SUPPLEMENTARY MATERIALS

The Amplex Red reagent (10-acetyl-3,7-dihydroxyphenoxazine; acetylresorufin) reacts with H_2O_2 with a 1:1 stoichiometry in the presence of peroxidase to produce the highly fluorescent oxidation product resorufin.[1] It is well-recognized that H_2O_2 can be reduced by AA in aqueous solution,[2] and there is evidence that this reaction can be accelerated by peroxidase in the presence of dyes with specific chemical structures, leading to an underestimate of [H_2O_2] assayed in the presence of AA.[3] However, previous studies suggest that AA does not interfere with Amplex red assay of H_2O_2 generated by the combination of glucose and glucose oxidase.[1] Furthermore, we confirmed that assay of H_2O_2 generated by the oxidation of 1 mM AA in oxygenated Holman's buffer at 37^o was unaffected by 5 min incubation with ascorbate oxidase (AAO, 0.1 U/ml), which rapidly destroys AA (Figure 1A). [4] We also confirmed that assay of authentic H_2O_2 (50 μM) was unaffected by addition of 200 μM BH_4 or 1 mM AA after its reaction in the Amplex red system, thus demonstrating that neither agent reduced the chromophore resorufin (Figure 1B).

Experiments performed in oxygenated deionized water confirmed that 1 μM Fe^{3+} or Cu^{2+} (as chlorides) catalysed the generation of H_2O_2 from 1 mM AA. Cu^{2+} was by far the more active cation in this respect, and the concentrations of H_2O_2 measured after 30 min incubation with either ion closely matched published data in the literature,[5] thereby providing additional validation of the Amplex red assay under the experimental conditions employed (Figure 1C).

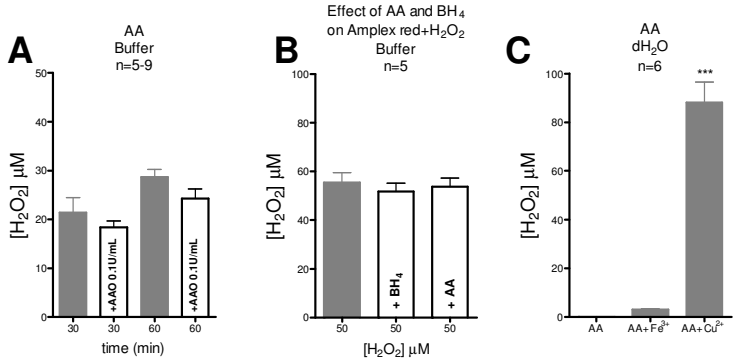

Figure 1

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3. Martinello F, da Silva EL. Ascorbic acid interference in the measurement of serum biochemical parameters: in vivo and in vitro studies. *Clin Biochem* 2006;**39**:396-403.
4. Kärkönen A, Fry SC. Effect of ascorbate and its oxidation products on H_2O_2 production in cell-suspension cultures of Picea abies and in the absence of cells. *J Exp Bot* 2006;**57**:1633-44.
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