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1018 — ACTIVATION OF OXYGEN BY METAL COMPLEXES AND ITS RELEVANCE TO AUTOXIDATIVE PROCESSES IN LIVING SYSTEMS *

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SUMMARY

Aspects of the kinetics and thermodynamics of the iron-catalysed Haber–Weiss reaction are discussed with special emphasis on the potential *in vivo* sources of iron. In addition, the properties of the iron chelates that inhibit the Haber–Weiss reaction are considered.

INTRODUCTION

Since the mid-1970s, when it was discovered that metal ions were an essential ingredient for the production of the hydroxyl radical in superoxide-generating systems [1,2], much effort has been devoted to the study of iron catalysis of the Haber–Weiss reaction.

$$O_2^{\cdot -} + \text{Fe(III) complex} \rightarrow O_2 + \text{Fe(II) complex} \quad (1)$$

$$\text{Fe(II) complex} + H_2O_2 \rightarrow OH^{\cdot} + OH^- + \text{Fe(III) complex} \quad (2)$$

$$O_2^{\cdot -} + H_2O_2 \rightarrow OH^{\cdot} + OH^- + O_2 \quad (3)$$

Four important points have been made:

(1) It takes only trace amounts of metal (micromolar levels) to render significant catalytic activity [3,4].

(2) The coordination environment of the metal can drastically alter its effectiveness as a catalyst [3].

(3) To reconcile the high reactivity of the hydroxyl radical produced in the metal ion-catalysed Haber–Weiss reaction with the occasional failure of scavengers to

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inhibit these reactions, the idea of *site-specific* damage in biological systems has been introduced, *i.e.* the metal is bound to sites in the immediate proximity of which the damage will be produced [5].

(4) An as yet unknown oxidant, occasionally referred to as *Crypto-OH* with kinetic properties similar to those of $OH^{\cdot}$ [6,7], rather than the free hydroxyl radical, may be formed in iron-containing superoxide-generating systems, such as xanthine–xanthine oxidase [8,9], as well as in paraquat radical–hydrogen peroxide solutions [6,10]. There is now evidence that one of the possible active oxygen–iron intermediates may involve both Fe(II) and Fe(III) [7,11–13]. The research in this area is described by Koppenol in this volume [14].

WHERE IS THE IRON?

In humans most of the iron in the body is continuously recycled, with 70% being present in haemoglobin and 20–25% present as storage iron. Thus, the most likely candidates as sources for catalytic iron are haemoglobin, ferritin, transferrin and perhaps haemosiderin. In addition, Tangeras *et al.* [15,16] identified a mitochondrial pool of iron that is bound neither to haem nor to iron–sulphur centres and it appears to be readily available for haem synthesis. This pool represents 1.1 nmol iron/mg protein and may be available to act as a catalyst under conditions of oxidative stress.

Haemoglobin

Sadrzadeh *et al.* [17] have shown that in the presence of a superoxide anion-generating system, free haemoglobin promotes hydroxyl radical formation. However, Gutteridge demonstrated that oxidation of haemoglobin by peroxides releases the iron and only then can it serve as an effective catalyst for $OH^{\cdot}$ production [18]. This suggests that it is the release of iron by haemoglobin when subjected to oxidative stress that is important. His data indicate that intact haemoglobin is not an effective catalyst for the Haber–Weiss reaction. However, it is well known that in a wound, blood or free haemoglobin in combination with bacteria can have lethal consequences. This is probably because the haemoglobin iron can support prolific bacterial growth as well as possibly serving as a biological Fenton reagent. Haptoglobin, a haem-binding protein, is able to act as a natural bacteriostat [19] in addition to its suppression of haemoglobin-mediated oxidation [17]. These findings have indicated an important role for haptoglobin in the prevention of haemoglobin-supported bacterial infection and haemoglobin-mediated oxidation [17,19].

Ferritin

Ferritin is a large iron storage protein (for reviews, see Refs. 20–23). It can store within the central core up to 4500 Fe(III) ions as micro-crystalline ferric-oxide–phosphate. Access to the core by small cations, anions and neutral species

can be gained by six channels in the protein shell. The hydrous ferric oxide core of horse spleen ferritin was shown to be reduced by one electron per iron atom with $U_{1/2} = -190$ mV at pH 7.0 and $U_{1/2} = -310$ mV at pH 8.0 (*versus* a n.h.e.) [24]. Thus, at physiological pH, cellular reducing agents such as ascorbate and superoxide can, on thermodynamic grounds, reduce the iron to Fe(II), which is then released. Indeed, ferritin in the presence of ascorbate has been shown to stimulate lipid peroxidation [25,26], and oxygen free radicals generated by xanthine oxidase were shown to depolymerize hyaluronic acid in the presence of ferritin [27]. In addition, $O_2^{\cdot -}$ was shown to release iron from ferritin [28–32] and bring about ferritin-promoted superoxide-dependent lipid peroxidation [31].

Our pulse radiolysis examination of the reaction of superoxide with ferritin yielded a second-order rate constant of 2×10^6 dm^3 mol^{-1} s^{-1} [33]. This corresponds to a rate constant of 3×10^5 dm^3 mol^{-1} s^{-1} for each of the six channels leading to the ferritin core. This rate is similar to that of $O_2^{\cdot -}$ reacting with Fe(III)–EDTA [34–36]. Thus, in the presence of reducing agents, such as superoxide and ascorbate, ferritin can provide the transition-metal catalyst for the Haber–Weiss reaction.

Transferrin

Transferrin is an iron-transport protein. It accounts for nearly all the iron in plasma, being $2–4 \times 10^{-5}$ *M* [37] with each transferrin able to hold two Fe(III) atoms. At physiological pH in aqueous solution, the solubility of iron Fe(III)aq, would be only of the order of 10^{-17} *M*. Using computer modelling of the various equilibria, May and Williams [38] estimated that in plasma [Fe(III)aq] = 10^{-23} *M*, and [Fe(III)–citrate] = 10^{-11} *M*. Thus, Fe(III)–citrate accounts for over 90% of the iron present as low molecular weight complexes, but transferrin contains the bulk of the iron in plasma.

Conflicting reports have appeared as to whether transferrin can be an effective catalyst for the iron-driven Haber–Weiss reaction. References 39–44 have provided evidence against the participation of transferrin while positive evidence for the participation of the diferric transferrin complex has been presented in Refs. 45–47.

The reduction of Fe(III)–transferrin leads to the rapid release of iron as Fe(II) [48], due to the very weak binding of Fe(II) by transferrin [49,50]. Thus, the reduction of Fe(III)–transferrin in the presence of H_2O_2 would neccessarily lead to $OH^{\cdot}$ radicals. Unfortunately, due to the high reactivity of $OH^{\cdot}$ it was necessary to use indirect methods to determine its presence in each of the studies on the possible involvement of transferrin in the Haber–Weiss reaction [39–47].

Fe(III)–EHPG (EHPG = ethylenebis [(*o*-hydroxyphenyl)glycine]) has been found to be an excellent model compound for the active site of transferrin [51], having similar ultraviolet difference [52] and Raman spectra [51]. In addition, the binding constants for both EHPG and transferrin for Fe(III) and Fe(II) are quite similar, thus they have the same reduction potential of +0.40 V [53]. Each shows a strong absorption at ≈ 470 nm in the presence of Fe(III), with essentially no absorbance at that wavelength with Fe(II) or without iron.

Using pulse radiolysis as a method of generating superoxide and formate radicals and kinetic U.V.–VIS spectroscopy, we looked directly at the reaction of superoxide and formate radicals with transferrin and the transferrin model compound Fe(III)–EHPG. As predicted on thermodynamic grounds, no bleaching of the 470 nm absorption band occurs in the presence of superoxide ($U_0' = -0.33$ V [54]), but the much more reducing $CO_2^{\cdot -}$ radical ($U_{0,\,\text{oxidation}} = +2.0$ V [54]) does react with transferrin and the Fe(III)–EHPG complex [55].

CHELATORS OF Fe(III)

Since it was first observed that chelating agents can drastically alter the efficiency of iron as a catalyst for the Haber–Weiss reaction [3], many different chelating agents have been examined for their ability to slow the reaction. They have been used extensively as tools to study the involvement of iron in superoxide-induced oxidative processes. Special interest has been given to DETAPAC (DTPA) and Desferal. Both appear to slow the reduction of Fe(III) by $O_2^{\cdot -}$ drastically [35,36] and are thus inefficient catalysts [56]. Desferal is of particular interest as it is a clinically approved drug for the treatment of iron overload. There is potential for its use in the amelioration of ischemia reperfusion injury [57,58].

EDTA

In the initial research on superoxide chemistry after the discovery of the superoxide dismutase activity of erythrocuprein [59,60], EDTA was a popular ingredient in enzymatic reaction mixtures used in the study of superoxide and superoxide dismutase. However, Fe(III)–EDTA is reduced quite rapidly by $O_2^{\cdot -}$, $k = 1.8 \times 10^6$ dm^3 mol^{-1} s^{-1} at pH 7 [34–36] with U_0' (reduction of Fe(III)–EDTA) $= 0.117$ V [61]. Also, Fe(II)–EDTA reacts readily with H_2O_2 to produce a highly oxidizing intermediate with similarities to the hydroxyl free radical [62,63]. Thus, EDTA enhances the catalytic activity of iron by solubilizing the iron [64] into a form which is readily reduced to Fe(II) by $O_2^{\cdot -}$, reaction (1). The Fe(II)–EDTA is then a willing participant in the Fenton reaction, reaction (2), if H_2O_2 is available.

The use of EDTA has been criticized by many as not representative of iron in biological system. However, it should be kept in mind that as a tool it has provided a wealth of valuable information about the role of iron in the Haber–Weiss reaction.

DETAPAC (DTPA)

DETAPAC (diethylenetriaminepentaacetic acid) is structurally related to EDTA, except that it chelates iron in a form that is not active in the Haber–Weiss reaction [4]. The poor catalytic efficiency of Fe(III)–DETAPAC is a result of significantly slowing the reduction of iron by $O_2^{\cdot -}$, $k < 10^4$ dm^3 mol^{-1} s^{-1} [35], reaction (1).

However, Fe(II)–DETAPAC appears to react readily with hydrogen peroxide in a Fenton process [35,65,66], reaction (2). Thus, $O_2^{\cdot -}$ is not a strong enough reductant to reduce Fe(III)–DETAPAC in the Haber–Weiss reaction, but the paraquat radical is able to reduce Fe(III)–DETAPAC to Fe(II)–DETAPAC, which in turn will participate in a Fenton reaction forming OH$^{\cdot}$ [66]. The reduction of Fe(III)–DETAPAC by $O_2^{\cdot -}$ appears not to be limited thermodynamically, U_0' reduction of Fe(III)–DETAPAC) = 0.03 V [54], but rather kinetically.

Desferal

Desferal is a relatively specific iron chelator ★ that is of major clinical importance in the treatment of iron overload. It also slows the reduction of iron by superoxide to render the iron essentially catalytically inactive in the Haber–Weiss reaction. The rate constant for the reduction of ferrioxamine B (Desferal chelated to iron(III) [67,68]) by $O_2^{\cdot -}$ has been found to be less than 2×10^5 dm^3 mol^{-1} s^{-1}, as determined by pulse radiolysis [36]. However, in ischemia reperfusion injury other cellular reductants must also be considered. The ability of these species to reduce ferrioxamine B has not yet been investigated. Thus, there is still much to do in this area of research in the design of an effective clinical modality.

Desferal (in the absence of iron) has been shown to react directly with $O_2^{\cdot -}$, but not as a superoxide dismutase [69,70]. Using data presented by Sinaceur *et al.* [69,70] and a rate constant of 2.5×10^5 dm^3 mol^{-1} s^{-1} (Butler *et al.* give 5.5×10^5 dm^3 mol^{-1} s^{-1} [71]) for the reduction of cytochrome *c* by $O_2^{\cdot -}$ at pH 7.8 [72], we can estimate a rate constant of 1.1×10^3 dm^3 mol^{-1} s^{-1} for the reaction of Desferal with $O_2^{\cdot -}$. This corresponds to Halliwell's experimental result of $k = 9.1 \times 10^2$ dm^3 mol^{-1} s^{-1} [73]. However, a pulse radiolysis study of this reaction by the French group has led them to conclude that the rate constant for this reaction is 1.4×10^6 dm^3 mol^{-1} s^{-1} [74]. This discrepancy remains to be explained.

Kanner and Harel have shown that Desferal can inhibit membrane lipid peroxidation initiated by peroxidative systems [75]. However, their experiments suggest that the mechanism of inhibition is not by the sequestering of iron, but rather by Desferal serving as an electron or a hydrogen atom donor (an antioxidant?). This aspect of Desferal's chemistry has not yet been considered and must be investigated in detail.

Phytate

Phytic acid is a major component of all plant seeds, constituting 1–3% by weight of many cereals and oil seeds accounting for 60–90% of total phosphorus. It has been suggested that phytate inhibits metal-catalysed free radical reactions [76–78],

★ The formation constant of the Fe(III)–Desferal complex is $10^{30.6}$, while that of Cu(II)–Desferal is 10^{14} and that of Ca(II)–Desferal is 10^2 [37,67,68].

thereby protecting seeds from oxidative damage during storage. Graf *et al.* provided one piece of evidence consistent with this hypothesis [76]. Using pulse radiolysis to generate $O_2^{\cdot -}$, we have estimated the rate constant for the reduction of Fe(III)–phytate by $O_2^{\cdot -}$ to be less than 5×10^4 dm^3 mol^{-1} s^{-1} (G.R. Buettner, unpublished observations) for solutions that are 5 m*M* in phytate and have Fe(III) at less than 0.2 m*M*. Thus, it would appear that phytate may render iron a poor catalyst for the Haber–Weiss reaction. However, more detailed studies need to be completed before a firm conclusion can be made.

CONCLUSIONS

Chelating agents have been found to alter the catalytic efficiency of iron drastically in the Haber–Weiss reaction. The investigations with chelating agents resulted in a good, though not complete, understanding of the role of catalytic metals in this reaction. The research in this area has also revealed observations that are not consistent with free OH$^{\cdot}$ always being produced. Thus, a new research front has been opened up that needs to be pursued. To date, the bulk of the research dealing with the role of metals in reactions (1)–(3) has centred on the slowing (or acceleration) of reaction (1). Graf *et al.* [76] proposed that the availability of a water coordination site on the Fe(III) complex is the key to the complex being efficiently reduced by $O_2^{\cdot -}$. Those Fe(III) complexes without this *open* site will result in reaction (1) being kinetically very slow. At present, all chelating agents which have been investigated appear to slow reaction (1), *i.e.* reaction (1) is the rate-determining step in the production of OH$^{\cdot}$ in a superoxide-generating system. It would seem reasonable to investigate reaction (2) as well, and perhaps develop an alternative route for the suppression of OH$^{\cdot}$ production.

There is still much to do, but the research that has been done on the treatment of iron overload provides a large data-base of useful information that can help researchers in the advancement of the understanding of the role of iron, copper, $O_2^{\cdot -}$, H_2O_2, organic peroxides, alkoxyl radicals and OH$^{\cdot}$ in human health problems related to oxidative stress.

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