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A simple liposome assay for the screening of zinc ionophore activity of polyphenols

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ABSTRACT

An efficient liposomal system for screening the zinc ionophore activity of a selected library consisting of the most relevant dietary polyphenols is presented. The zinc ionophore activity was demonstrated by exploring the use of zinc-specific fluorophore FluoZin-3 loaded liposomes as simple membrane tools that mimic the cell membrane. The zinc ionophore activity was demonstrated as the capacity of polyphenols to transport zinc cations across the liposome membrane and increase the zinc-specific fluorescence of the encapsulated fluorophore FluoZin-3. In addition, the zinc chelation strength of the polyphenols was also tested in a competition assay based on the fluorescence quenching of zinc-dependent fluorescence emitted by zinc–FluoZin-3 complex. Finally, the correlation between the chelation capacity and ionophore activity is demonstrated, thus underlining the sequestering or ionophoric activity that the phenolic compounds can display, thus, providing better knowledge of the importance of the structural conformation versus their biological activity. Furthermore, the assays developed can be used as tools for rapid, high-throughput screening of families of polyphenols towards different biometals.

1. Introduction

Zinc ions have long been known to mimic the actions of hormones, growth factors, neurotransmitters and cytokines, and it is believed that zinc may act on intracellular signaling molecules (Beyersmann & Haase, 2001; Colvin, Fontaine, Laskowski, & Thomas, 2003; Frederickson, 2003). In fact, zinc is a known inhibitor of protein tyrosine phosphatases (Brautigan, Bornstein, & Gallis, 1981) with a constant of inhibition in the nanomolar range (Maret, Jacob, Vallee, & Fischer, 1999). In addition, zinc affects the regulation of transcription factors, and can induce the expression of some genes, including those coding for molecules involved in zinc homeostasis, such as zinc transporters and metallothioneins (Palmiter & Huang, 2004). The gene expression of metallothioneins by zinc is regulated by metal response element-binding transcription factor-1 (Lichtlen & Schaffner, 2001). The chemical properties of zinc that differentiate it from other transition metals, such as copper and iron, which display several different oxidation states in biological systems, is that zinc exists as a redox inert Zn^{2+} cation, which does not undergo redox reactions at physiological redox potentials (Eide, 2011; Laitaoja, Valjakka, & Jänis, 2013). Additionally, zinc can induce the expression and maintain the levels of potential radical scavenging proteins such as metallothionein (MT), the major zinc binding protein associated with zinc homeostasis (Quesada et al., 2011), DNA protection, oxidative stress, and apoptosis (Higashimoto et al., 2009; Tapiero & Tew, 2003). Furthermore, it can act through stabilization of cell membranes (Powell, 2000) or as a structural component of anti-oxidant enzymes (Klotz, Kröncke, Buchczyk, & Sies, 2003).

On the other hand, recent studies have focused on dietary phenolic compounds as natural improvers of health and more than 8000 dietary polyphenols have been identified (Araújo, Gonçalves, & Martel, 2011). The growing interest in these compounds resides in the accumulating evidence regarding their ability to trigger several cellular pathways leading to the prevention and/or amelioration of pathological conditions, acting as antioxidants (Leopoldini, Russo, & Toscano, 2011), anti-carcinogenics (Ramos, 2008; Čipák, Rauko, Miadoková, Čipáková, & Novotný, 2003), anti-inflammatories (Bravo, 1998), neuroprotectors (Russo,

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Spagnuolo, Tedesco, Bilotto, & Russo, 2012), anti-lipidemic and vaso-relaxing agents (Araújo et al., 2011).

In recent decades, it has been demonstrated and understood that phenolic compounds interact with different metals, including zinc, and because of their distinctive chemical structure, they can easily form complexes through metal ion chelation (Hider, Liu, & Khodr, 2001) in a manner similar to that of other well-known metal chelators, such as the drug clioquinol (CQ), and also exert ionophore activity comparable to pyrithione (Pyr) (Cao et al., 2013, 2014). The first evidence of polyphenol–metal complexes was reported in 1970 between aluminum ions and flavonoids (Porter & Markham, 1970). Since then, more than 40 metal–flavonoid complexes have been investigated (Grazul & Budzisz, 2009).

One of the mechanisms by which flavonoids exert their antioxidant activity is via the chelation of redox-active transition metals (Thompson, Williams, & Elliot, 1976), which are known to catalyze many biological processes leading to the production of free radicals (Mladenka, Zatloukalová, Filipský, & Hrdina, 2010). The essential sites for metal chelation are hydroxyl groups, and the most suitable cations for chelation are Fe(II), Fe(III), Cu(II) and Zn (II) as they have high charge density, stimulating the interaction with the phenoxide groups that have a high negative charge density (Hider et al., 2001). The structure of the complexes formed depends on the type of flavonoid and metal ion involved, which in turn can influence its biological interactions that may be different from the native flavonoid (Afanas'eva, Ostrakhovitch, Mikhal'chik, Ibragimova, & Korkina, 2001; Aherne & O'Brien, 2000; Fernandez, Mira, Florêncio, & Jennings, 2002; Mira et al., 2002). Depending on the polyphenol and its potential binding sites, different structures could be formed with different stoichiometries, thus affecting the biological function of the complex (Wei & Guo, 2014). Experimental data has indicated that the chelated compounds are more effective free radical scavengers than flavonoids alone, suggesting that the Zn–polyphenol complexes not only exert singular biological properties, but can also enhance the effects of both compounds individually (Selvaraj, Krishnaswamy, Devashya, Sethuraman, & Krishnan, 2014).

Further studies have revealed that polyphenols not only interact with metal ions, but also deeply modulate expression of MTs, cellular zinc transporters, extracellular zinc carriers, and intracellular zinc accumulation which are key factors in zinc homeostasis (Quesada et al., 2011). Zinquin is a fluorescent zinc-specific indicator and an increase in zinquin-detectable cytoplasmic levels of zinc in a HepG2 cell line has been monitored when treated with phenolic compounds (Quesada et al., 2011). This increment in intracellular zinc levels has been reported to induce apoptosis of tumor cells (Ding, Liu, Vaught, Yamauchi, & Lind, 2005; Feng, Li, Guan, Franklin, & Costello, 2008), suggesting that zinc ionophores may serve as anticancer agents (Liang et al., 1999).

Although the ionophore activity of naturally occurring compounds has not been well established, there is strong evidence of their interaction and complex formation with zinc ions (Selvaraj et al., 2014), suggesting that they could be potential candidates as zinc ionophore molecules. The interaction of quercetin (QCT) and epigallocatechin-3-gallate (EGCG) with zinc, as well as their ionophore activity has been confirmed in a liposome model using the specific zinc indicator FluoZin-3 (Dabbagh-Bazarbachi et al., 2014). Luteolin (LUT) and naringenin (NAR) interact with zinc ions, forming complexes and exerting a biological function acting as strong radical scavengers (Chen, Wu, & Li, 2009; Wang, Yang, & Wang, 2006). The ability of genistein (GEN) to bind zinc ions has not been well elucidated, although its ability to bind iron is well known and these complexes exert a strong anti-oxidant role, and this suggests that it could have a similar action with other metals such as zinc (Harper, Kerr, Gescher, & Chipman, 1999). There is also evidence regarding the ability of catechin hydrate (CAT HYD), which is the one of the main bioactive components in green tea, to interact and form complexes with zinc ions (Bodini, del Valle, Tapia, Leighton, & Berrios, 2001; Le Nest et al., 2004), exerting an anti-oxidant activity, but also having an essential role in the treatment of different cancers, such as prostate cancer (Yu, Shen, & Yin, 2007). Several reports have confirmed that rutin (RUT) forms complexes with zinc (Bai et al., 2004), also by acting as a free radical scavenger, more effectively than the free flavonoid (De Souza & De Giovani, 2004). The anti-inflammatory activity of this bioflavonoid is also enhanced when complexed with zinc (Afanas'eva et al., 2001). Taxifolin (TAX) is also able to interact and form complexes with zinc ions, also acting as an effective radical scavenger (Donracheva et al., 2009). Most phenolic acids are good metal chelators, due to their structure with several catechol and/or galloyl moieties (Andjelkovic et al., 2006). To our knowledge, there are no reports to date on interactions and complex formation with zinc ions with phloretin (PHLO) or the stilbene resveratrol (RSV), although for RSV there is evidence regarding complex formation with copper, suggesting that potentially similar structures can be formed with other metal ions (Chiavarino et al., 2012). Catechol (CAT) is one of the simplest naturally occurring polyphenols, and also one of the most important moieties in a high variety of polyphenols, responsible for the interaction with metal ions. CAT forms complexes with Ruthenium, a rare transition metal, suggesting that it could have the same behavior with other transition metals like zinc (Almeida et al., 2007). Thus, a high proportion of polyphenols present some kind of interaction with zinc or other metal ions, although for the majority of polyphenols the ionophore activity is still undescribed.

The aim of this work was to evaluate the capacity of fourteen different phenolic compounds to bind and chelate zinc ions in solution and to demonstrate their ability to act as zinc ionophores. We focused on fourteen phenolic compounds grouped according to their chemical structure, including the flavonoids quercetin (QCT), epigallocatechin-3-gallate (EGCG), luteolin (LUT), naringenin (NAR), phloretin (PHLO), genistein (GEN), catechin hydrate (CAT HYD), rutin (RUT) and dihydroquercetin or taxifolin (TAX); the phenolic acids gallic acid (GAL), tannic acid (TAN) and caffeic acid (CAF); the stilbene resveratrol (RSV); and other polyphenols such as catechol (CAT). Two different zinc ionophore agents, clioquinol and pyrithione, were used to compare the ionophore activity of the selected polyphenols, as well as the zinc sequestrant molecule, TPEN (N,N,N′,N′-tetrakis(2-pyridylmethyl)ethylenediamine). The binding/chelation of the zinc ions by the polyphenols was evaluated using a competition assay based on the fluorescence quenching of zinc-dependent fluorescence emitted by FluoZin-3. In this competition assay the zinc chelation strength of each phenolic compound was correlated with the decrease in the fluorescence signal due to the dissociation of the zinc–FluoZin-3 complex as zinc cations are sequestered from the fluorophore complex by the polyphenol. In addition, we present a simple and rapid liposome assay for demonstrating the zinc ionophore activity of common dietary polyphenols. The method exploits the use of unilamellar liposomes loaded with the zinc-sensitive fluorophore FluoZin-3 as simple membrane models that mimic biological cell membranes to monitor the capacity of the phenolic compounds to transport zinc cations across the lipid bilayer. The zinc ionophore activity presented by the bioactive nutrients was compared with the strong, well-established synthetic pharmacological ionophores, such as clioquinol and pyrithione. The correlation between the chelation capacity and ionophore activity underlines the different behaviors that the phenolic compounds can display and the liposomal assays developed can be used as tools for the rapid, high-throughput screening of ionophore activity of families of polyphenols.