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Composition and Catalase-Like Activity of Mn(II)–Bicarbonate Complexes

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Abstract—The composition and catalase-like activity of Mn^{2+} complexes with bicarbonate were investigated with voltammetry and kinetic methods (by the rate of O_2 production from H_2O_2). Three linear sections were revealed on the dependence of the reduction potential of Mn^{2+} on logarithm of bicarbonate concentration ($\log C_{NaHCO_3}$) having slopes equal to 0 mV/$\log C_{NaHCO_3}$, –14 mV/$\log C_{NaHCO_3}$, and –59 mV/$\log C_{NaHCO_3}$, corresponding to Mn^{2+} aqua complex (Mn^{2+}_{aq}) and to Mn^{2+}–bicarbonate complexes of the composition $[Mn^{2+}(HCO_3^-)]^+$ (at concentration of HCO_3^- 10-100 mM) and $[Mn^{2+}(HCO_3^-)_2]^0$ (at concentration of HCO_3^- 100-600 mM). Comparison of HCO_3^- concentration needed for the catalase-like activity of Mn^{2+} with the electrochemical data showed that only electroneutral complex $Mn^{2+}(HCO_3^-)_2$ catalyzed decomposition of H_2O_2, whereas positively charged Mn^{2+}_{aq} complex and $[Mn^{2+}(HCO_3^-)]^+$ were not active. The catalase-like activity of Mn^{2+} did not appear upon substitution of anions of carbonic acids (acetate and formate) for HCO_3^-. The rate of O_2 production in the system Mn^{2+}–HCO_3^-–H_2O_2 (pH 7.4) is proportional to the second power of Mn^{2+} concentration and to the fourth power of HCO_3^- concentration that indicates simultaneous involvement of two $Mn^{2+}(HCO_3^-)_2$ complexes in the reaction of H_2O_2 decomposition.

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Key words: manganese, bicarbonate, hydrogen peroxide, catalase activity

Bicarbonate ions (HCO_3^-) are known to be necessary for the maximum activity of photosystem II (PS II) [1]. The stimulating effect of HCO_3^- on PS II has been described in detail for processes taking place on the acceptor side of PS II, where HCO_3^- binds to the non-heme Fe located between Q_A and Q_B and accelerates electron transfer between Q_A^- and Q_B and probably serves as a proton donor for the reduced Q_B [2-4].

Bicarbonate is needed also for stabilization and function of the water-oxidizing complex (WOC) of PS II [1]. After removal of all four manganese ions included in the WOC from subchloroplast membrane preparations of PS II, an effective photooxidation of exogenous Mn^{2+} cations [5-10] and reactivation of oxygen evolution [10-12] was observed only when stoichiometric amounts of Mn^{2+} (two-four Mn^{2+} atoms per reaction center of PS II) were added together with bicarbonate. At the same time, anions of carbonic acids (acetate, formate) had no similar effect. It was suggested that the stimulating effect of bicarbonate on reactivation of electron transfer on the donor side of PS II is related to the formation of easily oxidizable electroneutral Mn–bicarbonate complexes [13] as well as that bicarbonate ions together with Mn^{2+} can bind to PS II having no Mn-cluster (apo-WOC of PS II) to form the ternary complex [Mn–HCO_3^-–apo-WOC] capable of oxidation of other Mn^{2+} ions during reconstitution of Mn_4O_X-center of the WOC [12, 14]. The hypothesis that bicarbonate may be the substrate of oxidation instead of water in PS II was disproved in the recent works [15, 16], though the participation of bicarbonate as a cofactor of the WOC is not excluded.

Upon comparison of the light-induced Fourier-transformed IR spectra of the donor side of the oxygen-evolving preparations of PS II in the presence of $NaH^{12}CO_3$ or $NaH^{13}CO_3$ with the spectrum attributed to the S_1/S_2 transition, it was suggested that bicarbonate acts as a bridge ligand between redox-active Mn and Ca^{2+} within the WOC [17]. Data on the X-ray diffraction with

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Abbreviations: Mn^{2+}_{aq}) aqueous complex of Mn^{2+}; PS II) photosystem II; Q_A and Q_B) primary and secondary quinone acceptors of electrons in photosystem II; WOC) water oxidizing complex; apo-WOC) water oxidizing complex having no Mn-cluster.

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3.5 Å resolution of PS II complex isolated from cyanobacterium also suggests that HCO_3^- may be a bridge ligand between Ca^{2+} and Mn atom denoted in that work as Mn^4 [18], though in the last publication on the X-ray structure analysis of PS II, HCO_3^- ion was not revealed within the WOC [19].

The hypothesis that HCO_3^- binds with Mn^{2+} within the WOC has excited interest in investigation of the complexing of Mn^{2+} and Mn^{3+} with bicarbonate in model systems. In our works [13, 20], the potentials of oxidation of Mn^{2+} to Mn^{3+} in aqueous solutions of bicarbonate, formate, acetate, and oxalate were presented as the function of ligand concentration and compared to the ability of these ligands to stimulate photooxidation of Mn^{2+} with preparations of PS II depleted of Mn. Although all anions of carbonic acids considerably lower the oxidation potential of Mn^{2+}, only bicarbonate accelerates electron transfer from Mn^{2+} to the apo-WOC of PS II [13].

It is also known that Mn^{2+} ions in the presence of HCO_3^- catalyze disproportionation of hydrogen peroxide: $2H_2O_2 \rightarrow 2H_2O + O_2$ [21-26]. This catalase-like activity is apparently related to the formation of Mn^{2+}–bicarbonate complexes [22-26] of presumable composition $Mn^{2+}(HCO_3^-)_2$ [22] or $[Mn^{2+}(HCO_3^-)_3]^-$ [24]. It is known that Mn^{2+} aqua-cations practically do not catalyze the redox decay of H_2O_2 in neutral media [23]. Whether Mn cations within the WOC possess the catalase-like activity is not yet clear [27, 28].

The present work is devoted to determination of the composition and stability constants of Mn^{2+}–bicarbonate complexes with voltammetry as well as to examination of the possible correlation between the composition of the complexes, their catalase-like activity, and the ability of Mn^{2+} to donate electrons to the reaction centers of PS II.

MATERIALS AND METHODS

Electrochemical investigation of the complexes of Mn^{2+} was carried out with voltammetry as described earlier [29]. The dependence of the peak potential (E_p) of reduction of Mn^{2+} to Mn^0 on logarithm of concentration of HCO_3^- was measured on a stationary mercury cathode. Voltammetry curves were registered with a polarograph PA 3 (LP, Czechia) using a three-electrode cell. The working electrode was a hanging mercury drop with a diameter of 0.72 mm, which was renewed before each measurement. The counter electrode was a platinum plate of 1 cm^2 area. The potential was measured versus the saturated calomel electrode and then recalculated versus normal hydrogen electrode. The accuracy of the measurement was 2 mV. A 0.1-M $NaClO_4$ solution was used as a background. Agar (0.005%) was added to the medium for elimination of surface effects. Oxygen was removed from the medium by bubbling with argon before each measurement. The measurements were done at room temperature. The scan rate was 20 mV/sec.

Catalase-like activity was determined by the rate of evolution of oxygen formed upon decomposition of hydrogen peroxide: $2H_2O_2 \rightarrow 2H_2O + O_2$. Oxygen concentration in aqueous solution was measured with amperometric method using a Clark-type oxygen-measuring electrode (Hansatech, England) with the volume of 1 ml at 25°C.

The rate of O_2 evolution was determined by the slope of the kinetic curve of O_2 concentration in aqueous solution observed for 1-3 min after addition of H_2O_2 (or $MnCl_2$) (see further Fig. 2a). The order of the reaction on each component was determined from the dependence of logarithm of the rate of O_2 evolution from H_2O_2 on logarithm of concentration of the component of the reaction. The tangent of the slope of this dependence denoted the order of the reaction on the component, and presumably corresponded to the number of molecules involved in the reaction of H_2O_2 decomposition.

The addition of $NaHCO_3$ shifts the pH of the solution to alkaline side due to hydrolysis of $NaHCO_3$. Therefore after dissolution of $NaHCO_3$ the solutions were titrated by salt acid to the given pH value in the region pH 7.0-8.3. The pH value indicates the ratio of equilibrium concentrations HCO_3^-/H_2CO_3 by the equation:

$$pH = 6.35 + \log([HCO_3^-]/[H_2CO_3]). \qquad (1)$$

Tris-HCl buffer was used to maintain pH.

$NaHCO_3$ was from Merck (USA), Tris from GibcoBRL (USA), and H_2O_2 (3% solution) from Yaroslavskaya Farmfabrika (Russia).

RESULTS

Electrochemical measurements. Mn^{2+} cation is known to exist exclusively as hexaaquacation $[Mn^{2+}(H_2O)_6]^{2+}$ in aqueous solution [30]. In the presence of other ligands, formation of more stable complexes takes place. This process is accompanied by a shift in the potential of the reduction of Mn^{2+} to Mn^0 to the negative side because upon reduction Mn^{2+} is released from the ligands, which requires additional energy.

Lingane's equation was used for the calculations [31, 32]:

$$E_{1/2\,\mathrm{met}} - E_{1/2\,\mathrm{com}} = \frac{RT}{nF}\ln\beta + q\frac{RT}{nF}\ln C_X\,, \qquad (2)$$

where $E_{1/2\mathrm{met}}$ and $E_{1/2\mathrm{com}}$ are the midpoint potentials of the reduction of Mn^{2+}_{aq} and of its complex with the ligand X, respectively; β is the overall stability constant of the complex; C_X is concentration of the ligand X in the solution;

q is the number of ligands in the complex; n is the number of electrons involved in the redox reaction.

The number of ligands (q) in the complex can be found from the slope of the dependence of the reduction potential of Mn^{2+} to Mn^0 on logarithm of ligand concentration according to Eq. (3):

$$\Delta E_{1/2} \Big/ \Delta \log C_X = -q\frac{0.059}{n}. \qquad (3)$$

Upon extrapolation of the dependence to $\log C_X = 0$ ($C_X = 1$ M), the value E^0 of the complex and its β are determined:

$$E^0_{met} - E^0_{com} = \frac{0.059}{n}\log\beta. \qquad (4)$$

The voltage–current curves obtained on the stationary mercury electrode are registered as waves with a peak characterized by an E_p value. For the reversible two-electron process, the difference between E_p and $E_{1/2}$ is equal to 14 mV [33]. We used E_p experimental values instead of $E_{1/2}$ for plotting the dependence on Eq. (3).

It is seen from the dependence of E_p on log$C$ of bicarbonate at pH 8.3 (Fig. 1) that the addition of the first aliquots of bicarbonate does not change the reduction potential of Mn^{2+} to Mn^0. This indicates the presence of only the aquacation of Mn^{2+} in the solution. However, upon the increase in HCO_3^- concentration from 10 to 100 mM, a linear shift of the reduction potential of Mn^{2+} is observed corresponding to formation of a Mn^{2+}–bicarbonate complex. The slope of this section of the dependence of E_p on $\log C_{NaHCO_3}$ equal to −14 mV determines according to Eq. (3) (at $n = 2$) the number of ligands in the complex equal to $q = 0.5$, which indicates the formation of a complex with the ratio metal/ligand equal to 2 : 1. This may correspond to the formation of either a binuclear complex where two Mn^{2+} ions form two bonds with one HCO_3^- anion, i.e. $[Mn_2^{2+}(HCO_3^-)]^{3+}$ (as we suggested earlier [34]), or a mononuclear complex where one Mn^{2+} atom forms two bonds with one HCO_3^- ion, i.e., being a chelate compound where Mn^{2+} with one valency is bound to COO^- and with another – to non-dissociated OH-group within HCO_3^-. The EPR method failed to show the existence of binuclear complexes in the system Mn^{2+}–HCO_3^- [29]. Therefore, the most probable complex with value $q = 0.5$ may be a chelate complex $[Mn^{2+}(HCO_3^-)]^+$. This possibility is supported by EPR data [29] showing that in the complex $Mn^{2+}(HCO_3^-)_2$ one of the two (bi)carbonate ligands is bound in bidentate mode to Mn^{2+} ion, and that one or two water molecules are extruded from hexaaquacation $Mn^{2+}(H_2O)_6$ upon formation of the complex $[Mn^{2+}(HCO_3^-)]^+$. The stability constant β of the complex $[Mn^{2+}(HCO_3^-)]^+$ according to Eq. (4) is 10 M^{-1}.

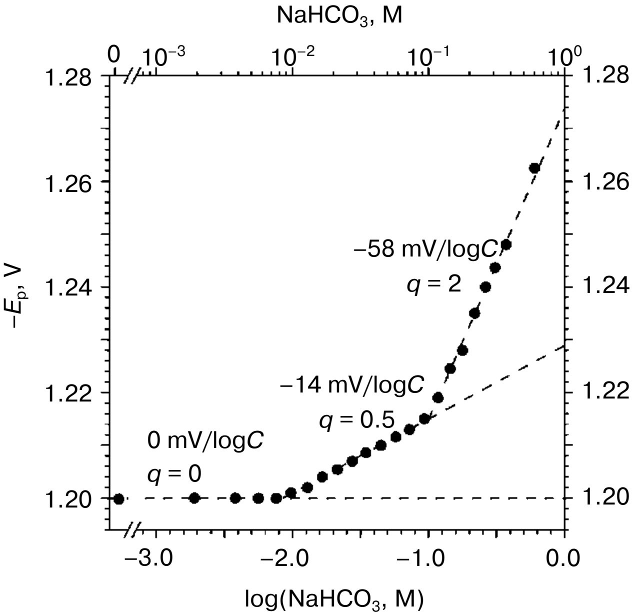


Fig. 1. Dependence of the peak potential of the reduction of Mn^{2+} at the stationary mercury cathode on concentration of $NaHCO_3$ in the medium containing 0.1 M $NaClO_4$, 0.476 mM $MnCl_2$, pH 8.3, at 21.5°C.

Upon further increase in $NaHCO_3$ concentration in the range from 100 to 600 mM, a second section on the dependence of E_p on $\log C_{NaHCO_3}$ is observed with the slope of −59 mV corresponding to $q = 2$ (at $n = 2$), i.e. to the formation of a complex with stoichiometry metal/ligand equal to 1 : 2 – $Mn^{2+}(HCO_3^-)_2$. Formation of the second complex, $Mn^{2+}(HCO_3^-)_2$, can be accounted for coordination of one HCO_3^- ion additionally to originally formed complex: $[Mn^{2+}(HCO_3^-)]^+ + HCO_3^- \leftrightarrow Mn^{2+}(HCO_3^-)_2$. The stability constant β of the complex $Mn^{2+}(HCO_3^-)_2$ according to Eq. (4) is 350 M^{-2}. Therefore, in the system Mn^{2+}–HCO_3^- in aqueous solution at pH 8.3, two species of the complexes are sequentially formed: in the range of HCO_3^- concentration from 0.01 to 0.1 M – a positively charged chelate complex $[Mn^{2+}(HCO_3^-)]^+$ and at $[HCO_3^-] > 0.1$ M – an electroneutral complex $Mn^{2+}(HCO_3^-)_2$. Similar results were obtained also at pH 7.4 in 50 mM Tris-HCl. In the presence of Tris, the dependence of the reduction potential of Mn^{2+} on the logarithm of HCO_3^- concentration also had three linear sections with the slopes of 0 mV/$\log C_{NaHCO_3}$ (at $NaHCO_3$ concentration in the range 0-25 mM), −14 mV/$\log C_{NaHCO_3}$ (at 25-90 mM $NaHCO_3$), and −59 mV/$\log C_{NaHCO_3}$ (at $NaHCO_3$ concentration higher than 90 mM) (data not presented), corresponding to the formation of aqua complex $[Mn^{2+}(H_2O)_6]^{2+}$ and of two bicarbonate complexes $[Mn^{2+}(HCO_3^-)]^+$ and $[Mn^{2+}(HCO_3^-)_2]^0$. The stability constants were equal to 6 M^{-1} for the complex $[Mn^{2+}(HCO_3^-)]^+$ and 240 M^{-2} for

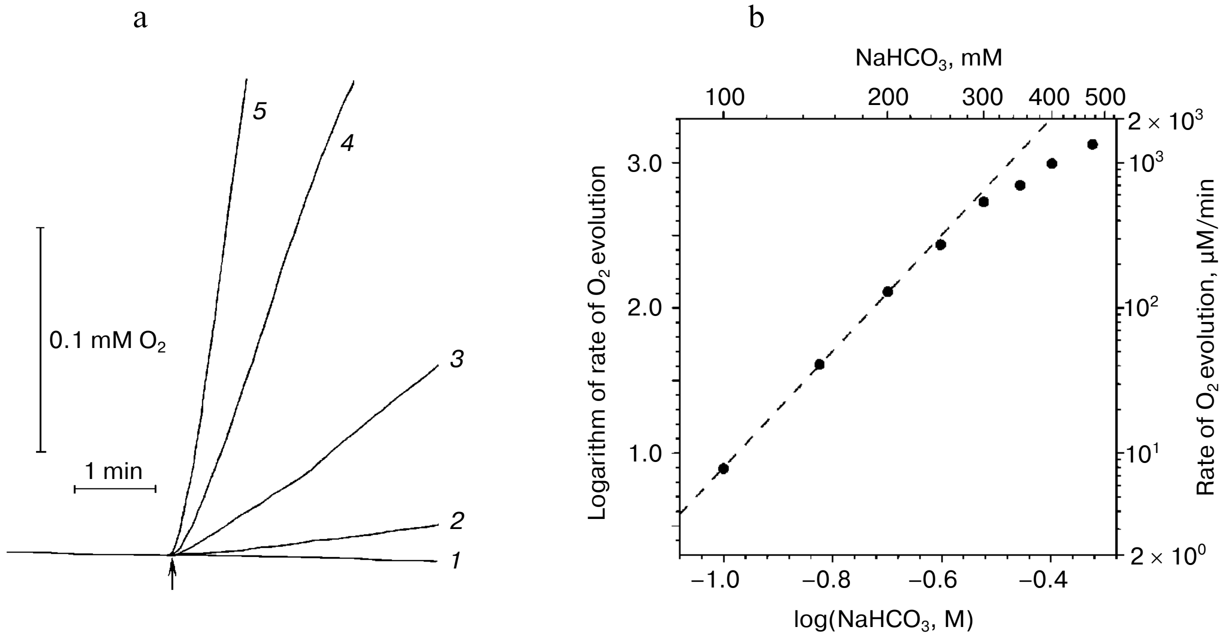


Fig. 2. a) Kinetic curves of oxygen concentration change in medium containing 10 mM H_2O_2, 10 µM $MnCl_2$, and $NaHCO_3$ at concentration (M) of: 0 (*1*), 0.1 (*2*), 0.15 (*3*), 0.2 (*4*), and 0.25 (*5*); ionic strength of the solution $I = 0.475$ ($NaHCO_3$ + $NaClO_4$); t = 25°C. The arrow shows the moment of the addition of 10 µM $MnCl_2$. b) Dependence of logarithm of the rate of O_2 evolution from H_2O_2 on logarithm of $NaHCO_3$ concentration in medium containing 10 µM $MnCl_2$ and 10 mM H_2O_2; ionic strength of the solution $I = 0.475$ ($NaHCO_3$ + $NaClO_4$); t = 25°C. The line corresponds to the function $y = ax^4$ in logarithmic scale.

the complex $[Mn^{2+}(HCO_3^-)_2]$. The lower values of the stability constants in Tris-HCl buffer (pH 7.4) as compared with those in non-buffered medium (pH 8.3) may be accounted for by competitive binding of Tris to Mn^{2+} as well as for the decrease in the equilibrium concentration of HCO_3^- upon the increase of the acidity of the medium.

Catalase-like activity. Figure 2a shows the kinetic curves of O_2 evolution in medium containing H_2O_2 and $MnCl_2$. In the absence of $NaHCO_3$ (curve *1*), O_2 evolution is not observed, which indicates the absence of the catalase-like activity of aqua complex Mn^{2+} (in agreement with literature data [22]). O_2 evolution starts to be registered only upon the increase in $NaHCO_3$ concentration up to 0.1 M (curve *2*), and upon further increase in $NaHCO_3$ concentration, the rate of O_2 evolution from H_2O_2 increases (curves *3*-*5*).

Figure 2b shows the dependence of logarithm of the rate of O_2 production from H_2O_2 on logarithm of $NaHCO_3$ concentration in the presence of 10 µM Mn^{2+}. The slope of this dependence shows that the order of the catalase-like reaction with respect to HCO_3^- is equal to 4 in the range of $NaHCO_3$ concentration from 100 to 300 mM. The order of the reaction with respect to HCO_3^- equal to 4 was observed both at pH 8.3 (Fig. 2) and at pH 7.4 (data not presented). The deviation from the theoretical dependence at high (>0.3 M) concentration of $NaHCO_3$ (Fig. 2) is probably related to the appearance of complex $MnCO_3$, which is experimentally confirmed with the formation of insoluble deposit in solution. The formation of $MnCO_3$ at high concentration of $NaHCO_3$ was noted earlier as well [22].

In Fig. 3, the dependence of the rate of O_2 evolution from H_2O_2 on logarithm of $MnCl_2$ concentration is shown in the presence of 20 mM bicarbonate at pH 7.4 (plot *1*). At concentrations of $MnCl_2$ from 0.1 to 1 mM, the reaction order with respect to Mn^{2+} is close to 2. The observed deviation from the theoretical curve at $[Mn^{2+}] > 1$ mM may be accounted for by the formation of insoluble $MnCO_3$. At pH 8.3, the order of the catalase-like reaction with respect to Mn^{2+} is equal to 1 (Fig. 3, plot *2*), and at pH 8.0 the order of the reaction is equal to 1.4 (not shown).

The rate of the studied catalase-like reaction depends on pH and on the composition of buffer solution. Figure 4 shows the dependence of the rate of O_2 evolution in the systems Mn^{2+}–HCO_3^-–H_2O_2 (curve *1*) on pH of the solution. At pH 6.5, the catalase-like activity of Mn^{2+}–bicarbonate complexes is negligible and appears only at concentration of Mn^{2+} higher than 1 mM (not shown). In the absence of buffer at pH 7.0-7.4, the catalase-like activity is low (2-4 µM of O_2/min); in the range of pH from 7.4 to 8.0 it increases approximately exponentially (up to 30 µM of O_2/min at pH 8.0); at pH > 8.0 the increase in the catalase-like activity decelerates, reaching a plateau (100 µM of O_2/min) at pH 8.5-9.0.

The presence of 50 mM Tris enhances the rate of O_2 production 3-40 times depending on pH. And the maximum effect of Tris is observed at pH 8.0 (Fig. 4, curve *2*).

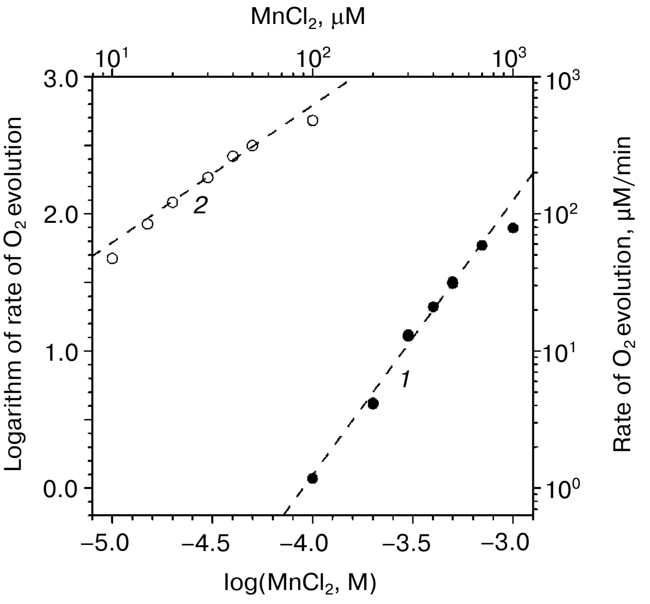


Fig. 3. Dependence of logarithm of the rate of O_2 evolution from H_2O_2 on logarithm of concentration of $MnCl_2$ in medium containing: *1*) 20 mM $NaHCO_3$, 100 mM Tris-HCl, pH 7.4, 2 mM H_2O_2; the line corresponds to the function $y = ax^2$ in logarithmic scale; *2*) 100 mM Tris-HCl, pH 8.3, 100 mM $NaHCO_3$, 2 mM H_2O_2; the line corresponds to the function $y = bx^1$ in logarithmic scale; t = 25°C.

At pH > 8.0, decrease in the rate of O_2 production from H_2O_2 is observed. Other buffers (HEPES, MES, phosphate) inhibited the catalase-like activity of the system Mn^{2+}–HCO_3^-: 0.2 M MES at pH 8.3 – by 30%; 0.2 M HEPES at pH 8.3 – by 70%. In our experiments, we did not observe the catalase-like activity of Mn^{2+} in the presence of acetate and formate ions in medium containing 50 mM sodium acetate or sodium formate, 20-100 μM $MnCl_2$, 100 mM Tris-HCl, pH 8.0.

DISCUSSION

It was shown earlier that aqua complex of Mn^{2+} does not catalyze redox decomposition of H_2O_2 in neutral aqueous media [22]. In the presence of bicarbonate, Mn^{2+} ions possess high catalase-like activity (the highest among low-molecular-weight complexes of Mn^{2+}) [21-24]. Nevertheless, the question about what is responsible for the ability of bicarbonate to stimulate decomposition of H_2O_2 with O_2 evolution in the presence of Mn^{2+} remains unresolved.

It was shown in the present work that the addition of $NaHCO_3$ to the solution of Mn^{2+} brings on successive formation of the chelate complex $[Mn^{2+}(HCO_3^-)]^+$ (in the range of $NaHCO_3$ concentration from 10 to 100 mM) and of the complex $Mn^{2+}(HCO_3^-)_2$ (at $[HCO_3^-] \geq 0.1$ M). It was also shown that the catalase-like activity of Mn^{2+} starts to be observed only at concentrations of $NaHCO_3$ higher that 0.1 M. Thus the comparison of the electrochemical data with the data on the dependence of the catalase-like activity of Mn^{2+} on $NaHCO_3$ concentration shows that only electroneutral complex $Mn^{2+}(HCO_3^-)_2$ is a catalytically active complex, whereas Mn^{2+}_{aq} and $[Mn^{2+}(HCO_3^-)]^+$ are not active.

The rate of O_2 evolution in the system Mn^{2+}–HCO_3^-–H_2O_2 (pH 7.4) is proportional to the second order of Mn^{2+} concentration and to the fourth order of HCO_3^- concentration, which may indicate the involvement of two molecules of the complex $Mn^{2+}(HCO_3^-)_2$ in the process of transfer of two electrons from one H_2O_2 molecule to another. We first determined the value of the reaction order with respect to Mn^{2+} equal to 2 and that with respect to HCO_3^- equal to 4. Earlier these values were determined to be equal to 1 and 2, respectively [21]. The dependence of the catalase-like activity on pH shows that, in subacidic media (at pH 6.5-7.0), the catalase-like activity is negligible (probably owing to the alkalinity of the solution to be too low to provide binding of two H^+ produced during H_2O_2 decomposition). Probably due to this cause, the order of the reaction on Mn^{2+} at pH 7.0 in work [21] was determined to be equal to 1 and that on HCO_3^- equal to 2. According to Stadtman and coauthors [24], the value of the reaction order with respect to Mn^{2+} is equal to 1 and that with respect to HCO_3^- is equal to 3 (pH 7.4-7.8). It is noted in work [21] that beginning from pH 7.6, $MnCO_3$ is formed in solution, which is not active

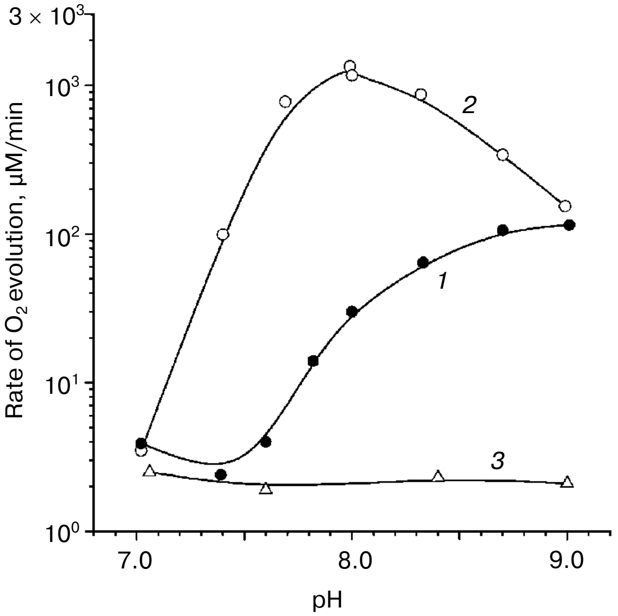


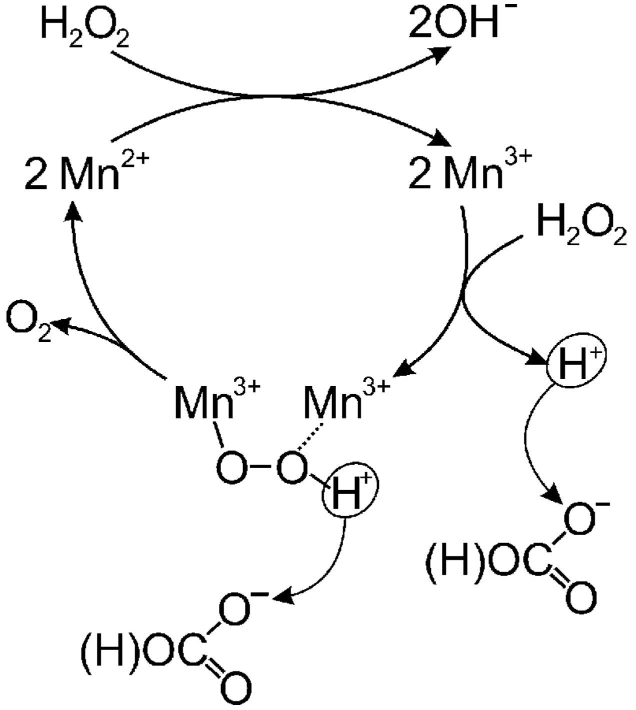
Fig. 4. Dependence of the rate of O_2 evolution from H_2O_2 on pH in medium containing 100 μM $MnCl_2$, 50 mM $NaHCO_3$, 0.5 M $NaClO_4$, and 10 mM H_2O_2 in the absence (*1*) or presence (*2*) of 50 mM Tris-$HClO_4$, as well as (*3*) in the absence of HCO_3^- and Tris; t = 25°C.

in the reaction of H_2O_2 decomposition. Thereby, as we suggest, the proportionality of the catalase-like activity to the concentration of Mn^{2+}–HCO_3^- complexes is disturbed, which probably brings about the reaction order with respect to Mn^{2+} close to 1 in work [24] and in the present work at pH 8.3 and pH 8.0. It is worth note that the reaction order with respect to HCO_3^- in our experiments was 4 both at pH 7.4 and at pH 8.3. This may indicate simultaneous involvement of two complexes $Mn^{2+}(HCO_3^-)_2$ in the reaction both at pH 7.4 and at pH 8.3 because the attachment of four HCO_3^- ligands to one Mn^{2+} ion seems unlikely.

In living organisms, decomposition of H_2O_2 is catalyzed by heme-containing catalase. It is known that the Fe^{3+} atom of heme forms a complex with one of the H_2O_2 molecules in catalytic cycle, which interacts with the second H_2O_2 molecule with subsequent formation of O_2 [33]. Some bacteria (*Lactobacillus plantarum* [35] and *Thermus thermophilus* [36]) have catalase containing in the catalytic center two Mn atoms linked by a carboxylic group of Glu70 residue as well as by oxygen atoms belonging to hydroxyl ion and to a water molecule, with the distance between Mn atoms equal to 3.14-3.18 Å [36]. The enzyme consists of six subunits with molecular weights equal to 34 kD. It is suggested [30, 37] that the interaction with the first H_2O_2 molecule transfers both Mn from the Mn^{2+}-Mn^{2+} state to the Mn^{3+}-Mn^{3+} state in which an oxo (or peroxo) bridge arises between the two Mn^{3+}. Interaction with the second H_2O_2 molecule brings to O_2 evolution and transfer of Mn to Mn^{2+}-Mn^{2+} state. It is suggested [30, 37] that one of the amino acid residues of the protein serves as a proton acceptor upon dissociation of a hydrogen peroxide molecule and as a proton donor upon formation of a water molecule.

According to contemporary concepts about the mechanism of H_2O_2 disproportionation in the presence of bicarbonate complexes of Mn^{2+} in aqueous solutions, Mn ions serve as an intermediate in electron transfer between two H_2O_2 molecules; it is also suggested that Mn reversibly takes on the oxidation states +2, +3, and +4 [23, 26]. Free radicals $O_2^{\cdot-}$, $^{\cdot}OOH$, and $^{\cdot}OH$ are probably included in the intermediates of H_2O_2 transformation (which distinguishes this reaction from the enzymatic one). It is suggested that, at the first stage of catalysis, the oxidation of Mn^{2+}–bicarbonate complex with a molecule of hydrogen peroxide occurs to produce a bicarbonate complex of Mn^{3+} [26] or Mn^{4+} [23]. In the following stages, complexes of Mn^{3+} or Mn^{4+} are reduced with the second H_2O_2 molecule to produce Mn^{2+}.

In the present work, in agreement with contemporary concepts [23, 26], we suggest that the primary act initiating H_2O_2 decomposition will be the oxidation of two Mn^{2+} ions to Mn^{3+} accompanied with the reduction of H_2O_2 to $2OH^-$ (see Scheme). At pH 7.40-8.35, the potential of two-electron reduction of H_2O_2 to H_2O and OH^- is equal to 1.14-1.12 V (versus the potential of the normal hydrogen electrode) [36]. Because the potential of one-electron oxidation of Mn^{2+} to Mn^{3+} is equal to 1.19 V in the range of pH 5.0-8.5 [13], the redox reaction between H_2O_2 and Mn^{2+} is suppressed. This explains the absence of catalytic activity of aqua complex Mn^{2+}_{aq}. Thus, the first necessary condition for participation of Mn^{2+} in the redox decay of H_2O_2 is lowering of the oxidation potential of Mn^{2+} owing to the complexing reaction.



Proposed mechanism of H_2O_2 decomposition with the involvement of Mn^{2+}–bicarbonate complexes (HCO_3^- ligands not shown)

Scheme

In our previous work [13] it was shown that the addition of HCO_3^- in the range of concentration from 3 to 63 mM brings linear lowering of the oxidation potential of aqua complex $[Mn^{2+}(H_2O)_6]^{2+}$ versus the logarithm of the bicarbonate concentration. Extrapolation of that dependence to the zero value of the logarithm of bicarbonate concentration gives the value of the standard potential (E^0) equal to 0.67 V for the reaction of Mn^{2+} oxidation in the presence of bicarbonate: $Mn^{2+} - e + 3HCO_3^- \leftrightarrow Mn^{3+}(HCO_3^-)_3$. In the same work, the possibility of successive formation of two bicarbonate complexes of Mn^{2+} was shown, and their standard oxidation potentials are 0.61 and 0.52 V. Thus in the presence of bicarbonate, the oxidation of Mn^{2+} with hydrogen peroxide becomes thermodynamically possible, which may explain the appearance of the catalase-like activity of Mn^{2+} in the presence of bicarbonate. On the other hand, as follows from our data [13] anions of carbonic acids (acetate, formate) as well as bicarbonate are able to shift the oxidation potential of Mn^{2+} up to values 0.69 V for acetate and 0.77 V for formate at which Mn^{2+} could become an electron donor for H_2O_2. However, as the data presented in this work show, only bicarbonate complex $Mn^{2+}(HCO_3^-)_2$, but not $[Mn^{2+}(CH_3COO^-)]^+$, $[Mn^{2+}(HCOO^-)]^+$ or $[Mn^{2+}(HCO_3^-)]^+$, possess the catalase-like activity. Hence, a suggestion appears that, for exhibition of the catalase-like activity of Mn^{2+}, besides the lowering of its oxidation potential it is necessary for a Mn^{2+}-complex to have no positive charge but to be electroneutral.

We suggest that the second stage of the catalysis of H_2O_2 disproportionation consists of the interaction of two formed Mn^{3+}–bicarbonate complexes with the second H_2O_2 molecule with formation of O_2 and regeneration of Mn^{2+}–bicarbonate complex (see Scheme). In an earlier proposed scheme [23], it is denoted that a deprotonated HO_2^- species undergoes oxidation. However, it is known that the exponent of dissociation constant (pK_a) for H_2O_2 is equal to 11.63 [38], therefore the formation of a complex of Mn^{2+} with HO_2^- is unlikely. More likely is that not Mn^{2+}, but Mn^{3+} (for which pK_a value of hydrolysis is equal to 1) is able to cause deprotonation of a H_2O_2 molecule and to attach it as a ligand with formation of a complex $Mn^{3+}(HCO_3^-)_2(OOH^-)$. For subsequent evolution of O_2, deprotonation of the OOH^--group is needed which, as follows from our data on the involvement of two Mn ions in the reaction, is accomplished with the second molecule of Mn^{3+}–bicarbonate complex. The simultaneous oxidation of $^-OO^-$ with two Mn^{3+} ions will lead to the evolution of O_2 and to the release of two protons causing the dependence of the catalase-like activity on pH. We suggest that bicarbonate herein plays the role of an acceptor of protons, having $pK_{a1} = 6.3$ and $pK_{a2} = 10.3$ [39] and so promoting the oxidation of H_2O_2, unlike acetate (pK_a = 4.8), formate ($pK_a = 3.7$), and MES ($pK_a = 6.1$) unable to attach protons at pH 7.4-9.0. It remains possible that a polarizing effect of Mn^{3+} ion lowers pK_{a2} value of bound bicarbonate to 8-9; this may explain the existence of the catalase-like activity of Mn–(bi)carbonate complexes at pH 7.4-9.0. The catalase-like activity of Mn^{2+}-complexes will increase in the presence of additional acceptors of protons in solution, which is indeed observed upon the addition of Tris having pK_a 8.1 (Fig. 4). Inhibition of the catalase-like activity in the presence of other buffer systems occurs probably due to reduction of Mn^{3+} ions with buffer molecules (MES, HEPES) or due to formation of insoluble compounds with Mn^{2+}/Mn^{3+} (phosphate). It is suggested [30, 37] that, in Mn-containing catalase from thermophilic bacterium *Thermus thermophilus*, the role of ligands containing a nitrogen atom with a lone electron pair consists of the reversible binding of protons like the proximal histidine of heme catalase [40].

Thus we suggest that the appearance of the catalase-like activity of Mn^{2+} upon the addition of bicarbonate ions is due to formation of an electroneutral complex $Mn^{2+}(HCO_3^-)_2$ and to the lowering of the oxidation potential of Mn^{2+} (as a result of which Mn^{2+} becomes capable of redox interaction with H_2O_2), as well as to the presence of the proton-binding properties in bicarbonate.

The comparison of the data obtained in the present work with the experiments on the effect of bicarbonate on the activity of PS II of plants [5-13] suggests which of complexes of Mn with bicarbonate can be responsible for the stimulation by bicarbonate of the electron donation from Mn^{2+} to manganese-depleted preparations of PS II and for the exhibition of the catalase-like activity of Mn^{2+}. It was earlier shown [5-13] that the electron donation from Mn^{2+} to the reaction center of PS II is stimulated by concentrations of HCO_3^- (20 µM-5 mM) at which, as follows from the electrochemical data (Fig. 1 in this work), a prior formation of Mn^{2+}–bicarbonate complexes does not occur. That means that the electron donation to the reaction center of PS II occurs upon the oxidation of aqua complex Mn^{2+}_{aq} to Mn^{3+} in the presence of bicarbonate. In this case the role of bicarbonate may consist of binding to Mn^{3+} formed as a result of the photooxidation of Mn^{2+} in the apo-WOC of PS II which leads to the lowering of the oxidation potential of Mn^{2+}: $Mn^{2+} - \bar{e} + 3HCO_3^- \leftrightarrow Mn^{3+}(HCO_3^-)_3$, or in binding of protons released as a result of oxidation of water [13, 41]. As is known [13], the complexes of Mn^{3+} have significantly higher stability constants compared with the complexes of Mn^{2+}. This may explain the requirement of HCO_3^- at low concentrations (20 µM-5 mM) for exhibition of the stimulating effects in the WOC. And the catalase-like activity is shown only by the complex $Mn^{2+}(HCO_3^-)_2$ formed at $[HCO_3^-] \geq 0.1$ M.

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