Desferrioxamine and membrane oxidation: radical scavenger or iron chelator?

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Oxidative membrane damage can be both initiated (reviewed in [1–3]) and propagated [4, 5] by radical reactions catalysed by low molecular mass complexes of non-haem iron.

The iron chelator desferrioxamine complexes ferrie ions with a binding constant of \(10^{13}\) [6]. It has also been shown to interact with the superoxide radical [7, 8], the peroxyl radical [9] and to inhibit peroxidation in membranes by acting as an electron donor [10].

Abnormal iron-containing species associated with the erythrocyte membrane in sickle-cell anaemia have been described [11–13]. These have been implicated in the catalysis of radical reactions leading to the oxidative membrane damage characteristic of this disease [12]. In particular, the oxidative modifications to the cytoskeletal proteins [14] may contribute towards the development of morphologically altered cytoskeletons in irreversibly sickled cells [15].

Recent reports suggest that these membrane-associated iron species are bound to the cytoskeleton [16], are mostly non-haem in nature and are composed of two components: a less accessible component which may be contained within ferritin or haemosiderin and a more accessible component [17, 18]. Haem iron is a minor component of membrane-associated iron.

Abbreviation used: DMPO, 5,5-dimethyl-1-pyrroline N-oxide.

Here we have used electron spin resonance (e.s.r.) spectroscopy to assess the nature and catalytic ability of the iron associated with sickle-cell membranes and the subsequent responses on incorporation of desferrioxamine.

Erythrocyte membranes were prepared from sickle and normal erythrocytes at pH 7.4 as described in [12]. To assess the ability of membrane-associated iron species to catalyse the breakdown of hydroperoxides, membranes (1.7 mg of protein/ml) were mixed with tert-butyl hydroperoxide (10 mM). The alkoxyl and peroxyl radical products were detected, using the spin trap DMPO (40 mM), with a Bruker ESR 300 e.s.r. spectrometer. Hyperfine coupling constants were measured directly from the field scans. Total membrane-associated iron species and the specific components were quantified as previously described [18].

The addition of sickle erythrocyte membranes initiated the breakdown of tert-butyl hydroperoxide to alkoxyl and peroxyl radical species. Typical e.s.r. spectra of alkoxyl-DMPO and peroxyl-DMPO radical adducts are shown in Fig. 1.

The initial rate of alkoxyl and peroxyl radical spin adduct generation from tert-butyl hydroperoxide induced by erythrocyte membranes of different sickle patients was found to correlate \((r=0.95, n=7)\) with the levels of the more accessible component of non-haem iron associated with these membranes. The addition of normal erythrocyte membranes also initiated the breakdown of tert-butyl hydroperoxide to alkoxyl and peroxyl radical species, but to a considerably lesser extent.

The catalytic activity of the normal erythrocyte membranes can be accounted for by the low levels of membrane-associated haem iron. Since the levels of haem iron are
similar in the normal and sickle membrane preparations [18], the increased activity of the latter can be ascribed to the non-haem iron species, in particular, to the more accessible non-haem iron component. Ferritin (dialysed) at appropriate concentrations did not have any catalytic activity.

Preincubation of sickle erythrocyte membranes with 90 μM-desferrioxamine significantly reduced the levels of alkoxyl and peroxyl radical spin adducts produced in the t-butyl hydroperoxide system and signals from the desferrioxamine nitroxide radical [7] were also detected (Fig. 1). On similar treatment of the normal membranes no modification was observed.

Higher concentrations (10 mM) of desferrioxamine had a more pronounced effect on reducing the levels of alkoxyl and peroxyl radical adducts in both sickle and normal membranes, while the signals corresponding to the desferrioxamine nitroxide radical increased.

These results suggest that at low concentrations (90 μM) desferrioxamine appears to be acting mainly as an iron chelator, binding the more accessible component of non-haem iron, thus partially inhibiting the catalysis of the hydroperoxide breakdown. At high (>1 mM) concentrations, desferrioxamine is also acting as a scavenger of peroxy and alkoxyl radicals.

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