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Comment

Comment on "Free-Radical Formation by the Peroxidase-like Catalytic Activity of MFeO (M = Fe, Ni and Mn) Nanoparticules"

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Comment on "Free-radical formation by the peroxidase-like catalytic activity of MFe_2O_4 (M = Fe, Ni and Mn) nanoparticules"

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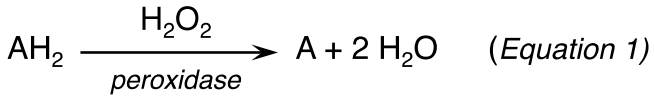
The peroxidase-like activity of magnetite and its connection with a neurodegenerative disease such as Alzheimer's disease has been recently questioned by our group,[1] despite claims by Gao *et al.*[2] Since reproducibility is the essential factor in experimental sciences, the article of Moreno Maldonado *et al.* on the peroxidase-like activity of magnetite attracted our attention.[3] As a matter of fact this new report is providing additional evidences on the absence of peroxidase activity of magnetite derivatives. The following comments are not a question of semantics, but are driven by the necessity to correctly

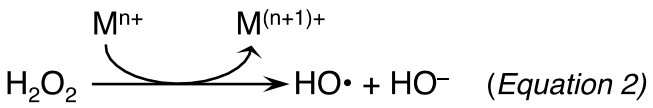
describe the different possible origins of the oxidative stress involved in many neurodegenerative pathologies.

First question: are the authors really observing a "peroxidase-like catalytic activity" as claimed in the title? Peroxidases are enzymes able to perform the 2-electron oxidation of a substrate AH_2 using hydrogen peroxide as oxidant (see equation 1).



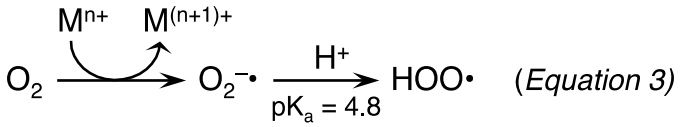
(*Equation 1)*

Mechanistic details concerning peroxidases and biomimetic models are available in text-books.[4,5] The authors did not report the catalytic oxidation of a peroxidase substrate, but they detected by spin-trap methods the presence of two radicals, HO• or HOO•, derived from the reaction of hydrogen peroxide or molecular oxygen, respectively, with low-valent metal ions. The hydroxyl radical HO• is the one-electron reduction product of hydrogen peroxide, generated by a low-valent metal ion in a Fenton-type reaction (see equation 2).



(*Equation 2)*

The hydroperoxyl radical HOO• is the protonated form of the superoxide anion $O_2^{\bullet -}$, which is the result of the one-electron reduction of molecular oxygen by a low-valent metal ion (equation 3). The protonation of superoxide anion with a pKa value of 4.9 generates the neutral radical HOO• (named hydroperoxyl or perhydroxyl). [6]



(*Equation 3)*

These two oxygen-centered radicals are clearly not the products of a peroxidase-type reaction.

Second question: are both oxygen-centered radicals generated by a catalytic reaction? Peroxidases are highly efficient catalysts. For instance, a $2x10^{-11}$ M concentration of the paradigm horseradish peroxidase (HRP) achieves oxidation of $4x10^{-5}$ M of tetramethylbenzidine within 5 min, in acetate buffer pH 3.5, thus giving rise to a turnover number of 2 millions of catalytic cycles within this short period of time, which corresponds to a turnover rate of 7000 cycles per second.[1] In the article by Moreno Maldonado *et al.*, the total production of HO• + HOO• with magnetite was slightly below 2000 nM (lanes "Fe-MNP", Figure 4 of the ref. 3) for an initial concentration of Fe(II) of 1600 μM (120 μg of $Fe^{II}_{0.81}Fe^{III}_{2.19}O_4$ in 260 μL of solution, Experimental

Methods). So the yield of these two oxyl radicals was only 0.13% with respect to Fe(II), a sub-stoechiometric number, far from a catalytic activity. The reported initial concentration of H_2O_2 was 0.49 M (Experimental Methods). The production of 2 μM of HO• + HOO• therefore corresponds to the conversion of 0.0004%, a very small fraction of the starting amount of H_2O_2. So, the very low and non-catalytic yield of HO• + HOO• with respect to Fe(II)] is probably the result of a Fenton-type chemistry induced by adventitious redox-active iron impurities contained in the isolated nanoparticles or generated by traces of reductants.

In conclusion, the experimental results reported by Moreno Maldonado *et al.*, provide an additional proof that magnetite nanoparticles has no peroxidase activity. In addition, the authors mentioned that "no radical formation was evidenced for any sample at pH = 7.4", a value corresponding to physiological pH. So, the biomedical claims are not consistent with the reported results. Consequently, the title of the article and all comments concerning a "peroxidase-like catalytic activity" (and putative biomedical claims) of magnetite and other ferrite nanoparticles, did not resist to a careful examination of experimental results. All the reported results can be summarized by the following sentence: "*No peroxidase activity is observed with MFe_2O_4 (M = Fe, Ni and Mn) nanoparticules and only traces of hydroxyl and hydroperoxyl radicals are observed at pH = 4.8. At physiological pH, these radicals are not detected*".

References

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