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# Differential effects of oleuropein, a biophenol from *Olea europaea*, on anionic and zwiterionic phospholipid model membranes

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**Abstract**

Oleuropein (Ole) is the major phenolic constituent of the olive leaf (*Olea europaea*) and it is also present in olive oil and fruit. In the last years several compounds from olive tree, oleuropein among them, have shown a variety of biological activities such as antimicrobial or antioxidant. A phospholipid model membrane system was used to study whether the Ole biological effects could be membrane related. Ole showed a significant partition level in phospholipid membranes, i.e. 80%, at lipid-saturating conditions. Moreover, fluorescence quenching experiments indicated a shallow location for Ole in membranes. Ole promoted weak effects on zwiterionic phospholipids such as phosphatidylcholine or phosphatidylethanolamine. In contrast, differential scanning microcalorimetry, light scattering and fluorescence anisotropy pH titration studies revealed strong effects on anionic phospholipids such as phosphatidylglycerol at physiological pH and salt conditions. These effects consisted on perturbations at the phospholipid membrane surface, which might involve specific molecular interactions between Ole and the negatively charged phosphate group and therefore modify the phospholipid/water interface properties. It is proposed that Ole induces lipid structures similar to the gel–fluid intermediate phase (IP) described for PG membranes, in a similar way than low ionic strength does. These effects on phosphatidylglycerol may account for the antimicrobial activity of Ole.


*Keywords:* Oleuropein; Phospholipid membranes; Phosphatidylglycerol; DSC; Fluorescence; Gel–fluid intermediate phase; Antimicrobial

*Abbreviations:* $\langle r \rangle$, steady-state fluorescence anisotropy; 16-NS, 16-doxyl-stearic acid; 5-NS, 5-doxyl-stearic acid; DEPE, 1,2-dielaidoyl-*sn*-glycero-3-phosphoethanolamine; DMPA, 1,2-dimyristoyl-*sn*-glycero-3-phosphate; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DMPE, 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine; DMPG, 1,2-dimyristoyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)]; DMPS, 1,2-dimyristoyl-*sn*-glycero-3-[phospho-L-serine]; DPH, 1,6-diphenyl-1,3,5-hexatriene; DPPG, 1,2-dipalmitoyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)]; DSC, differential scanning calorimetry; $H_{II}$, inverted hexagonal-$H_{II}$ phase; HPLC, high performance liquid chromatography; IP, gel–fluid intermediate phase; $K_P$, phospholipid/water partition coefficient; LUVs, large unilamellar vesicles; MLVs, multilamellar vesicles; Ole, oleuropein; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PS, phosphatidylserine; $T_c$, onset temperature of the gel to liquid-crystalline phase transition; $T_m$, gel to liquid-crystalline phase transition temperature; $T_m^{off}$, offset temperature of the IP region; $T_m^{on}$, onset temperature of the IP region; VHSV, viral haemorrhagic septicaemia virus

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## 1. Introduction

Leaves and drupes from olive tree, *Olea europaea*, are rich in olive biophenols, such as oleuropein, verbascoside, ligstroside, tyrosol and hydroxytyrosol, which have exhibited antioxidant and antimicrobial properties. Oleuropein (Ole) is the major biophenol in olive leaf and fruit (Benavente-García et al., 2000) (Fig. 1). This compound is a phenolic secoiridoid glycoside with hydroxyaromatic functionality deriving from the shikimate and phenylpropanoid metabolism. Hydroxytyrosol and oleuropein, have been shown to be potent radical scavengers (Visioli et al., 1998; Saija et al., 1998; Benavente-García et al., 2000; Gordon et al., 2001; Briante et al., 2001; Saija and Uccella, 2001; Paiva-Martins et al., 2003). In addition, the presence of some biophenols in olive oil and drupes, among other factors, has been related to the prevention of coronary artery disease and atherosclerosis because of their capability to inhibit platelet aggregation (Carluccio et al., 2003), arachidonic acid metabolism modulation (Kohyama et al., 1997) and to inhibit LDL peroxidation (Visioli et al., 1995, 2002; Visioli and Galli, 2002).

Furthermore, some of these compounds have demonstrated to have antimicrobial activity by inhibiting the growth of a wide variety of bacteria (Aziz et al., 1998; Bisignano et al., 1999), fungi (Tassou et al., 1991; Aziz et al., 1998) and viruses (Renis, 1969; Hirschman, 1972; Fredrickson, 2000; Ma et al., 2001). In particular, oleuropein has exhibited antibacterial activity against a variety of Gram-positive and Gram-negative human pathogenetic bacterial strains (Bisignano et al., 1999). Besides, oleuropein has shown antiviral activity mostly against enveloped virus (Bisignano et al., 1999; Ma et al., 2001; Fredrickson, 2000; Micol et al., 2005). It is also proposed that the lower antimicrobial capacity of oleuropein in comparison to hydroxytyrosol might be due to its lower capability to penetrate the cell membrane due to its glycosidic structure (Saija and Uccella, 2001).

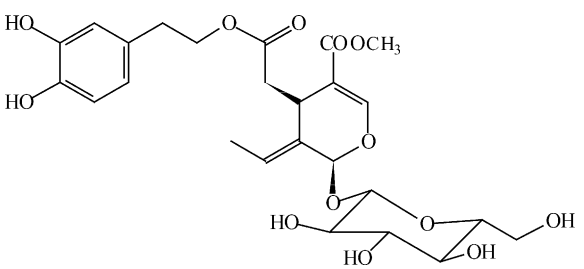


Fig. 1. Chemical structure of the secoiridoid oleuropein.

The putative molecular mechanism of the wide biological activity of Ole has been pointed out only in a few cases. This compound, when digested by β-glucosidases present in separate plant leaf compartments or deriving from intestinal bacteria, yields a glutaraldehyde-like structure which exhibits protein-denaturing, protein-crosslinking and lysine-alkylating properties (Konno et al., 1999).

Some Ole effects may be also related to its capacity to interact with biological membranes. It is presumed that this compound, being a water and lipid soluble molecule, can get through cell membranes in some extent reaching intracellular targets in a similar way than hydroxytyrosol does (Kohyama et al., 1997). In fact, it has been shown that a significant amount of Ole reaches intracellular targets in isolated erythrocytes (Saija and Uccella, 2001). Ole partition coefficient has been previously determined in octanol/water and oil/water systems through UV absorbance or HPLC measurements (Saija et al., 1995, 1998; Gordon et al., 2001; Paiva-Martins et al., 2003) but it has not yet been determined in a phospholipid model membrane system. In addition, the association of Ole with biological membranes may promote changes in the membrane physical properties and therefore modulate membrane-dependent biological processes. To this respect, we have recently shown that anthraquinones, some of them bearing a glucoside moiety, promote important changes on the surface of membranes containing negatively charged phospholipids which could account for their antimicrobial activity (Alves et al., 2004).

Some authors have previously studied the interaction of Ole with model membranes containing phosphatidylcholine and linoleic acid postulating that Ole may act as an internal radical scavenger against lipid peroxidation within biomembranes (Saija et al., 1998). In contrast, other authors have recently proposed a superficial location for Ole in phospolipid bilayers where this compound would play a role as an effective antioxidant (Paiva-Martins et al., 2003).

In this work, phospholipid model membranes were used first to determine the phospholipid/water partition