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ORIGINAL PAPER

Determination of antioxidant activity using oxidative damage to plasmid DNA – pursuit of solvent optimization

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Oxidative stress plays a key role in the pathophysiology of many diseases. Hydroxyl radical is the oxidative species most commonly causing damage to cells. The aim of this work was to optimize the method for antioxidant activity determination on a model lipophilic geranylated flavanone, diplacone. This method uses protection of plasmid DNA from oxidation by a hydroxyl radical generated by the Fenton reaction involving oxidation of metal ions using H_2O_2 and ascorbate. The method was optimized for lipophilic compounds using several solvents and co-solvents. It was found that (2-hydroxypropyl)-β-cyclodextrin (0.1 mass % aq. sol.) is the best co-solvent for our model lipophilic compound to measure the antioxidant activity by the method presented. Other solvents, namely dimethyl sulfoxide, Cremophor EL® (0.1 mass % aq. sol.), ethanol, and methanol, were not suitable for the determination of the antioxidant activity by the method described. Tween 80 (0.1 mass % aq. sol.) and a mixture of 10 vol. % ethanol and 9 mass % bovine serum albumin (aq. sol.) significantly decreased the antioxidant activity of the model lipophilic compound and thus were not suitable for this method.

Keywords: antioxidant activity, plasmid DNA, Fenton reaction, solubility, diplacone

Introduction

Aerobic organisms take advantage of oxidative phosphorylation and therefore gain much more energy from one molecule of glucose compared to anaerobic organisms. The price paid is the production of reactive oxygen species (ROS). ROS consist of various small molecules derived from oxygen, including oxygen radicals (such as hydroxyl radical $^{\bullet}OH$) and certain non-radicals (such as hydrogen peroxide; H_2O_2). Cells use numerous antioxidant enzymes and defensive molecules to avoid the overproduction of ROS; however, if the production of ROS exceeds the capacity of the cellular antioxidant system, the cell has to face a state called oxidative stress. Oxidative stress plays a key role in the pathophysiology of many diseases such as neurodegeneration, cardiovascular diseases, and cancer (Ma, 2010).

The method presented for antioxidant activity determination is based on the generation of hydroxyl radicals which then cause oxidative damage to plasmid DNA. The hydroxyl radical can be produced by the Fenton reaction Eq. (1). Originally, the metal ion reacting with H_2O_2 was iron but also copper reacts with H_2O_2 (Que et al., 1980). Ascorbate is an excellent antioxidant; however, it also shows a pro oxidant activity due to its reducing character, Eq. (2) (Chiou, 1983). Chiou (1984) reported that H_2O_2 alone (without metal ions) does not show any visible cleavage of DNA, which makes it an important intermediate in the reaction.

There are various ways of DNA damage; it can be fragmented (single- or double-strand breaks) or cross-linked (intra- or inter-strand). The hydroxyl radical also causes base oxidation (e.g., formation of 8-hydroxyguanine or thymine glycol). If not repaired, these DNA alterations lead to mutations or cell death (Cooke et al., 2006). The production and effects of hy-

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