

Determination of antiradical and antioxidant activity: basic principles and new insights

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Although the term "antioxidant" is used very frequently, there are problems with the definition of antioxidants and estimation of antioxidant activity. The distinction between antioxidant and antiradical activities is not always obvious. This minireview discusses critically the principles, advantages and limitations of the most frequently used methods of estimation of antiradical and antioxidant activities.

Keywords: antioxidant, antiradical, DPPH, ABTS, hydroxyl radical

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CURRENT STATE OF THE ART

"It is difficult these days to open a popular science magazine or medical journal without seeing an article about the role of free radicals in human diseases" (Gutteridge & Halliwell, 1994). This sentence written in 1994 by the leading scientists in the field of free radicals and antioxidants, John Gutteridge and Barry Halliwell is true today as well. Another statement of those authors that "antioxidant is a term widely used but rarely defined" (Halliwell & Gutteridge, 1999), has also remained true. A Google search for "antioxidants definition" gives more than 600 000 entries! Halliwell and Gutteridge propose to define an antioxidant as "any substance that, when present at low concentration compared with those of an oxidisable substrate, significantly delays or prevents oxidation of that substrate" (Halliwell & Gutteridge, 1999). This definition covers all oxidation processes, both radical and non-radical ones. But, as noted elsewhere, "a generic definition of an antioxidant is not experimentally constructive unless it is associated with the notion of the oxidant that has to be quenched" (Azzu *et al.*, 2004). Moreover, the validity of the term "antioxidant" depends on the environment of its action, viz. whether we consider an *in vitro* or *in vivo* action. In this context a precise definition of conditions and processes in which antioxidant action is studied becomes crucial. Outside this context, a statement that some compound is an antioxidant may not bring any biologically meaningful information.

The literature of the last decade concerning free radical reactions *in vivo* shows that our understanding of these processes in the organism, both under normal conditions and in pathological situations, has changed considerably. Free radicals and reactive oxygen species in general are no longer seen only as destructive factors but also (and perhaps first of all) as messengers involved in intracellular and intercellular signalling (Bartosz, 2005; 2009; Halliwell, 2006). The revision of the ideas on the

role of free radical reactions in the functioning of cells and organisms has led to a new concept of redox equilibrium. According to this hypothesis, oxidative stress is a modulation of thiol redox reactions, involved mainly in signalling pathways. Therefore, non-radical oxidants (enzymatically generated hydrogen peroxide, other peroxides, quinones, etc.) play a basic role in the oxidation of thiols for the sake of signalling, without the necessity of formation of free radical intermediates (Ghezzi *et al.*, 2005; Jones, 2006; 2008).

Similar changes are taking place with respect to our understanding of the role of vitamin E (α -tocopherol) in living processes. For a long time it was believed that the main function of vitamin E is its antioxidant action in biomembranes. Within the last few years it has become clear that the antioxidant activity of vitamin E is not the only one (and perhaps not the most important) of its physiological functions (Atkinson *et al.*, 2008; Engin, 2009; Jones, 2008; Ricciarelli *et al.*, 2001). The common belief of the beneficial health-improving action of plant phenolics has also been revised (Halliwell, 2007).

In view of the substantial changes in the understanding of the role of reactive oxygen species and antioxidants in living systems, a critical re-evaluation of the methods of determination of the antioxidant activity is also necessary.

ANTIOXIDANT AND ANTIRADICAL ACTIVITY

The general methods of determination of antioxidant activity are summarized in many reviews, including (Frankel & Finley, 2008; Huang *et al.*, 2005; Sanchez-Moreno, 2002). Due to their practical significance much attention is paid to studies of natural products and food supplements (Davalos *et al.*, 2003; Moon & Shinamoto, 2009). Numerous studies have demonstrated that the antioxidant activity measured depends substantially on the test system used (Bauzaite *et al.*, 2003; Janaszewska & Bartosz, 2002) and recommended to base any conclusions on at least two different test systems (Moon & Shinamoto, 2009).

Most of the methods of determination of total antioxidant activity characterize the ability of the tested compound or product to scavenge free radicals and/or to complex metal ions driving the oxidation process.

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