Comparative antioxidant activity of tocotrienols and the novel chromanyl-polyisoprenyl molecule FeAox-6 in isolated membranes and intact cells

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Abstract

Oxidative stress plays a pivotal role in the pathogenesis of several chronic diseases and antioxidants may represent potential tools for the prevention of these diseases. Here, we investigated the antioxidant efficiency of different tocotrienol isoforms (α-, δ-, γ-tocotrienols), and that of FeAox-6, a novel synthetic compound which combines, by a stable covalent bond, the chroman head of vitamin E and a polyisoprenyl sequence of four conjugated double bonds into a single molecule. The antioxidant efficiency was evaluated as the ability of the compounds to inhibit lipid peroxidation, reactive oxygen species (ROS) production, heat shock protein (hsp) expression in rat liver microsomal membranes as well as in RAT-1 immortalized fibroblasts challenged with different free radical sources, including 2,2′-azobis(2-amidinopropane) (AAPH), tert-butyl hydroperoxide (tert-BOOH) and H₂O₂. Our results show that individual tocotrienols display different antioxidant potencies. Irrespective of the prooxidant used, the order of effectiveness was: δ-tocotrienol > γ-tocotrienol = α-tocotrienol in both isolated membranes and intact cells. This is presumably due to the decreased methylation of δ-tocotrienol chromane ring, which allows the molecule to be more easily incorporated into cell membranes. Moreover, we found that FeAox-6 showed an antioxidant potency greater than that of δ-tocotrienol. Such an efficiency seems to depend on the concomitant presence of a chromane ring and a phytlyl chain in the molecule, which because of four conjugated double bonds, may induce a greater mobility and a more uniform distribution within cell membrane. In view of these results, FeAox-6 represents a new potential preventive agent in chronic diseases in which oxidative stress plays a pathogenic role. (Mol Cell Biochem 287: 21–32, 2006)

Key words: FeAox-6, hsp70 and hsp90, lipid peroxidation, ROS, tocotrienols

Introduction

In recent years there has been considerable interest in the role of dietary compounds in human health, especially in view of the concept that health promotion has become a legitimate part of health care and chronic imbalance of important nutrients may be involved in the etiology of important degenerative diseases. One of the areas that has attracted...
great deal of attention is the involvement of dietary antioxidants in the control of age-related chronic diseases in which oxidative damage has been implicated as one of the possible pathogenic mechanisms [1–2]. In fact, the oxidation of biological molecules, such as lipids, proteins and DNA, is recognized to be involved in the development of numerous pathological events, such as neurodegeneration, cancer, cardiovascular diseases, atherosclerosis and age-related macular degeneration [3–4]. Such oxidative damage in lipids usually proceeds by a free radical-mediated chain mechanism and chain breaking antioxidants have been reported to suppress the oxidation besides protecting biological molecules and tissues from oxidative damage [5–8].

It is reported that one of the most effective chain breaking antioxidants is vitamin E [9–10]. Vitamin E is a generic term that represents a family of chemically-related compounds that is subdivided into two subgroups called tocopherols and tocotrienols [11]. Tocopherols and tocotrienols have the same basic chemical structure characterized by a long phytanyl chain attached at the 1-position of a chromane ring [12–13], α-, β-, γ-, and δ-species of both tocopherols and tocotrienols differ with regard to the number and positions of methyl groups on the chromanyl ring. In addition, the structure of tocotrienols differs from that of tocopherols by the presence of three trans double bonds in the phytanyl tail. The antioxidant property of vitamin E, as a chain breaking donor molecule, is exerted through the phenolic hydroxyl group, which readily donates its hydrogen atom (H) to the lipid peroxyl (PUFAOO•) radical, resulting in the formation of a stable lipid species. In donating the H, vitamin E becomes a relatively unreactive free radical, as the unpaired electron becomes delocalised into the aromatic ring. The efficiency of this protection depends on two factors: first the mobility of the molecule in membranes, which is determined by the aliphatic tail; second the number of methyl species on the chromanyl ring, where each methyl group confers additional antioxidant capacity in view of the electron donor properties. In addition, the proximity of the methyl species to the hydroxyl group is also important. Thus, α-homologues, which have the greatest number of methyl species, and in which these methyl groups flank the hydroxyl group, are thought to be more effective than the other homologues.

It has been shown that tocotrienols are potent antioxidants [14, 15] and display greater bioactivity than tocopherols [16, 17]. A reason suggested for this greater effectiveness is the presence of conjugated C–C double bonds. The isoprenoid tail of tocotrienols has a stronger disordering effect on membranes than tocopherols. This property leads to a greater mobility and more uniform distribution within the membrane. NMR studies have also shown that the chromanol ring of α-tocotrienol is situated closer to the membrane surface. These factors may contribute to a greater ability of tocotrienols to interact with radicals and may allow for better recycling of the molecule to its active reduced form [16, 17]. However, though there are many reports on the antioxidant properties of tocopherols, only few studies are available for tocotrienols. In particular, not much is known on the antioxidant efficiency of the different tocotrienol isoforms in biological models.

We have recently shown that FeAox-6, a novel synthetic compound which combines, by a stable covalent bond, the chromanyl head of vitamin E and a sequence of four conjugated double bonds into a single molecule, acted as a strong antioxidant in in vitro models [18].

In this study, we have compared the antioxidant efficiency of different tocotrienols (α-, γ-, and δ-tocotrienols) with that of FeAox-6 in preventing oxidative stress caused by different sources of free radicals in both isolated membranes and intact cells. The difference between tocotrienols and FeAox-6 is that the former possess an unsaturated isoprenoid chain, whereas the latter has an unsaturated but conjugated polyene chain.

Materials and methods

Chemicals

tert-BOOH, butylated hydroxytoluene (BHT), dimethyl sulfoxide (DMSO), 1,1,3,3-tetramethoxypropane, ammonium acetate and 2-thiobarbituric acid (TBA) were obtained from Sigma, Chemical Co (St Louis, MO). Trichloroacetic acid (TCA) and hydrochloric acid were obtained from Fisher Scientific Co. (Fairlawn, NJ). AAPH was obtained from Polysciences, Inc. (Warrington, PA). H2O2 and tetrabutyl ammonium dihydrogen phosphate were purchased from Aldrich Chemical Co (St Louis, MO). Hexane, methanol, isobutyl alcohol, isopropanol and ethanol were HPLC grade and obtained from Fluka Chemika-Biochemika (Buchs, Switzerland). α-Tocotrienol, γ-tocotrienol and δ-tocotrienol were obtained from tocotrienol-enriched extract. Briefly, 1.5 g of palm oil extract (Tocomin 50%⃝ Polichimica, Bologna) were purified by several passages on silica gel column chromatography, using as eluents, mixtures of petroleum ether and ethyl acetate (9:5:0.5). After collection and evaporation under vacuum of appropriate fractions, the following amounts were obtained: 0.2 g α-tocotrienol, 0.24 g γ-tocotrienol, 0.1 g δ-tocotrienol.

FeAox-6, ((±)-(E/Z)-2,5,7,8-tetramethyl-2-(4,8,12-trimethyl-trideca-1,3,5,7,11-pentanyloxy)chroman-6-ol) was synthesized as described before [18].

All the prooxidants used were dissolved in distilled water. To minimize autooxidation process, FeAox-6 in DMSO was stored under nitrogen in sealed containers at –20°C and used within a week. On the other hand, stock solutions of tocotrienols in DMSO were prepared immediately before each experiment.