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Fast Kinetic Studies of Dioxygen-Derived Species and Their Metal Complexes [and Discussion]

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Fast kinetic studies of dioxygen-derived species and their metal complexes

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The role of metals in the reactivity of HO_2/O_2^- with compounds of biological interest is discussed. A scheme that illustrates the various reactions that a transition metal complex can undergo when reacting with HO_2/O_2^- is presented in terms of ligand and pH effects.

The decomposition of hydrogen peroxide catalysed by ferrous ion is reviewed in terms of new rate data for the reactions of ferric ion with perhydroxyl (HO_2) and superoxide (O_2^-) radicals. The new results support a mechanism proposed by Barb and his coworkers (W. G. Barb, J. H. Baxendale, P. George & K. R. Hargrave, *Trans. Faraday Soc.* **47**, 462–500 (1951)) and negates the occurrence of the Haber–Weiss reaction in this system.

In the presence of Mn^{II} complexes, O_2^- reacts to form MnO_2^+ transients and Mn^{III} complexes. Their reactivities with ascorbate, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and NADH–NADPH is discussed.

Introduction

While the chemical and physical properties of the superoxide radical (O_2^-) and its conjugate acid, the perhydroxyl radical (HO_2), were first studied solely to elucidate their roles in the radiolysis of oxygen-containing aqueous solutions, the discovery in the late 1960s that O_2^- plays an important role in living systems (McCord & Fridovich 1969) resulted in renewed research efforts and a shift towards the investigation of the reactivity of these oxy species with biological compounds. A major question that arose at the time concerned the nature and origin of various deleterious effects observed when O_2^- and H_2O_2 are present together in a biological system. The suppression of such effects by addition of catalase, which destroys hydrogen peroxide as follows:

$$2\,H_2O_2 \xrightarrow{\text{catalase}} 2\,H_2O + O_2, \tag{1}$$

or superoxide dismutase (SOD), a class of enzymes that accelerates the disproportionation of superoxide radicals,

$$2\,O_2^- + 2\,H_2O \xrightarrow{\text{SOD}} H_2O_2 + 2\,OH^- + O_2, \tag{2}$$

or both catalase and SOD led to the belief that powerful oxidizing species such as the hydroxyl radical (OH) or singlet molecular oxygen ($^1O_2$) were generated from peroxide and O_2^- by the so-called 'Haber–Weiss' reaction (Haber & Weiss 1934)

$$HO_2 + H_2O_2 \rightarrow {}^{\cdot}OH + H_2O + O_2 \tag{3}$$

and by the disproportionation of HO_2/O_2^- respectively. A number of reported rate measurements indicate a very low reactivity between HO_2/O_2^- and H_2O_2 (k values range from 10 to 10^{-4} dm^3 mol^{-1} s^{-1}; Weinstein & Bielski (1979) and references therein), thus putting in

question reaction (3) as an efficient source of OH radicals under physiological conditions. Recent studies on $^1O_2$ formation in the disproportionation of HO_2/O_2^- have also shown that this species is not formed (Arudi *et al.* 1984).

As the reported toxic effects of superoxide radicals are unquestionably real and because the Haber–Weiss reaction and singlet molecular oxygen production were shown to be ineffective only under extremely clean conditions, the role of metal catalysis became the overriding question. The possibility that metal catalysis may play a major role in the deleterious effects caused by HO_2/O_2^- would not be surprising in view of the ubiquity of metals in living cells.

The approach in this laboratory to these queries is twofold. The first aspect involves basic research on the reactivities of metals with HO_2/O_2^- in isolation, to determine if metal–HO_2/O_2^- complexes are formed and, if they are formed, to determine their chemical properties. Secondly, we have been measuring the reactivity of such metal–oxy species and metals themselves with compounds of biological interest.

Metal–HO_2/O_2^- *complexes*

Metal–dioxygen complexes can be formed by two well-documented methods (Buxton & Sellers 1977); the reaction between a reduced metal ion and molecular oxygen,

$$M^{n+} + e^-_{aq} \rightarrow M^{(n-1)+}, \tag{4}$$

$$M^{(n-1)+} + O_2 \rightarrow MO_2^{(n-1)+}; \tag{5}$$

or the reaction between a metal ion in a stable oxidation state and an active oxygen species,

$$HO_2 \rightleftharpoons O_2^- + H^+, \tag{6}$$

$$M^{n+} + HO_2 \rightarrow MOOH^{n+}, \tag{7}$$

$$M^{n+} + O_2^- \rightleftharpoons MO_2^{(n-1)+}. \tag{8}$$

The first thorough investigation of reaction (7) involved the reaction of HO_2 with ferrous ion near pH 1 (Jayson *et al.* 1969, 1973). Despite the complexity of the system, they succeeded in resolving the spectra of transient complexes and showed that their kinetic measurements were consistent with the following reaction mechanism (k values are given at 25 °C):

$$HO_2 + Fe^{2+} \rightarrow Fe^{3+}HO_2^-; \quad k_9 = 1.2 \times 10^6 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}, \tag{9}$$

$$Fe^{3+}HO_2^- + Fe^{2+} \rightleftharpoons Fe^{3+}HO_2^-Fe^{2+}; \quad K_{10} = 27 \pm 2 \text{ M}^{-1}, \tag{10}$$

$$Fe^{3+}HO_2^- \rightarrow Fe^{3+} + HO_2^-; \quad k_{11} = 1.8 \times 10^3 \text{ s}^{-1}, \tag{11}$$

$$Fe^{3+}HO_2^-Fe^{2+} \rightleftharpoons Fe^{3+} + Fe^{2+}HO_2^-; \quad k_{12} = 2.5 \times 10^4 \text{ s}^{-1}, \tag{12}$$

$$H^+ + Fe^{2+}HO_2^- \rightarrow Fe^{2+} + H_2O_2. \tag{13}$$

To investigate further the reactivity of HO_2/O_2^- radicals with the Fe^{II}/Fe^{III} system, experiments were designed (Rush & Bielski 1985) that used a 100 ms time window (Stuglik & Zagorski 1981) that exists under certain pulse-radiolytic conditions and represents the timespan between formation and onset of precipitation of Fe^{III}. Because of the rapid oxidation of Fe^{II}_{aq} near neutrality, oxygen-containing ferrous sulphate solutions were prepared by rapid mixing of anaerobic ferrous sulphate solutions with oxygen-saturated formate solutions. The solutions exiting from the jet mixer were transferred to the radiation cell, where they were

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

pulse-irradiated within five seconds of mixing. The ferrous iron concentration and radiation doses were adjusted so that $[O_2^-] > [Fe^{2+}]$. By taking into consideration that Fe^{III} is present in a hydrolysed form at pH 7.2, where these experiments were performed, the mechanism can be described as follows:

$$H_2O \overset{O_2,\ HCOONa}{\sim\!\sim\!\sim\!\sim\!\sim\!\sim\!\sim\!\sim\!\rightarrow} O_2^-, \tag{14}$$

$$Fe^{2+}_{aq} + O_2^- \xrightarrow{H^+} Fe(OH)^{2+}_{aq} + H_2O_2; \quad k_{15} = 10^7\ dm^3\ mol^{-1}\ s^{-1}, \text{ (Rush \& Bielski 1985)}, \tag{15}$$

$$FeOH^{2+}_{aq} \rightleftharpoons Fe(OH)^{+}_{aq} + H^+; \quad pK = 3.3\ mol, \text{ (Schneider 1984)} \tag{16}$$

$$Fe(OH)^{+}_{2aq} + O_2^- \longrightarrow Fe^{2+}_{aq} + O_2 + 2OH^-; \quad k_{17} = 1.5 \times 10^8\ dm^3\ mol^{-1}\ s^{-1} \text{ (Rush \& Bielski 1985).} \tag{17}$$

Competition studies of HO_2/O_2^- with Fe^{III}/Ce^{III} in the acid range led to the determination of the rates of reaction between HO_2/O_2^- and $FeSO_4^+$.

$$FeSO_4^+ + HO_2 \rightarrow Fe^{2+} + O_2 + HSO_4^-; \quad k \approx 10^3\ dm^3\ mol^{-1}\ s^{-1}, \text{ (Rush \& Bielski 1985)} \tag{18}$$

$$FeSO_4^+ + O_2^- \rightarrow Fe^{2+} + O_2 + SO_4^{2-}; \quad k = 1.5 \times 10^8\ dm^3\ mol^{-1}\ s^{-1} \text{ (Rush \& Bielski 1985).} \tag{19}$$

The exceptionally high rate at which O_2^- reduced $FeSO_4^+$ makes this the predominant pathway by which the HO_2/O_2^- radical pair reacts with Fe^{III} even at pH = 0.

Haber & Weiss (1934) found in their classic study of the decomposition of hydrogen peroxide catalysed by ferrous ion that at high H_2O_2 concentrations the amount of peroxide consumed and oxygen formed was much greater than the amount of Fe^{II} oxidized. They suggested that this stoichiometric imbalance might result from reaction (3). Although they were unaware at the time that reaction (3) is insignificant, Barb *et al.* (1951) concluded after a careful reinvestigation of this system that the overall mechanism can be described by the following equations.

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\cdot} + OH^-; \quad k_{20} = 53.0 \pm 0.7\ dm^3\ mol^{-1}\ s^{-1}, \text{ (Barb } et\ al. \text{ 1951)} \tag{20}$$

$$Fe^{2+} + OH^{\cdot} \rightarrow Fe^{3+} + OH^-; \quad k_{15} = 3.2 \times 10^8\ dm^3\ mol^{-1}\ s^{-1}, \text{ (Stuglik \& Zagorski 1981)} \tag{15}$$

$$H_2O_2 + OH^{\cdot} \rightarrow H_2O + HO_2; \quad k_{21} = 4.5 \times 10^7\ dm^3\ mol^{-1}\ s^{-1}, \text{ (Schwarz 1962)} \tag{21}$$

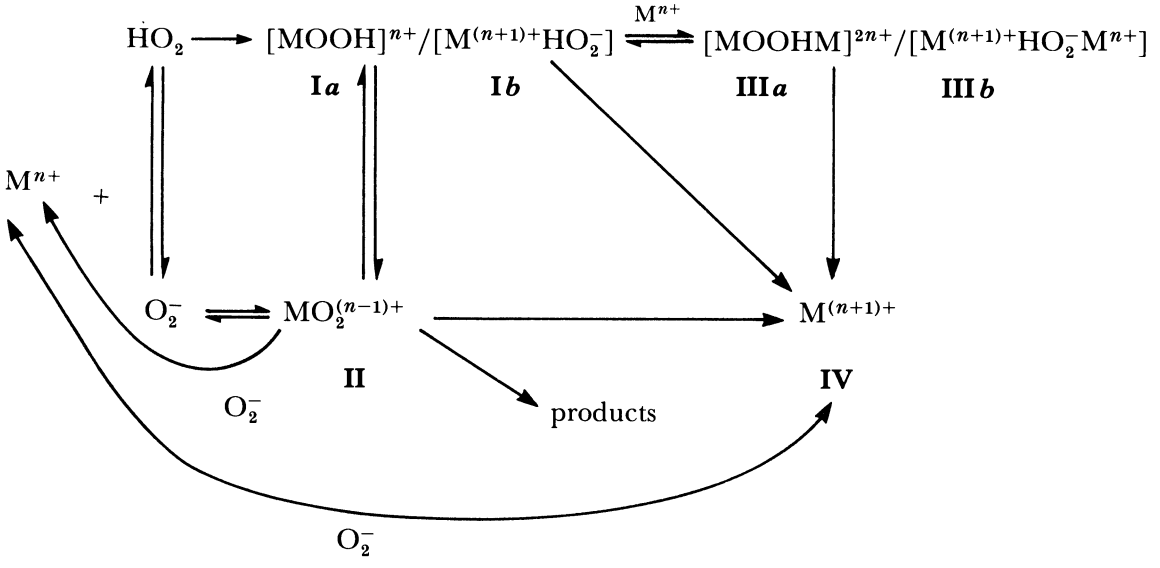
$$Fe^{2+} + HO_2 \rightarrow Fe^{3+} + HO_2^-; \quad k_9 = 1.2 \times 10^6\ dm^3\ mol^{-1}\ s^{-1}, \text{ (Jayson } et\ al. \text{ 1973)} \tag{9}$$

$$Fe^{3+} + O_2^- \rightarrow Fe^{2+} + O_2; \quad k_{19} = 1.5 \times 10^8\ dm^3\ mol^{-1}\ s^{-1} \text{ (Rush \& Bielski 1985).} \tag{17}$$

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

This early study examined the competition of Fe^{II} with Fe^{III} for HO_2/O_2^-, and measured the ratio of these reactions over the pH range from 1 to 2.65. By using their ratio and the more recently obtained values of $K_{HO_2} = 1.6 \times 10^{-5}$ mol (Bielski *et al.* 1985) and $k_9 = 1.2 \times 10^6$ dm^3 mol^{-1} s^{-1} (Jayson *et al.* 1975) the rate of reaction between Fe^{III} and O_2^- can be calculated. This gives $k_{19} = 2.5 \times 10^8$ cm^3 mol^{-1} s^{-1}. The close agreement of this rate to $k_{19} = 1.5 \times 10^8$ dm^3 mol^{-1} s^{-1} (see below) corroborates the correctness of their mechanism. It should be noted that if the ratio of H_2O_2/Fe^{II} is initially high, this mechanism accounts for the results observed by Haber & Weiss (1934) without involving the direct interaction of HO_2 with hydrogen peroxide. This leaves the Fenton reaction (reaction (20)) as the sole OH$^\cdot$ radical source in this system.

Studies of the reactions between Mn^{II} complexes and HO_2/O_2^- radicals (Cabelli & Bielski 1984*a*, *b* and references therein), taken in conjunction with the aforementioned Fe^{II} system, led to the formulation of an overall mechanism (scheme of reactions) that illustrates the various reactions a transition metal complex can undergo when reacting with HO_2 or O_2^-.



Scheme 1

The various reactions and equilibria shown in scheme 1 represent competitive pathways. The reaction mechanism of a particular metal complex with HO_2/O_2^- is affected by the nature of its ligands and the degree by which one pathway predominates over another. Superimposed upon these effects is the pH effect, which not only controls the distribution of HO_2/O_2^- but also the form(s) of the metal complex in the medium.

As illustrated in scheme 1, the reactions can be divided into those involving predominantly protonated species and those involving $MO_2^{(n-1)+}$ and O_2^- itself. The $pK_{HO_2} = 4.8$ is well established and experimental evidence indicates that upon formation of metal–HO_2/O_2^- complexes a shift towards a lower pK occurs (p$K \approx 2.0$–3.5). The protonated complexes **I*a*/I*b*** and **III*a*/III*b*** have been discussed for both Mn^{II} and Fe^{II} reactions with HO_2. For Fe^{II}, intermediates were described as outer sphere complexes (**I*b*/III*b***) where the electron had already been transferred and the transients were Fe^{III}–peroxy species, a conclusion reached on the basis of spectral evidence (Jayson *et al.* 1969, 1973). Studies of HO_2 with Mn^{II}

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

complexes suggest, however, that although the reaction pathway is analogous to that of the Fe^{II} system, the transients appear to be inner sphere perhydroxyl complexes, again on the basis of spectral evidence (Cabelli & Bielski 1984*a*, *b*). The species formed in the more physiologically relevant pH range include the unprotonated metal–O_2^- complexes. Such $MO_2^{(n-1)+}$ species have been reported previously for the following metals and complexes: Fe^{2+} (Jayson *et al.* 1973); Fe^{II}–EDTA (Halliwell 1975; Butler & Halliwell 1982; Ilan & Czapski 1977; McClune *et al.* 1977; Bull *et al.* 1982); Mn^{2+} (Pick-Kaplan & Rabani 1976; Bielski & Chan 1978; Götz & Lengfelder 1983; Cabelli & Bielski 1984*a*, *b*); Mn^{II}–EDTA and Mn^{II}–NTA (Lati & Meyerstein 1978); Ba^{2+}/Ca^{2+} (Bray *et al.* 1977); Ni^{2+}, Cr^{2+} (Sellers & Simic 1976; Ilan *et al.* 1975; V^{5+}, Ti^{4+}, Ce^{3+}, Zr^{4+}, U^{6+}, Mo^{6+} (Samuni & Czapski 1970). A study of Mn^{II} complexes (sulphate, formate, phosphate, pyrophosphate) with O_2^- showed that MnO_2^+ formation and disappearance is very sensitive to the nature of the ligand. In the presence of sulphate and formate, the transient disappears without ever forming Mn^{III} while both Mn^{II} phosphate and pyrophosphate ultimately give the corresponding Mn^{III} complexes.

As the reactivity of metals and metal–O_2^- complexes with biological compounds is of great interest and because the MnO_2^+/Mn^{III} system is well characterized, we have some preliminary results on the reactivity of these species with ascorbic acid, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and NADPH/NADH.

Reactivity of HO_2/O_2^- *and* MnO_2^+/Mn^{III}) *with ascorbic acid and Trolox*

Ascorbic acid is not only an excellent HO_2/O_2^- scavenger (Cabelli & Bielski 1983), but also a well-known synergistic agent for vitamin E. As these compounds may well play important roles in the protection of membranes against oxy-radical attack, they have been the subject of intensive research.

Although the reactivity of ascorbic acid with numerous transition metals has been studied at great length in the acid pH range, fewer such studies have been performed near neutrality because of the instability of some metal ions (Fe^{2+}/Fe^{3+}, Mn^{2+}/Mn^{3+}) in such media. In particular, the reactions between Mn^{III} and ascorbic acid have been studied in very acidic solutions where Mn^{III} is stable in the presence of Mn^{II}. In 0.5–2.0 mol dm^{-3} perchloric acid, ascorbic acid reacts with Mn^{3+}_{aq} and $Mn(OH)^{2+}_{aq}$ at rates $k = 6 \times 10^3$ and 5.3×10^4 dm mol^{-1} s^{-1} respectively (Pelizzetti *et al.* 1978). As illustrated in table 1, the various Mn^{III} and ascorbate (AH^-) complexes, with the exception of Mn^{III}–pyrophosphate, are much more reactive than HO_2 and O_2^-. The Mn^{III} complexes were studied in isolation in N_2O-saturated solutions, where they were generated pulse radiolytically from the corresponding Mn^{II} complexes. Similarly the HO_2/O_2^- radicals and MnO_2^+–formate complex were generated in solutions containing, in addition to ascorbate, appropriate amounts of formate, oxygen and Mn^{II}.

Earlier studies of the reactivity of HO_2/O_2^- with Trolox (Bielski 1983) had shown that while HO_2 reacts with this vitamin E analogue at a rate of 2×10^5 dm^3 mol^{-1} s^{-1}, the superoxide radical reacts with a rate constant $k < 0.1$ dm^3 mol^{-1} s^{-1} (see table 1 and figure 1).

The reactivity of MnO_2^+–formate with Trolox was studied in a stopped-flow spectrophotometer equipped with two jet mixers in tandem and an O_2^--generating plasma lamp. The MnO_2^+–formate complex that was generated from O_2^- and Mn^{II}–formate in the first mixer was then mixed with Trolox solutions in the second mixer and scanned for absorbance changes at 390 nm. All experiments were done in 60% ethanol and at 23.5 °C, where the spontaneous decay of the

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Table 1. The reactivity of HO_2/O_2^- and MnO_2^+/Mn^{III} with ascorbic acid/ascorbate (AH_2/AH^-), Trolox (TH, T^-) and NADH/NADPH at 23.5 °C

reactants	pH	k/(dm^3 mol^{-1} s^{-1})	references
HO_2/AH_2	2.0	1.6×10^4	Cabelli & Bielski (1983)
O_2^-/AH^-	7.0	5.0×10^4	Cabelli & Bielski (1983)
MnO_2^+–formate/AH^-	7.4	3.5×10^5	Cabelli & Bielski (1985)
Mn^{3+}_{aq}/AH_2	−0.3	6.0×10^3	Pelizzetti *et al.* (1977)
$MnOH^{2+}_{aq}/AH_2$	0.3	5.3×10^4	Pelizzetti *et al.* (1977)
Mn^{III}–sulphate/AH^-	5.6	1.8×10^6	Cabelli & Bielski (1985)
Mn^{III}–phosphate/AH_2/AH^-	4.7	1.4×10^6	Cabelli & Bielski (1985)
Mn^{III}–pyrophosphate/AH^-	7.0	1.4×10^4	Cabelli & Bielski (1985)
HO_2/TH	2.0	2.0×10^5	Bielski (1983)
O_2^-/TH/T^-	10.0	0.1	Bielski (1983)
MnO_2^+–formate/TH	5.4	1.1×10^5	this paper
Mn^{III}–pyrophosphate/TH	2.0	2.0×10^5	this paper
Mn^{III}–pyrophosphate/TH	7.8	5.0×10^1	this paper
HO_2/NADH	5.0	1.3×10^5	Nadezhdin & Dunford (1979)
O_2^-/NADH	8.6	27.0	Land & Swallow (1971)
Mn^{3+}–pyrophosphate/NADH	5.0	0.8	Bielski (1984)
Mn^{3+}–pyrophosphate/NADPH	5.0	0.3	Bielski (1984)
Mn^{3+}–sulphate/NADPH	5.4	3.1×10^6	this paper
HO_2/LDH–NADH	5.0	2.0×10^6	Bielski & Chan (1976)
O_2^-/LDH–NADH	8.0	10^5	Bielski & Chan (1976)

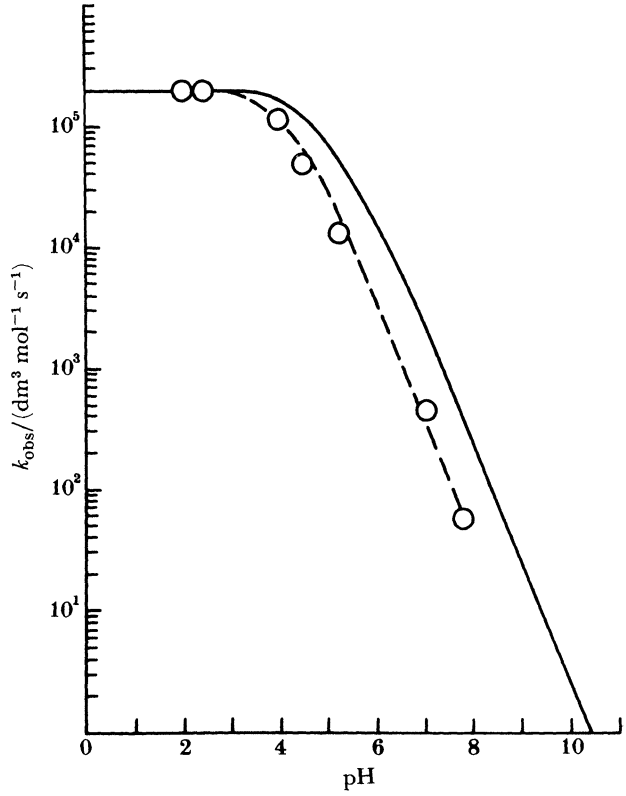


Figure 1. The pH profile for the reactions of Trolox with HO_2 (——) and Mn^{3+}–pyrophosphate (– – –).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

MnO_2^+–formate complex is identical to its decay in aqueous solutions. The observed rate for

$$MnO_2^+\text{–formate} + \text{Trolox} \rightarrow Mn^{II}\text{–formate} + \text{T-semiquinone} + O_2 \quad (22)$$

at pH 5.4 is $k_{22} = (1.1 \pm 0.2) \times 10^4$ dm^3 mol^{-1} s^{-1}.

The oxidation of Trolox by Mn^{III}–pyrophosphate was studied in 60% ethanolic solutions as a function of pH under pseudo-first-order conditions by the stopped-flow method.

$$Mn^{III}\text{–pyrophosphate} + \text{Trolox} \rightarrow \text{T-semiquinone} + H^+ + Mn^{II}\text{–pyrophosphate}. \quad (23)$$

The corresponding rate constants were calculated from observed optical changes at 390 nm, the absorption maximum for Trolox semiquinone. The results are shown in figure 1 (broken line) where the plateau region represents reaction (23); $k_{23} = (2.0 \pm 0.4) \times 10^5$ dm^3 mol^{-1} s^{-1} at 23.5 °C. The profile of the curve is described by the simple kinetic equation $k_{obs} = k_{23}/(1 + K_c/H^+)$, where

$$K_c = [Mn(HP_2O_7)_2^{3-}]\,[H_4P_2O_7]/[Mn(H_2P_2O_7)_3^{3-}] = 5.63 \times 10^{-5} \text{ mol}; \quad \text{(Davies 1969)}.$$

The reactivity of HO_2/O_2^- *and* Mn^{III}) *complexes with* NADH/NADPH

The involvement of the NADH/NADPH cofactors in the metabolic chemistry of O_2^- is well documented. For example, it has been observed (Mayewsky 1974, 1975) that animals exposed to hyperbaric oxygen show first an immediate increase in O_2^- level that is followed by a drop in cellular NADPH, suggesting that some NADPH depletion mechanism is operational. Also, it is believed (Babior 1981) that during phagocytosis the crucial reaction is the reduction of dioxygen to O_2^- at the expense of NADH/NADPH.

In vitro experiments have shown that an NADH-depletion mechanism can be initiated by O_2^- in presence of NADH-activating enzymes (Bielski & Chan 1976) or certain metal complexes (Curnutte *et al.* 1976). In an effort to elucidate the basic mechanism for the Mn^{II}-catalysed chain oxidation of NADPH by O_2^- proposed by Curnutte *et al.* (1976), pulse-radiolysis and stopped-flow experiments have been done in the presence of different Mn^{II} complexes. As can be seen from table 1, while Mn^{III}–pyrophosphate is unreactive towards NADPH, Mn^{III}–sulphate reacts at a rate of $k_{26} = 3.1 \times 10^6$ dm^3 mol^{-1} s^{-1}. The latter value was obtained in a pulse radiolysis study in which Mn^{III}–sulphate was generated from the Mn^{II} salt by $OH^\cdot$ radical oxidation (pH 5.4) as follows.

$$H_2O \overset{N_2O}{\sim\!\sim\!\rightarrow} OH^\cdot, \quad (24)$$

$$OH^\cdot + Mn^{II}\text{–sulphate} \rightarrow Mn^{III}\text{–sulphate} + OH^-, \quad (25)$$

$$Mn^{III}\text{–sulphate} + \text{NADPH} \rightarrow Mn^{II}\text{–sulphate} + \text{NADP}^\cdot, \quad (26)$$

$$2\,\text{NADP}^\cdot \rightarrow (\text{NADP})_2. \quad (27)$$

It should be noted that in spite of the fast rate of reaction (26) this mechanism does not involve a catalytic cycle.

Preliminary pulse-radiolysis studies of the Mn^{II}–phosphate/NADH system in presence of dioxygen, led to the observation of a chain reaction that corroborates the Curnutte *et al.* (1976) mechanism. An additional step observed in this study, which was beyond the time resolution

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

of the original experiments, shows that a short-lived transient MnO_2^+–phosphate complex is formed which yields Mn^{III}–phosphate that reacts with NADH as follows.

$$H_2O \xrightarrow[HCOO^-,\,O_2^-]{Mn^{II}\text{–phosphate}} O_2^-, \tag{28}$$

$$O_2^- + Mn^{II}\text{–phosphate} \rightarrow MnO_2^+\text{–phosphate}, \tag{29}$$

$$MnO_2^+\text{–phosphate} \rightarrow Mn^{III}\text{–phosphate}, \tag{30}$$

$$Mn^{III}\text{–phosphate} + NADH \rightarrow Mn^{II}\text{–phosphate} + NAD^{\cdot} + H^+, \tag{31}$$

$$NAD^{\cdot} + O_2 \rightarrow NAD^+ + O_2^-. \tag{32}$$

The kinetics of this system have not yet been completely resolved because of complex reactions that involve, in addition to NADH, the various complexes NADH forms with Mn^{II}.

As is apparent from these results the overall reaction schemes for such systems are strongly dependent upon the ligands present in the medium and the nature of the various metal complexes.

Conclusions

Our kinetic results taken in conjunction with the mechanism proposed by Barb *et al.* (1951) led to the following conclusions. First, because the only source of OH radicals in an aqueous solution containing $Fe^{2+}/Fe^{3+}/H_2O_2/HO_2/O_2^-$ is the Fenton reaction, there is a high probability that the Haber–Weiss reaction does not occur in aqueous solutions under any conditions. Secondly, a metal-mediated generation of OH radicals from hydrogen peroxide by the superoxide radical appears possible if, as for ferric ion, the O_2^- radical can transform the metal ion or complex to an oxidation state in which it will react with H_2O_2 by a Fenton-type reaction.

Although the research on the Mn^{II}–HO_2/O_2^- systems is at a much more preliminary state, two basic trends have been observed. If O_2^- is allowed to react with Mn^{II}–complexes to yield either MnO_2^+–transients or, ultimately, Mn^{III} complexes, the reactivity of these metal species towards some biological compounds is greatly enhanced. Also, because Mn^{III}–complexes have much longer lifetimes than O_2^- in the pH range 4–7, they could have potentially much more deleterious effects even if their reactivity towards some organic compounds is not increased from that of free O_2^-.

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Discussion

R. L. Willson (*Department of Biochemistry, Brunel University, Uxbridge, Middlesex*). I am very interested in Dr Bielski's studies on O_2^- and $HO_2^\cdot$ with NADH in the absence and presence of the dehydrogenase enzyme. When he published this work some time ago he attributed the observed increase in the rate constant to a change in the conformation of NADH when it is bound to the enzyme. Is this still totally his view, or is there any possibility that a local environment in the enzyme can cause the equilibrium $HO_2^\cdot/O_2^-$ to be shifted in favour of $HO_2^\cdot$?

B. H. J. Bielski. Although I have no evidence that the increased rate of NADH oxidation in the presence of dehydrogenases is a result of a conformational change in the cofactor, the magnitude of the observed rate constants suggests enzyme activation of NADH; $k(HO_2 + NADH) = 1.8 \times 10^5$ dm^3 mol^{-1} s^{-1} (Nadezhdin & Dunford 1979); $k(HO_2 + LDH–NADH) = 2.0 \times 10^6$ dm^3 mol^{-1} s^{-1} and $k(HO_2 + GAPDH–NADH) = 2.0 \times 10^7$ dm^3 mol^{-1} s^{-1} (Bielski & Chan 1976, 1980). Of course it is quite possible that the observed increase in the rate of reaction is a result of a combination of enzyme activation of NADH and a local pH shift in favour of HO_2.

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R. L. Willson. Has Dr Bielski studied the reaction of organic peroxy radicals, $RO_2^\cdot$, with NADH? Unlike $HO_2^\cdot$, in many instances peroxy radicals are unlikely to be dissociated at pH 7 and are therefore more likely to react with NADH. Has he any rate data concerning such organic peroxy radical reactions or any evidence that they do occur?

B. H. J. Bielski. Unfortunately I never studied the reactivity of $RO_2^\cdot$ radicals with NADH.

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