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A proposed role FOR MN(II)/Bicarbonate CLusters in THE Evolutionary Origin and Functioning of the Water-Oxidizing/Oxygen-evolving Complex of Photosystem II

[1]Klimov V.V., [1]Kozlov Yu. N., [1,2]Baranov S.V., [2]DasGupta J., [2]Tyryshkin A., [1]Kazakova A.A. and [2]Dismukes G.C.

[1]Institute of Basic Biological Problems, Russian Academy of Sciences, Pushchino, Moscow Region, Russia, 142290; FAX : 7(096)779-0532; E-mail: klimov@issp.serpukhov.su

[2]Department of Chemistry, Hoyt Laboratory, Princeton University, Princeton, NJ 08544; FAX: (609)258-1980; E-mail: dismukes@Princeton.EDU

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Introduction.

It is well established that bicarbonate ions (BC) are required for the maximum activity of photosystem II (PSII) (for recent review see (van Rensen et al., 1999) and references therein). However, the interpretation of the stimulating effect of BC («bicarbonate effect») on PSII activities remains controversial. Initially the site of action was thought to be in the water-oxidizing complex (WOC) and a model including BC as a mediator for the photosynthetic water oxidation had been suggested which, however, seemed to be in contradiction with the results of isotopic experiments . On the other hand, strong evidence for the action of BC on the electron acceptor side of PSII, providing efficient reoxidation of the first plastoquinone electron acceptor Q_A , has been presented , and the idea was supported by a number of data (for review see (van Rensen et al., 1999)). The non-heme Fe between Q_A and the secondary plastoquinone electron acceptor Q_B has been shown to play an essential role in BC binding. More recently, Klimov's group and their collaborators found strong evidence for involvement of BC in stimulating electron donation from Mn^{2+} to apo-WOC-PSII, in which the inorganic core was first removed (for review see (Klimov and Baranov, 2001) and references there in). Importantly, they found that BC increases the binding affinity and photooxidation rate of the first two Mn^{II} ions, implicating a high affinity pair of Mn^{II} ions involved in BC-dependent electron transfer from Mn^{II} They also found definitive evidence for BC enhancing the rate of light-induced charge separation within the intact holo-WOC-PSII at a site on the donor side of PSII, as well as for stabilizing the holo-WOC-PSII against thermal deactivation. The Pushchino group has proposed a direct role for BC as an intrinsic cofactor involved in stimulation of water oxidation within the WOC. However, direct spectroscopic evidence identifying the location and characteristics of the binding site for BC in the WOC (rather than alternatively delivering OH^-) is still lacking. In this paper we shall focus on a possible role of bicarbonate ions in the evolutionary origins and functioning of the WOC.

Results and Discussion.

Recently, we have obtained several lines of evidence showing that BC specifically alters the speciation of Mn^{2+} ions in solution and its redox properties. To illustrate the change in speciation, we show in Fig. 1 representative electrochemical data for the reduction of Mn^{2+} to Mn^0 at a Hg electrode as a function of BC concentration (pH 8.3) (Kozlov et al., submitted).

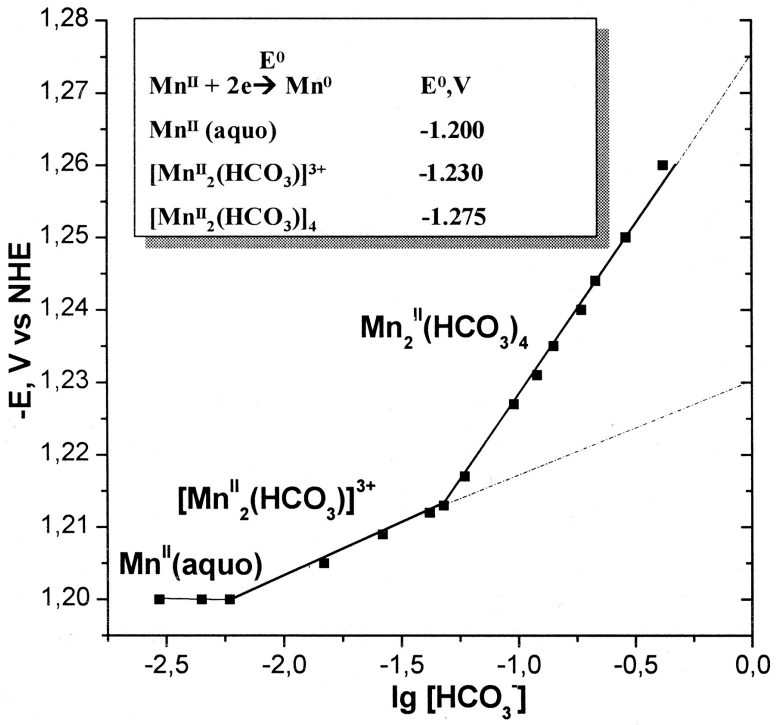

Fig.1. Representative electrochemical data for the reduction of a solution of Mn^{II} (2.5×10^{-4} M $MnSO_4$) to Mn^0 at a Hg electrode as a function of the concentration of bicarbonate (pH 8.3, 0.1 M $LiCLO_4$). Voltage scanning at 50 mV s^{-1}. Inset shows the standard reduction potentials and formulas of the Mn^{II} species that form. .

One observes two transitions corresponding to the formation of two species having different BC binding constants. From the slopes of these plots (14 mV and 60 mV), one is able to obtain directly the stoichiometry of binding, while the intercepts provide the standard potentials and the formation constants. The slopes show that the two species that form between Mn^{2+} and BC have stoichiometries equal to 2:1 and 1:2. These correspond to complexes with empirical formulas, $Mn^{II}_2(HCO_3)^{3+}$ and $[Mn^{II}(HCO_3)_2]n$. The remaining coordination sites in the first coordination shell will be occupied by water molecules, by analogy to Mn^{II}_{aq}, which coordinates six water molecules in the first shell. The formation constants (K_b) that were obtained for these complexes are given in Table 1. By contrast, only the 1:1 complexes form with acetate or formate, and these have a lower affinity (Kozlov et al., submitted). These data show that BC specifically induces formation of manganese clusters having apparent dimanganese composition.

Electrochemical oxidation of the Mn^{II} clusters to the Mn^{III} state was also performed as a function of BC concentration and provided the standard potentials (E_0) given in Table 1. We found that oxidation of the Mn^{II} -BC clusters in the presence of excess BC leads to the

Table 1. Manganese(II)-bicarbonate equilibrium speciation in water

Mn(II) species	K_B, M^{-1}	E_0, V, NHE	Catalase activity†
Mn^{2+}_{aq}	NA	1.18‡,§	Zero
$Mn^{II}_2(HCO_3)^{3+}$	11-60, 10‡,§	0.61	?
$[Mn^{II}_2(HCO_3)_4]_n$	4-20, 34‡,§	0.52	Active

†Catalysis of peroxide dismutation: $2H_2O_2 \rightarrow 2H_2O + O_2$. From Stadtman et al., 1990; Sychev and Isac, 1993 ; ‡From Smith and Martell, 1976 ; § From Kozlov et al., 1997.

formation of Mn^{III} –BC clusters at potentials (E $_0$ = 0.61- 0.52 V) that are far lower relative to Mn^{II}_{aq} (E $_0$ = 1.18 V). These potential shifts are sufficiently large that they would enable Mn-BC clusters to function as electron donors to anoxygenic phototrophs. However, the electrochemical data do not reveal whether BC complexes to manganese or delivers hydroxide to form the corresponding Mn-hydroxo/oxo species. Evidence in the literature shows that Mn-BC solutions catalyze the multi-electron dismutation of hydrogen peroxide ($H_2O_2 \leftrightarrow O_2 + 2H_2O$), so-called catalase activity (Sychev and Isac, 1993). Stadtman additionally showed that the rate of Mn-dependent dismutation depended on the BC concentration to the third power, suggesting the formation of an active species with 1:3 Mn/BC stoichiometry (Stadtman et al., 1990). We now know from the studies described herein that their experimental conditions lead to formation of Mn^{II} -BC clusters as the active species. It is also known that manganese catalases found in biology contain exclusively dimanganese centers, and that efficient abiotic manganese catalase model complexes contain di- or multi-manganese centers. Considering these results, we may attribute the catalase activity of Mn-BC solutions to the formation of Mn-BC clusters, possibly $Mn^{II}_2 (HCO_3)_4$ produced from $Mn^{II}_2(HCO_3)^{3+}$. Both complexes are oxidizable by peroxide by forming the Mn_2 (III, III) oxidation state as intermediate in the two-electron/two-proton-coupled dismutation reaction.

Additional evidence in support of formation of Mn-BC oligomers in solution comes from variable temperature electron paramagnetic resonance (EPR) experiments on samples that do not contain an electrolyte ($LiClO_4$) but include 60% glycerol as glassing agent. Fig. 2 gives a plot of the EPR intensity for the Mn^{II}_{aq} ion as a function of BC concentration (six-line spectrum in *Inset*). This signal is well known to be caused by the monomeric Mn^{II}_{aq} ion. Upon addition of BC, the Mn^{II}_{aq} signal disappears and is replaced by an unstructured broad signal from a second Mn^{II} species (Fig. 2) whose intensity grows and saturates in reciprocal proportion to the loss of Mn^{II}_{aq} . The EPR titration data are compared with the solid line showing the fit to an equilibrium binding reaction: $n\ Mn^{II}_{aq} + 2n\ HCO_3^- \Leftrightarrow Mn(HCO_3)_{2n}$. Other stoichiometries gave poorer fits to the data. All titration data were recorded at a fixed time after mixing because the system was found to be time-dependent because of slow precipitation of $MnCO_3$ crystals. The broad EPR signal may be attributable to the precursor(s) to solid $MnCO_{3(s)}$. Unlike Mn^{II}_{aq} the BC-induced Mn^{II} EPR signal is detectable only below 70 K, exhibits a non-Curie temperature dependence of signal intensity and a temperature-dependent g-value, and undergoes rapid spin relaxation.

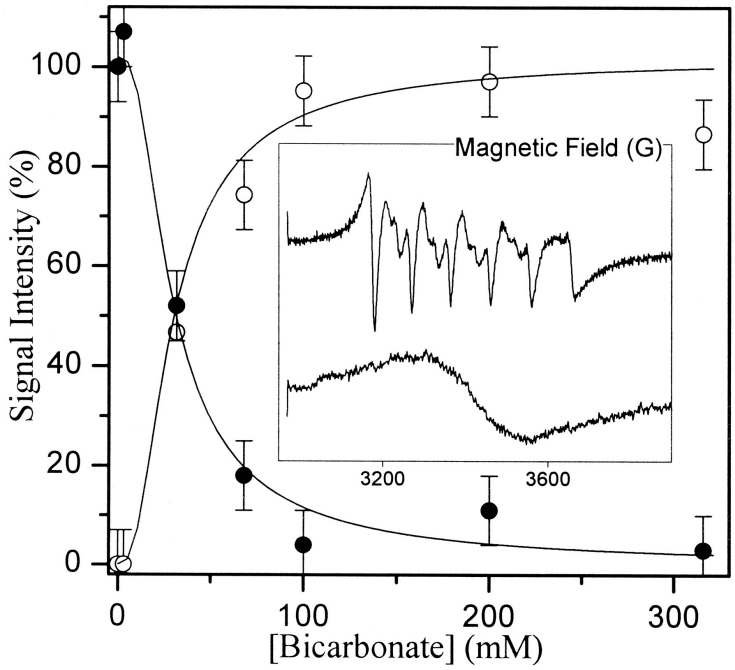

Fig. 2. Titration of the EPR signal intensities as function of bicarbonate concentration for the Mn II aq species (g2 six-line spectrum, *Inset*) and the broad signal that replaces it upon addition of bicarbonate (broad signal, *Inset*). The broad signal is detectable only below 70 K, and it exhibits strong spin relax-ation and a temperature-dependent linewidth and a *g* value indicative of formation of a Mn^{II}_{x}-bicarbonate oligomer.

These properties differ greatly from isolated Mn^{II} ions and indicate that the new species corresponds to an oligomer of Mn^{II} ions that interact via both electronic exchange (chemical bonding) and magnetic dipolar interactions. Comparisons to simple dimanganese complexes and extended solids indicate that the Mn ions must share one or more bridging ligands, presumably BC, hydroxide, or carbonate ions. These EPR data are consistent with the electrochemical data in showing that Mn^{II}_{aq} forms a Mn^{II} oligomer upon complexation or reaction with BC rather than remaining monomeric. The composition of the Mn-BC oligomer is under investigation.

The electrochemical data above indicate that dilute solutions of Mn^{II} in water above a concentration of 10 mM bicarbonate exist primarily as Mn-bicarbonate (or hydroxide) clusters of 2:1 and 1:2 composition. The estimated stability constants for their formation (Table 1) together with estimates for the Mn^{II}_{aq} concentration in the Archean ocean (see Dismukes et al., 2001) indicate that these Mn^{II} clusters would have represented the dominant form of soluble Mn^{II} present in the Archean ocean, unlike today where the speciation favors the monomeric aquo ion Mn^{II}_{aq}. The pKa of Mn^{II}_{aq} is 10.5. Hence, at the pH of the contemporary ocean (≈8), the fractional concentration of $Mn(OH)^{+}$ would be vanishing small ($10^{-2.5} \times Mn^{II}_{aq}$) if there was no BC to serve as hydroxide source. In the Archean ocean (pH ≈ 6.5–7), it would have been even smaller. Thus, bicarbonate, not free hydroxide, is the major source of hydrolytic species formed from Mn^{II}_{aq}, including $Mn(OH)^{+}$, in both the contemporary and Archean oceans. Figure 3 (upper panel) compares the standard potentials (per electron) for oxidation of water, BC, and the dimanganese BC complex $Mn_2(HCO_3)_4$ vs. the reaction center pigments found in cyanobacteria and higher plants (P680; Chl-a), purple and green nonsulfur bacteria (P870; BChl-a), and green sulfur bacteria and heliobacteria (P840; BChl-a) shows that the Mn-bicarbonate complexes fall much closer to the potential generated by anoxy-genic bacterial reaction centers than to the PSII oxygenic reaction center of cyanobacteria. Thus, the

Mn-bicarbonate clusters would be readily oxidizable substrates for photosynthetic bacteria, even though free bicarbonate or water are thermodynamically inaccessible as reductants. Additional evidence has accumulated showing that Mn-BC precursors are highly efficient in the assembly of the tetramanganese-oxo core of PSII during biogenesis (see Klimov and Baranov, 2000).

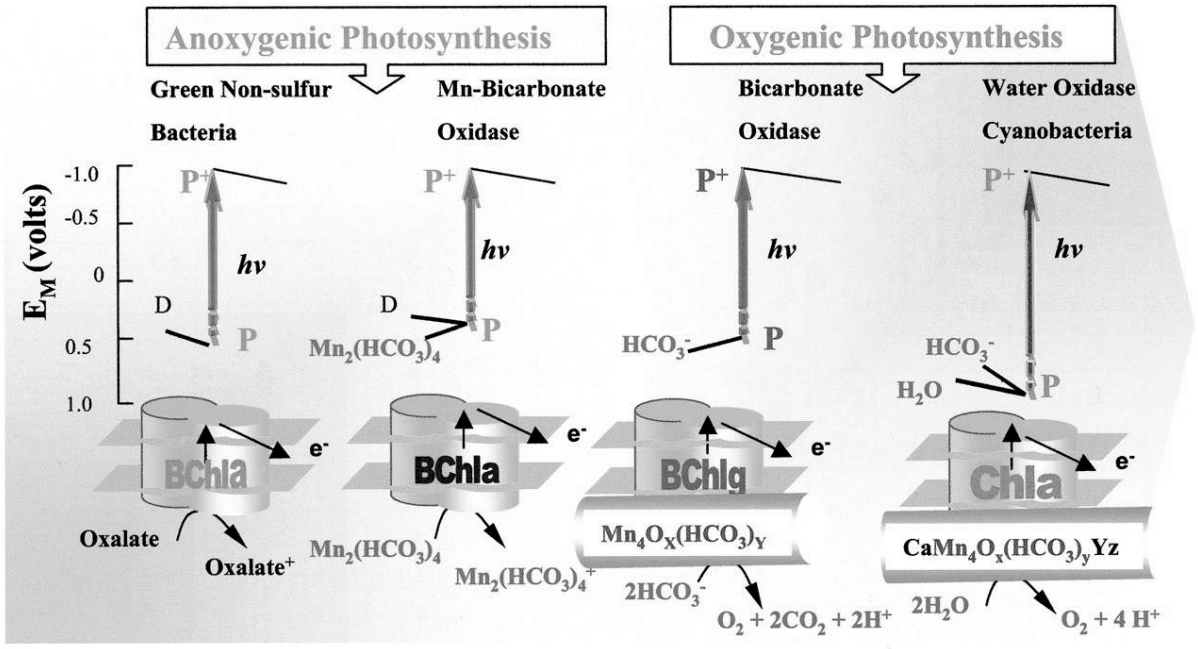

Fig.3. (*Lower*) Proposed evolutionary stages of development of type II bacterial reaction centers towards cyanobacterial (oxygen-evolving) reaction centers in the Archean period. (*Upper*) Electrochemical potentials of the reaction center photooxidant (P) and terminal substrates (D = formate, oxalate, etc.).

In Fig. 3, we give a hypothesis for the sequence of events that may describe how the inorganic core of the WOC-PSII was first created and evolved from anoxygenic bacteria. We suggest that preformed, abiotic dimanganese-bicarbonate clusters served initially as terminal substrates to primitive anoxygenic phototrophs, such as the green nonsulfur bacteria (Mn-bicarbonate oxidase, stage 1). Mn-bicarbonate clusters would have been feasible, although inefficient, electron donors to phototrophic bacteria owing to the mismatch in electrochemical potentials. In the next stage, two features may have been adopted in the Archean period that characterize the ‘‘missing link’’ in evolutionary development: (i) mutations in the reaction center proteins occurred that favored binding of a tetramanganese-bicarbonate cluster derived from the Mn-bicarbonate clusters present in the environment, and (ii) evolution of a higher potential photooxidant, such as BChl-g, the suggested evolutionary precursor pigment to Chl-a (Xiong et al., 2000). These developments would have enabled bicarbonate to serve as an inefficient terminal substrate for the concerted four-electron oxidation to O_2. Hence we call this stage the bicarbonate oxidase stage (Dismukes et al., 2001). The most recent proposed stage of development represents the emergence of cyanobacteria and is denoted as the water oxidase stage. This stage was brought on by the enormous reduction of atmospheric CO_2 in the post-Archean period. Although it is unclear how this transition occurred, it would have required the evolution of a stronger inorganic catalyst and a stronger photooxidant to split water efficiently. We suggest that this developmental stage may correspond to the incorporation of calcium as integral cofactor within the tetramanganese cluster (see Dismukes et al., 2001). The calcium cofactor boosts the electrochemical potential of the tetramanganese

core in the contemporary WOC, thus permitting weaker reductants such as water to serve as terminal substrates. Also, the adoption of a stronger photooxidant such as Chl-a would have greatly increased the quantum efficiency of water oxidation, owing to its considerably higher potential. It is possible that this final stage of development also included the incorporation of basic amino acid residues in the reaction center protein environment to serve as proton acceptors, thus replacing the lost function of BC as hydroxide buffer during assembly of the inorganic core.

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