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Does isoprene protect plant membranes from thermal shock? A molecular dynamics study

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Abstract

The question of why plants release isoprene when heat stressed has been continuously debated for more than half a century. In this work we use molecular dynamics simulation techniques to directly investigate the interaction between isoprene and a model phospholipid membrane in atomic detail. It is found that isoprene partitions preferentially in the center of the membrane and in a dose dependent manner enhances the order within the membrane without significantly changing the dynamical properties of the system. At a concentration of 20 mol% isoprene (16 isoprene molecules per 64 lipid molecules) the effect of the addition of isoprene on the membrane order is equivalent to a reduction in temperature of 10 K, rising to a reduction of 30 K at 43 mol% isoprene. The significance of the work is that it provides for the first time direct evidence that isoprene stabilizes lipid membranes and reduces the likelihood of a phospholipid membrane undergoing a heat induced phase transition. Furthermore it provides a clear mechanistic picture as to why plants specifically utilize isoprene for this purpose.

Keywords: Isoprene; Molecular dynamic; Membrane; Dimyristoylphosphatidylcholine; Simulation; Plant; Temperature; Thermotolerance

1. Introduction

The characteristic blue haze over the evergreen forests of the Australian Blue Mountains or the Blue Ridge Mountains of North Western Carolina in the USA on hot summer days in part results from the scattering of light by microscopic particles composed of isoprenoids (i.e. monoterpenes and isoprene). Above a critical temperature some plants release up to 50% of the photosynthetically assimilated carbon into the atmosphere in the form of isoprenoids. Isoprene (2-methyl-1,3-butadiene) is one of the most common biogenic volatile organic compounds (BVOCs) with the worldwide annual release by plants being in the order of 5×10^{14} C g/year [1]. Decomposition of isoprene and other BVOCs reduces the concentration of the atmospheric OH radicals required for degradation of the greenhouse gas methane [2,3]. The breakdown of isoprene and other BVOCs in the presence of NO_x also leads to the formation of ozone in the troposphere [4] aggravating air pollution where [NO_x] is high, such as in industrial areas.

Isoprene is primarily emitted by woody plants of which genera Quercus (oaks) and *Populus* (poplars) [5] have the highest rate of emission. However, particular species of conifers (i.e. *Picea*) as well as Eucalyptus trees [6], mosses, ferns and even marine algae [7] also emit significant amounts of isoprene. Isoprene is generally found at low concentration in the atmosphere (<10 ppb). During short, acute temperature shocks the production and emission of isoprene can increase dramatically (Fig. 1). Above 303 K significant quantities of isoprene are produced, ceasing again when the temperature falls.

Despite the importance of isoprene in atmospheric chemistry, the cellular function of isoprene, if any, is uncertain. One

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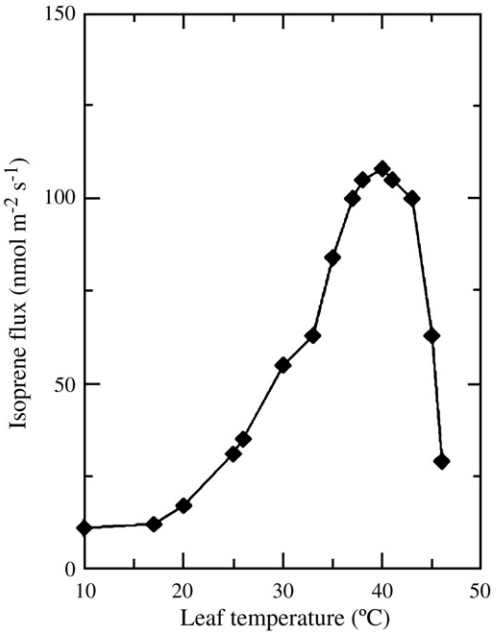

Fig. 1. Isoprene emission (measured as a flux) in oak leaves as a function of temperature. Taken from [46,47].

proposal is that isoprene production is simply a byproduct of rapid photosynthesis, just as acetone can be a byproduct of fatty acid metabolism [8] or methanol is emitted as a product of pectin methyl esterase [9]. Other proposals to explain isoprene emission include a flowering hormone [10] , an antioxidant [11], and as a means to rid the plant of excess carbon [5,12]. However, the most widely accepted hypothesis proposed originally by Sharkey and Singsaas [13–16] is that isoprene plays a role in thermotolerance. Sharkey and Singsaas argued that isoprene protects the photosynthetic system from thermal damage. For example isoprene may prevent the formation of non-lamellar aggregates or help stabilize the photosynthetic complexes anchored in thylakoid membrane [17]. They assessed the temperature tolerance of photosynthesis by keeping leaves either in the dark or in a nitrogen atmosphere to control endogenous isoprene synthesis. They showed that adding isoprene to the air surrounding the leaves led to an increase in the temperature at which irreversible damage began to occur from 308 K to 318 K. Logan and Monson [18] in contrast found that photosynthetic activity of leaf discs was unaffected until 318 K in the presence or absence of isoprene.

Due to its high volatility the residence time of isoprene in the thylakoid membrane will be very short. This suggests that isoprene may primarily protect plant membranes against transient exposure to high temperatures, an idea supported by studies in which leaves are exposed to a short high-temperature shock instead of continuous heating [19].

Here we use molecular dynamics (MD) simulation techniques to study the molecular details of the interaction between a model lipid membrane and isoprene. Our aim is to answer two questions. First, how does isoprene interact with a phospholipid bilayer? Second, does isoprene affect the temperature dependent dynamic and thermodynamic properties of lipid bilayers?

Over the last decade MD simulations have been used successfully to study the properties of lipid membranes, and the interaction of membranes with a variety of other molecules including cholesterol [20], sugars [21,22], drugs [23], and peptides [24]. Biological membranes are, however, very complex systems. Our ability to model specific aspects such as exact lipid composition is limited. For this reason the results presented are more qualitative than quantitative. In this work the membrane is modeled by a bilayer consisting of either 64 DMPC (dimyristoylphosphatidylcholine) or 64 POPC (1-palmitoyl-2-oleoyl-phosphatidylcholine) lipid molecules. DMPC is a fully saturated phospholipid. POPC is mono-unsaturated at the sn-2 chain. DMPC was chosen for the initial stages of this study because DMPC membranes are well studied experimentally. However, chloroplast membranes contain a high proportion of unsaturated fatty acids. For this reason simulations were also performed using POPC, a common unsaturated lipid to gain insight into the effect of unsaturation on the interaction of the lipids with isoprene.

Although the change in the rate at which isoprene is released into the surrounding environment as a function of temperature is readily determined experimentally [13] the actual concentration of isoprene within the membrane is unknown. Determination of this quantity is difficult due to the volatility of the compound and as a consequence the short residence time of isoprene in the membrane. In this work simulations have been performed at two different concentrations of isoprene (20 and 43 mol% equivalent to isoprene:lipid ratios of 16:64 and 48:64 respectively) over a range of physiologically relevant temperatures. Using the simulations we demonstrate that isoprene preferentially accumulates at the center of the membrane bilayer and results in a reduction of the order of the acyl chains without significantly affecting the dynamic properties of the system. The effect of isoprene on the order of the membrane is similar to that induced by lowering the temperature. The implications of these results in terms of the thermotolerance hypothesis are discussed.

2. Results

2.1. Partitioning of isoprene within the membrane

Isoprene is small and hydrophobic. Starting from a configuration in which the isoprene molecules are dissolved in the water, the isoprene molecules spontaneously partition into the membrane, remaining there during the time scale of the simulation (tens of nanoseconds). The preference of the isoprene molecules for the bilayer interior over the water phase is consistent with the value of the partition coefficient between octanol and water ($\log P_{o/w} = 2.30$ [25]) of isoprene and the poor solubility (<1 mg L^{-1}) of isoprene in water [25]. A typical snapshot of the equilibrated bilayer consisting of 64 DMPC and 16 isoprene molecules (20 mol%) is shown in Fig. 2. The distribution of the various types of atoms in the system is quantified in Fig. 3, which shows the averaged density profile along the direction normal to the bilayer surface. This specific system was simulated at 353 K, the highest temperature

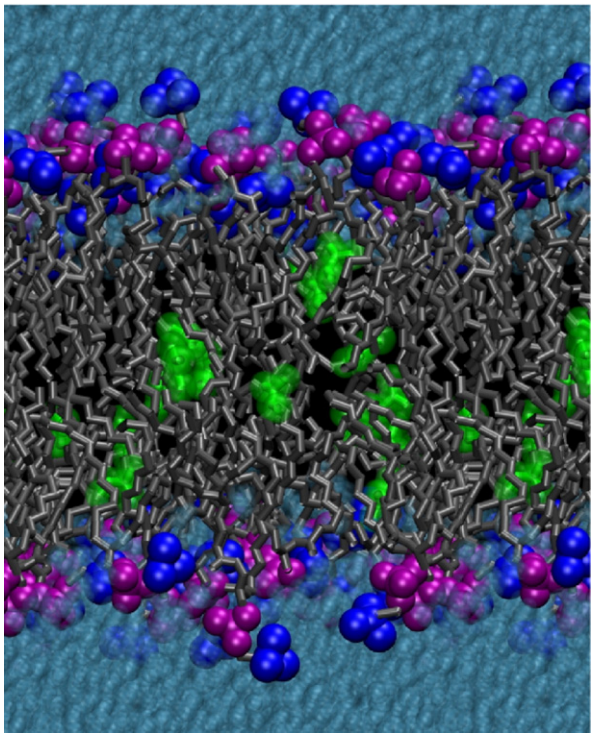

Fig. 2. Equilibrium structure of a DMPC bilayer containing 20 mol% (isoprene:lipid 16:64) isoprene at $T=353$ K. The lipid headgroups are shown as blue (choline) and purple (phosphate) spheres, the lipid tails as grey sticks, and the water in semi-transparent blue. The isoprene molecules, represented by green spheres, intercalate between the lipid tails. The highest isoprene density is found at the bilayer center. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

studied. Systems simulated at lower temperatures show similar distributions. In the temperature range studied (303 K–353 K), the lipid membrane is in the physiologically relevant fluid, or liquid–crystalline phase, characterized by disordered lipid tails and fast lateral lipid diffusion. Figs. 2 and 3 both show that the isoprene molecules preferentially locate at the bilayer center. This is for two reasons. First, the bilayer center has the lowest lipid density and therefore the most free (available) volume to accommodate the isoprene molecules. Second, the bilayer center is furthest away from the hydrophilic interfacial region. The distribution is wide, however, and isoprene molecules are found across the entire volume occupied by the lipid tails, up to the position of the carbonyl groups of the glycerol ester at the junction between the hydrophobic interior and the hydrophilic interfacial region. The isoprene molecules are highly mobile. Fast exchange between different locations in the membrane is observed on a nanosecond time scale. The qualitative behavior is very similar for the two different concentrations studied. Even at 43 mol%, all of the isoprene is accommodated within the interior of the membrane. At this concentration the accumulation of isoprene in the middle of the bilayer results in a local drop in density of the lipid tails (see Fig. 3). Simulations of isoprene in a POPC bilayer showed that the effect of chain unsaturation has little or no effect on the distribution of isoprene within the lipid membrane.

The effect of temperature and concentration on the structure of the DMPC bilayers was characterized by calculating the area per lipid and the bilayer thickness. These are given in Table 1 and compared to experimental data determined from ^{2}H-NMR spectroscopy measurements [26]. The best agreement between the experimentally determined area per lipid and the data obtained from the simulations was for temperatures around 323 K. The temperature dependence of the area per lipid appears somewhat smaller in the simulations, however. The area per lipid in the simulations increases by 0.01 nm^2 per 10 K. The expansion in area is a direct consequence of an increased disorder of the lipid tails, which is entropically favorable at

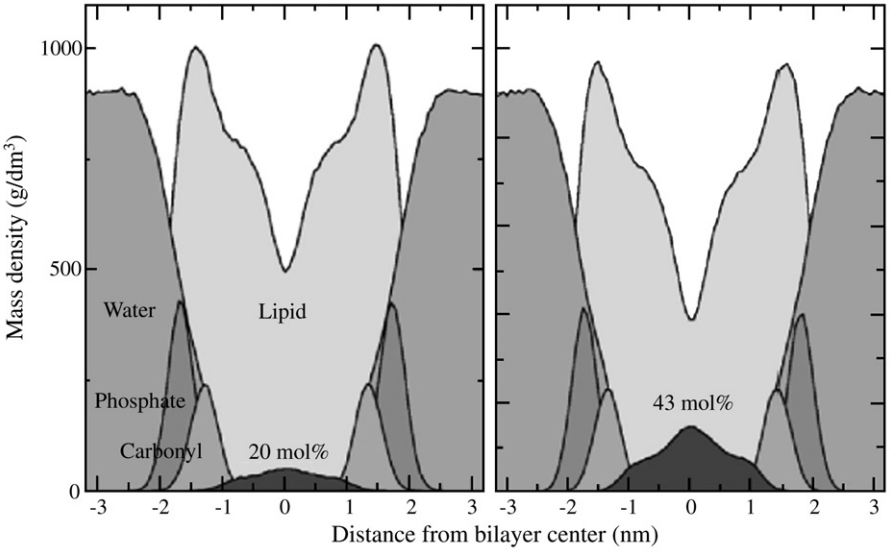

Fig. 3. Mass density profiles of the individual constituents of a DMPC bilayer containing 20 mol% (isoprene:lipid 16:64) (left) and 43 mol% (isoprene:lipid 48:64) (right) isoprene at $T=353$ K. The densities are plotted as a function of the distance from the bilayer center along the membrane normal. The constituents of the system are indicated on the plot. The filled black area denotes isoprene.

Table 1
Comparison of structural properties of a pure DMPC bilayer and different DMPC/isoprene systems

System	T (K)	Area per lipid (nm2)	$\langle S_{CD} \rangle$	D_{P-P} (nm)
Pure DMPC	303	0.63	0.174	3.4
	323	0.66	0.165	3.3
	333	0.67	0.162	3.4
	343	0.68	0.155	3.3
	353	0.69	0.150	3.4
DMPC+16 isoprene	303	0.64	0.196	3.5
	323	0.67	0.178	3.5
	333	0.68	0.168	3.4
	343	0.69	0.161	3.3
	353	0.70	0.154	3.3
DMPC+48 isoprene	303	0.68	0.197	3.4
	323	0.70	0.180	3.5
	353	0.73	0.165	3.2
From ^{2}H NMR experiment	303	0.60	0.180	–
for pure DMPC (Petrache et al., 2000)	323	0.65	0.140	–
	338	0.68	0.127	–
From X-ray scattering	303	0.61	–	3.5
for pure DMPC (Kučerka et al., 2005)	323	0.66	–	–
	353	0.73	–	–

$\langle S_{CD} \rangle$ is the order parameter averaged over carbons 3 to 13, averaged over both chains. D_{P-P} is a measure of membrane thickness. It is the distance between the maxima in the phosphate density distribution along the membrane normal. The standard error is smaller or equal to 0.001 for all values of $\langle S_{CD} \rangle$, 0.003 for the area/lipid, and 0.1 for the membrane thickness.

higher temperatures. The presence of isoprene inside the membrane results in a somewhat larger area as compared to the pure DMPC system. At 323 K the area per lipid is 0.01 nm^2 larger for the system containing 16 isoprenes compared to the area of pure DMPC. For the system containing 48 isoprene molecules the expansion in area is about 0.04 nm^2. The thickness of the DMPC bilayer remains approximately constant over the temperature range studied and the incorporation of isoprene into the membrane appears to have little effect on the thickness.

2.2. Isoprene induced order

The fluidity of the lipid membrane is an important physical property directly related to biological activity. One measure of fluidity is the lipid tail order parameter S_{CD}, which can be obtained experimentally from ^{2}H-NMR. It can also be obtained from the simulations. Fig. 4 shows S_{CD} as a function of the position of the carbon vector along the lipid tail, averaged over the two chains. Perfect alignment of the lipid chains with the bilayer normal is indicated by $|S_{CD}|=0.5$, a random ordering is indicated by $|S_{CD}|=0$. The order parameters obtained from the simulations agree very well with the experimentally determined order parameter profile. The order parameter profile shows decreasing order along the lipid tails. It is high close to the headgroup region, where the density is high, and low towards the ends of tails. In the pure bilayer the shape of the profile is similar over the entire temperature range, but the average order decreases as the temperature increases (see also Table 1) indicating the membrane becomes more disordered. The addition of isoprene does not effect the shape of the profile, but the average order parameter increases indicating the membrane becomes more ordered.

3. Discussion

Molecular dynamics simulations suggest that isoprene molecules intercalate between the lipid tails in the membrane interior resulting in an increase in the order of the lipid tails.

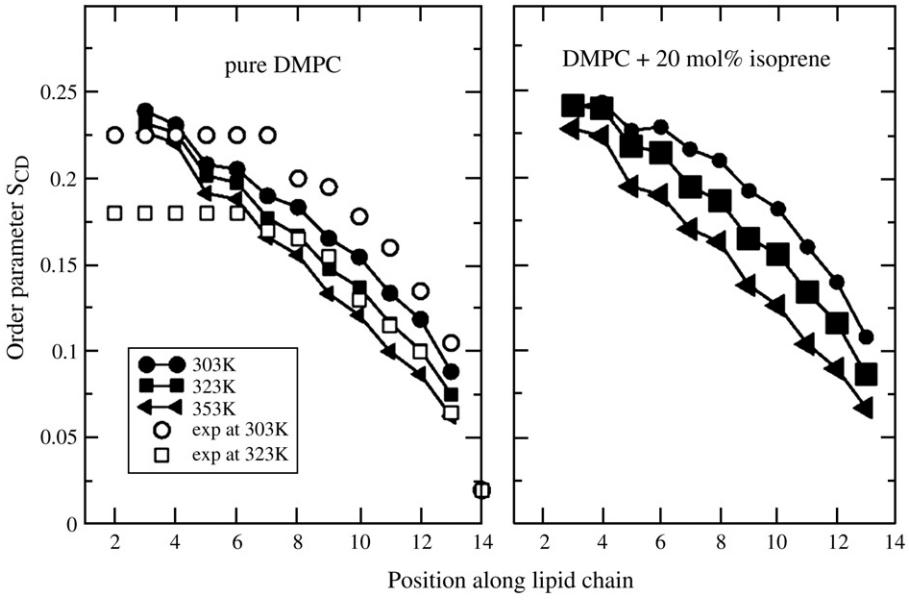

Fig. 4. Lipid tail order parameters as a function of position along the alkyl tails. Carbon one is the carbonyl carbon closest to the headgroup. The order parameters of each position are averaged over the two chains. The left panel shows the order parameters as a function of temperature for pure DMPC, the right panel for the system containing 20 mol% isoprene. Statistical significance of the results is indicated by the size of the symbols, representing standard errors. The standard error is at most 0.005. The experimentally determined order parameters for pure DMPC at 303 K and 323 K are also shown [26,48].

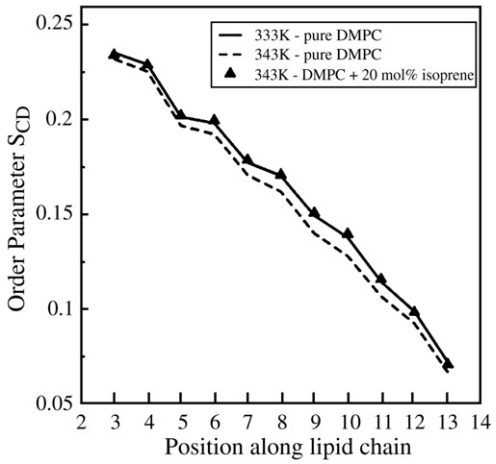

Fig. 5. Comparison of lipid tail order parameters for a pure DMPC system at 333 K and 343 K as well as for a DMPC system containing 20 mol% isoprene at 343 K. The standard error is at most 0.005.

Our simulations thus suggest that isoprene acts directly to stabilize the membrane. In this way isoprene counteracts the increase in disorder associated with increasing temperature. This is illustrated in Fig. 5 where the order parameter profiles for a pure DMPC bilayer and a DMPC bilayer containing 20 mol% isoprene molecules at a temperature 10 K higher are compared. As can be seen the profiles are very similar. In the case of a DMPC bilayer with 20 mol% isoprene the increase in order is equivalent to lowering the temperature by 10 degrees. At 43 mol%, the observed increase in order is equivalent to lowering the temperature of the DMPC bilayer by almost 30 K (data not shown). Similar results were obtained for the POPC bilayer, indicating that isoprene induces similar ordering with saturated or unsaturated lipids. Analysis of trans/gauche isomerizations corroborate these results (not shown). The average fraction of trans dihedral angles rises about 0.005 every 10 K for pure DMPC. In the presence of isoprene the average fraction of trans dihedral angles decreases by 0.005 or equivalent to a drop in temperature of 10 K lower.

An alternative way to characterize the fluidity of the membrane is in terms of its dynamical parameters. In Fig. 6 the autocorrelation function of the orientation of the vector between the 9th and 11th carbon of the lipid tails w.r.t. the Z axis (normal to the membrane) is plotted for pure DMPC systems at 333 K, 343 K and a system containing 20% isoprene at 343 K (see methods). The autocorrelation function, which reflects rate of reorientation or rotational motion of the lipid tails, decays within 2–5 ns towards a constant residual value. The residual value reflects the long time order of the system. Note that the residual values for the pure membrane at 333 K and the isoprene-containing membrane at 343 K are similar, in correspondence with the analysis of the order parameter. However, the decay times, which were obtained by fitting the curves in Fig. 6 to a double exponential decay, were different. The decay times for the fast τ_1 and slow τ_2 components were as follows: τ_1=24 (±1) and τ_2=285 (±10) ps for pure DMPC bilayer at 333 K, τ_1=22 (±1) and τ_2=229 (±9) ps for pure DMPC at 343 K, and τ_1=21 (±2) and τ_2=224 (±9) ps for DMPC bilayer enriched by 20% of isoprene also at 343 K. As can be seen the short and the long time scale delay is not affected by the presence of isoprene. Thus while isoprene increases lipid order, it has little effect on the local dynamics of the membrane.

Overall our results support the hypothesis that isoprene is thermoprotective [13–16,19]. Isoprene production would allow plants to transiently modulate the order of essential membranes without affecting the dynamics thus maintaining photosynthetic activity under thermal stress. As noted previously isoprene is highly volatile. Thus, there will be a dynamic equilibrium between isoprene production and loss by evaporation. As the ambient temperature drops, and isoprene production decreases, the isoprene will be readily lost thus preventing freezing of the membrane. In this way plants can maintain constant membrane order and photosynthetic activity over a wide temperature range.

It is worth mentioning that there are other protective compounds like disaccharides (trehalose, sucrose, glucose) which also have a stabilizing and protective effect on biological membranes. Experimental studies by Crowe et al. [27] have shown their efficiency as protectants against freezing (as cryoprotectants) and freezedrying (as lyoprotectants). According to the water-replacement hypothesis [28], sugars can substitute water molecules around the polar and charged groups present in phospholipid membranes and proteins. In that way they can preserve the native membrane structure in the absence of water. Theoretical studies on this subject confirm this hypothesis [21,22]. Sugar molecules are observed to act as a bridging unit between adjacent lipids filling the gap formed after dehydration. Instead of interacting with the headgroups, isoprene embeds within the hydrophobic part of bilayer, and affects the order of the lipid acyl chains. In this respect it is similar to cholesterol. Cholesterol, like isoprene, increases the ordering and molecular packing of lipid tails [29] but unlike of isoprene, cholesterol substantially affects the dynamics of the lipids reducing lateral diffusion [30].

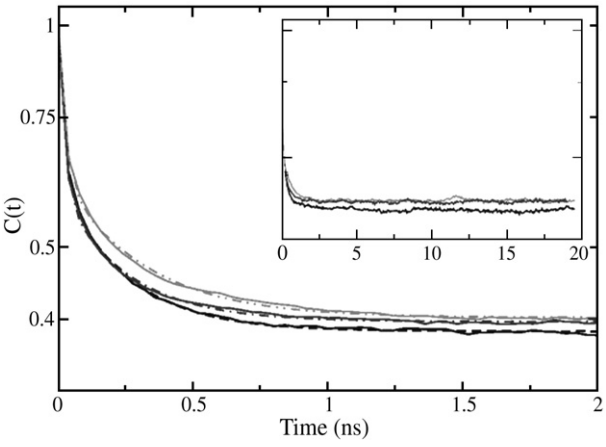

Fig. 6. Double-exponential fits to the rotational autocorrelation functions for a pure DMPC system at 333 K (doubledot-dashed line) and 343 K (dashed line) as well as for a DMPC system containing 20 mol% isoprene at 343 K (dot-dashed line). Solid lines represent the rotational correlation functions at consecutive temperatures. The small graph shows the convergence of the dynamics of the system with isoprene towards that of pure DMPC at the lower temperature.

Isoprene is well suited to act as a transient modulator of membrane order. Being small and hydrophobic it readily migrates to the interior of the membrane and occupies the available free volume (i.e. volume not occupied by the lipid tails) [31,32]. At low temperature lipids adopt what is known as the lipid gel phase in which the lipid tails are highly ordered and there is very little free volume. At higher temperatures they adopt the physiologically relevant liquid–crystalline phase in which entropic effects give rise to trans/gauche isomerization of the lipid tails. The tails become less ordered, they pack at a lower density and the membrane becomes fluid. In the liquid–crystalline phase there is a significant amount of free volume in the form of small, irregularly shaped pockets. Isoprene occupies this free volume thereby increasing the adhesive forces in the membrane interior, acting as a molecular glue. At higher temperatures, the amount of free volume increases and the affinity of the membrane interior for isoprene is increased. Unlike other modulators of membrane order such as cholesterol, isoprene is readily lost as the temperature decreases preventing the membrane from freezing. Summarizing, the three essential properties of isoprene that it is small, hydrophobic, and irregular make isoprene well suited for its role as a transient stabilizer of membranes because (i) it fits well into the available pockets of free volume inside the membrane, (ii) it adds cohesiveness amplifying the membrane packing, while not affecting the dynamics, (iii) it is relatively easily expelled from the membrane as the temperature drops, and (iv) being highly volatile it readily escapes from the plant after a heat shock.

The final issue that must be addressed is how realistic the simulations are and whether they can be directly compared to the situation *in vivo*. Based on the nominal concentration of isoprene in air within leaves of ~20 ppm and the partition constant of isoprene in octanol one would predict an isoprene: lipid ratio of approximately 1:200. However, there are several reasons why the actual concentration within the cell membrane might be much larger and close to the concentrations used in the simulations (1:4, 3:4). First, isoprene was found to bind specifically at the center of the membrane. The partition constant of isoprene in DMPC is thus expected to be higher than that in octanol. Second, isoprene is synthesized continuously at high temperature. The transient concentrations within the cell and hence within the cell or thylakoid membranes are likely to be far from equilibrium and higher than that inferred from the concentration of isoprene within the air inside leaves. Finally, the estimate of the concentration of isoprene within the air within leaves is also open to question as it is based on several assumptions including that the transport behavior of water and isoprene within leaf are similar. Although our study has been limited to simple model membrane systems, the basic underlying principles are expected to be applicable to membranes of a wide range of composition. The thylakoid membrane consists of ~50% protein and ~50% lipid with a high proportion of unsaturated fatty acids. Qualitatively similar results were obtained using both saturated (DMPC) and unsaturated (POPC) lipids. The simulations suggest that concentrations of ~20 mol% isoprene can induce a decrease in order equivalent to a drop of ~10 K in the effective temperature. This prediction could be directly tested by measuring lipid order parameters in the presence of isoprene using deuterium NMR.

4. Conclusion

Molecular dynamics simulations of phospholipid bilayers with and without isoprene suggest that isoprene enhances the packing of lipid tails. The small, irregularly shaped isoprene molecules partition into the free volume at the center of the bilayer inducing an increase in order of the lipid tails counteracting in part the effect of an increase in temperature. A concentration of 20 mol% isoprene results in a decrease in the effective temperature of about 10 K in the case of a pure DMPC bilayer. Interestingly, the dynamics of the tails is not affected. Based on our work we propose that isoprene is synthesized by plants in order to maintain a constant level of membrane cohesiveness that is assumed to be required for biological activity, thereby protecting the plant against thermal shock.

5. Methods

5.1. Force field parametrization

The force field for the lipid molecules was based on that of Berger et al. [33]. The SPC [34] model was used for water. This combination has been shown to reproduce a range of experimental data, such as the area per lipid and C–D order parameters well [33,35].

The geometry and definition of the atom types of isoprene are described in Fig. 7. As there was no force field available for isoprene, we based it on the isoprene like unit found in retinol which is parameterized in GROMOS96 force field. The GROMOS96 force field is a united atom model in which the aliphatic hydrogens are incorporated into the carbon atom to which they are attached. The equilibrium bond lengths, bond angles, and dihedral angles were taken from literature *ab initio* calculations [36]. Force constants for the bonds and angles were taken from the GROMOS96 force field for similar bonds in aromatic compounds. Parameters for the torsion around the central bond were obtained by fitting the profile from *ab initio* calculations [36] using 2 cosine functions. The bonded parameters for isoprene are given in Table 2.

There are no specific parameters for a terminal CH2 group in the GROMOS96 force field. Using the standard CH2 parameters for the terminal carbons failed to reproduce the density and heat of vaporization of isoprene with sufficient accuracy. For this reason a new atom type (DH2) was introduced to describe the terminal CH2 groups. The Lennard–Jones parameters for this group were obtained by fitting the density and heat of vaporization calculated for a box of 500 isoprene molecules at 298 K to the experimental values [37]. The Lennard–Jones parameters for the other atom types defined in the GROMOS96 force field were not changed. Density and heat of vaporization were calculated

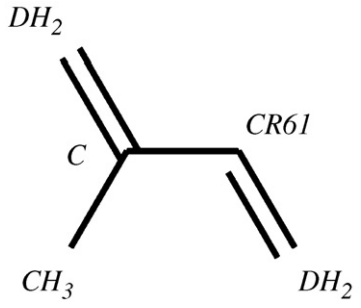

Fig. 7. Geometry and definition of atom types of isoprene. Note that the hydrogens are not modeled explicitly.

Table 2
Bonded force field parameters for isoprene

Structural parameter	Bond length, b_0 (nm)	Force constant, k_b (kJ mol^{-1} nm^{-2})	
R(DH2═══C)	0.1346	418400.	
R(DH2═══CR61)	0.1343	418400.	
R(CR61–C)	0.1479	334720.	
R(C–CH3)	0.1508	334720.	
	Bond angle, θ_0 (degree)	Force constant, k_θ(kJ mol^{-1} rad^{-2})	
∠ (DH2═══C–CR61)	120.0	418.0	
∠ (C–CR61═══DH2)	125.0	418.0	
∠ (DH2═══C–CH3)	122.0	418.0	
∠ (CR61–C–CH3)	118.0	418.0	
	Dihedral angle, ϕ_0 (degree)	Force constant, k_ϕ(kJ mol^{-1})	Multiplicity, n
∠ (DH2═══C–CR61═══DH2)	−136.0	9.0	2
∠ (DH2═══C–CR61═══DH2)	0.0	8.0	1
	Improper dihedral angle, ξ_0 (degree)	Force constant, k_ξ (kJ mol^{-1} rad^{-2})	
∠ (C═══DH2–CR61–CH3)	0.0	167.0	

using the same cutoff values as in the force field used for the lipids [33], to ensure compatibility. The calculated density at 298 K was 0.674 g cm^{-3}, compared to the experimental density of 0.676 g cm^{-3}. The calculated heat of vaporization was 27.0 kJ mol^{-1}, compared to the experimental value of 24.0 kJ mol^{-1}. The non-bonded parameters used for isoprene are given in Table 3.

5.2. Simulation procedure

All simulations and analysis were performed using the GROMACS simulation package (version 3.0) [38,39]. A time-step of 4 fs was used for integrating the equations of motion. The non-bonded interactions were evaluated using a twin-range cutoff scheme. All interactions within a short-range cutoff of 1.0 nm were evaluated every step. Electrostatic interactions outside the short-range cutoff but inside the long-range cutoff of 1.4 nm were evaluated every 10 steps, together with the neighbor list. To correct for the truncation of longer range electrostatic interactions beyond the long range cutoff a reaction-field correction [40] was applied, with a value of 54 for the dielectric constant corresponding to the SPC water model [41]. Each component of the system (lipids, water, isoprene) was coupled separately to a temperature bath using the Berendsen thermostat [42]. Simulations were performed at 6 different temperatures 303, 313, 323, 333, 343 and 353 K. Pressure coupling was applied using a Berendsen barostat [42] with a coupling constant of 1 ps, with a reference pressure of 1 bar in all directions. The lateral and longitudinal dimensions were coupled independently.

A bilayer of dimyristoylphosphatidylcholine (DMPC) in water was prepared by spontaneous aggregation at 323 K, starting from a random solution of lipids in water [43]. The system consisted of 64 DMPC molecules and a number of 35 water molecules per lipid. According to the work by de Vries et al. [44] a number of structural and dynamical properties are converged already for a bilayer containing 36 lipids. An equilibrated bilayer containing 32 lipids in each leaflet was formed after 30 ns of simulation. The configuration at 30 ns served as the starting point for the various simulations performed at different temperatures. Each trial simulation performed in the absence of isoprene was 20 ns, with the final 10 ns being used for analysis. To add isoprene to the system all water molecules were first removed. Then, 16 or 48 isoprene molecules were randomly placed in the box, avoiding overlap with the lipid molecules and the system was resolvated. The final systems consisted of 64 DMPC molecules, 16 or 48 isoprene molecules and approximately 2200 water molecules. The system containing 16 isoprene molecules was simulated for 50 ns at 303, 323, 333, 343 and 353 K. The systems with 48 isoprene molecules was simulated at 303, 323 and 353 K. A similar procedure was followed for the systems containing palmitoyl-oleoyl-phosphatidylcholine (POPC). In all simulations, the isoprene molecules had entered the bilayer within 30 ns. The simulations were continued for least 20 ns after the last isoprene molecule had entered the bilayer in each case to ensure equilibration. The final 10 ns were used for analysis. Some of the simulations were extended for 40 ns.

Table 3
Non-bonded force field parameters for isoprene

Atom name	C6 (kJ mol^{-1} nm^6)	C12 (kJ mol^{-1} nm^{12})
DH2	0.87286E-02	0.27800E-04
C	0.23402E-02	0.33740E-05
CR61	0.55132E-02	0.15120E-04
CH3	0.98765E-02	0.16150E-04

5.3. Analysis

5.3.1. Area per lipid

The area per lipid was calculated from the box dimensions in the lateral directions. To check statistical significance standard errors were calculated using block-averaging, assuming statistically independent blocks of 2 ns. Mass density profiles were calculated by dividing the system in 100 slabs in the stacking direction and counting the mass density associated with each atom in a particular slab.

5.3.2. Order parameter

The order parameter profile was calculated as S_{CD} (i)=<3cos$^2\theta_i$−1>/2, where θ_i is the angle between the C–D bond of methylene group i and the applied magnetic field which was assumed to be perpendicular to the plane of the membrane (Z-axis of the box). The brackets denote an ensemble average. The absolute value of the order parameters of the methylene segments is reported. Because there are no explicit H-atoms in the simulations the order parameters were calculated from the positions of the C-atoms along the chain [45]. The standard error for S_{CD} was obtained by considering time averaged value <S_{CD}> for each of the 64 DMPC molecules independently. The rotational correlation function, $C(t)$, provides a measure of the rotational motion of the C–H vectors in the tail segments of lipids. Because our model does not include methylene hydrogens, $C(t)$ is calculated as the autocorrelation function of the vector defined by the ninth and eleventh carbon atoms and the normal Z to the membrane. These two carbons were chosen as they show the most substantial differences in the order parameter profile (see Fig. 5). The correlation functions were calculated using 40 ns represented trajectories in each case. The resulting curves could be represented over their entire range by fitting a double exponential to the first 2 ns of the correlation function. The relaxation times cited in the text for short and long time processes correspond to the parameters from this fit. The standard error was

calculated using a block-averaging procedure assuming four statistically independent blocks of 16 lipids.

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