Free Radical Biology and Medicine 108 (2017) 94–109

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Original article

Labile iron potentiates ascorbate-dependent reduction and mobilization of ferritin iron

Charles Badu-Boateng[a], Sofia Pardalaki[a], Claude Wolf[b], Sonia Lajnef[c], Fabienne Peyrot[c,d], Richard J. Naftalin[a,*]

[a] Cardiovascular Division, British Heart Foundation Centre of Research Excellence and Physiology Department, King's College London, 150 Stamford Street, London SE1 9NH, UK
[b] APLIPID Sarl, 75016 Paris, France
[c] Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques (UMR CNRS 8601), Université Paris Descartes, 75006 Paris, France
[d] ESPE de l'académie de Paris, Université Paris Sorbonne, 75016 Paris, France

ABSTRACT

Ascorbate mobilizes iron from equine spleen ferritin by two separate processes. Ascorbate alone mobilizes ferritin iron with an apparent $K_{m\ (ascorbate)} \approx 1.5$ mM. Labile iron > 2 μM, complexed with citrate (10 mM), synergises ascorbate-dependent iron mobilization by decreasing the apparent $K_{m\ (ascorbate)}$ to ≈270 μM and raising maximal mobilization rate by ≈5-fold. Catalase reduces the apparent $K_{m(ascorbate)}$ for both ascorbate and ascorbate + iron dependent mobilization by ≈80%. Iron mobilization by ascorbate alone has a higher activation energy ($E_a = 45.0 \pm 5.5$ kJ/mole) than when mediated by ascorbate with labile iron (10 μM) ($E_a = 13.7 \pm 2.2$ kJ/mole); also mobilization by iron-ascorbate has a three-fold higher pH sensitivity (pH range 6.0–8.0) than with ascorbate alone. Hydrogen peroxide inhibits ascorbate's iron mobilizing action.

EPR and autochemiluminescence studies show that ascorbate and labile iron within ferritin enhances radical formation, whereas ascorbate alone produces negligible radicals. These findings suggest that iron catalysed single electron transfer reactions from ascorbate, involving ascorbate or superoxide and possibly ferroxidase tyrosine radicals, accelerate iron mobilization from the ferroxidase centre more than EPR silent, bi-dentate two-electron transfers. These differing modes of electron transference from ascorbate mirror the known mono and bidentate oxidation reactions of dioxygen and hydrogen peroxide with di-ferrous iron at the ferroxidase centre. This study implies that labile iron, at physiological pH, complexed with citrate, synergises iron mobilization from ferritin by ascorbate (50–4000 μM). This autocatalytic process can exacerbate oxidative stress in ferritin-containing inflamed tissue.

1. Introduction

Iron is an essential mineral required for synthesis of iron-containing proteins, including haemoglobin, myoglobin and cytochromes [1]. In a typical 70 kg adult human male, around 30% of his total iron (6 g) is sequestered in ferritin. Free "labile" iron activity within body fluids is normally very low and present mainly as hydrated complexes in proteins, such as albumin, or as low molecular weight chelates with citrate or glutathione [2]. In the plasma of patients suffering from iron overload, the amount of redox active and chelatable non-transferrin bound iron (NTBI) is in the range of 1–20 μM [3–5]. Ascorbic acid is highly concentrated in a few tissues, e.g. brain, adrenal glands, and cartilage [6], but low in others, e.g. liver and pancreas [7]. Under these circumstances, a complex tissue-differentiated regulation of the iron storage/release from ferritin must be considered.

Ferritin is a multimeric iron storage protein consisting of a mixture of 24 heavy (FHC) and light (FLC) polypeptide chains with combined weights of 474 kDa that form a spherical shell with the capacity to store oxidised ferric iron. Only the FHC chains possess ferroxidase activity (i.e. the capacity to catalyse oxidation of Fe(II) to Fe(III)); FLC-rich ferritins, such as equine spleen ferritin, have slower iron oxidation rates, but promote nucleation and storage of ferric iron into the ferritin

Abbreviations: Asc, ascorbic acid; Asc Ox, ascorbic acid oxidase; $AscH^-$, ascorbate anion; Asc^{2-}, ascorbate dianion; $AscH^{\bullet}$, ascorbyl radical; AFR or $Asc^{\bullet-}$, ascorbate radical; Cat., catalase; CL, chemiluminescence; DHA, dehydroascorbate; EPR, electron paramagnetic resonance; FHC, ferritin heavy chain; FLC, ferritin light chain; FOC, ferroxidase centre; respectively, K_m, $V_{max\ ascorbate}$, Michaelis Menten coefficients in mM and nmol mg^{-1} ferritin min^{-1}; mT, milliTesla; MOPS, 3-(N-morpholine)propanesulfonic acid; NTBI, non-transferrin bound iron; ROS, reactive oxygen species; SOD, superoxide dismutase; XO, xanthine oxidase.

* Corresponding author.
E-mail address: richard.naftalin@kcl.ac.uk (R.J. Naftalin).

http://dx.doi.org/10.1016/j.freeradbiomed.2017.03.015
Received 14 October 2016; Received in revised form 29 January 2017; Accepted 15 March 2017
Available online 21 March 2017

core [8]. The numbers of iron ions stored as crystalline solid per ferritin cage normally vary from 500 to 2500. In iron-overload conditions, this number can reach 3000–4000 [9].

Nonpolar channels, predominantly lined with leucine lie within the six four-fold subunit intersections of the ferritin shell [10,11]; whereas hydrophilic channels, lined predominately with anionic aspartate and glutamate side-chains, are present in all eight of the three-subunit intersections. Ferrous iron Fe(II) enters and leaves ferritin through these 3-fold hydrophilic channels and a gateway iron binding site at the channel entrance [12]. Iron(II) entry via the threefold channels can be blocked by Zn(II) [13].

1.1. Iron uptake into ferritin via multiple oxidation processes

The initial reaction of ferrous iron with ferritin involves dioxygen reacting with two vicinally bound ferrous ions at the ferroxidase centre (FOC), present only in FHC, in a sequential electron transfer process to reduce dioxygen to hydrogen peroxide [14–16] to produce diferric-peroxo precursor (2FeOOH), protons and hydrogen peroxide (Eqs. (1) and (2)) and then to the diferric oxo product. Similar processes occur in other members of the ferritin-like superfamily, e.g. ribonucleotide reductase, R2 proteins, where a di-iron ligand pair is bridged by glutamate carboxyl residues [17,18].

$$2\text{Fe(II)-P} + O_2 + 2H^+ \rightarrow 2\text{Fe(III)}\,(O_2) + 2H^+ \rightarrow 2\text{Fe(III)-P} + H_2O_2 \quad (1)$$

where Fe(II)2-P represents the protein complex with iron [19,20].

The unstable bridged oxy-diferric complex undergoes hydrolysis, which results in proton release and is the basis of the mineralization process. As iron loading proceeds, a higher proportion of oxygen from water takes part in the reaction and net hydrogen peroxide production is reduced to zero:

$$4\text{Fe(II)} + O_2 + 6H_2O \rightarrow 4\text{FeOOH-P} + 8H^+ \quad (2)$$

At still higher iron loads [11], hydrogen peroxide is consumed in a two-electron transfer reaction to convert the diferrous to diferric complex at the FHC, thereby consuming the potentially toxic hydrogen peroxide.

$$2\text{Fe(II)} + H_2O_2 + 2H_2O \rightarrow 2\text{FeOOH-P} + 4H^+ \quad (3)$$

This differs from the Fenton reaction in which water solvated ferrous iron is oxidised by hydrogen peroxide to produce ferric iron and hydroxyl radical.

$$\text{Fe(II)} + H_2O_2 \rightarrow \text{Fe(III)} + HO^{\bullet} + OH^- \text{ [21]} \quad (4)$$

The one-electron reduction of O_2 by Fe(II) produces superoxide [18].

$$\text{Fe(II)} + O_2 \rightarrow \text{Fe(III)} + O_2^{\bullet -} \quad (5)$$

The sum of reactions in Eq. (4) and the reversed reaction in Eq. (5) [22] in aqueous solutions is termed the Haber-Weiss reaction [22,23] Eq. (6),

$$H_2O_2 + O_2^{\cdot -} \xrightarrow[\Leftrightarrow]{Fe(II),\ Fe(III)} HO^{\cdot} + OH^- + O_2 \quad (6)$$

This process is facilitated when ferric iron is chelated with citrate or adenine nucleotides or EDTA [5] and citrate is used as the iron buffer in all experiments described in this paper. At aqueous interfaces, the most favoured reaction appears to be a two-electron oxidation [24].

$$\text{Fe(II)} + H_2O_2 \rightarrow \text{Fe(IV)}O^{2+} + H_2O \quad (7)$$

Or

$$\text{Fe(II)} + O_3 \rightarrow \text{Fe(IV)}O^{2+} + O_2 \quad (8)$$

and this reaction may also occur within the ferritin core.

It has been reported that ferric citrate complexes can auto-reduce and take part in redox cycling [25].

1.2. Release of iron from ferritin

Although lysosomal proteolytic degradation of the ferritin shell with consequent iron release occurs [26], this is an uncontrolled and potentially toxic process; normally, a more controlled mechanism is thought to occur [26,27]. Elucidating this buffering role by ferritin of cytoplasmic labile iron and reactive oxygen species (ROS) is the current priority. Iron mobilization from the ferritin core involves reduction of the insoluble ferric to soluble ferrous state. Electron transfer between the cytosol and the core is essential to this release process. The liberated core ferrous ions are hydrated, solubilised and exit via ferritin's threefold polar channels with water [18]. Electron donors, such as superoxide ($O_2^{\bullet -}$) or ascorbate anion ($AscH^-$), or ascorbate radical, $Asc^{\bullet -}$ (AFR) [28], may pass directly via the wall channels to the ferritin core to reduce iron by one-electron transfer [29–31].

$$AscH^- + \text{Citrate-Fe(III)} \rightarrow Asc^{\bullet -} + \text{Citrate-Fe(II)} + H^+ \quad (9)$$

A subsequent one-electron reduction of ferric to ferrous iron and also producing dehydroascorbate (DHA) can be obtained by oxidation of AFR [4,23].

$$Asc^{\bullet -} + \text{Citrate-Fe(III)} \rightarrow \text{DHA} + \text{Citrate-Fe(II)} \quad (10)$$

An alternative mechanism may involve electron transference by tunnelling through the protein via an electron transport chain. Haem, too large to pass into the ferritin via the pores, can act as an electron donor [29,32]. Electrons can also be transferred to ferritin by passing an electric current directly from electrodes [33].

1.3. Effects of ascorbate: role of complexed metal ions in ascorbate oxidation

The spontaneous rate of ascorbate oxidation

$$Asc^{2-} + O_2 \rightarrow Asc^{\bullet -} + O_2^{\bullet -} \quad (11)$$

is slow ($300\ M^{-1}\ s^{-1}$), but in the presence of traces of iron or copper ascorbate oxidation is notoriously rapid [21,34,35]. Ascorbate can readily donate electrons to metal ions such as iron and copper [36–39] as seen in Eqs. (9) and (10).

Ascorbate is generally considered as an efficient antioxidant of nearly every oxidizing radical, e.g. $R^{\bullet} + AscH^- \rightarrow Asc^{\bullet -} + RH$ [40].

This reaction can be either retarded or accelerated by complexation with reagents such as desferrioxamine or citrate, respectively [23,41].

The mechanisms by which combinations of metal ion, dioxygen, and ascorbate produce superoxide, hydrogen peroxide, and hydroxyl radicals are contentious [21,37–39,42]. Metal ion catalysis of superoxide production results in extensive redox cycling when iron, oxygen, and ascorbate are together in solution, (see Eqs. (5)–(11)) [43]. The review paper by Frei and Lawson [37] indicates that metal ion-catalysed oxidation of ascorbate produces ascorbate radicals as a consequence of the transient intermediate formation of superoxide radicals that disproportionate to hydrogen peroxide and dioxygen. In turn, the hydrogen peroxide formed can react with superoxide in the Haber-Weiss reaction (Eqs. (5) and (6)) to produce hydroxyl radical and may be the basis of ascorbate's observed pro-oxidant effect.

These secondary reactions initiated by ascorbate, oxygen, and metal ions can be inhibited by superoxide dismutase and catalase and thereby can alter the rates of ferritin iron mobilization [44].

Many studies have shown that ascorbate increases the rate of iron release from ferritin by reducing core ferric to ferrous iron [45–47]. The apparent K_m for this complex ascorbate-dependent iron mobilization process from ferritin is in the range 1.5–2.0 mM [44,47,48]. In this paper the apparent $K_{m\ (\text{ascorbate} \pm \text{Fe})}$ refers to the half-maximal concen-