Fixing the Classical 2-Deoxyribose Assay

Genaro-Mattos, T.C.[(1,2)]; Dalvi L.T.[(1,3)]; Bicalho, J.M.[(1,3)]; and Hermes-Lima, M[(1)].
[1]Oxyradical Research Group, Biologia Celular, UnB; [2]Instituto de Química, UnB;
[3]PPG Nutrição, UnB, Brasília, 70910-900, Brazil.

In the past 2-3 decades many works (> 350) employed the 2-deoxyribose (2-DR) degradation assay in order to study anti/pro-oxidant properties of pure compounds or plant extracts. Previous reports from our group showed that the blank assays used to study Fenton or Fe(II) autoxidation reactions were not appropriated (SBBq 2007, abstract T-31). Our results demonstrated that Fe(III), a product of these reactions, reacts with 2-DR to form malonaldehyde (MDA), creating an artifact. This artifact accounts for 20-90% of Fe(III)-induced interference, depending on the assay conditions. We also showed that Fe(III)-interference is not influenced by buffer composition, or by the addition of antioxidants or iron-chelators. In this work we further characterize the Fe(III)-interference to establish a new blank for the 2-DR assay. We demonstrate that increasing concentrations of H_2O_2 (0 to 1.0 mM) have no effect on Fe(III)-interference (50 µM iron). Moreover, HPLC determinations show that Fe(III), added before or after thiobarbituric acid (followed by 15 min boiling), generates an amount of MDA – a major product of 2-DR degradation – that is consistent with spectrophotometric determinations. These results confirm that Fe(III) does react with 2-DR to produce MDA and that this reaction occurs in the analytical phase of method. We also demonstrate that the efficiency several antioxidants (evaluated by the 2-DR assay) are underestimated without the proper blank corrections. For example, I-50 values for Nasturtium leaf extracts (in Fenton induced 2-DR damage) were 0.04 and 0.08 mg/mL, with and without blank-correction, respectively. The mechanism of 2-DR reaction with Fe(III) is now under investigation. **Acknowledgments:** CNPq, Redoxoma-CNPq, Sandra Arruda, Fernanda Rosa. **Keywords:** Fenton, Fe(II)-autoxidation, TBARs.