Reevaluation of the 2-deoxyribose assay for the study of metal-mediated free-radical formation.

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The 2-deoxyribose (2-DR) degradation assay is a simple and widely used test for determining anti/pro-oxidant properties of molecules and plant extracts. Most reports use reaction blanks omitting 2-DR or TBA during assays. However when studying Fenton or Fe(II) autoxidation reactions, we verified that these blanks are not appropriated. Ferric ion - the product of these reactions – causes a relevant artifact in the assay, where 2-DR is oxidized by a Fe(III)-mediated process. It was observed that Fe(III) added after or before TBA generates considerable amounts of malonaldehyde (2-DR degradation product) in comparison with assays employing Fenton reagents or Fe(II) autoxidation. Moreover, the addition of antioxidants such as catalase and thiourea has no effect on Fe(II)-induced 2-DR degradation, indicating that ROS are not involved in this process. This Fe(III)-mediated 2-DR damage is dependent on the concentration of iron and 2-DR, but it is not influenced by buffer composition or iron-chelators. Depending on the assay conditions the Fe(III)-interference accounts for 20-50% of 2-DR degradation mediated by Fe(II). A new blank reaction is proposed herein – based on the use of Fe(III) - for the 2-DR assay. The lack of such correction has caused the underestimation of antioxidant activity of many compounds in hundreds of previous studies. The mechanistic aspects of this Fe(III)-interference are under determination.
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