial. In the early 1970s the effect was ascribed to the donor side of PSII [2]. However, Wydrzynski and Govindjee clearly showed [3] that reoxidation of the first plastoquinone electron acceptor, Q_A, by the plastoquinone pool is impaired by BC removal, which was confirmed by a number of data (for review see [1]). The non-heme Fe between Q_A and Q_B has been shown to play an essential role in BC binding [4]. On the other hand, there are data indicating that BC may affect both the electron donor and acceptor sides of PSII [5,6]. El-Shintinawy and Govindjee [6] confirmed that BC has two sites of action: between Q_A and Q_B, and between the secondary electron donor Y_Z and Q_A (however, the site between the primary electron acceptor, pheophytin, and Q_A was speculated for the latter case). Recently [7–10] we have presented strong evidence for BC requirement for the donor side of PSII, and participation of BC in the formation of the Mn-cluster capable of water oxidation was suggested.

Experiments on PSII photoinhibition in CO_2/BC-depleted samples also provided contradictory results. There are data showing that in thylakoids BC protects the photosynthetic apparatus of PSII against photoinhibition [11,12]. On the other hand, depletion of BC in thylakoid membranes (by formate treatment) [11,12] or in green algae (using BC-depleted medium) [13,14] results in a lower susceptibility of PSII to photoinhibition, which is consistent with the idea of blocking the electron transfer between Q_A and Q_B in BC-depleted samples since similar protection against photoinhibition is reached upon DCMU addition.

In this work we present evidence for the protective action of BC against both photoinhibition and thermoinactivation of the donor side of PSII in subchloroplast membrane fragments, which confirms the idea of BC requirement for stability of the Mn-containing water-oxidizing complex (WOC).

2. Materials and methods

Subchloroplast PSII membrane fragments ('BBY particles') were isolated as described earlier [15]. Complete (>95%) removal of Mn from the membrane fragments was carried out using 1 M Tris-HCl (pH 8.0) plus 0.5 M $MgCl_2$ treatment [16]. The preparations were stored in liquid nitrogen at a chlorophyll (Chl) concentration of 2 mg/ml after the addition of 10% glycerol. Bicarbonate removal from BBY preparations was achieved as described [7,8] by a 200-fold dilution of concentrated PSII preparations into a medium (50 mM MES-NaOH buffer, pH 6.2, 35 mM NaCl, 2mM $MgCl_2$) depleted of endogenous BC by means of 60 min flushing with air depleted of CO_2 by passage through a solution of 50% NaOH and a 20 cm layer of ascarite. The sample was subsequently incubated in this medium for 10 min at 4°C.

Photoinhibition was done by means of continuous illumination with heat-filtered red light (120 W/m^2 for untreated and 55 W/m^2 for Mn-depleted BBY preparations) at a Chl concentration of 10 μg/ml in a tightly closed 1-cm cuvette at 20°C. Thermoinactivation was carried out at 40°C in the dark. All the photoinhibition and thermoinactivation procedures were done in medium depleted of CO_2 to which $NaHCO_3$ was added where indicated. Before the measurements of PSII activities the concentration of bicarbonate was equalized in all samples by adding 5 mM $NaHCO_3$.

The kinetics of the photoinduced reduction of 2,6-dichlorophenol-indophenol (DCPIP) and changes of chlorophyll fluorescence yield (ΔF) were measured in a 1-cm cuvette using the phosphoroscopic set-up as described earlier [7,8].

3. Results

3.1. Photoinhibition

Fig. 1 (curves 1 and 2) shows that even after a dark incubation the rate and the magnitude of the photoinduced in-

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Abbreviations: BC, bicarbonate; PSII, photosystem II; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DCPIP, 2,6-dichlorophenol-indophenol; ΔF, photoinduced change of chlorophyll fluorescence yield; WOC, water-oxidizing complex of PSII

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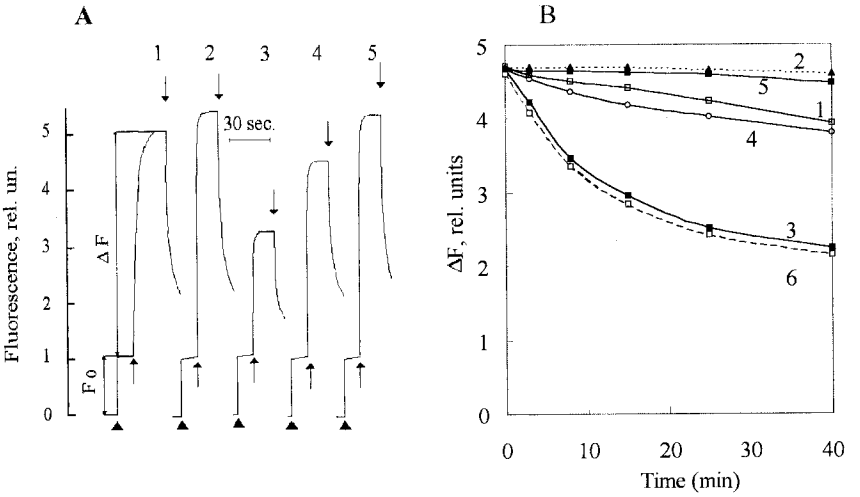

Fig. 1. Effect of bicarbonate on photoinhibition of photoinduced change of chlorophyll fluorescence yield (ΔF) in untreated (O_2-evolving) PSII membrane fragments (BBY particles) in medium first depleted of CO_2 (A, kinetics of ΔF after a 15-min incubation; B, dependence of the ΔF magnitude on the incubation time): 1, in the dark in the absence of additions; 2, in the dark in the presence of 5 mM $NaHCO_3$; 3, under inhibitory illumination in the absence of additions; 4, under inhibitory illumination in the presence of 5 mM $NaHCO_3$; 5, under inhibitory illumination in the presence of 100 μM $MnCl_2$. Before the ΔF measurements, 5 mM $NaHCO_3$ was added to all samples except those already containing 5 mM $NaHCO_3$ during the pre-incubation. Curve 6 (B) is the same as curve 3 except 5 mM $NaHCO_3$ was not added before the ΔF measurements. Photoinhibition was done by illumination with heat-filtered red light at 220 W/m^2. The medium contained 50 mM MES-NaOH buffer, pH 6.2, 2 mM $MgCl_2$ and 35 mM NaCl; $[C]_{Chl}$ = 10 μg/ml; ▲, switching on the measuring light (480 nm, 0.15 W/m^2); ↑ and ↓, actinic light (λ > 600 nm, 100 W/m^2) on and off, respectively.

crease of chlorophyll fluorescence yield were lower in the sample kept in the absence of $NaHCO_3$ (in spite of the fact that the $NaHCO_3$ concentration in the samples was made the same before the measurements).

The protecting effect of added bicarbonate was especially pronounced if these two samples were exposed to the inhibitory illumination (curves 3 and 4); a 40-min illumination in the absence of $NaHCO_3$ led to a 50% inhibition of ΔF while in the presence of 5 mM $NaHCO_3$ only a 15–20% inhibition was observed. A similar protecting effect was revealed if 100 μM $MnCl_2$ (instead of $NaHCO_3$) was added to the sample before the photoinhibition (curve 5). (Note that 2 mM $MgCl_2$ is present in the buffer used in all experiments.) On the other hand, if $MnCl_2$ was added after the photoinhibition made in the absence of BC, there was no restoration of ΔF (data not shown).

Bicarbonate added to the sample photoinhibited in the absence of $NaHCO_3$ also was not efficient to restore ΔF (compare curves 3 and 6, Fig. 1B). It is important that in all these experiments the level F_0 (fluorescence induced by the measuring light in the absence of actinic light) was practically not changed.

A protecting effect of $NaHCO_3$ presence during photoinhibition was also observed for DCPIP photoreduction (Fig. 2). The rate of this photoreaction was decreased by a factor of 5 after a 20-min photoinhibition in the absence of bicarbonate while in the presence of 5 mM $NaHCO_3$ the inhibition was 2.5 times lower (curves 3 and 4).

It has been shown earlier [16] that a complete removal of Mn from PSII membrane fragments results in a 15–20-fold decrease of photoinduced ΔF which can be restored by Mn^{2+} added at a catalytic (0.1–0.2 μM) concentration. The capability of Mn^{2+} to restore electron flow on the donor side of PSII is lost as a result of a short-term illumination of the Mn-depleted preparations while Mn^{2+} prevented the photoinhibition [17]. Fig. 3 shows that the capability of added Mn^{2+} to restore photoinduced ΔF in Mn-depleted BBY membrane fragments is dramatically lowered by a 2–5-min pre-illumination with actinic light. If the photoinhibition is made in the absence of added Mn^{2+} and BC the magnitude of ΔF is decreased by a factor of 2 or 10 upon a 1-min or a 10-min pre-illumination, respectively (Fig. 3, curve 2). Note that the intensity of the inhibiting light is 2 times lower than that used for photoinhibition of untreated Mn-containing membrane fragments. $MnCl_2$ added before the photoinhibition at a concentration of 0.2 μM (which corresponds to 4 Mn atoms per PSII reaction center) decreases the rate of photoinhibition so that nearly 35% of the activity remains after a 10-min illumination (curve 4). Much higher protection against the photo-

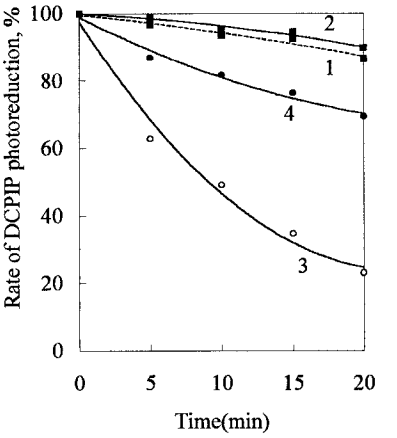

Fig. 2. Dependence of the rate of DCPIP photoreduction in BBY membrane fragments on the photoinhibition time in medium first depleted of CO_2: 1 and 2, dark incubation in the absence (1) and presence (2) of 5 mM $NaHCO_3$; 3, photoinhibition in the absence of added bicarbonate; 4, photoinhibition in the presence of 5 mM $NaHCO_3$. Before the measurements, 5 mM $NaHCO_3$ was added to samples 1 and 3. The experimental conditions were the same as in Fig. 1 except pH 6.5 was used instead of pH 6.2 and 20 μM DCPIP was added before the measurements.

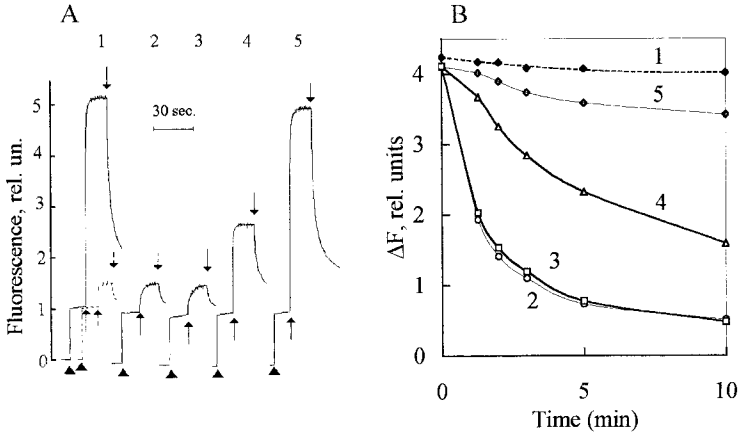

Fig. 3. Effect of bicarbonate on photoinhibition of photoinduced ΔF in Mn-depleted BBY particles in the medium depleted of CO_2 (A, kinetics of ΔF after a 10 min incubation; B, dependence of ΔF magnitude on the incubation time): 1, after dark incubation in the absence of additions (5 mM $NaHCO_3$ and 10 μM $MnCl_2$ were added before the ΔF measurements); 2, after photoinhibition in the absence of additions (5 mM $NaHCO_3$ and 10 μM $MnCl_2$ were added before the ΔF measurements); 3, same as 2 except the photoinhibition was made in the presence of 5 mM $NaHCO_3$; 4, same as 2 except the photoinhibition was made in the presence of 0.2 μM $MnCl_2$ (4 Mn/RC); 5, same as 2 except the photoinhibition was made in the presence of 0.2 μM $MnCl_2$ and 5 mM $NaHCO_3$. The dashed line (A) shows ΔF after a 10-min incubation in the dark in the absence of additions, 5 mM $NaHCO_3$ only was added before the ΔF measurement. Photoinhibition was done by illumination with heat-filtered red light at 100 W/m^2 at 20°C. The experimental conditions were the same as in Fig. 1.

inhibition is achieved if 0.2 μM $MnCl_2$ is added in combination with 5 mM $NaHCO_3$: nearly 85% of ΔF remains after the 10-min photoinhibition (curve 5). On the other hand, the addition of 5 mM $NaHCO_3$ alone before the photoinhibition does not produce any protecting effect (compare curves 2 and 3). Note that during the ΔF measurements all samples contained the same concentration of $MnCl_2$ (10 μM) and $NaHCO_3$ (5 mM).

3.2. Thermoinactivation

Fig. 4 shows that the rate of thermoinactivation of PSII also depends on the presence of bicarbonate in the medium. If the incubation is done in BC-depleted medium the ΔF amplitude is decreased by a factor of 3 after a 10-min incubation of BBY membrane fragments at 40°C in the dark and it is close to 0 after a 30-min heat treatment (curves 2). However, if 5 mM $NaHCO_3$ is added to the medium before the heat treatment, the rate of thermoinactivation is considerably decreased (curve 3) so that nearly 70% of the activity remains even after 30-min thermoinactivation. The protecting effect is also observed if 100 μM $MnCl_2$ (or $MnCl_2$ plus $NaHCO_3$) is added to the medium before the thermoinactivation (curves 4 and 5). The addition of $MnCl_2$ after thermoinactivation results in a considerable restoration of ΔF (Fig. 4A; dotted traces) and the effect is especially pronounced if the heat treatment is carried out in the absence of BC, thus showing that electron donation to PSII reaction centers is damaged during the thermoinactivation.

4. Discussion

Depending on the circumstances the initial photoinhibition

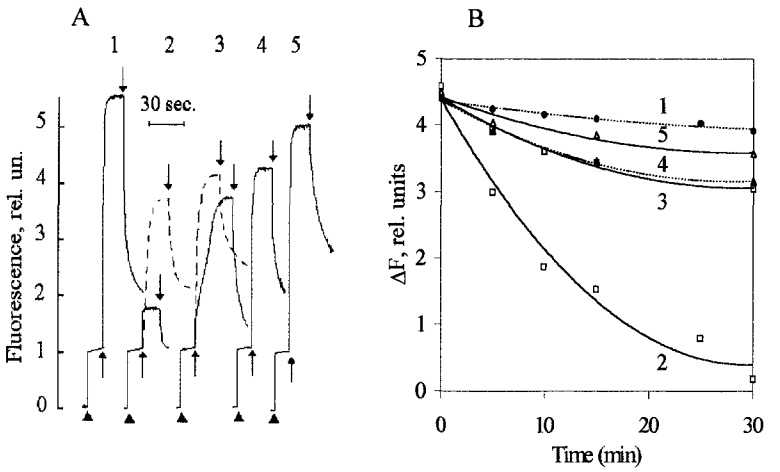

Fig. 4. Effect of bicarbonate on thermoinactivation of photoinduced ΔF in untreated (O_2-evolving) BBY preparations (A, kinetics of ΔF after a 15-min incubation; B, dependence of ΔF magnitude on incubation time): 1, after incubation at 20°C in the absence of additions; 2, after incubation at 40°C in the absence of additions; 3, same as 2 except the thermoinactivation was made in the presence of 5 mM $NaHCO_3$; 4, same as 3 except the thermoinactivation was made in the presence of 100 μM $MnCl_2$; 5, same as 3 except the thermoinactivation was made in the presence of 100 μM $MnCl_2$ and 5 mM $NaHCO_3$. Before the ΔF measurements, 5 mM $NaHCO_3$ was added to all samples except those already containing 5 mM $NaHCO_3$ during the pre-incubation. The dashed lines 2 and 3 (Fig. A) are the same as the corresponding solid lines, except 100 μM $MnCl_2$ was added before the ΔF measurements. The heat treatment was done by incubation of the samples at 40°C in the dark. The experiment conditions are same as in Fig. 1.

of PSII electron transfer can occur by two distinct mechanisms (for review see [18]). It can be initiated on the reducing side of PSII due to double reduction and protonation of the primary electron acceptor, Q_A, which subsequently leaves the Q_A binding site. Under these conditions recombination of the radical pair [$P680^+$ $Pheo^-$] leads to generation of the triplet state of P680, which in turn reacts with oxygen to form singlet oxygen, $^1O_2$, a highly toxic state of O_2 which can destroy P680 leading subsequently to degradation of the RC proteins (reviewed in [18]).

The donor side mechanism occurs under the conditions when the electron flow from water cannot compete with the electron withdrawal on the acceptor side. This can result in an increase in the life time of $P680^+$ which, with a redox potential of 1.12 V [19], is capable of oxidation of its environment causing irreversible damage of both the pigments and proteins [17,18,20,21]. The donor side mechanism is especially efficient after the complete removal of Mn from PSII membrane fragments and the vulnerability of PSII to damage by light is considerably lowered by addition of Mn or other electron donors [17,20,21].

Photoinhibition of photoinduced ΔF in BBY preparations made in bicarbonate-depleted medium (Fig. 1) is typical for irreversible impairment of electron transfer on the donor side of PSII since: (1) the sum $F_0+\Delta F$ is decreased as a result of the photoinhibition at the expense of ΔF without considerable changes in the F_0 value; (2) $MnCl_2$ prevents the inhibition if added before the light treatment while its addition after the photoinhibition does not restore ΔF. The protecting effect of $NaHCO_3$ addition (which is similar to that of $MnCl_2$ addition) reveals that bicarbonate restores electron donation to PSII reaction centers lost due to incubation in the bicarbonate-depleted medium. This idea is confirmed by the data on a decreased rate of photoinduced ΔF even after dark incubation in the medium depleted of bicarbonate (compare curves 1 and 2, Fig. 1A) and it is in agreement with our previous data [7–10] showing that in the absence of bicarbonate the Mn-containing WOC becomes unstable and loses its capability to donate electrons to PSII reaction centers.

Our previous publications demonstrated the direct 'bicarbonate effect' (stimulation of electron transfer upon the addition of BC). Here the inhibitory effect of temporary incubation in the medium depleted of bicarbonate is reported. In this case, subsequent addition of bicarbonate does not restore the activity while bicarbonate added before the inhibitory illumination prevents the inhibition.

The protecting effect of bicarbonate related to stabilization of the Mn center functioning on the donor side of PSII is especially pronounced if Mn-depleted PSII preparations are used. Our results confirm the earlier data [17] showing that Mn^{2+} (together with other electron donors to PSII) considerably decreases the rate of photoinhibition in Mn-depleted PSII preparations due to restoration of electron transfer to oxidized PSII reaction centers. The present work demonstrates that the protecting effect of added Mn^{2+} is much higher if bicarbonate is present in the medium during the photoinhibition. These results are consistent with our earlier data [7–10] on the requirement for bicarbonate to restore both electron transfer and oxygen evolution with Mn^{2+} in Mn-depleted PSII.

Stabilization of the WOC by bicarbonate is clearly demonstrated in the thermoinactivation experiments (Fig. 4). It is known that high temperature treatment results mainly in inhibition of the donor side of PSII due to destruction of the Mn cluster [22–25]. Our data on the protecting effect of Mn^{2+} added before the thermoinactivation as well as on restoration of ΔF with Mn^{2+} added after the thermoinactivation support this idea. Moreover, our results show that bicarbonate added before the thermoinactivation prevents decomposition of the WOC, which is again in line with the idea that bicarbonate is an essential constituent of the WOC, which becomes unstable in its absence.

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