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Free radicals, natural antioxidants, and their reaction mechanisms

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The normal biochemical reactions in our body, increased exposure to the environment, and higher levels of dietary xenobiotics result in the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS). The ROS and RNS create oxidative stress in different pathophysiological conditions. The reported chemical evidence suggests that dietary antioxidants help in disease prevention. The antioxidant compounds react in one-electron reactions with free radicals *in vivo/in vitro* and prevent oxidative damage. Therefore, it is very important to understand the reaction mechanism of antioxidants with the free radicals. This review elaborates the mechanism of action of the natural antioxidant compounds and assays for the evaluation of their antioxidant activities. The reaction mechanisms of the antioxidant assays are briefly discussed (165 references). Practical applications: understanding the reaction mechanisms can help in evaluating the antioxidant activity of various antioxidant compounds as well as in the development of novel antioxidants.

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1. Introduction and background

Antioxidants are molecules that inhibit or quench free radical reactions and delay or inhibit cellular damage.¹ Though the antioxidant defenses are different from species to species, the presence of the antioxidant defense is universal. Antioxidants exist both in enzymatic and non-enzymatic forms in the intracellular and extracellular environment.

Normal biochemical reactions, increased exposure to the environment, and higher levels of dietary xenobiotics result in the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS).² ROS and RNS are responsible for the oxidative stress in different pathophysiological conditions.³ Cellular constituents of our body are altered in oxidative stress conditions, resulting in various disease states. The oxidative stress can be effectively neutralized by enhancing cellular defenses in the form of antioxidants.^{4,5} Certain compounds act as *in vivo* antioxidants by raising the levels of endogenous antioxidant defenses. Expression of genes encoding the enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSHPx) increases the level of endogenous antioxidants.⁶

Antioxidants can be categorized in multiple ways. Based on their activity, they can be categorized as enzymatic and non-enzymatic antioxidants. Enzymatic antioxidants work by breaking down and removing free radicals. The antioxidant

enzymes convert dangerous oxidative products to hydrogen peroxide (H_2O_2) and then to water, in a multi-step process in presence of cofactors such as copper, zinc, manganese, and iron. Non-enzymatic antioxidants work by interrupting free radical chain reactions. Few examples of the non-enzymatic antioxidants are vitamin C, vitamin E, plant polyphenol, carotenoids, and glutathione.⁷

The other way of categorizing the antioxidants is based on their solubility in the water or lipids. The antioxidants can be categorized as water-soluble and lipid-soluble antioxidants. The water-soluble antioxidants (e.g. vitamin C) are present in the cellular fluids such as cytosol, or cytoplasmic matrix. The lipid-soluble antioxidants (e.g. vitamin E, carotenoids, and lipoic acid) are predominantly located in cell membranes.

The antioxidants can also be categorized according to their size, the small-molecule antioxidants and large-molecule antioxidants. The small-molecule antioxidants neutralize the ROS in a process called radical scavenging and carry them away. The main antioxidants in this category are vitamin C, vitamin E, carotenoids, and glutathione (GSH). The large-molecule antioxidants are enzymes (SOD, CAT, and GSHPx) and sacrificial proteins (albumin) that absorb ROS and prevent them from attacking other essential proteins.

To understand the mechanism of action of antioxidants, it is necessary to understand the generation of free radicals and their damaging reactions. This review elaborates the generation and damages that free radicals create, mechanism of action of the natural antioxidant compounds and assays for the evaluation of their antioxidant properties. The reaction mechanisms of the antioxidant assays are discussed. The scope of this article

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