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Membrane perturbing properties of natural phenolic and resorcinolic lipids

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Abstract The effects induced by natural phenolic and resorcinolic lipids on membrane permeability were investigated. All of the compounds tested perturbed the phospholipid bilayer and stabilized erythrocytes against hypoosmotically induced hemolysis. Dipalmitoylphosphatidylcholine liposomes with two preincorporated fluorescent dyes (1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatrien *p*-toluenesulfonate (TMA-DPH) and *N*-(-nitrobenz-2-oxa-1,3-diazol-4-yl)-1,2-dihexadecanoyl-*sn*-glycero-3-phosphoetanolamine triethylammonium salt (NBD-PE)) were used to determine the effects of tested compounds on the core and surface of the bilayer. Resorcinolic lipids from rye and cardol increased the polarization of TMA-DPH fluorescence more than that of NBD-PE, but anacardic acid, methylocardol, and alkylphenol increased NBD-PE dye fluorescence.

Keywords: Antihemolysis; Fluorescence polarization; Hemolysis; Permeability; Phenolic lipids; Resorcinolic lipids

1. Introduction

Phenolic lipids constitute a heterogeneous group that includes simple phenols and polyphenols as well as their derivatives [1].

Cashew nut shell liquid (CNSL) is a unique source of material useful both in industrial technology and in biological/pharmaceutical applications. Technical CNSL containing cardol and cardanol is widely used in the production of polymeric friction dusts and in certain polymeric/surface coating applications [2]. Anacardic acid is a potent inhibitor of histone acetyltransferase, prostaglandin synthase and lipoxygenase [3], and it also has anti-microbial properties [4]. Cardanol derivatives were found to have many biological activities [5–7].

Resorcinolic lipids are also common in cereal grains, e.g. rye, wheat, barley, and millet [8]. Alkylresorcinols were reported to have antiparasitic, anticancer, antifungal, antimicrobial, and antioxidant effects suggesting involvement in the regulation of cell metabolism [1,9]. Alkylresorcinols present in daily diet have been found to be absorbed by rats, pigs [10], and humans [11,12], but little is known about their metabolism. Previous in vitro experiments showed that resorcinolic lipids exhibit strong amphiphilic character [13], and show high affinity for lipid bilayers as well as for biological membranes [14–17].

In the present study we focused on the characterization of the membrane properties of resorcinolic and phenolic lipids with respect to biological as well as liposomal membranes.

2. Materials and methods

2.1. Materials

All phospholipids were purchased from Lipid Products (Nutfield, Surrey, GB). All fluorescent probes were from Molecular Probes (Eugene, OR, USA).

2.2. Isolation of phenolic and resorcinolic lipids

All tested compounds were isolated as described previously [36]. The purity of the tested compounds was assessed by HPLC method and was above 98%.

2.3. Critical micelles concentration (CMC) measurements

The CMC of the resorcinolic lipids was determined by surface pressure changes [37].

2.4. Preparation of erythrocytes

Erythrocytes were isolated from sheep blood as described earlier [27].

2.5. Hemolytic activity of phenolic lipids

The hemolytic effect of the phenolic lipids on erythrocyte membrane and the effect of divalent cations on phenolic lipids-induced hemolysis were studied as described earlier [16].

2.6. The effect of phenolic lipids on hyposomotically induced hemolysis

The experiments were done as described earlier [27].

2.7. Effect of phenolic and resorcinolic lipids on liposomal membranes

Leakage of entrapped calcein and the effect of the phenolic and resorcinolic lipids on liposomal surface charge and mobility of lipids were studied as described earlier [27].

3. Results and discussion

The phenolic lipids used in the present study were isolated from CNSL from *Anacardium occidentale* (cardol,

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Abbreviations: CMC, critical micelles concentration; CNSL, cashew nut shell liquid from *Anacardium occidentale*; DGDG, digalactosyldiacylglycerol; DPPC, dipalmitoylphosphatidylcholine; LUVs, large unilamellar vesicles; MGDG, monogalactosyldiacylglycerol; NBD-PE, *N*-(-nitrobenz-2-oxa-1,3-diazol-4-yl)-1,2-dihexadecanoyl-*sn*-glycero-3-phosphoetanolamine triethylammonium salt; PC, phosphatidylcholine; PE, phosphatidyletanolamine; PS, phosphatidylserine; SM, sphingomyelin; TMA-DPH, 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatrien *p*-toluenesulfonate; SUVs, small unilamellar vesicles.

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methylcardol, cardanol, and anacardic acid) and rye grain. From the chemical point of view, cardol, methylcardol, and homologues from rye grain are resorcinolic lipids and cardanol and anacardic acid are alkylphenolic lipids.

3.1. Hemolytic and antihemolytic activity of the phenolic lipids

Phenolic lipids from both rye (Fig. 1) and CNSL (Fig. 2) exhibited hemolytic activity against sheep erythrocytes with EH_{50} values dependent on the length and saturation of the hydrocarbon chain present in the molecules and/or the chemical structure of the polar heads of the molecules (Table 1). The EH_{50} values (but not c_{sol} values) for the resorcinolic lipids from rye were determined earlier [17]: here they are shown for comparison only. We used hemolytic curves to obtain c_{sat} and c_{sol} values (Table 1). All the hemolytic curves for the agents from CNSL were biphasic, contrary to what was demonstrated for the resorcinolic lipids from rye bran [17] (Fig. 3). All the investigated compounds from CNSL had lower EH_{50} values than the resorcinolic lipids from rye bran, especially those for anacardic acid and cardol. The EH_{50} values of cardol, methylcardol, and alkylphenol show significant differences. This effect may depend on small differences in the chemical structure of the polar part of the molecules since the structures of their hydrocarbon chains are identical (Fig. 2). All the investigated compounds from CNSL had c_{sol} values much lower than that of pentadecylresorcinol (a homologue with an identical number of carbon atoms in its hydrocarbon chain), but these differences are significantly greater than the differences between the EH_{50} values. Anacardic acid and cardol show differences in c_{sat} and EH_{50}, but the c_{sol} values are almost identical. This means, that similar amount of molecules are necessary to start erythrocytes lysis, even though the localization of its molecules in the phospholipid bilayer is different. It was shown earlier [18,19] that a conical molecule

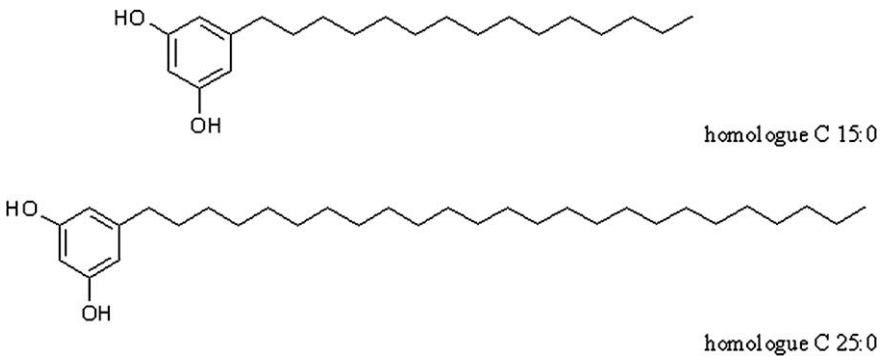

Fig. 1. The structures of resorcinolic lipids from rye grain (C15:0 and C25:0 as an example).

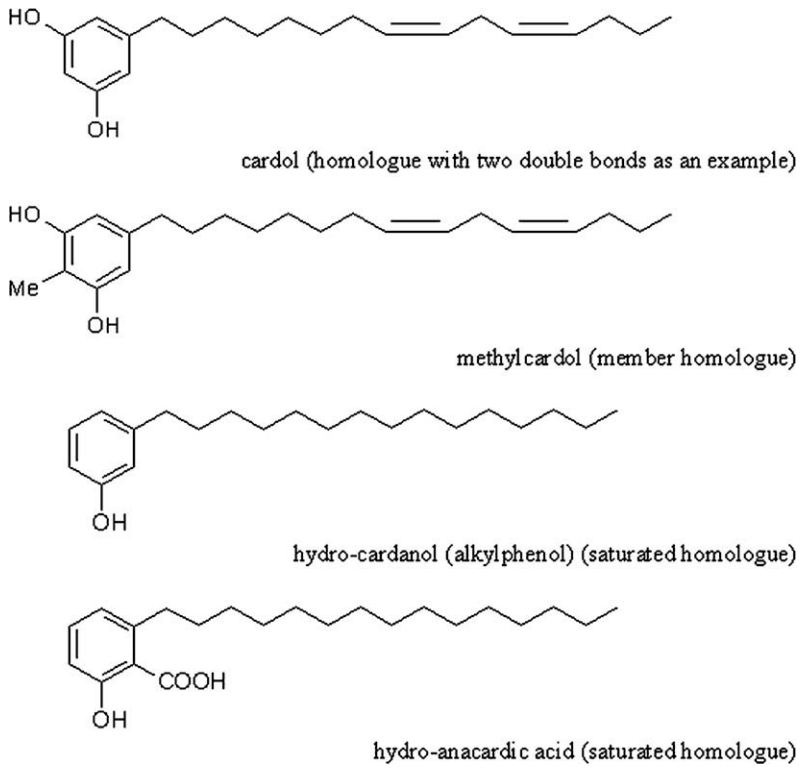

Fig. 2. The structures of resorcinolic and alkylphenolic lipids from *Anacardium occidentale*.