



## Configuration of polyisoprenoids affects the permeability and thermotropic properties of phospholipid/polyisoprenoid model membranes

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### ABSTRACT

The influence of  $\alpha$ -*cis*- and  $\alpha$ -*trans*-polyprenols on the structure and properties of model membranes was analyzed. The interaction of Ficaprenol-12 ( $\alpha$ -*cis*-Prenol-12,  $\alpha$ -Z-Prenol-12) and Allopprenol-12 ( $\alpha$ -*trans*-Prenol-12,  $\alpha$ -E-Prenol-12) with model membranes was compared using high performance liquid chromatography (HPLC), differential scanning calorimetry (DSC) and fluorescent methods. L- $\alpha$ -Phosphatidylcholine from egg yolk (EYPC) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) as the main lipid components of unilamellar (SUVs) and multilamellar (MLVs) vesicles were used. The two-step extraction procedure (n-pentane and hexane, respectively) allowed to separately analyze the fractions of polyprenol as non-incorporated (Prenol<sub>NonInc</sub>) and incorporated (Prenol<sub>Inc</sub>) into liposomes. Consequently, distribution coefficients,  $P$ , describing the equilibrium of prenol content between phospholipid (EYPC) membrane and the aqueous phase gave different  $\log P$  for  $\alpha$ -*cis*- and  $\alpha$ -*trans*-Prenol-12, indicating that the configuration of the  $\alpha$ -terminal residue significantly alters the hydrophobicity of the polyisoprenoid molecule and consequently the affinity of polyprenols for EYPC membrane. In fluorescence experiments  $\alpha$ -*trans*-Pren-12 increased up to 1.7-fold the permeability of EYPC bilayer for glucose while the effect of  $\alpha$ -*cis*-Pren-12 was almost negligible. Considerable changes of thermotropic behavior of DPPC membranes in the presence of both prenol isomers were observed.  $\alpha$ -*trans*-Pren-12 completely abolished the pretransition while in the case of  $\alpha$ -*cis*-Pren-12 it was noticeably reduced. Furthermore, for both prenol isomers, the temperature of the main phase transition ( $T_m$ ) was shifted by about 1 °C to lower values and the height of the peak was significantly reduced. The DSC analysis profiles also showed a new peak at 38.7 °C, which may suggest the concomitant presence of more than one phase within the membrane.

Results of these experiments and the concomitant occurrence of allopprenols and ficaprenols in plant tissues suggest that *cis/trans* isomerization of the  $\alpha$ -residue of polyisoprenoid molecule might comprise a putative mechanism responsible for modulation of the permeability of cellular membranes.

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### 1. Introduction

Polyprenols are a well known subgroup of polyisoprenoid alcohols described already in the 1960s (Stone et al., 1967). The highest accumulation of polyprenols has been noted in plant photosynthetic tissues, but they have also been detected in wood, seeds, flowers and in bacterial cells (reviewed in Swiezewska and Danikiewicz, 2005). Polyisoprenoid alcohol chains are built of 5–100 and more isoprenoid units creating polymers that differ in the chain-length and/or geometrical configuration. With respect to the structure, the hydrocarbon chain of polyprenols is built of an  $\omega$ -terminal isoprenoid residue followed by 2 or 3 internal *trans* residues and a stretch of *cis* residues; typical polyprenols – ficaprenols are finally decorated with an  $\alpha$ -*cis*-terminal residue (Fig. 1A). In 2007 our group described a new type of polyisoprenoid alcohols of plant origin – allopprenols (Fig. 1B) (Ciepichal et al., 2007).

**Abbreviations:** Pren-12, polyprenol composed of 12 isoprene units; PrenInc, incorporated prenol; PrenNonInc, non-incorporated prenol.

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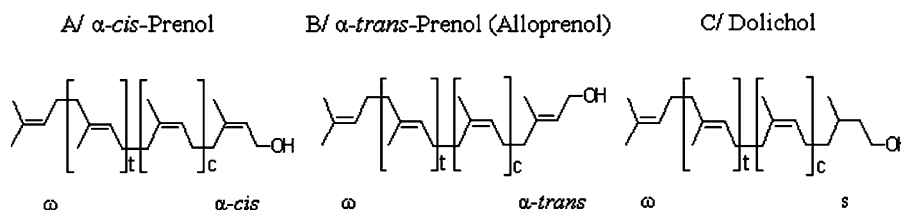
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**Fig. 1.** Structure of A/ $\alpha$ -cis-prenol, B/ $\alpha$ -trans-prenol, C/dolichol; t and c indicate the number of internal *trans*- and *cis*-isoprenoid residues, respectively;  $\omega$ ,  $\alpha$  and s stand for  $\omega$ -,  $\alpha$ -terminal and saturated isoprenoid residues, respectively.

In contrast to typical  $\alpha$ -cis polyprenols (e.g. ficaprenols), alloprenols possess the  $\alpha$ -terminal residue in a *trans* configuration. Interestingly, the proportion of the amounts of  $\alpha$ -cis vs.  $\alpha$ -trans polyprenols is 1 to 1 in the leaves of *Allophylus caudatus* and eleven other plant species (Marczewski et al., 2007). It should be also mentioned that  $\alpha$ -dihydro-polyprenols (syn. dolichols, Fig. 1C) are common constituents of animal and yeast cells.

In contrast to polyisoprenoid phosphates, functioning as cofactors in the biosynthesis of bacterial peptidoglycan and eukaryotic glycoproteins and substrates for protein prenylation (Gutkowska et al., 2004), the role of free polyisoprenoid alcohols is still uncertain. High hydrophobicity of polyisoprenoids causes their localization in cellular membranes, e.g. mitochondria, chloroplast envelopes, Golgi membranes (Swiezewska et al., 1993). However, explanation of the presence of polyisoprenoid molecules within the biological membranes has remained for many years a question of debate due to their length which exceeds the thickness of the bilayer. The first molecular model of a dolichol (Murgolo et al., 1989) suggested the dimensions of Dolichol-19 (Dol-19) as 53.07 Å (length)  $\times$  30.94 Å (width), and the molecule was proposed to consist of three geometrical regions, a central coiled segment and two flanking regions. According to the recent model the dimensions of Dol-19 were decreased to 31.87 Å (length)  $\times$  15.41 Å (width). These findings also revealed that the 3D conformations of Dol-19, Dol-19P (Dolichyl-19-Monophosphate), and Pren-11P (Prenyl-11-Monophosphate) were nearly identical with their three coiled, helical domains (Zhou and Troy, 2003) arranged as a central segment and two flanking arms.

Such a model explains well the possible orientation of the mainly-cis polyisoprenoid alcohols in model membranes (Zhou and Troy, 2005). It is also in line with the earlier observations showing that polyprenols, dolichols and their phosphorylated derivatives alter the structure of the phospholipid bilayer by promoting the formation of a nonlamellar (inverted hexagonal, Hex II) structure. These structural changes explained well the effect of polyisoprenoids on the increase of the fluidity, permeability and fusogenicity of the membranes observed earlier (Chojnacki and Dallner, 1988 and references therein). It has been reported (voltammetric methods) that incorporation of polyprenols into a model lipid membrane (dioleoylphosphatidylcholine, DOPC) decreased the activation energy and increased membrane conductance and membrane permeability coefficient (Janas et al., 2000). However, in all the studies only typical polyisoprenoids with di-*trans*-poly-*cis* or tri-*trans*-poly-*cis* structure were taken into consideration and the effect of discrete structural changes (configuration of the  $\alpha$ -*trans*-residue within the prenol molecule) has not been studied yet. This question seems intriguing in the light of the undisclosed biological function of alloprenols.

In this work the influence of  $\alpha$ -cis-Prenol-12 and  $\alpha$ -trans-Prenol-12 (Allopreenol-12), occurring concomitantly in plant cell membranes, on the properties of the phospholipid bilayer was analyzed. As model membranes unilamellar (SUVs) and multilamellar (MLVs) vesicles from natural (L- $\alpha$ -phosphatidylcholine, EYPC) or synthetic (1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine, DPPC) lecithin, were prepared. Efficiency of incorporation of  $\alpha$ -cis- and

*trans*-prenols into unilamellar phospholipid vesicles was examined using quantitative HPLC-UV analysis following the extraction procedure. The effect of polyisoprenoid alcohols on the permeability of model lipid bilayers for glucose, as an example of a low-molecular-weight electrically neutral metabolite, and for protons was monitored by using of fluorescence probes while the influence of polyprenols on thermotropic properties of phospholipid bilayers was followed using differential scanning calorimetry (DSC). Our results indicate that configuration of the polyisoprenoid chain affects the behavior of polyprenols in the bilayer. The occurrence of the additional  $\alpha$ -*trans* double bond results in a decreased degree of incorporation of polyprenols into the membrane and at the certain membrane concentration it also results in its enhanced permeability. Interestingly, such a configurational change seems not to be crucial for the formation of lipid domains within the membrane.

## 2. Materials and methods

### 2.1. Chemicals

L- $\alpha$ -Phosphatidylcholine from egg yolk (EYPC) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Prenol-12 – natural  $\alpha$ -cis-isomer isolated from the leaves of *Magnolia kobus* was from the Collection of Polyprenols (Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland). All solvents of spectral purity and chromatographic materials were from Merck (Darmstadt, Germany). 6-Carboxyfluorescein and valinomycin were from Sigma-Aldrich and pyranine was from Molecular Probes (Eugene, USA). All other chemicals were purchased from Sigma-Aldrich and were of p.a. grade.

### 2.2. Synthesis of $\alpha$ -trans-Pren-12 (Allopreenol-12)

Aldehyde of Prenol-12 was prepared as earlier (Ciepichal et al., 2007) with some modifications. Briefly: 60 mg of Polyprenol-12 in 1 ml of dichloromethane was stirred with 150 mg of pyridinium chlorochromate for 20 min at room temperature. Aldehyde was isolated by column chromatography (Florisil, dichloromethane). Fractions containing aldehyde (55 mg, mixture of  $\alpha$ -cis/*trans* aldehydes, 9:1 ratio) were pooled, evaporated under nitrogen (30°C) and dissolved in 3 ml of methanol. Enhanced isomerization of the  $\alpha$ -unit of aldehyde was achieved by the incubation of Prenal-12 in the presence of sodium carbonate (molar ratio of aldehyde:sodium carbonate, 1:15) at room temperature for 16 h (60% conversion). Further reduction of Prenal-12 (mixture of  $\alpha$ -cis/*trans* aldehydes) with sodium borohydride (molar ratio of aldehyde:sodium borohydride, 2:1) in the same solution (40 min, room temperature) yielded a mixture of  $\alpha$ -cis- and  $\alpha$ -trans-Pren-12 (99% conversion). Mixture of  $\alpha$ -cis- and  $\alpha$ -trans-Pren-12 was extracted (hexane:water; 1:1), concentrated and products were separated by column chromatography (silica gel 60 column, linear gradient of diethyl ether in hexane from 0 to 10%). Each step of preparation of  $\alpha$ -trans-Pren-12 was followed by TLC (silica gel plates, ethyl acetate:toluene, 1:9

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