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The resistance of transferrin, lactoferrin and caeruloplasmin to oxidative damage

The recent paper of Winterbourn & Molloy [1], reporting that apolactoferrin and apotransferrin lose some of their ability to bind iron after exposure to an excess of hypochlorous acid (HOCl), prompts us to report some of our unpublished experimental observations and to comment on the general significance of these studies.

Iron ions attached to the two high-affinity binding sites of transferrin or lactoferrin are unable to catalyse damaging free radical reactions such as lipid peroxidation or formation of hydroxyl radicals ($^{\cdot}OH$) from superoxide (O_2^-) and H_2O_2 [2–4]. Thus addition of these proteins can inhibit iron-dependent radical reactions [4,5]. We found that when purified human lactoferrin or transferrin (obtained as in [4]) were incubated for 30 min with up to a 20-fold molar excess of HOCl (1 μM-protein, 20 μM-HOCl) and subsequently added to radical generating systems, the preincubation with HOCl did not affect the ability of the proteins to prevent formation of $^{\cdot}OH$ from H_2O_2 plus O_2^- (or ascorbate and H_2O_2) in the presence of $FeCl_3$ or ferritin. The assay systems used are as described in [4]. Thus, even though HOCl treatment may weaken the binding of iron to the proteins, so that it is lost on dialysis [1], the initial binding that does occur is still sufficient to inhibit iron-dependent radical reactions. These results essentially confirm and strengthen the proposal [1] that lactoferrin and transferrin will tend to retain their iron-binding capacity at sites of inflammation. The increased resistance of the iron-loaded proteins to attack by HOCl [1] is in agreement with previous reports that the iron-loaded proteins are much more resistant to denaturation than the apoproteins [6,7]. The ability of lactoferrin and transferrin to resist attack by HOCl, H_2O_2 and organic peroxides [8] supports the proposal [9–11] that they function as antioxidants at sites of inflammation, by inhibiting iron-dependent radical reactions.

It has been suggested that caeruloplasmin also functions as a major antioxidant in extracellular fluids, partly by its ferroxidase activity [12]. Caeruloplasmin is usually regarded as labile, readily releasing some of its copper ions and undergoing proteolytic modification; this lability has hampered isolation of the intact protein (reviewed in [12]). It is therefore interesting to note that caeruloplasmin is fairly reistant to oxidative attack. Thus incubation of 1 μM purified human caeruloplasmin with 100–200 μM-HOCl at 37 °C and pH 7.4 for 20 min caused only a 31% loss of oxidase activity (our unpublished results). Gutteridge [8] found that treatment with 0.48 mM-H_2O_2 or organic hydroperoxide had no effect on the ferroxidase action of human caeruloplasmin, whereas Winyard *et al.* [13] found only a 25% loss of the ferroxidase activity of this protein after incubation with 0.4 mM-H_2O_2 and 0.1 mM-copper, a system generating reactive species such as $^{\cdot}OH$ and/or copper(III) [10]. Indeed, caeruloplasmin may be able to scavenge H_2O_2 [14]. Thus the ability of transferrin, lactoferrin and caeruloplasmin to withstand oxidative stress would make them good candidates to act as antioxidants at sites of inflammation, as has been proposed [9,10].

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Halliwell and his colleages [1] agree with our conclusion [2] that apolactoferrin and apotransferrin are relatively resistant to inactivation by myeloperoxidase-derived hypochlorous acid (HOCl). However, in our study we did observe some loss of binding capacity (approx. 70%