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Desferrioxamine

by

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Abbreviations:

DFO- Desferrioxamine
EPR- Electron paramagnetic resonance
FAB-MS- Fast atom bombardment mass spectrometry
HPLC- High pressure liquid chromatography
M_w- Molecular weight
UV/Vis- Ultraviolet/Visible spectrometry

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Abstract

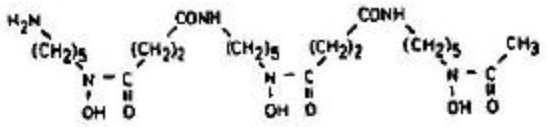
Desferrioxamine is a chelator of iron, aluminum and other metals. It is a hexadentate ligand that binds with an extremely favorable stability constant. This property of desferrioxamine makes it ideal for treating diseases such as thassalemia, in which the body is overloaded with iron. Desferrioxamine can also be oxidized to give a nitroxide radical, which leads to some interesting chemistry. The properties, reactions and biological activities of desferrioxamine will be discussed.

Introduction

Iron is essential for life, but as is often the case, it is possible to get too much of a good thing. If that happens, desferrioxamine could be used to sponge up the excess. Desferrioxamine is a hexadentate chelating ligand, which binds to Fe^{3+} with a stability constant near 10^{31} [1]. It is somewhat selective, in that it binds to Fe^{2+} with a stability constant of 10^{7} [2] and other first row transition metals in the range of 10^{10} to 10^{15} [3]. Desferrioxamine is a large molecule, composed of one acetic acid, two succinic acids, and three molecules of 1-amino-5-hydroxylaminopentane, giving a M_w of 560 a.u. Desferrioxamine was first extracted from *Streptomyces pilosus*, where it was a natural iron chelator. Its basic character and ability to form inorganic salts derives from its terminal free amino group [4], and desferrioxamine has a pK_a of 9.2 [5].

When desferrioxamine is combined with iron, it is called ferrioxamine. To further complicate matters, desferrioxamine is referred to by many names in the literature. Some are alternate spellings: desferioxamine, deferrioxamine, and deferioxamine, while others are totally different, like Desferal™. Because the molecule is not ferocious or savage, it is not called feral when combined with iron.

The structure of desferrioxamine is shown below, in both chain form and chelated to Fe^{3+}.



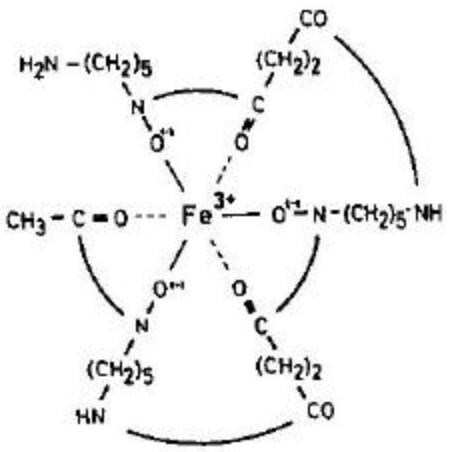


Figure 1- Above, desferrioxamine in the chain form, and, at right, ferrioxamine chelated to Fe^{3+}. Notice the complicated arrangement around the Fe atom. Adapted from [4].

Stability of desferrioxamine complexes

Desferrioxamine is selective towards Fe^{3+} because of their extremely favorable binding constant, nearly 10^{31}. The binding constants of other metals are shown below, in Table 1. This was adapted from [18].

Metal	Fe^{3+}	Fe^{2+}	Ni^{2+}	Cu^{2+}	Zn^{2+}	Cd^{2+}	Al^{3+}	La^{3+}	Yb^{3+}
Stability Constant [M-DFO]/[M][DFO] (log K =)	30.7	7.2	10.9	14.1	10.1	7.9	23.1	10.9	16.0

The most stable compound, by far, is Fe^{3+}-DFO. Its nearest neighbor is Al^{3+}-DFO. The preference of DFO for Fe^{3+} rather than Fe^{2+} is clearly shown above.

Desferrioxamine in biological systems

There are several biological uses for desferrioxamine. Thassalemia is a disease caused by iron overload, and a series of subcutaneous desferrioxamine injections are the primary treatment [6]. Free radicals are produced during the ischemia/reperfusion sequence of all open-heart operations. Transition metals are responsible for some of the radicals produced, and DFO can inhibit their action [7]. A DFO derivative, hydroxyethyl-starch desferrioxamine, has been used to delay the onset of diabetes mellitus in rats. Once again, the mechanism was believed to involve the chelation of iron, but only seemed to work on male rats. Further studies are ongoing to elucidate this discrepancy [8].

Desferrioxamine can also be used to help cells survive high levels of H_2O_2. When DFO is present, enough iron is chelated to prevent the harmful breakdown of H_2O_2, and

cells could deal with it by normal means, such as catalase [9]. Iron isn't the only metal chelated by DFO. The presence of DFO-Mn(III), an SOD mimic, inhibits cataract formation in rabbits by decreasing production of $O_2^{\bullet-}$ and $HO^{\bullet}$ [10].

Desferrioxamine can be used to inhibit lipid peroxidation. In systems containing metmyoglobin and methemoglobin, lipid peroxidation was inhibited almost completely by the addition of 10 μM DFO. Interestingly, another chelator, EDTA, was used and had very little effect. The results are pictured in the graph below, taken from [17].

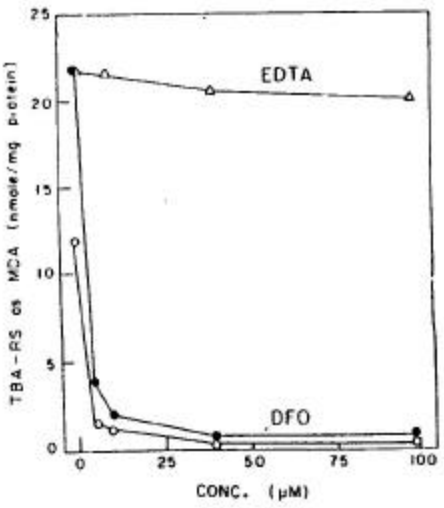


Figure 2- The effect of DFO and EDTA concentration on membranal lipid peroxidation by H_2O_2-activated metmyglobin/methemoglobin. DFA drastically reduces peroxidation by this system.

Nitroxide Radical

Although desferrioxamine is an inhibitor of iron dependent free radical reactions, its prolonged use is far from benign. Actually, DFO can be oxidized by horseradish peroxidase [11], neutrophils [12], or other enzymes, and the resulting nitroxide radical can initiate radical chemistry in a system.

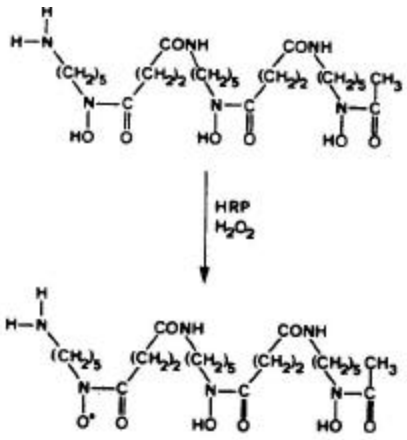


Figure 3- The nitroxide radical produced from the reaction of DFO with HRP and H_2O_2. The resolution of the resulting EPR spectrum is too poor to determine which hydroxamic acid is attacked. Adapted from [11].

The nitroxide radical is only formed at relatively high concentrations of DFO, greater than 1 mM. This means that DFO should not be used in biological systems with peroxidases present [11]. Neutrophils contain myeloperoxidase, which oxidizes DFO and causes problems with the removal of radicals from infection sites [12]. The nitroxide radical may also affect the activity of other enzymes, such as Ca^{2+}-ATPase. This enzyme is found in the sarcoplasmic reticulum, and can act in the same manner as a peroxidase. Metabolites, such as desferrioxamine nitroxide radical, inhibit the enzyme by interrupting the Fenton reaction [13].

Detection of DFO Radical

Once desferrioxamine has been oxidized by a peroxidase, the EPR spectrum is obtainable, since the radical is rather stable. A sample spectrum, adapted from [11] is pictured below:

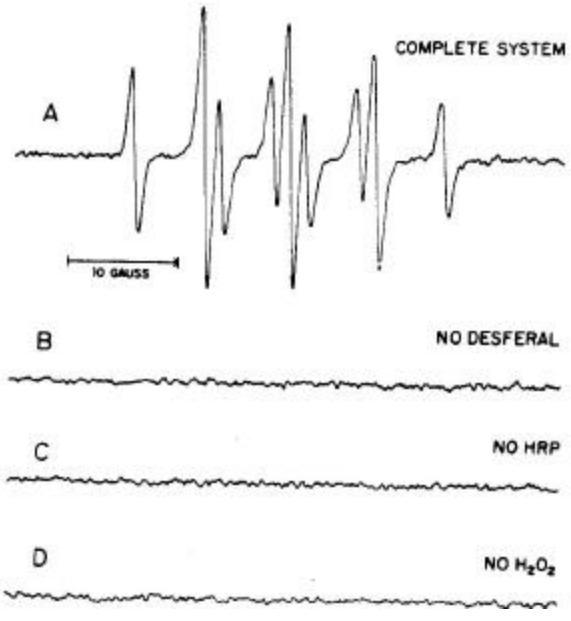


Figure 4- The one-electron oxidation of DFO by HRP. The reaction mixture, A, contained 0.1 mM Desferal, 0.1 mg/mL HRP, and 50μM H_2O_2 in a 100 mM phosphate buffer at pH 7.4. B-D were control experiments, each having 1 essential reactant removed. The g-value is 2.0065, with a^N = 7.85 G and a^H = 6.35 G.

Vladimirov *et al.* discovered a fantastic method for determining the free iron concentration in tissue homogenates. They used both DFO and 1,10-phenanthroline to chelate iron in both the (II) and (III) states. Then, a combination of EPR and UV/Vis techniques measured the concentration of free iron in rat liver [17].

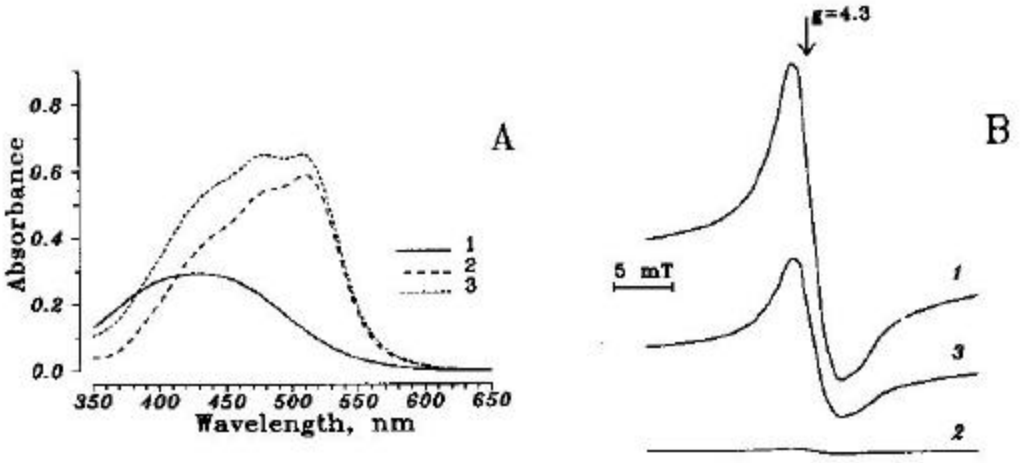


Figure 5- Absorption spectra (A) and EPR signals (B) of solutions containing 50 μM Fe(II) and 50 μM Fe(III) in the presence of desferrioxamine (1), 1,10-phenanthroline (2), or a desferrioxamine-phenanthroline mixture (3). In A.3, an additive effect is observed, whereas in B.3 there is destructive interference. The EPR signal in B is centered at g = 4.3, indicating the presence of Fe(III). Adapted from [17].

Ferrioxamine absorbs at 430 nm (ε = 2.48 mM^{-1} cm^{-1}) [14], while desferrioxamine is a colorless crystal, so alternate methods are used to detect it. Other methods for detection of DFO include HPLC and FAB-MS. Reverse phase HPLC has been used to detect the amount of DFO available iron in rabbit kidneys. The kidneys were processed, with ferrioxamine and desferrioxamine eluting at different times. This experiment was done to check the total amount of iron which could be chelated by DFO, thus depriving other harmful enzymes, such as bleomycin, which uses iron to degrade DNA, of using iron to harm cells [15]. Finally, FAB-MS was used to test the urine of DFO-treated

humans looking for the signature *m/z* of 613 [16]. This is probably one of the easiest methods of detection.

Conclusion

In summation, desferrioxamine is extremely effective at sponging up excess iron in biological systems. This is due to its high stability constant, as well as properties that make it friendly to most cells. In high concentrations however, a nitroxide radical may be formed, causing damage to the local environment. There are many methods used to detect desferrioxamine, including EPR and UV/Vis. These methods can also be used to discover interesting features of the cell, such as the iron content.

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