Mechanistic Studies on the Oxidation of Glyoxylic and Pyruvic Acid by a $[Mn_4O_6]^{4+}$ Core in Aqueous Media: Kinetics of Oxo-Bridge Protonation

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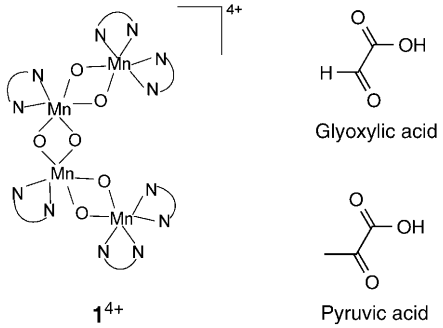
In aqueous media (pH 2.5–6.0), the Mn^{IV} tetramer $[Mn_4(\mu\text{-}O)_6(bipy)_6]^{4+}$ (**1**$^{4+}$; bipy = 2,2′-bipyridine) oxidizes both glyoxylic and pyruvic acid to formic and acetic acid, respectively, under formation of CO_2. Kinetics studies suggest that the species **1**$^{4+}$, its oxo-bridge protonated form [**1**H]$^{5+}$, *i.e.*, $[Mn_4(\mu\text{-}O)_5(\mu\text{-}OH)(bipy)_6]^{5+}$, the reducing acids (RH) and their conjugate bases (R^-) all take part in the reaction. The oxo-bridge protonated oxidant [**1**H]$^{5+}$ was found to react much faster than **1**$^{4+}$. Thereby, the gem-diol forms of the α-oxo acids (especially in the case of glyoxylic acid) are the possible reductants. A one-electron/one-proton electroprotic mechanism operates in the rate-determining step.

Introduction. – The unique manganese-oxo aggregate (OEC) present in photosystem II (PS-II) catalyzes the light-driven oxidation of H_2O to O_2 [1–6] resulting in the O_2-rich atmosphere encountered on Earth. The OEC cycles through five redox states, S_0 – S_4, the index of which refer to the number of oxidizing equivalents stored [7][8]. The overall four-electron (4 e^-) oxidation of two H_2O molecules leading to O_2 is associated with transfer of four H^+: $2\,H_2O \rightarrow O_2 + 4\,H^+ + 4\,e^-$.

Ligands derived from H_2O (O^{2-} or OH^-) are present as bridges between Mn-atoms along with carboxylato moieties in the catalytic site [9–11]. Successive redox reactions at the Mn site are definitely associated with a substantial change in the oxo-bridge basicity [1][9][10], and it is likely that a change in the protonation state of the bridged metal cluster also occurs [12]. The observed decrease in the exchange coupling between Mn^{IV} in a model tetranuclear system [13][14], resulting from protonation of the oxo bridges, has important implications in interpreting the changes in magnetic behavior of the OEC upon S-state advancement and changes in configuration. Oxo-bridge protonations also cause a substantial increase in the Mn–Mn distance in multinuclear Mn complexes, and an increase in reduction potential [14–18]. Besides these physical effects, investigations on chemical aspects resulting from oxo-bridge protonation have hardly been studied, expect in a few reports where it was observed that catalase activity of $[Mn(salpn)(\mu\text{-}O)]_2$ (salpn = 1,3-bis(salicylideneamineto)propane) is inhibited by a single protonation on the oxo-bridge [18], whereas disproportionation of a $(Mn^{III})_2$ complex requires oxo-bridge protonation [19]. It is also of note that oxo-bridge protonation in multinuclear higher-valent Mn complexes sometimes leads to cluster breakup [16][20], rendering their redox chemistry H^+-coupled. The acid–base chemistry resulting from oxo-bridge protonation is also well-studied, along with the kinetic stabilities of oxo bridged species [15].

The complex investigated, $[Mn_4(\mu\text{-}O)_6(bipy)_6]^{4+}$ ($\mathbf{1}^{4+}$; bipy = 2,2′-bipyridine), was originally prepared by *Girerd* and co-workers [16]. On the basis of the oxidation states of its metal centers, $\mathbf{1}^{4+}$ formally corresponds to an S_3 or S_4 state of OEC [2], and is stable in aqueous solution. The one-electron-reduced, mixed-valent $(Mn^{IV})_3Mn^{III}$ form of $\mathbf{1}^{4+}$ is one of the best EPR spectroscopic models for the S_2 state [16]. Mechanistic studies of the reduction of $\mathbf{1}^{4+}$, thus, appeared interesting.



$\mathbf{1}^{4+}$

The present investigation deals with the kinetics and mechanistic aspects of the oxidation of two small carboxylic acids, glyoxylic acid (= oxoacetic acid) and pyruvic acid (= 2-oxopropanoic acid), which are of biological relevance [21–23]. Examples of the oxidation of such α-oxo carboxylic acids by metal oxidants are not too scarce [24]. Interestingly, these acids and their anions co-exist in equilibrium with their hydrated geminal-diol forms in aqueous solution [25][1]). However, so far, not even a single report is available in the literature that clearly describes the actual species involved in the redox process, if not apportioned. An attempt has been made through this investigation to search the mechanistic pathways of the oxidation of these substrates and to detect the possible species involved in the reduction of the title Mn complex. In this study we also gained strong kinetic evidence of oxo-bridge protonation. The reactivity of the oxo-bridge-protonated oxidant is much higher than that of its conjugate base in oxidizing the title reducing agents. We further note that most of the Mn enzymes, including PS-II, work in combination with radicals [4], and in this study, we will show that the title Mn complex reacts with the α-oxo acids *via* one-electron steps generating acyl radicals.

Results and Discussion. – *Equilibrium Measurements.* The measured pK_a values of glyoxylic and pyruvic acid are 3.13 and 2.50 in H_2O, and 3.40 and 2.75 in 95% D_2O, respectively. These values are the averages of at least ten independent measurements, the experimental uncertainty being well within 0.10 pK_a unit. These values compare well with the literature data [26][27] (*Table 1*). Intuitively, one would expect that, due to the electron-pushing effect of the Me group, glyoxylic acid is the stronger acid than pyruvic acid, just as is the case with formic *vs.* acetic acid. The observed reversal in the expected order of acid strength (*Table 1*) is in line with the predominant hydra-

[1]) The hydration constants of the reducing species as found in [25] are as follows: glyoxylic acid, 1.0×10^2–1.4×10^3; glyoxylate, 6.5–260; pyruvic acid, 0.61–3.1; pyruvate, 6.4×10^{-2} – 0.21.

tion of glyoxylic acid in aqueous solution [25], which leads to the gem-diol species $HC(OH)_2CO_2H$; the corresponding hydro form is, at best, weak in the case of pyruvic acid [25]. Interestingly, we note that progressive replacement of the α-H-atom of acetic acid (AcOH) increases the acidity of the species thus formed. The pK_a values of CH_3CO_2H, $CH_2(OH)CO_2H$, and $HC(OH)_2CO_2H$ are 4.57 [28], 3.62 [28], and 3.13 (this work), all values referring to 25.0° and an ionic strength I of 1.0M. It may, thus, be concluded that dissociation mainly occurs from the hydrated form of glyoxylic acid, but from the regular (non-hydrated) form of pyruvic acid. The two OH groups in $HC(OH)_2CO_2H$ possibly make the bonded C-atom less electropositive, and the pK_a value is, therefore, a strong indication of the diol form.

Table 1. *p*K_a *Values of Glyoxylic and Pyruvic Acid in Aqueous Media.* $T = 25.0°$; ionic strength I in M.

Reducing agent	H_2O	Ref.	D_2O/H_2O 95:5
Glyoxylic acid	3.13 ± 0.10[a])	t.w.[a])	3.40 ± 0.10[a])
	2.91 ($I = 1.0$)	[26a]	
	3.20 ($I = 1.0$)	[24h]	
	2.98 ($I = 0.5$)	[26b]	
	3.46 ($I = 0$)	[26c]	
Pyruvic acid	2.50 ± 0.10[a])	t.w., [24i]	2.75 ± 0.10[a])
	2.35 ($I = 0.5$)	[27]	
	2.60 ($I = 0$)	[26c]	

[a]) This work (see text and *Exper. Part*).

Stoichiometric Analyses. Quantitative ($96 \pm 6\%$) evolution of CO_2 (in accordance with *Eqns. 1* and *2*) was found in both the α-oxo acid oxidations, along with HCO_2H and CH_3CO_2H, respectively, for the redox reaction of glyoxylate and pyruvate. These stoichiometric results, thus, confirm that the reducing species are, overall, two-electron-transfer agents. The likely Mn^{II} species under the experimental conditions are $[Mn^{II}(bipy)]$ complexes [29]. The UV/VIS spectra of the product solutions were superimposable to that of a mixture of $Mn(NO_3)_2$ and bipy under the reaction conditions. Thus, the oxidation of glyoxylic and pyruvic acid is represented by:

$$\mathbf{1}^{4+} + 4\ HC(O)COOH + 4\ H^+ \rightarrow 4\ Mn^{2+} + 6\ bipy + 4\ CO_2 + 4\ HCOOH + 2\ H_2O \quad (1)$$

$$\mathbf{1}^{4+} + 4\ MeC(O)COOH + 4\ H^+ \rightarrow 4\ Mn^{2+} + 6\ bipy + 4\ CO_2 + 4\ MeCOOH + 2\ H_2O \quad (2)$$

Kinetics. In the presence of excess reducing agent, the reactions followed excellent first-order kinetics for at least four half-lives; and any initial drop in absorbance for faster reactions could be estimated by the observed first-order rate constants k_0 (*Table 2*) and the estimated time difference (*ca.* 2 s) between mixing of the reagents and UV/VIS measurement in the photometer's kinetics mode. The following changes in reaction conditions had, within experimental uncertainty, no effect on the observed first-order rate constants: presence or absence of dissolved O_2, diffuse light, change in monitoring wavelength in the range 380–530 nm. At a fixed reducing-agent concentration, k_0 increased with increasing acidity for the oxidation of both reducing agents

Table 2. *Some Representative First-Order Rate Constants for the Oxidation of Glyoxylic and Pyruvic Acid by the Title Complex*. Conditions: $[\mathbf{1}^{4+}]=0.10$ mM, $T=25.0°$, $I=1.0$M ($NaNO_3$). Values in parentheses were calculated from *Eqn. 10* with the rate constants reported in *Table 3*. C_{bipy} ($=[(bipy)H)^+]+[bipy]$) and T_R ($=RH+R^-$) in mM.

Glyoxylic acid				Pyruvic acid			
pH	T_R	C_{bipy}	$10^3\,k_0$ [s^{-1}]	pH	T_R	C_{bipy}	$10^4\,k_0$ [s^{-1}]
2.89	2.0	3.0	110 (103)	2.54	2.0	3.0	172 (181)
3.07	2.0	3.0	78.0 (79.3)	3.29	2.0	3.0	44.7 (42.5)
3.60	2.0	3.0	32.0 (33.4)	3.73	2.0	3.0	16.9 (17.0)
4.23	2.0	3.0	9.91 (10.2)	4.09	2.0	3.0	8.44 (8.20)
4.48	2.0	3.0	6.91 (6.47)	4.50	2.0	3.0	4.14 (3.91)
5.14	2.0	3.0	2.69 (2.50)	5.33	2.0	3.0	1.65 (1.55)
5.69	2.0	3.0	1.77 (1.45)	5.92	2.0	3.0	1.21[a]) (1.25)
5.96	2.0	3.0	1.45 (1.52)	4.03	4.0	3.0	18.5 (18.5)
5.18	4.0	3.0	5.09 (4.80)	4.00	6.0	3.0	28.5 (29.4)
5.18	6.0	3.0	7.71 (7.20)	4.04	8.0	3.0	34.1 (36.2)
5.19	8.0	3.0	9.88 (9.50)	4.04	10	3.0	43.6 (45.2)
5.19	10	3.0	12.0 (11.9)	4.14	2.0	1.0	8.02 (7.44)
5.49	2.0	1.0	1.95 (1.87)	4.12	2.0	10	8.18 (7.74)
5.43	2.0	3.0	1.95[b]) (1.94)	4.11	2.0	30	8.24 (7.89)
5.50	2.0	10	1.96 (1.85)	4.10	2.0	60	8.46 (8.04)
5.51	2.0	30	1.98 (1.84)	3.63	2.0	3.0	30.3[c])
5.24	2.0	3.0	4.38[c])	3.54	2.0	3.0	59.7[d])
5.26	2.0	3.0	6.29[d])				

[a]) $10^4\,k_0=1.24$ s^{-1} in the absence of added bipy. [b]) $10^3\,k_0=1.91$ s^{-1} in the absence of added bipy. [c]) $I=0.5$M ($NaNO_3$). [d]) $I=0.1$M ($NaNO_3$).

(*Figure*). Both reactions show good linear plots of k_0 *vs.* T_R, and there was no T_R-independent term in either redox process[2]). Interestingly, both the reactions are independent on C_{bipy} ($=[(bipy)H)^+]+[bipy]$) in the range 1–60 mM (*Table 2*). Thus, the weak influence of the concentration of the 2,2′-bipyridine ligands on the reaction rates refutes the importance of the equation equilibrium (*Eqn. 3*):

$$[Mn_4(\mu\text{-}O)_6(bipy)_6]^{4+}+H^+ +2\ H_2O \rightarrow [Mn_4(\mu\text{-}O)_6(bipy)_5(H_2O)_2]^{4+}+[(bipy)H]^+ \quad (3)$$

The observed dependence of k_0 on H^+ could not be fitted satisfactorily to any type of reaction with only one protic equilibrium (*Eqns. 4* or *5*). Both need to be considered simultaneously, as outlined in *Eqns. 6–9*. Thereby, the approximation $1>>K_1[H^+]$ was made, which leads to the rate-law presented in *Eqn. 10* (where RH is glyoxylic or pyruvic acid).

$$\mathbf{1}^{4+} + H^+ \overset{k_1}{\rightleftharpoons} [\mathbf{1}H]^{5+} \quad (4)$$

[2]) Convention: $T_R=[RH]+[R^-]$, where RH are the reducing agents (acids).

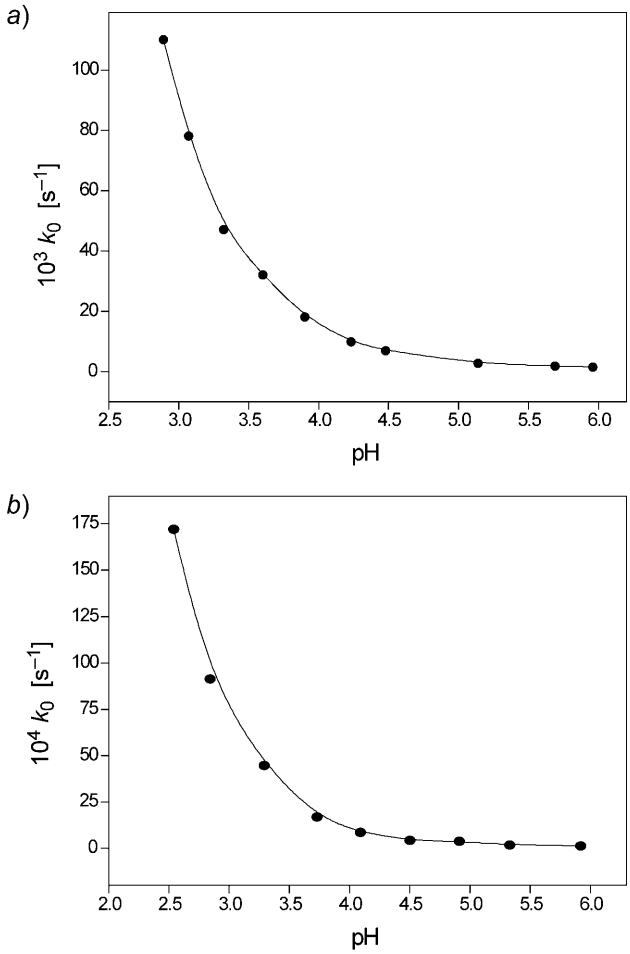


Figure. *Plot of the rate constant* k_0 vs. *pH for glyoxylic acid* (a) *and pyruvic acid* (b). Conditions: [complex] = 0.10 mM, T_R = 2.0 mM, C_{bipy} = 3.0 mM, T = 25.0°, I = 1.0M ($NaNO_3$). The solid lines were drawn on the basis of the rate constants reported in *Table 3* in combination with *Eqn. 10*. The actually observed rate-constant values are represented by circles.

$$RH \overset{k_a}{\rightleftharpoons} R^- + H^+ \tag{5}$$

$$[\mathbf{1}H]^{5+} + RH \overset{k_1}{\rightleftharpoons} \text{products} \tag{6}$$

$$[\mathbf{1}H]^{5+} + R^- \overset{k_2}{\rightleftharpoons} \text{products} \tag{7}$$

$$\mathbf{1}^{4+} + RH \overset{k_3}{\rightleftharpoons} \text{products} \tag{8}$$

$$\mathbf{1}^{4+} + R^- \overset{k_4}{\rightleftharpoons} \text{products} \tag{9}$$

$$k_0(K_a+[H^+])/T_R = k_1K_1[H^+]^2 + (k_2K_1K_a + k_3)[H^+] + k_4K_a \quad (10)$$

Eqn. 10, along with the non-linear least-squares values for k_1K_1, $(k_2K_1K_a+k_3)$, and k_4, as reported in *Table 3*, reproduced the observed k_0 values acceptably well (within 7%).

Table 3. *Rate Constants for the Reduction of* $\mathbf{1}^{4+}$ *by α-Oxo Acids in Aqueous Media.* Conditions: $C_{bipy}=3.0$ mM, $T=25.0°$, $I=1.0$ M ($NaNO_3$).

Reaction path	H_2O		D_2O/H_2O 95 : 5	
	Glyoxylic acid	Pyruvic acid	Glyoxylic acid	Pyruvic acid
k_1K_1[a])	$(1.60\pm0.05)\times10^4$	$(1.70\pm0.07)\times10^3$	$(1.10\pm0.06)\times10^4$	$(9.00\pm0.07)\times10^2$
$k_2K_1K_a+k_3$[b])	60 ± 5	14 ± 1	50 ± 5	9.6 ± 0.6
k_4[b])	0.68 ± 0.04	0.057 ± 0.003	0.50 ± 0.03	0.045 ± 0.003

[a]) In $M^{-2}s^{-1}$; [b]) In $M^{-1}s^{-1}$.

Since long it is known [25] that hydration of the two α-oxo acids and their anions forms the respective gem-diols, the extent of hydration decreasing in the order glyoxylic acid > glyoxylate > pyruvic acid > pyruvate. However, as the spread of the reported values for the hydration of each species is sufficiently high [25] (though the above-mentioned order is maintained), no quantitative evaluation of rate parameters was attempted to confirm the participation of dehydrated or hydrated reducing species.

Table 3 clearly demonstrates that $[\mathbf{1}H]^{5+}$ is kinetically superior than $[\mathbf{1}]^{4+}$ in oxidizing the acids RH or their dissociated forms R^-. However, it is not possible to conclusively compare the reactivities of RH or R^- in reducing $[\mathbf{1}H]^{5+}$ or $[\mathbf{1}]^{4+}$ as the k_2 and k_3 paths are 'proton-ambiguous'. Having a common view that deprotonated reductants normally react faster [30] than their neutral conjugate acids, we may assume $10k_3 \le k_4$, and it results $k_2K_1 \ge 4k_1K_1$ (for glyoxylic acid), and $k_2K_1 \gg 2.5k_1K_1$ (for pyruvic acid). For $k_1K_1 > k_2K_1$, it follows that $k_3 \ge 50$ (for glyoxylic acid) or $k_3 \ge 10$ (for pyruvic acid). A reasonable conclusion regarding the relative contributions of the k_2 and k_3 paths is, thus, not possible, yet significant contribution of the k_2 path in either redox reaction is anticipated, as the reaction rates were found to increase significantly with decreasing ionic strength of the reaction medium (*Table 2*), which indicates reaction between oppositely charged species. The dominance of the k_1 path may be attributed to H-bonding involving the protonated oxo-bridge and the reducing species that renders a close proximity of the redox agents, in addition to the higher positive charge of the protonated species $[\mathbf{1}H]^{5+}$ compared to $\mathbf{1}^{4+}$.

The oxidation rates of glyoxylic and pyruvic acid by periodate were found to increase with increasing pH. We, thus, suggested that the reactions proceed through nucleophilic attack of periodate on the C=O group of the α-oxo acids, where the hydrated forms are non-reactive [24d]. In the present case, however, we found a reverse trend: increase in rate with decreasing pH. This might be rationalized by the weaker reactivity of the reducing anions in comparison with their parent acids, along with the superior reactivity of $[\mathbf{1}H]^{5+}$ over $[\mathbf{1}]^{4+}$. Furthermore, we noted that glycolic acid

($HO-CH_2-CO_2H$) does basically not react with the Mn oxidant[3]). This observation supports our assumption that the gem-diol forms of the reducing α-oxo acids are the actual reactive species.

In deriving the rate law of *Eqn. 10*, we assumed $K_1[H^+] \ll 1$ (so that $T_{Mn} \approx [\mathbf{1}^{4+}]$). From this inequality, along with the k_1K_1 values in *Table 3*, we can fix a range for K_1 between 10^{-6} and 10. Though we are unable to more precisely estimate K_1, a low value is anticipated as we got no evidence for any UV/VIS spectroscopic changes of the tetranuclear Mn complex (even at pH 2.0) recorded at pH 6.0. Reports so far available [13][31] for the very low basicities of oxo bridges in aqueous solution of a number of multinuclear Mn^{IV} complexes support the approximation[4]) $K_1[H^+] \ll 1$, as well as $K_1 \ll 1$.

Instead of the above description of reaction sequences, where $\mathbf{1}^{4+}$ and $[\mathbf{1}H]^{5+}$ react with RH and R^-, a sequence of parallel reactions of $\mathbf{1}^{4+}$, $[\mathbf{1}H]^{5+}$, and $[\mathbf{1}H_2]^{6+}$ ($[\mathbf{1}H]^{5+} + H^+ \rightleftharpoons [\mathbf{1}H_2]^{6+}$; K_2) with R^- may equally be in agreement with the observed kinetics. The 'proton ambiguity', however, is resolved in the way that for being $[\mathbf{1}H_2]^{6+}$ to be a reactive species, its second-order rate constant, while reacting with R^-, would have to exceed far diffusion control (calculated data not shown), as $K_2 \ll K_1$, based on charge considerations. This alternative reaction path is, thus, not considered.

Table 3 also demonstrates that among the k_i rate constants ($i = 1-4$), k_1 is the highest, k_2 possibly also being high, while k_4 is rather low. It appears that the $[\mathbf{1}H]^{5+}$ species kinetically dominates over $\mathbf{1}^{4+}$. In fact, the very high reactivity of protonated oxo-bridged dinuclear Mn^{IV} species has been reported [15], which are quickly reduced to lower states in the presence of acids, illustrating that the Mn^{IV} oxidation level is destabilized upon protonation of the oxo bridge.

Mechanism. In *Eqns. 6–9*, we presume that an initial rate-determining step is followed by rapid reactions wherein the intermediate mixed-valent Mn species $(Mn^{IV})_3Mn^{III}$ are quickly reduced by the excess reducing agents or by the radicals produced from the one-electron oxidation of them. Polymerization of acrylonitrile put forward a definite evidence of the involvement of free radicals in the reaction course. Glyoxylate radicals produced from one-electron oxidation of glyoxylate have already been established by EPR measurements [24f], and pyruvate radicals have also been proposed elsewhere [24j,k]. The follow-up reactions must be rapid for the following

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[3]) After 3 h, 0.02M glycolic acid decreased the absorbance of the title Mn oxidant by less than 1% (conditions: H_2O (pH 3.0), 25.0°, $I = 1.0$M ($NaNO_3$), $C_{bipy} = 3.0$ mM).

[4]) The so far known acidity values of protonated oxo bridges in multinuclear Mn^{IV} complexes clearly demonstrate that when the ligands are anionic and have stronger donor ability (as compared to pyridine and related ligands like bipy), this leads to more electron density on the metal centers. Therefore, the oxo bridges are not required to donate as much electron density to the metal centers and can be involved in proton-acceptor chemistry. For example, the pK_a of $[(Mn^{IV})_2(salpn)_2(\mu\text{-}O)(\mu\text{-}OH)]^+$ (salpn = 1,3-bis(salicylideneamineto)propane) in H_2O is *ca.* 6 [15], whereas that for $[(Mn^{IV})_4(\mu\text{-}O)_5(\mu\text{-}OH)(bpea)_4]^{5+}$ (bpea = *N*,*N*′-bis(2-pyridylmethyl)ethanamine) is *ca.* −6 [13]. This shows that the bridging oxo groups in the latter complex, comparable to that used in our study, are much less 'proton-hungry' than the former. From the inequality $K_1[H^+] \ll 1$ at pH *ca.* 2, an estimated upper limit for K_1 lies around 1, and the lower limit lies at *ca.* 10^{-6}, assuming that the maximum k_1 is in the range one expects for diffusion-controlled processes.

reasons: *1*) the reducing agent may be very reactive radical species, in addition to the pure reducing agents. *2*) The most likely one-electron-reduced species of $[\mathbf{1}]^{4+}$ are highly reactive ($[(Mn^{IV})_3Mn^{III}(\mu\text{-}O)_6(bipy)_6]^{3+}$ is very unstable even at low temperature [20]). *3*) In the electrochemical reduction of $\mathbf{1}^{4+}$ at 0.50 V (*vs.* SCE) just before the first one-electron reduction wave to attempt a single electron reduction, it was not possible to stop the process at the stage of the one-electron-reduced species. Color fading of the solution and EPR spectroscopic evidence indicated Mn^{II} at the final state [16]. These observations clearly indicate the immediate collapsing nature of the $(Mn^{IV})_3Mn^{III}$ species. *4*) A cluster breakup is expected once one of the Mn-atoms is reduced to the Mn^{II} state [13][32]. *5*) Finally, tri- or dinuclear species formed from $\mathbf{1}^{4+}$ should bear two to four H_2O molecules in their coordination sphere. Such aqua complexes are known to possess a very high kinetic activity towards redox processes [33]. The correct formulation of the one-electron-reduced species of $\mathbf{1}^{4+}$ should have a protonated oxo bridge, as the basicity of the bridge must increase remarkably when one Mn^{IV} is reduced to Mn^{III} [34]. Of the four Mn centers, one of the two terminal ones is expected to be reduced first as these two Mn^{IV} centers should have a higher formal positive charge than the two other central ones due to the coordination of four oxo ligands to the two central Mn^{IV} sites.

The Mn^{IV} tetramer $\mathbf{1}^{4+}$ is coordinatively saturated, and an inner-sphere attachment of reducing species is, thus, unlikely. Redox reactions of multinuclear higher-valent oxo-bridged Mn and Fe complexes are often associated with H^+ transfer [32][35–38]. The oxo bridges in the one-electron-reduced Mn oxidant in this investigation, $(Mn^{IV})_3Mn^{III}$, must have increased basicity, and fast H^+ transfer from bulk solvent is expected. The slow rate limiting the one-electron reduction of the tetramer may, thus, be coupled with simultaneous H^+ transfer to the oxo bridge and, in fact, we found the rate constants k_1 to k_4 (*Table 3*) determined in 95% D_2O to be significantly lowered [39]. This clearly supports the occurrence of H^+-coupled electron transfer as a key step in the overall redox reaction.

Glyoxylate oxidation by high-valent metal centers is generally faster [24] than pyruvate oxidation. The relative strengths of C(O)–COOH bonds in these two reducing species may be responsible for this observation. The presence of an electron-donating Me group in pyruvic acid increases the electron density at the adjacent C=O C-atom, which reduces similar charge repulsion between the C=O and the COOH C-atoms. The calculated C–C bond distances, however, in glyoxylic and pyruvic acids, estimated with the aid of AM1, PM3, and ZINDO/1 semiempirical calculations, showed a reverse result: the bond length in glyoxylic acid was determined as 1.505 (AM1), 1.524 (PM3), and 1.449 Å (ZINDO/1). For pyruvic acid, the values were 1.510 (AM1), 1.530 (PM3), and 1.458 (ZINDO/1). These calculations, when applied to the hydrated form of glyoxylic acid, results in a bond length of 1.527 (AM1), 1.550 (PM3), and 1.469 Å (ZINDO/1). Similarly, hydrated pyruvic acid gives rise to values of 1.534 (AM1); 1.558 (PM3), and 1.473 Å (ZINDO/1). These results indirectly support the involvement of hydrated glyoxylic and non-hydrated pyruvic acid as reactive species, respectively. The lower C(O)–COOH bond energy in hydrated glyoxylic acid compared to that in regular pyruvic acid may, thus, be a driving force for faster initial electron transfer leading to faster overall oxidation of glyoxylic acid. Though we find it difficult to exactly pinpoint a single act in the rate-determining step of the title redox system, formation

of a hydrated acyl radical ($RC^{\bullet}(OH)_2$) accompanied by one-electron reduction of the oxidant, and simultaneous H^+ transfer to the oxo bridge of the one-electron-reduced oxidant, may define the rate-determining step. Formation of acyl radicals by one-electron oxidants like Mn^{III} [40] or V^V [24] has been proposed earlier.

The initial decarboxylation of glyoxylic acid to oxalic acid, followed by the oxidation of oxalic acid to CO_2, seems unlikely, as we verified that oxalic acid reacts with $\mathbf{1}^{4+}$ at a rate at least ten times slower than the oxidation of glyoxylic acid under comparable conditions. Again, it is known that acetaldehyde, on oxidation by a metal ion in mineral acid, yields both formic and acetic acid [41][42]. However, in the present investigation, we found only acetic acid as the oxidation product of pyruvic acid. Initial decarboxylation of pyruvic acid, followed by oxidation of acetaldehyde, is, thus, ruled out. Moreover, we noted that acetaldehyde does not react with the Mn oxidant when used in concentrations similar to those used for the present reducing agents.

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Experimental Part

Materials. The complex salt hydrate $[Mn_4(\mu\text{-}O)_6(bipy)_6][ClO_4]_4 \cdot 2\ H_2O$ was prepared according to a literature procedure [16]. Note that one H_2O molecule is easily lost [16], which explains why the elemental analysis of the material matched with that calculated for the monohydrate (anal. calc. for $C_{60}H_{50}Cl_4Mn_4N_{12}O_{23}$: C 43.16, H 2.99, N 10.07; found C 43.39, H 3.03, N 10.00); the tetramer $\mathbf{1}^{4+}$ used in all the experiments, thus, appears to be the sufficiently pure monohydrate. The preparation, standardization, and storage of glyoxylic acid, pyruvic acid, and sodium nitrate were described earlier [24k][37]. 2,2′-bipyridine (bipy; *Sigma*) was used as received. D_2O (99.9%) was purchased from *Sigma* or *Merck*; all other chemicals were of reagent grade. Doubly distilled, deionized and freshly distilled H_2O was used throughout.

Equilibrium Measurements. The dissociation constants K_a of glyoxylic and pyruvic acid were determined by titration with CO_2-free NaOH in both H_2O and D_2O/H_2O 95:5 at $25.0 \pm 0.1°$ ($I = 1.0$M ($NaNO_3$)) using a *Metrohm 736-GP-Titrino* autotitrator, as described earlier [38].

Stoichiometric Measurements. The generation of gaseous CO_2 as oxidation product of both glyoxylic and pyruvic acid was quantitatively established by GC analysis, as described earlier [43]. Formation of formic and acetic acid, respectively, from the oxidation of glyoxylic and pyruvic acid was secured with the aid of chromotropic acid and the $La(NO_3)_3/I_2$ test [44]. Mn^{2+} and bipy did not interfere.

Physical Measurements and Kinetics. UV/VIS Absorbance spectra were recorded on a *Shimadzu 1601-PC* spectrophotometer using 1-cm quartz cells. The kinetics were monitored *in situ* in the 'kinetics mode' of the instrument, and in a thermostated ($25.0 \pm 0.1°$) cell housing (*CPS-240A*) at 420 nm, where all the reaction partners (except the Mn oxidant; $\varepsilon = 7500\ M^{-1}\ cm^{-1}$) are transparent. A few runs were also performed at other wavelengths: 380, 450, and 500 nm. The ionic strength was normally maintained at 1.0M with $NaNO_3$. Excess of the 2,2′-bipyridine concentration, C_{bipy} ($=[(bipy)H^+] + [bipy]$), was in the range 1–60 mM, and acted as a good buffer[5]) in the pH range studied (2.5–6.0), as measured with an *Orion-Ross* combined-electrode system (*Model 81-02*), before and after the reaction. The electrode

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[5]) Excess bipy present in soln. nicely buffers the reaction within 0.03 pH units above pH 3.2. Below pH 3.0, an increase in pH of *ca.* 0.20–0.26 was observed at the end of the reactions. This might be due to the removal of H^+ from the reaction medium by the released bipy. Plots of $\log_{10}$(absorbance) *vs.* time in this low-pH region were found to be slightly curved upwards after *ca.* 60–70% completion of the reaction. The first-order rate constants in such situations were computed from the slope of the initial linear plots.

was calibrated to read $-\log_{10}[H^+]$ with the help of a calibration curve constructed by plotting the pH-meter reading against $-\log_{10}[H^+]$ [45]. For reactions in D_2O, the pD was calculated as pD = pH + 0.40 [46]. All the kinetics runs were carried out with reducing agents in excess. To monitor faster reactions (especially in the low-pH region), the calculated volume of the reducing agent of known strength (pre-adjusted to the desired temperature, pH, and ionic strength) was quickly mixed with the oxidant kept in the spectrophotometer cuvette and pre-equilibrated at the desired conditions.

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