Redox interaction of Mn–bicarbonate complexes with reaction centres of purple bacteria

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It is found that dark reduction of photooxidized primary electron donor P870 in reaction centres from purple anoxygenic bacteria (two non-sulphur Fe-oxidizing Rhodovulum iodosum and Rhodovulum robiginosum, Rhodobacter sphaeroides R-26 and sulphur alkalophilic Thiornodospira sibirica) is accelerated upon the addition of Mn2+ jointly with bicarbonate (30–75 mM). The effect is not observed if Mn2+ and HCO3- have been replaced by Mg2+ and HCO3-, respectively. The dependence of the effect on bicarbonate concentration suggests that formation of Mn2+-bicarbonate complexes, Mn(HCO3)2+, is required for re-reduction of P870 with Mn2+. The results are considered as experimental evidence for a hypothesis on possible participation of Mn–bicarbonate complexes in the evolutionary origin of oxygenic photosynthesis in the Archean era.

**Keywords:** manganese–bicarbonate complexes; reaction centres; purple bacteria

1. INTRODUCTION

It is known that bicarbonate is required for maximal activity of both electron acceptor and electron donor sides of photosystem II (PSII; for recent review, see Van Rensen & Klimov (2005)). The stimulating effect of bicarbonate on electron transfer on the donor side of PSII is especially pronounced during reassembly of the inorganic core of the Mn-containing water-oxidizing complex (WOC; Klimov et al. 1997, 2004; Baranov et al. 2000, 2004; Klimov & Baranov 2001). It was shown that after the removal of all four manganese ions from subchloroplast PSII preparations, an effective reactivation of electron transfer (Allakhverdiev et al. 1997; Klimov et al. 1995a,b, 1997; Hulsebosch et al. 1998) and oxygen evolution (Allakhverdiev et al. 1997; Baranov et al. 2000, 2004) was observed only if a nearly stoichiometric amount of Mn2+ (2–4 atoms of Mn2+ per PSII reaction centre) was added together with bicarbonate. Changes in redox properties of manganese ions upon formation of complexes with bicarbonate evidently play a key role in the effect described above. In fact, upon the addition of NaHCO3 to an aqueous solution of Mn2+, the potential for oxidation of Mn2+ to Mn3+ was shifted from 1.18 to 0.52–0.68 V at pH 8.3 as a result of formation of bi(carbonate) complexes (Kozlov et al. 1997, 2004; Dasgupta et al. 2006). Formation of Mn complex with carboxylates (formate, acetate) induced similar lowering of the oxidation potential; however, only bicarbonate stimulated the electron transfer from Mn2+ to PSII reaction centres (RCs; Kozlov et al. 2004). On the basis of the electrochemical and electron paramagnetic resonance (EPR) data, it was proposed that the unique capability of Mn2+-bicarbonate complexes to be photooxidized by PSII could be due to four possible reasons: (i) significantly larger decrease in the oxidation potential of Mn3+ (down to 0.52 V), (ii) electroneutrality of the functional electron transfer complex, (iii) the more favourable energetics reflected in the two pK values for H2CO3/HCO3- and HCO3-/CO3- and greater number of proton transfer sites, and (iv) multiple composition possibilities for the Mn3+ photoproduction such as Mn3+(HCO3)3, Mn3+(HCO3)(CO32-) and Mn3+(HCO3)2(OH-) (due to the high Lewis acidity of Mn3+; pK<1; Kozlov et al. 2004; Dasgupta et al. 2006).

The oxidation potential of Mn–bicarbonate complex (0.52–0.67 V) is close to the midpoint redox potential of the primary electron donor in the RCs of anoxygenic bacteria (Kozlov et al. 2004) and therefore it has been suggested (Dismukes et al. 2001) that the capability of bicarbonate to form easily oxidizable complexes with manganese ions might be critical to the evolutionary origin of the first O2-evolving cyanobacteria from a non-oxygenic bacterial precursor in the Archean period (>2.2 Gyr ago) when the concentration of bicarbonate dissolved in water was 30–30 000 times greater than today.

Recently, it has been shown that as a result of a set of single mutations of amino acid residues near P870, the primary electron donor in the RCs of purple anoxygenic bacterium Rhodobacter sphaeroides R-26, the P+/P midpoint potential (equal to 0.5 V in the initial strain) was increased to 0.58–0.765 V, and exogenous Mn2+ added together with bicarbonate enabled electron donation to P+ in RCs isolated from these mutants (Kalman et al. 2003). In the present work, we investigated the possibility of electron donation from Mn2+-bicarbonate complexes to oxidized P in wild-types of anoxygenic photosynthesizing bacteria. Three types of purple bacteria (belonging to the class of

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Proteobacteria containing type II of RCs; Gupta et al. 1999) were used: (i) two non-sulphur Fe-oxidizing bacteria Rhodovulum iodosum and Rhodovulum robiginosum (isolated from sediment of the North Sea, Germany) that use Fe$^{2+}$ as an electron donor for anoxygenic photosynthesis (Straub et al. 1999) and so can be good candidates as possible users of the easily oxidizable Mn$^{2+}$–bicarbonate complexes as electron donors, (ii) the purple sulphur alkaliophilic bacterium Thiorhodospira sibirica (received from the collection of Prof. V.M. Gorlenko, Institute of Microbiology, RAS, Moscow), found in ‘soda’ lake Malyy Kasitui (pH of water is 9.5); its optimal growth is at pH 9.0, at a carbonate concentration of 45 mM; the bacterium is resistant to high concentrations of NaCl (up to 1 M) and Na$_2$CO$_3$ up to 740 mM (Bryantseva et al. 1999), and (iii) the widely used non-sulphur bacterium Rh. sphaeroides R-26.

The results presented in this work show that the formation of Mn$^{2+}$–bicarbonate complexes favours electron donation from Mn$^{2+}$ to type II RCs from at least four contemporary purple bacteria.

2. MATERIAL AND METHODS

(a) Isolation of pigment–protein complexes B890

Cell cultures of Rh. iodosum and Rh. robiginosum, and Th. sibirica were grown as described earlier (Straub et al. 1999; Bryantseva et al. 1999; respectively) at 25–30°C and continuous irradiance of 2000 lux. The cells were washed twice in distilled water, suspended in 10 mM HEPES (pH 7.8) and after sonication pigment–protein complexes B890 were isolated as described earlier (Qian et al. 2000) with some modifications. The chromatophore membranes (OD$_{650}$ = 50 cm$^{-1}$) were incubated in 10 mM HEPES buffer (pH 7.8) with 2% dodecyl-$b$-d-maltoside (DM) for 30 min at 4°C in the dark. The suspension was centrifuged (10 000g), and the supernatant was loaded onto a stepwise sucrose density gradient (0.3, 0.5, 0.6 and 1.2 M) and centrifuged (200 000g) at 4°C for 20 h. The pigment–protein complexes B890 appearing between the 0.5 and 0.6 M sucrose layers were collected and loaded onto a 20 cm layer of ascarite. The RCs from Rh. sphaeroides R-26 were isolated as described earlier (Shuvakov & Duyens 1986). The concentration of RCs was determined as described previously (Clayton & Wang 1971).

(b) Measurements of absorbance changes related to reversible photooxidation of P$_{870}$

Kinetics of photoinduced absorbance changes, related to reversible photooxidation of P$_{870}$, were measured in a 10 mm cuvette at 20°C using a phosphoroscopy set-up (Klimov et al. 1982). Accumulation of the oxidized primary electron donor, P$_{870}^+$, was reached by illumination of pigment–protein complexes B890 with red light ($\lambda>600$ nm, 1900 mumol photon s$^{-1}$ m$^{-2}$) for 30 s. The difference ‘light–dark’ absorption spectrum was measured by a spectrophotometer (Hitachi-U-2800, Japan).

(c) Removal of bicarbonate from complexes B890

Partial removal of bicarbonate from pigment–protein complexes was achieved by a 50-fold dilution of the samples in the medium depleted of the endogenous bicarbonate by 60 min flushing with air freed of CO$_2$ by passage through a solution of 50% NaOH and a 2 cm layer of ascarite.

3. RESULTS

To study the possibility of redox interaction of Mn$^{2+}$–bicarbonate complexes with RCs of purple bacteria, we used the pigment–protein complexes B890 isolated from Rh. iodosum, Rh. robiginosum and Th. sibirica. The complex B890 from purple bacteria is known to be the light-harvesting complex (that is termed LH1) tightly associated with the RC in fixed stoichiometry 1:1 (Law et al. 2004). Figure 1a illustrates the absorption spectrum of the pigment–protein complexes B890 isolated from Rh. iodosum which is similar to that isolated previously from other types of purple bacteria (Law et al. 2004; Moskalenko et al. 2005).

Kinetics of photoaccumulation of the primary electron donor P$_{870}$ in the oxidized form P$^+$ and its dark re-reduction, measured at 790 nm ($\Delta_{A790}$) corresponding to the electrochromic blue shift of absorption band at 800 nm due to oxidation of P$_{870}$, are shown in figure 1b. After a 30 s illumination at pH 8.2, nearly 50% of RCs remain in a ‘long-lived’ oxidized state: their re-reduction occurs in minute range. Using redox titration of the amplitude of the photoinduced $\Delta_{A790}$ (upon the addition of K$_4$[Fe(CN)$_6$] and K$_4$[Fe(CN)$_4$]), the midpoint redox potential ($E_m$) of the pair P$^+/P$ in Rh. iodosum, Rh. robiginosum and Th. sibirica was found to be equal to 450 ± 30 mV (which is close to $E_m$ of P in other purple bacteria; Prince et al. 1976; Klimov et al. 1977; Lin et al. 1994).

The dark reduction of P$^+$ is considerably accelerated if 0.1 mM K$_4$[Fe(CN)$_6$] (a known artificial electron donor to P$^+$) is present in the medium so that only 15% of P were in the oxidized state after 30 s of darkness (figure 1b, curve 2). Interestingly, an acceleration of P$^+$ reduction is also observed upon the addition of 0.5 mM MnCl$_2$ together with 50 mM NaHCO$_3$ (figure 1b, curve 3). The effect was clearly seen for the long-lived component of P$^+$ relaxation: a 50% decay of this component was at 110, 63 and 5 s for the samples without additions, in the presence of 0.5 mM MnCl$_2$ together with 50 mM NaHCO$_3$ and in the presence of 0.1 mM K$_4$[Fe(CN)$_6$], respectively. In further experiments on the effect of Mn$^{2+}$ on the redox state of P$^+$, the difference light–dark absorption spectra of P$^+$ corresponding to the long-lived state of photooxidized P$_{870}$ were measured. A similar approach for revealing the redox interaction of Mn$^{2+}$ with bacterial RCs was used earlier (Kalman et al. 2003). The spectra were measured after a 2.5 min dark incubation of the samples illuminated with red light ($\lambda>600$ nm, 1900 mumol photon s$^{-1}$ m$^{-2}$) for 30 s.

The spectrum (figure 1c) is typical of photooxidation of P in pigment–protein complexes B890 of purple bacteria (Shuvakov & Klimov 1976; Moskalenko et al. 2005). It includes the bleaching of a broad absorption band of P with maximum at 870 nm accompanied by a
Rh. iodosum

sulphur Fe-oxidizing purple bacterium

pigment–protein complexes B890 isolated from the non-

additions (1) and after the addition of 0.1 mM K₄[Fe(CN)₆]

difference light

D

incubation in the dark. The measurements of

HEPES (pH 8.2) depleted of CO₂/HCO₃

(2), 0.5 mM MnCl₂ and 50 mM NaHCO₃ (3). Up and down

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Figure 1. (a) Absorption spectrum, kinetics of photoinduced

absorbance changes at 790 nm (ΔA₇₉₀) (b) related to

photooxidation of the primary electron donor P₈₇₀ in

pigment–protein complexes B890 isolated from the non-
sulphur Fe-oxidizing purple bacterium Rh. iodosum and

(c) difference light–dark absorption spectra. (c) Spectra

were measured after a 30 s illumination with actinic light

(λ>600 nm, 1900 µmol photons s⁻¹ m⁻²) and 2.5 min

incubation in the dark. The measurements of ΔA₇₉₀ and

difference light–dark absorption spectra were made without

additions (1) and after the addition of 0.1 mM K₄[Fe(CN)₆]

(2), 0.5 mM MnCl₂ and 50 mM NaHCO₃ (3). Up and down

arrows indicate actinic light on and off, respectively. The

measurements were done in the medium containing 50 mM

HEPES (pH 8.2) depleted of CO₂/HCO₃⁻ (see §2).

characteristic shift of the absorption band at 800 nm.

The amplitudes of both the bleaching at 870 nm and the

shift at 800 nm are decreased upon the addition of

either 0.1 mM ferricyanide (figure 1c, curve 2) or

0.5 mM MnCl₂ added jointly with 50 mM NaHCO₃,

(figure 1c, curve 3) indicating the acceleration of dark

re-reduction of P⁺. For a detailed investigation of the

specificity of the pair Mn²⁺+bicarbonate in the

acceleration of re-reduction of P⁺ in purple bacteria,

the difference ΔA₇₉₀−ΔA₈₁₀ (ΔA₇₉₀–₈₁₀ corresponding

to the blue shift of the absorption band at 800 nm

due to oxidation of P) was used as a measure of the

fraction of RCs with oxidized P₈₇₀ (to avoid possible

absorbance changes related to photobleaching of the

antenna BChl).

Figure 2a shows that the addition of 0.5 mM MnCl₂

together with 50 mM NaHCO₃ to pigment–protein

complex B890 from Rh. iodosum accelerates re-reduction

of P⁺ (closed circles), while 0.5 mM MnCl₂ or

bicarbonate added alone does not increase the rate of

dark relaxation of ΔA₇₉₀–₈₁₀ (filled inverted triangles

and filled squares in figure 2a, respectively). The

bicarbonate requirement for reduction of P⁺ with

added Mn²⁺ is in agreement with the data reported

earlier (Kozlov et al. 2004) on lowering of the oxidation

potential of Mn²⁺ upon formation of complexes with

bicarbonate.

Mg²⁺ (in contrast to Mn²⁺) added jointly with

bicarbonate does not increase the rate of re-reduction

of P⁺ (figure 2d). Similarly, replacement of bicarbon-

ate by formate (without change in Mn²⁺ concen-

tration) also leads to the loss of the effect observed in

the presence of Mn²⁺ and bicarbonate (figure 2d).

This clearly demonstrates that Mn²⁺–bicarbonate

complexes are important for the acceleration of P⁺

re-reduction.

A similar effect of the increase of the rate of dark

reduction of P⁺ upon addition of the Mn²⁺ jointly

with bicarbonate is observed in pigment–protein

complex B890 isolated from two other purple bacteria:

Fe-oxidizing Rh. robiginosum (figure 2b) and sulphur

alkaliphilic Th. sibirica (figure 2c).

The acceleration of dark reduction of P⁺ upon the

addition of Mn²⁺ depended on the concentration of

added bicarbonate: at an Mn²⁺ concentration equal to

0.5 mM, the effect was not observed at bicarbonate

concentrations of 10 and 15 mM; it is clearly seen

starting from a bicarbonate concentration of 30 mM

and is saturated at 50–70 mM (figure 3a). The

dependence of the effect on Mn²⁺ concentration

shows that in the presence of 50 mM NaHCO₃, the

acceleration of reduction of P⁺ is observed already at

Mn²⁺ concentration of 10 µM, and the effect is

maximal at 0.3–0.5 mM MnCl₂ (figure 3b).

It has been shown earlier (Kalman et al. 2003) that in

RCs isolated from the initial strain of Rh. sphaeroides R-26 (the P⁺/P midpoint potential = 0.5 V), Mn²⁺ added jointly with 15 mM bicarbonate is not able to accelerate the dark reduction of P₈₇₀.

Figure 4 demonstrates that under the conditions used

in our experiments (photoaccumulation of P⁺ at pH

8.2, addition of 0.5 mM MnCl₂ together with 50 mM

NaHCO₃), the rate of re-reduction of P⁺ in RCs

isolated from the initial strain of Rh. sphaeroides is

considerably increased upon the addition of the pair

Mn²⁺+bicarbonate so that in their presence the

concentration of P⁺ remaining after a 30 s incuba-

tion of illuminated samples in the dark becomes 3.5

times lower.
4. DISCUSSION

Dismukes et al. (2001) put forward a hypothesis that Mn\(^{2+}\)-bicarbonate complexes could play a key role in the evolutionary origins of the WOC due to the use of Mn complexes as transient electron donors (and ‘building blocks’) for anoxygenic photosynthetic bacteria in the Archean Ocean when the content of CO\(_2\) and HCO\(_3^-\) was high enough (a few orders higher than nowadays) to produce easily oxidizable Mn\(^{2+}\) complexes.

The results obtained in our work show that the formation of Mn\(^{2+}\)-bicarbonate complexes favours electron donation from Mn\(^{2+}\) to the RC of purple bacteria. Such a conclusion has been made on the basis of spectrophotometric measurements of \(\Delta A\) related to reversible photooxidation of the primary electron donor, P. As in the case with ferrocyanide (a known exogenous electron donor to P\(^+\)), Mn\(^{2+}\) in the presence of bicarbonate accelerated the re-reduction of P\(^+\). The midpoint potential for the P\(^+\)/P of anoxygenic purple bacteria is 0.45–0.50 V (Prince et al. 1976; Klimov et al. 1977; Lin et al. 1994) and a similar \(E_m\) is found in our work for the pair P\(^+\)/P in RCs isolated from \(Rh.\) iodosum. Therefore, the RC of these bacteria cannot act as an oxidant for Mn\(^{2+}\) (the oxidation potential of aqua ions of Mn\(^{2+}\) is 1.18 V). On the basis of electrochemical measurements, it was demonstrated that the formation of complexes of Mn\(^{2+}\) with bicarbonate greatly favours the electrochemical oxidation of Mn\(^{2+}\) to Mn\(^{3+}\) so that the oxidation potential of Mn\(^{2+}\) becomes 0.52–0.67 V (Kozlov et al. 2004). Therefore, in response to the formation of Mn\(^{2+}\)-bicarbonate complexes, the oxidation potential of Mn\(^{2+}\) becomes so low that it is possible to expect the oxidation of Mn\(^{2+}\) by RCs of anoxygenic purple bacteria, and that is what we see in our experiments. The replacement of Mn\(^{2+}\) by Mg\(^{2+}\) or bicarbonate with formate leading to the loss of the effect observed with the Mn–bicarbonate system confirms the

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Figure 2. Kinetics of the long-lived component of dark relaxation of photoinduced \(\Delta A\) related to the reversible photooxidation of P\(_{770}\) in pigment–protein complexes isolated from (a) \(Rh.\) iodosum, (b) \(Rh.\) robiginosum, (c) \(Th.\) sibirica in the absence of additions (open squares) and in the presence of 0.1 mM K\(_4\)Fe(CN)\(_6\) (open triangles), 0.5 mM MnCl\(_2\) (filled inverted triangles), 50 mM NaHCO\(_3\) (filled squares), 0.5 mM MnCl\(_2\) and 50 mM NaHCO\(_3\) (filled circles). The difference \(\Delta A_{790}\)–\(\Delta A_{810}\) (corresponding to the blue shift of absorption band at 800 nm due to oxidation of P\(_{770}\) taken from difference light–dark absorption spectra measured after a 30 s illumination (for details see figure 1) was used as a measure of the fraction of RCs with oxidized P\(_{770}\). The first spectrum was measured 30 s after switching the actinic light off. (d) Kinetics of the long-lived component of dark relaxation of photoinduced \(\Delta A\) related to the reversible photoreduction of P\(_{770}\) in pigment–protein complexes isolated from \(Rh.\) iodosum in the absence of additions (open squares) and presence of 0.5 mM MgCl\(_2\) and 50 mM NaHCO\(_3\) (filled diamond); 0.5 mM MnCl\(_2\) and 50 mM NaHCO\(_3\) (right-faced filled triangle). The results are an average of three experiments.
specificity of Mn–bicarbonate complexes in this reaction (figure 3d). Recently, it has been found that carboxylates (formate and acetate) are also capable of producing complexes with Mn\(^{2+}\) that lead to a lowering of the oxidation potential of Mn\(^{2+}\); the oxidation potential of Mn\(^{2+}\)–formate complexes is approximately 0.76 V (Kozlov et al. 2004). The relatively high oxidation potential of Mn\(^{2+}\)–formate complexes (in comparison with Mn\(^{2+}\)–bicarbonate complexes) can explain the inability of the pair Mn\(^{2+}\)–formate to accelerate re-reduction of P\(^{+}\) in purple bacteria. In addition, bicarbonate (in contrast to formate) can form electro-neutral complexes with Mn\(^{2+}\) and Mn\(^{1+}\) (Mn\(^{4+}\)(HCO\(_3\))\(_2\) and Mn\(^{3+}\)(HCO\(_3\))\(_3\) (Kozlov et al. 2004) that can also favour the redox interaction of Mn\(^{2+}\) with the RC of purple bacteria. It is interesting that PSII RCs are also unable to oxidize Mn–formate complexes (Kozlov et al. 2004) though the oxidation potential of P\(_{\text{es}}\)(>1.1 V) is enough for their oxidation. Evidently, the multiple composition possibilities for the Mn\(^{2+}\) photoproduct, such as Mn\(^{3+}\)(HCO\(_3\))\(_3\), Mn\(^{3+}\)(HCO\(_3\)^\(-\)) and Mn\(^{3+}\)(HCO\(_3\))^\(-\)(OH\(^-\)) (Kozlov et al. 2004), are important for the redox interaction of Mn–bicarbonate complexes with RCs of both PSII and anoxygenic bacteria.

The acceleration of re-reduction of P\(^{+}\) in the presence of Mn\(^{2+}\) begins from 30 mM of added bicarbonate and the effect is maximal at 50 mM. Electrochemical and EPR measurements (Kozlov et al. 2004; Dasgupta et al. 2006) revealed the presence of 1 : 1 (Mn(HCO\(_3\))\(^{2-}\)) and 1 : 2 (Mn(HCO\(_3\))\(_2\)) complexes in water solution of Mn\(^{2+}\) and bicarbonate. The oxidation potentials for the 1 : 1 and 1 : 2 Mn\(^{2+}\)–bicarbonate complexes (0.61 and 0.52 V, respectively; Kozlov et al. 2004) are much lower than those for the aqua complex of Mn\(^{2+}\) (1.18 V). Using the equilibrium constants of Mn\(^{2+}\)–bicarbonate complexes presented earlier (Kozlov et al. 2004; Dasgupta et al. 2006), we estimated the ratio of Mn\(^{2+}\)–bicarbonate complexes in the solution at different concentrations of added bicarbonate. In the presence of 15 mM bicarbonate at pH 8.2, the total Mn\(^{2+}\) in the solution is represented by a mixture of 69% of ions Mn\(^{2+}\) aqua, approximately 23% of [Mn(HCO\(_3\))]\(^{2+}\) and 8% of Mn(HCO\(_3\))\(_2\). The content of Mn(HCO\(_3\))\(_2\) increases with increase in the bicarbonate concentration and at 50 mM the mixture of total Mn\(^{2+}\) is 29% of Mn\(^{2+}\) aqua, approximately 31% of [Mn(HCO\(_3\))]\(^{2+}\) and 40% of Mn(HCO\(_3\))\(_2\). Most probably, the bicarbonate concentration dependence of the acceleration of re-reduction of P\(^{+}\) with Mn\(^{2+}\) (figure 3a) reflects the increase in the content of the complex Mn(HCO\(_3\))\(_2\) most active in the redox interaction with P\(^{+}\). It was shown (Kalman et al. 2003) that only RCs from the mutant cells of Rh. sphaeroides in which the P\(^{+}\)/P\(_{\text{es}}\) ratio rises from 2.0 to 2.4 in the redox interaction of Mn–bicarbonate complexes.
midpoint potential was increased to 0.58–0.765 V were able to oxidize Mn$^{2+}$ in the presence of 15 mM bicarbonate, while oxidation of Mn$^{2+}$ by RCs from the initial strain (where the P$^-$/P midpoint potential was 0.45–0.5 V) was not observed. (Our experiments confirm that if the concentration of added bicarbonate is lower than 30 mM, Mn$^{2+}$ does not accelerate the reduction of P$^+$.). One can suggest that the domination of the complex [Mn(HCO$_3$)$_2$]$^{+}$ and, as a consequence, the low the concentration of the complex Mn(HCO$_3$)$_2$, were responsible for the lack of redox interaction of Mn–bicarbonate complex with the RCs of Rh. sphaeroides in the experiments reported earlier (Kalman et al. 2003).

The effect of Mn$^{2+}$–bicarbonate complexes on the redox state of P$^+$ was also obtained in pigment–protein B890 isolated from another non-sulphur Fe-oxidizing purple bacterium Rhodovulum robiginosum and from a purple sulphur alkaliphilic bacterium Th. sibirica (figure 2b,c). Thus, the results demonstrate that even without pre-modifications of the redox properties of the RCs, at least four contemporary purple bacteria (containing type II RCs) are capable of using Mn–bicarbonate complexes as electron donor for oxidized P and this capability could be typical of most purple bacteria with a P$^-$/P midpoint potential near 0.45–0.5 V. Therefore, under certain conditions (appropriate pH, enhanced concentration of bicarbonate) favourable for the formation of the easily oxidizable Mn$^{2+}$–bicarbonate complexes, the purple bacteria could use the Mn$^{2+}$–bicarbonate complexes as a source of electrons; according to the hypothesis (Dismukes et al. 2001) this eventually could have led to the origin of the WOC in the Archean era.

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in the oxygen evolving complex of photosystem II. Biochimica et Biophysica Acta 1040–1044. (doi:10.1016/j.bbabio.2004.09.001) A photosystem II-associated carbonic anhydrase catalyzes more efficient oxygen evolution (especially for the S3 and S4 reaction centers) than that observed in the wild-type enzyme. Interestingly, the results obtained from the CAH3 lacking mutant of PSII–associated carbonic anhydrase (CA; Villarejo et al. 2002). Interestingly, the results obtained from the CAH3 lacking mutant of PSII particles, it remains bound to PSII and its content (per Chl basis) increases (Villarejo et al. 2002). H. Dau (Freie University, Berlin, Germany). I have a question. Tatiana Shutova (Laboratory of Klimov, Pushinov, Russia, and G. Samuelsson, Umeå, Sweden) carried out measurements in our laboratory using a time-resolved delayed fluorescence approach (Grabolle & Dau 2005; Buchta et al. 2007) to study the donor-side processes in PSII preparations from wild-type cells and mutants lacking the PSII-associated carbonic anhydrase (CA; Villarejo et al. 2002). Interestingly, the results obtained for the CAH3 free mutant suggest that proton release in the S2→S3 and S3→S4 transition is indeed accelerated by bicarbonate addition. This finding supports that bicarbonate facilitates more efficient proton removal from the Mn complex.

W. Hillier (Australian National University, Australia). I would like to comment. PSII is often found to contain carbonic anhydrase activity. We have quantified this activity in a number of different samples, and surprisingly for the PSII from Th. elongatus there is practically no carbonic anhydrase activity associated with PSII.

J. Barber (Imperial College London). I would like to point out that the electron density tentatively assigned to carbonate in the OEC by Ferreira et al. (2004) is in part occupied by CO32−. I have two questions. Was the effect of bicarbonate specific to the enzyme from Chlamydomonas and can you relate any role of bicarbonate to a specific S-state transition? V. V. Klimov. Yes. The effect of bicarbonate was specific for the enzyme from Chlamydomonas; the effect was not seen upon the addition of a bovine carbonic anhydrase. We consider bicarbonate as an available base with an appropriate pK of 6.4 which can be used as an acceptor for protons released from the water oxidation centre (especially for the S3→S4→S0 transition; Shutova et al. Submitted).