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Mini-review

How to boost antioxidants by lipophilization?

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ABSTRACT

Covalent modification of antioxidants through lipophilization is an important field of research aiming at developing antioxidants with improved efficacy. However, due to insufficient knowledge on how hydrophobicity affects antioxidant activity, lipophilization strategies have been largely based on empirism. Often, the resulting lipophilized antioxidants were not optimal. Here we described how the body of knowledge regarding hydrophobicity has been dramatically redefined as unexpected results were recently published. Using a broad range of lipophilized antioxidants assessed in dispersed lipids models and cultured cells, it has been demonstrated that the antioxidant activity increases progressively with increasing chain length up to a critical point, beyond which the activity of the compounds dramatically decreases. Taking into account this nonlinear phenomenon, also known as cut-off effect, antioxidant drug designers now have to seek the critical chain length to synthesize the optimal drug in a rational manner. Here, we briefly presented three putative mechanisms of action to try to account for the cut-off effect.

1. Introduction

Intrinsic chemical reactivity of antioxidant molecules towards free radicals has been extensively explored and largely elucidated for two decades. However, the determinant factors underlying their mechanism of action and, above all, their real efficiency in a given medium remain to be determined. Nowadays, even though we know how antioxidants behave in theory, we are still unable to accurately predict their antioxidant capacity in real situations often because we do not understand the physical behaviour of antioxidants.

Concerning the intrinsic chemical reactivity of antioxidants towards free radicals, it is well admitted that number and position of hydrogen-donating groups (Ph-OH, OH, SH) are of prime importance. Since phenolics are the most abundant antioxidants on earth, most of the structure–activity relationship (SAR) studies have been performed on these molecules. The occurrence of an *o*-dihydroxy group (catechol) is a crucial trait for phenolic reactivity. Any structure that extends the conjugation of phenolic hydroxyls also improves antioxidant reactivity. This is the case with the C2–C3 double bond in flavonols or the double bond between the acid group and the phenolic ring of hydroxycinnamic acids. These unsaturations enlarge the π-system wherein the unpaired electron is delocalized and thus stabilized. The larger the delocalization area and the lower the bond dissociation energy (BDE) are, the higher is the ability to reduce free radicals [1].

However, we should keep in mind that reactivity of antioxidants is not the only parameter that affects their efficiency. Antioxidant reactivity can be regarded as a virtual *potential* which needs to be physically *expressed*. Indeed, a highly reactive antioxidant needs to be mobile enough and to diffuse easily to the site of action. It also needs to be properly positioned in compartmentalized systems. Diffusion and location of antioxidants are two key parameters which dictate the extrinsic *expression/fulfilment* of a given intrinsic *potential* (antioxidant reactivity). In turn, these two parameters are mainly governed by the antioxidant hydrophobicity. Rice-Evans et al. [2] did not say anything else when stating that "the partition coefficients of the flavonoids as well as their rates of reaction with the relevant radicals define the antioxidant activities in the lipophilic phase".

In compartmentalized systems such as emulsions, membranes, and living cells, surface-activity (hence hydrophobicity) is generally regarded as advantageous. Surface-activity allows the antioxidant to be fusogenic and to have a good affinity with lipid–water interfaces. One of the most reliable methods to improve antioxidant activity is to incorporate properly positioned lipophilic groups.

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Since it is difficult to manipulate the polar group, the traditional lore is that the antioxidant has the polar group in the correct position and that antioxidant activity is improved by correctly positioned lipophilic groups [3]. Accordingly, lipophilization appears more and more as a crucial step in the design of new antioxidant additives and drugs. Basically, lipophilization consists in grafting lipophilic moiety(ies) to a given molecule to make it more surface-active. The molecules so prepared, with fine-tuned lipophilicity have improved bioavailability *in vivo* compared to their parent antioxidants. They also show greater miscibility and incorporation into lipid phases and lipocarriers offering an advantage for their use in drug delivery systems, foods, and cosmetic formulations. These functionalized antioxidants can be prepared by a wide range of lipophilization strategies using lipases or acidic resins to carry out esterification, transesterification, amidation, and etherification. To date, a multitude of lipophilized antioxidants have been synthesized [4]. Phenolic acids, flavonoids, and tocopherols have been extensively modified by the grafting of fatty alcohols [5–9], triacylglycerols [10] or phospholipids [11] to obtain lipophilized phenolics called "phenolipids" (Fig. 1). Ascorbic acid has also been hydrophobized by various aliphatic chains [12–14] including polyunsaturated fatty acids [15].

Although hydrophobicity is generally considered as advantageous, it is worth asking whether increasing hydrophobicity necessarily leads to a more efficient antioxidant. This mini-review aimed at covering the complex role of hydrophobicity on antioxidant properties. This is all the more difficult since, until recently, there was no general theory describing the impact of hydrophobicity in a great variety of physico-chemical and biological systems. For their part, physico-chemists developed the concept of the polar paradox stating in dispersed lipids such as micelles, emulsions, and membranes that lipophilic antioxidants are more active than their hydrophilic counterparts [16–18]. Independently, biologists and pharmacologists developed the consensual opinion that hydrophobicity benefits to the antioxidant activity in biological media, even though no clear evidence has been brought so far for reasons that are explained hereafter.

In this mini-review, we describe an alternative idea according to which the relationship between hydrophobicity and activity is not linear at all and follows a parabolic-like trend. Antioxidant activity rises with an increase of hydrophobicity until a threshold is reached, and beyond which, any lengthening of the hydrophobic chain leads to a collapse in antioxidant activity [19–21]. This nonlinear phenomenon, called cut-off effect, has been mainly observed with various antioxidants incubated in numerous biological (cultured cells) and physico-chemical (emulsions and liposomes) systems. Through the cut-off effect, lipophilization finally appears as a double-edge sword technique: if the grafted aliphatic chain is too short or too long, the resulting antioxidant activity will not be optimal. Here is described a novel and rational strategy to boost antioxidants by grafting the proper hydrophobic chain.

2. How does hydrophobicity impact antioxidant activity? The unanswered question

In lipid dispersions, the role of hydrophobicity on antioxidant activity has been first covered by the polar paradox in 1980's [16]. According to Porter et al. [17], nonpolar antioxidants would be more effective than their polar analogues in oil-in-water emulsions and membranes. The polar paradox has been the prevalent paradigm for approximately two decades and broadly used for predicting antioxidant capacity in such heterogeneous systems. Indeed, numerous studies often performed on few molecules confirmed the accuracy of this model. In 1994, Frankel et al. [18] provided to the polar paradox a mechanism of action in introducing the concept of interfacial oxidation and antioxidation. Accordingly, the higher efficiency of hydrophobic antioxidants in oil-in-water emulsions would be due to their tendency to

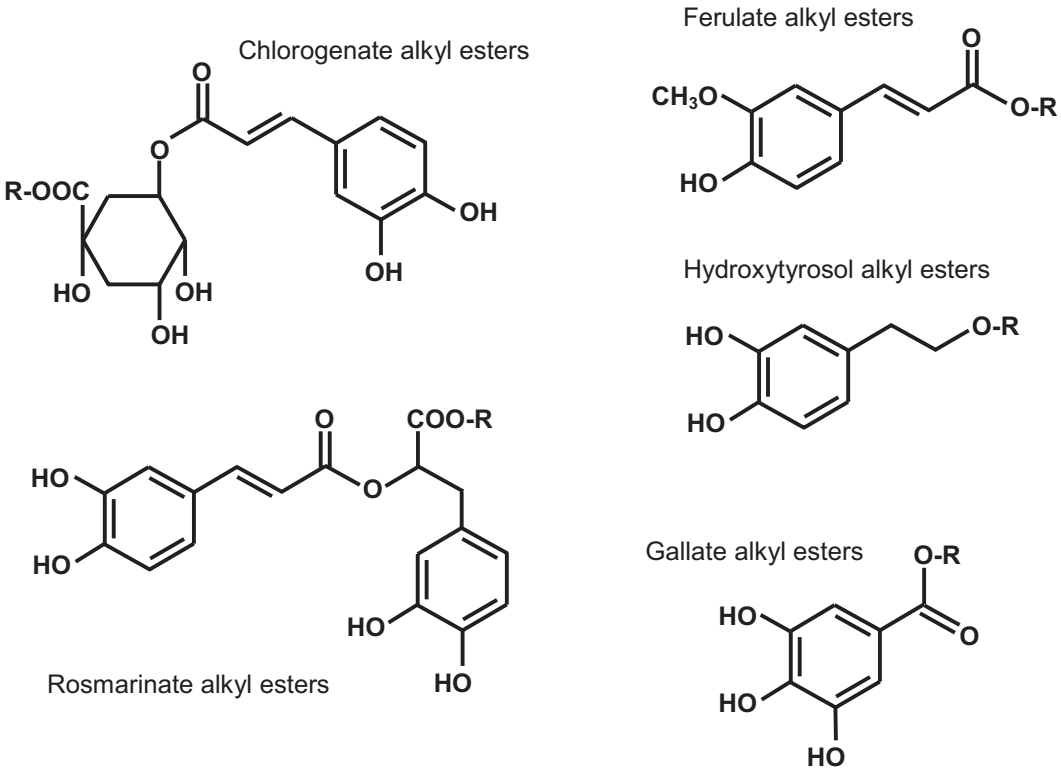

Fig. 1. Chemical structures of the main phenolipids mentioned in this mini-review.