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Computational study of nisin interaction with model membrane

L. Lins [a], P. Ducarme [b], E. Breukink [c], R. Brasseur [b,*]

[a] *INSERM U10, Hôpital Bichat, 170 bd Ney, 75018 Paris, France*

[b] *Centre de Biophysique Moléculaire Numérique, Faculté Universitaire des Sciences Agronomiques de Gembloux, Passage des Déportés 2, 5030 Gembloux, Belgium*

[c] *Department Biochemistry of Membranes, Centre for Biomembranes and Lipid Enzymology, Institute of Biomembranes, Utrecht University, Padualaan 8, 3584 Utrecht, Netherlands*

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Abstract

Nisin is a 34-residue lantibiotic widely used as food preservative. Its mode of action on the bacterial cytoplasmic membrane is unclear. It should form ion channels but a molecular description of the interaction between nisin and phospholipids is lacking. The interactions between nisin and a membrane and the influence of phospholipids are here analysed by molecular modelling. The NMR structures of nisin in a micellar environment were previously determined (Van den Hooven et al., Eur. J. Biochem. 235 (1996) 382–393) Those structures were used to start with. They were refined by running a Monte Carlo procedure at a model lipid/water interface. It was shown that nisin is adsorbing onto the interface, with its N-terminal moiety more deeply inserted in lipids than the C-end, indicating distinct hydrophobic properties of the N- and C-domains. Therefore, we suggest that the N-terminal part is implied in the insertion of nisin in lipids, while the C-terminal moiety could be involved in the initial interaction with the membrane surface. Modelling the interaction of nisin with different neutral or anionic phospholipids shows that it disturbs the lipid organisation. The disturbance is maximal with phosphatidylglycerol. In this system, nisin curves the surface of phosphatidylglycerol layer round suggesting it could induce micelle formation. This could be a preliminary step to pore formation. It suggests that phosphatidylglycerol could have a direct action on nisin insertion and on ion channel formation. Appearance of a curvature also agrees with the 'wedge model' proposed in the literature for the nisin pore formation.

Keywords: Protein; Membrane; Insertion; Lantibiotic; Wedge model

1. Introduction

Nisin is a lantibiotic (i.e. contains lanthionine residues) produced by *Lactococcus lactis*. It acts against Gram-positive bacteria. There are two variants of the peptide, nisin A and Z, which differ only by the mutation $His^{27} \rightarrow Asn$. Out of the 34 residues of nisin, 13 are modified after translation, leading to the formation of five rings (Fig. 1). The first three rings (A–C) form the N-terminal domain of the peptide, while the C-terminal moiety is made of the D and E rings. The NMR structure of nisin A has been determined in the presence of micelles of dodecylphosphocholine and sodium dodecylsulphate as well as in water [1–4].

Nisin activity is not well understood. Its microbial action is due to an interaction with the phospholipid membrane [5–8], but it does not seem to require a

* Corresponding author. Fax: +32-81-622-522; E-mail: brasseur.r@fsagx.ac.be

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specific membrane receptor. The phospholipid composition of the membrane influences nisin activity [8–12]. The activity is increased in the presence of a trans-negative membrane potential [8,13]. Since nisin induces membrane leakage, it should disrupt the bilayer and it has been suggested to form an ion channel [14]. The molecular details of the pore-forming mechanism of nisin remain unclear. For example, the roles of the N- and C- terminal moieties in the different steps leading to pore-formation (e.g. binding, insertion and aggregation) are unknown.

In this paper, we analysed by molecular modelling, on the one hand the interaction of nisin with a rigid model membrane and, on the other hand, the interaction of nisin with different kinds of lipids. Our simulations on membranes suggest that nisin adsorbs to a bilayer surface, with its N-terminal domain more deeply inserted in the membrane. Analysis of lipid–nisin interaction further supports that nisin could modify the packing of acyl chains of some lipids. A direct action of phosphatidylglycerol (PG) on nisin insertion and pore formation is suggested.

2. Materials and methods

Calculations were performed on RAMSES (Rapid Analysis Master/Slaves Extensible System), a parallel hardware of 21 Tracor Europa Pentium Pro PC cadenced at 180 MHz connected by a 100-Mbytes Network and controlled by a HP Vectra VA Pentium Pro cadenced at 200 MHz. The calculation softwares have been developed in our laboratory [15,16]. Molecular views and hydrophobicity potentials were drawn with WinMGM 2.0 [17] (Ab Initio Technology, Obernai, France).

2.1. Energies used in the molecular modelling procedure

2.1.1. Van der Waals energy

The London–Van der Waals energy of interaction between all pairs of non-mutually bonded atoms can be described by the function of the Lennard–Jones energy function (Eq. 1):

$$E\mathrm{vdw}_{ij} = -\frac{A}{D_{ij}^{6}} + \frac{B}{D_{ij}^{12}} \quad (1)$$

where D_{ij} is the distance between atoms i and j and A and B are coefficients assigned to atom pairs.

2.1.2. Electrostatic energy

The energy of electrostatic interactions is given by Coulomb's law (Eq. 2):

$$E\mathrm{elec}_{ij} = \frac{q_i q_j}{\varepsilon D_{ij}} \quad (2)$$

where D_{ij} is the distance between charges of atoms i and j; q_i and q_j are their respective fractions of unit charges and ε the dielectric constant of the medium. ε variation is simulated as a linear function of the distance between i and j atoms (D_{ij}).

2.1.3. Potential energy of rotation of torsion angles

This potential is described by Eq. 3:

$$E\mathrm{tor} = U_{ij}/2(1 + \cos\Phi_{ij}) \quad (3)$$

where U_{ij} is the energy barrier in the eclipsed conformation during the rotation.

2.1.4. Hydrophobic/hydrophilic interactions

Free energy of solvation can be calculated assuming that, in a condensed system, each atom is completely surrounded by close neighbours. Various authors [18,19] have proposed that the free energy of transfer of a molecule, defined as the solvation free energy, is a linear function of the solvent accessible surface of its atomic constituents. Based upon this concept, we developed an empirical equation (Eq. 4) to describe free energy of solvation between atoms i and j [20]:

$$E\mathrm{tr}_{ij} = \delta_{ij}(|(E_{\mathrm{tr}i}f_{ij}| + |E_{\mathrm{tr}j}f_{ji}|)\exp((r_i + r_j - d_{ij})/2r_{\mathrm{sol}}) \quad (4)$$

where δ_{ij} is -1 when atoms i and j are either both hydrophobic or both hydrophilic and +1 otherwise. $E_{\mathrm{tr}i}$ and $E_{\mathrm{tr}j}$ are the atomic free energies of transfer from a hydrophobic to a hydrophilic phase, r_i and r_j are the radii of atoms i and j, respectively, d_{ij} is the distance between atomic centres, and r_{sol} the radius of a solvent molecule. f_{ij} is the fraction of atom i covered by atom j and is between 0 and 1 [21].

Use of this corrective term in the energy field allows to take implicitly water molecules into account and thus to obtain a better structure, as demon-

strated for small molecules [22] and for the folding of small soluble proteins [21,23].

2.2. IMPALA procedure

2.2.1. Description of the interface

The medium is described as continuous. The interface function, C, has already been described in a previous work [16]. For bilayers, C varies only along the z-axis, normal to the bilayer surface and has its origin ($z=0$) at the centre of the membrane (Eq. 5):

$$C_{(z)} = \frac{1}{1 + e^{a(|z|-z_0)}} \quad (5)$$

with z_0 and α are determined so that:

$$C_{(z>18, z<-18)} \approx 1$$

$$C_{(-13.5<z<13.5)} \approx 0$$

Those parameters were previously tested for peptide insertion in bilayer [16].

2.2.2. lipid/nisin interaction

The lipid/nisin interaction is estimated by an empirical equation described below [16]. The estimator contains two terms (Eq. 6).

$$\text{Estimator} = E_{\text{int}} + E_{\text{lip}} \quad (6)$$

The first one, E_{int}, (Eq. 7) simulates the hydrophobic effect and depends upon the accessible surface of nisin, S_i, the hydrophobicity, $E_{\text{tr}i}$, and the position, z_i, of each atom i:

$$E_{\text{int}} = -\sum_{i=1}^{N} S_{(i)} E_{\text{tr}(i)} C_{(zi)} \quad (7)$$

This term constraints medium-accessible hydrophobic atoms to get into the membrane, whereas medium-accessible hydrophilic atoms will not.

The second term, E_{lip}, accounts for the solvophobic effect in the lipid phase (Eq. 8):

$$E_{\text{lip}} = a_{\text{lip}} \sum_{i=1}^{N} S_i C_{(zi)} \quad (8)$$

where a_{lip} is an empirical constant. This term tends to minimise the interactions between nisin and the lipids if they are not as favourable as lipid/lipid interactions are.

2.2.3. Selection between NMR structures

The NMR file of nisin in a micelle-like environment has 15 different structures (Table 1). Among them, the structure displaying the best interaction with the membrane interface was determined by running a Monte Carlo (MC) procedure of 10^4 steps at 310 K. The starting position was calculated using the TAMMO procedure [16]. This algorithm calculates the position of molecules at a single planar water/lipid interface taking into account the hydrophobic and the hydrophilic centres of the molecules calculated as described elsewhere [15].

Each of the 15 structures of nisin remains stable and three degrees of freedom were tested (two rotations and one translation along the z-axis). An extensive MC simulation of 10^5 steps leads to identical results as with a simulation of 10^4 steps, in agreement with the few parameters tested. Maximal rotations of 5° and translations of 1 Å were allowed. The structure with the best estimator was kept for further calculations.

2.2.4. Nisin/bilayer interaction

To refine the structure of nisin interacting with a bilayer, a second Monte Carlo was run to look for the position and torsion angles of the pre-selected structure (see above). In this simulation, Van der Waals, torsion and electrostatic energies were taken into account. Not all dihedral angles were allowed to move. Indeed, due to the presence of many rings in nisin, several torsion angles of the backbone and of the cycling lateral chains are heavily constrained [3]. We assumed that those angles are almost identical in micelle-associated and in bilayer-associated nisin. The simulation was divided into three phases: two of 10^5 steps, each with rotations $<5°$ and translations <1 Å. In the first one E_{lip}, E_{int} and temperature are multiplied by 50. In the second one, those terms are multiplied by 5. In the last phase, the same number of steps are run, but rotations are $<0.5°$ and translations are <0.1 Å. In that last phase, E_{lip}, E_{int} and temperature are multiplied by 1.

2.3. Matching of nisin with different phospholipids by the Hypermatrix procedure

The influence of lipid composition was analysed by the Hypermatrix method. This procedure is derived from that used to surround drugs with lipids [15] and was successfully used to study the interaction between different molecules and lipids [24–27]. In the Hypermatrix procedure, the lipid/water interface is taken into account by linearly varying the dielectric constant ε between 3 (above the interface) and 30 (below the interface).

The position and orientation of nisin with respect to lipids are those defined by the IMPALA procedure (see above). The initial position and orientation of lipids molecules are defined using the TAMMO procedure [15].

The position of nisin is constant and parallel to the y-axis while the first lipid molecule translates towards the nisin molecule along the x-axis by l steps of 0.05 nm. It rotates by steps of 30° around its z'-axis and around the x-axis: l is the number of positions tested along the x-axis, m is the number of rotations around nisin and n is the number of rotations around the lipid itself. For each set of l, m and n values, the energy of interaction between nisin and lipid is calculated as the sum of Van der Waals, electrostatic and hydrophobic terms. Then, for each set of values l, m and n, the lipid molecule moves by step of 0.05 nm along the z'-axis perpendicular to the interface and the angle of z'-axis bends $\pm 5°$ with respect to the z-axis. The energy values together with the coordinates of all assemblies are stored in a matrix and classified according to decreasing values. The most stable matching is used to decide of the position of the first lipid. The position of the second lipid is then defined as the next most energetically favourable orientation stored in the hypermatrix, taking sterical and energetic constraints due to the presence of the first lipid molecule into account. To further minimise the energy of the tri-complex, the position of both lipid molecules is alternatively modified according to the energy classification of the hypermatrix. For the next lipid molecule, the same process is repeated, but the positions of all surrounding molecules are modified alternatively in order to find the lowest energy state. In these calculations, the energy of interaction between all lipids is minimised. The process ends when nisin is completely surrounded with lipids.

The mean area occupied by one lipid in the complex is estimated by projection on the x–y plane using a grid of 1 Å square.

2.4. Molecular hydrophobicity potential (MHP) calculations

Hydrophobicity of a molecule is usually defined by a single parameter measuring its partition coefficient between water and octanol. For analysis of complex interactions between lipids and proteins, the concept of helical hydrophobic moment was developed by Eisenberg et al. [28]. However, this concept does not enable to visualise the hydrophobic/hydrophilic environment of a polypeptide.

The calculation of the MHP [29] is based upon a concept introduced for small molecules [30] and extended to plot the 3D envelope of the hydrophobicity isopotential surfaces around a peptide. We assumed that the hydrophobicity potential of an atom decreases exponentially with distance according to Eq. 9:

$$\mathrm{MHP} = \sum_{i=1}^{N} E_{\mathrm{tr}i} \mathrm{e}^{(r_i - d_i)} \tag{9}$$

where $E_{\mathrm{tr}i}$ is the transfer energy of atom i, r_i and d_i are the radius of atom i and the distance between atom i and a point M, respectively. All points M, corresponding to isopotential values are joined to draw the isopotential hydrophobic and hydrophilic surfaces. Transfer energies for individual atoms were calculated from the molecular transfer energies compiled by Tanford [31] and are listed elsewhere [29].

A modification of the program was developed to visualise the electronic isopotential surfaces [32].

3. Results and discussion

3.1. Orientation of nisin towards the lipid/water interface

Among the 15 available NMR structures, the conformation with the best interaction with the mem-

brane was first selected by running the IMPALA procedure described in Section 2. All structures end mostly parallel to the lipid interface. Nisin-216 has the minimal IMPALA estimator value (Table 1) and was therefore selected.

Five independent optimisations of nisin-216 gave similar results (Fig. 2). The root mean square deviations (RMSD) of calculated structures with respect to the original structure ranged from 2.0 to 3.8 Å. The RMS between all calculated structures varied from 1.1 to 2.6 Å. Prior to the refinement, nisin-216 was globally adsorbed at the interface (Fig. 2, red ribbon). The most relevant change is the adsorption of the C-terminal extremity initially in bulk water. The N-terminal moiety (1–19) is inserted in the hydrophobic core of the bilayer and none of its lateral chains is in contact with the water phase. Conversely, residues of the C-terminal moiety (i.e. residues 20–34) Asn^{20}, Lys^{22}, β-methyllanthionine$^{23-26}$, Asn^{27}, His^{31}, dehydroalanine^{33} and Lys^{34} are outside of the membrane whereas Met^{21}, Ala^{24}, β-methyllanthionine$^{25-28}$, Ser^{29}, Ile^{30} and Val^{32} are near the interface. This indicates that both sides of nisin have distinct hydrophobic properties and suggests different structural roles. The difference between the N and C domains appears clearly when MHP are calculated around nisin-216 (Fig. 3). Calculations of MHP previously allowed to differentiate between types of peptides interacting with lipids, by visualising the different distribution and magnitude of the hydrophobic domains along their axis [29]. In Fig. 3, the C-terminal moiety is amphipathic with a segregation of hydrophobic and hydrophilic sides, in agreement with the interfacial position. The N-terminal part is more obviously hydrophobic. The loop (Asn^{20}, Met^{21}, Lys^{22}) separating both domains forms a hydrophilic pocket (arrow in Fig. 3).

The results of nisin orientations are in agreement with NMR studies of nisin in DPC micelles using spin-labelled fatty acids. This study indicated that nisin is inserted mostly parallel to the micelle interface [4].

Nisin binding and insertion measurements [33] further support our model. They showed that the binding of a negatively charged mutant ($Val^{32} \rightarrow Glu$) of nisin to membrane was low, whereas the surface pressure (i.e. the membrane insertion) remained high as compared to the wild type. Therefore, the authors suggested that the N-terminal part of nisin is involved in insertion within lipids. Furthermore, when a His-tag was added to the C-terminal end of the peptide, no modification on the lipid insertion was observed [37], while the addition of few residues in the N-terminal moiety drastically reduces the insertion in lipids [10].

When nisin is mutated at different locations (residues 1, 17, or 32 [34] or Ile^{30} [35]) with tryptophan, it was shown that the Trp residues are near the interface, in a non-aqueous environment. The 1 W mutant exhibited the largest shift in tryptophan fluorescence spectrum, indicating a deeper penetration in the lipid phase. From fluorescence depth experiments using spin-labelled lipids and the 1, 17 or 32 Trp-mutated nisin, it was concluded that the peptide is tilted towards the membrane interface with the N-terminus more deeply inserted as compared to the C-terminus [34], in agreement with our calculations.

Up to now, two hypotheses were put forward concerning nisin adsorption. Sahl [14] suggested that only the charged and polar side chains come in contact with the lipid polar heads while the other chains are exposed to water. Alternatively, Van den Hooven et al. [4] proposed a model where hydrophobic residues are immersed in the membrane. Our simulation clearly supports the second hypothesis since the amphipathy of the C-terminus tends to maintain this domain at the interface with the hydrophobic resi-

Table 1
Estimators of the interaction of the 15 NMR structures of nisin with a model membrane

Structure	Estimator
216	14.18
294	14.38
002	14.67
224	14.91
004	14.96
003	15.00
189	15.19
045	15.26
041	15.32
240	15.40
229	15.70
042	15.71
060	15.92
052	16.25
049	16.40

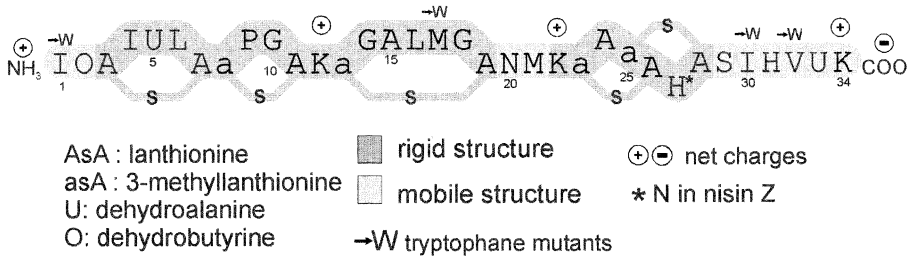

Fig. 1. Structure of nisin (see text for details).

dues buried in the hydrophobic phase of the bilayer.

It is well established that a *trans*-negative potential enhances the cell leakage induced by nisin [7,13,36]. However, the potential has no effect on the binding of nisin. Therefore, an effect on the topology of the protein associated with the membrane was suggested [33]. In view of our data, we propose that the N-terminal moiety is first pulled into the membrane because of its hydrophobicity. The presence of two positive charges in that domain (from the N-terminal extremity and Lys^{12}) that should not be compensated by the negative charges of the lipid headgroups also favour that hypothesis. Conversely, the C-terminal part, at the interface has two lysines that could interact with the lipid negative charges and could therefore be less influenced upon the occurrence of a transmembrane potential.

3.2. Influence of phophoslipids

Nisin activity is depending upon lipids [6,8,10–12,34]. It was demonstrated that nisin associates with negatively charged lipids and the presence of phosphatidylglycerol (PG) is essential for nisin binding, insertion and pore formation [34]. This could be due to electrostatic attraction between lysine residues and negatively charged polar lipid heads. This would increase the nisin binding resulting in a direct role of lipids in the nisin insertion and pore formation. Taking those observations into account, we analysed the assembly of nisin with different phospholipids by the Hypermatrix procedure described in Section 2. This method allows a molecule to be surrounded with lipids, taking into account the presence of the lipid/water interface. It was used to define the mode of association between lipids and various molecules, such as gramicidin [26], alkanols [25], aminoglycosides [27] or apolipoproteins [24].

Different types of phospholipids were used: phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS), plus phosphatidic acid and cardiolipin (CL). The acyl chains are two palmitic chains (C16), except for POPC (palmitoyloleylphosphatidylcholine) and DOPC (dioleylphosphatidylcholine).

Fig. 4A and B show that nisin binds to the lipid surface, as expected from the first calculations with IMPALA, but is more embedded in lipids as compared to the nisin structure from Fig. 2, especially for DPPG. This difference could be due to specific atomic interactions between nisin and lipids that are not taken into account in the IMPALA procedure. The effect observed for the nisin/DPPG association is in agreement with experiments using spin-labelled lipids and Trp mutated nisin, showing that nisin is more deeply inserted in lipids when the content of PG increases [34]. It is worth noting that the theoretical area occupied by nisin in PG monolayer is 850 $Å^2$, close to the area (925 $Å^2$) experimentally determined in the same lipid (Breukink, unpublished results).

Table 2 gives the different parameters measured on the calculated nisin/lipid aggregates: the number of lipids around the nisin molecule, the energy of interaction of the entire aggregate (lipids+peptide), of a small aggregate (a nisin and one molecule of lipid), and the area occupied by one molecule of lipid in the assembly. Those parameters are also given for pure lipids assembled by the same procedure (columns 5 and 7).

The number of lipids (12–19) is close to the number of lipids theoretically covering one nisin molecule, equal to 14 [33,38].

The energy of interaction between lipid molecules themselves is significantly lowered when nisin is inserted into lipids, whatever the phospholipid is. Fur-

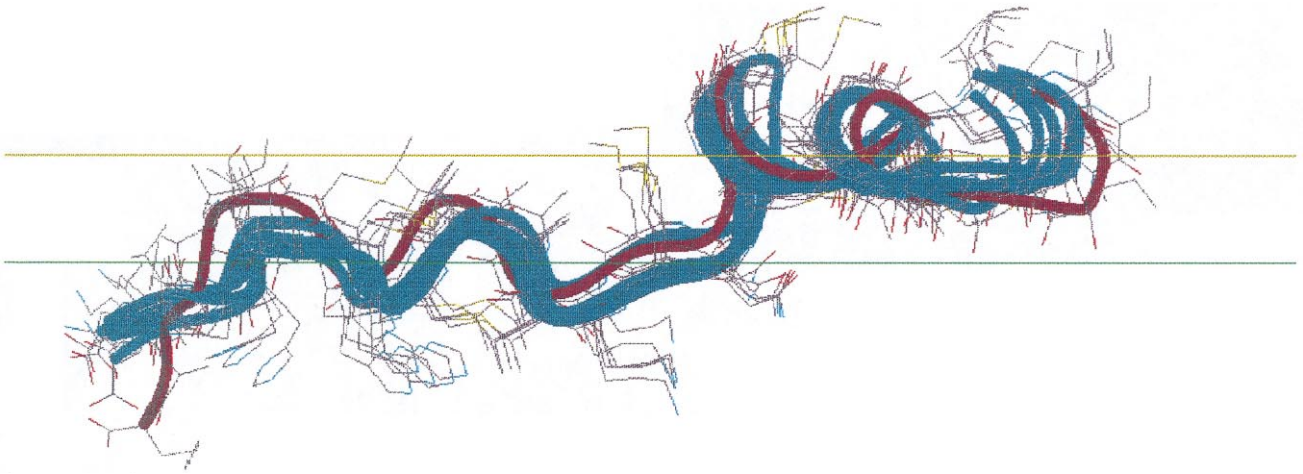

Fig. 2. Monte Carlo calculation of the interaction of nisin with a model membrane. Yellow line, $z = 13.5$ Å; green line, $z = 18$ Å. The environment is completely hydrophilic below the green line and completely hydrophobic above the yellow line. Red ribbon, calculated conformation of nisin without internal structure change; blue ribbons, five calculated structures of nisin with torsion angle modifications allowed during simulations.

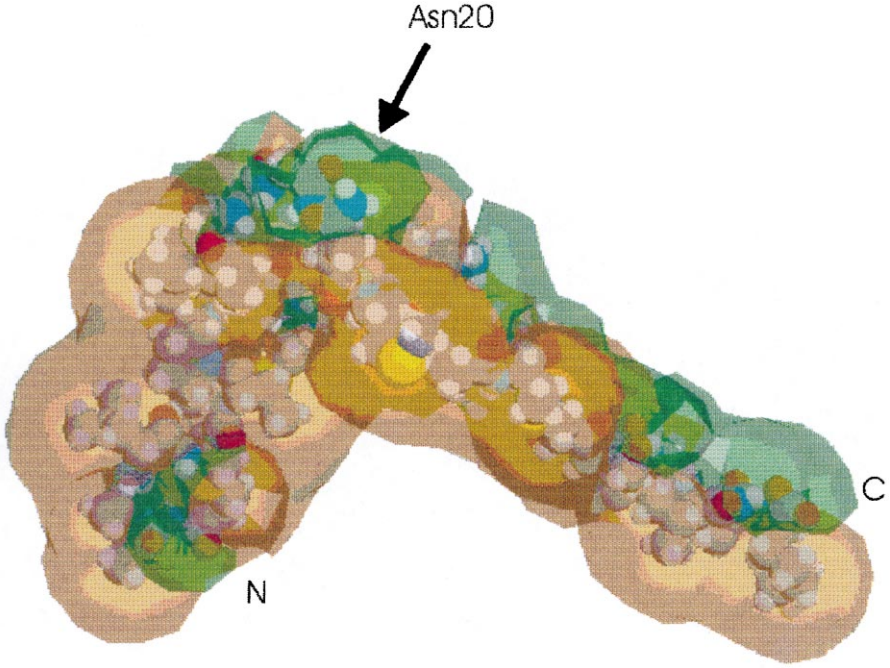

Fig. 3. Molecular hydrophobicity potential (MHP) around nisin which is represented in CPK. The nisin molecule is rotated by 90° as compared to Fig. 2. Green envelopes represent hydrophilic potentials and orange envelopes, the hydrophobic ones. The arrow underlines Asn^{20} belonging to the loop separating the ABC rings from the DE rings.

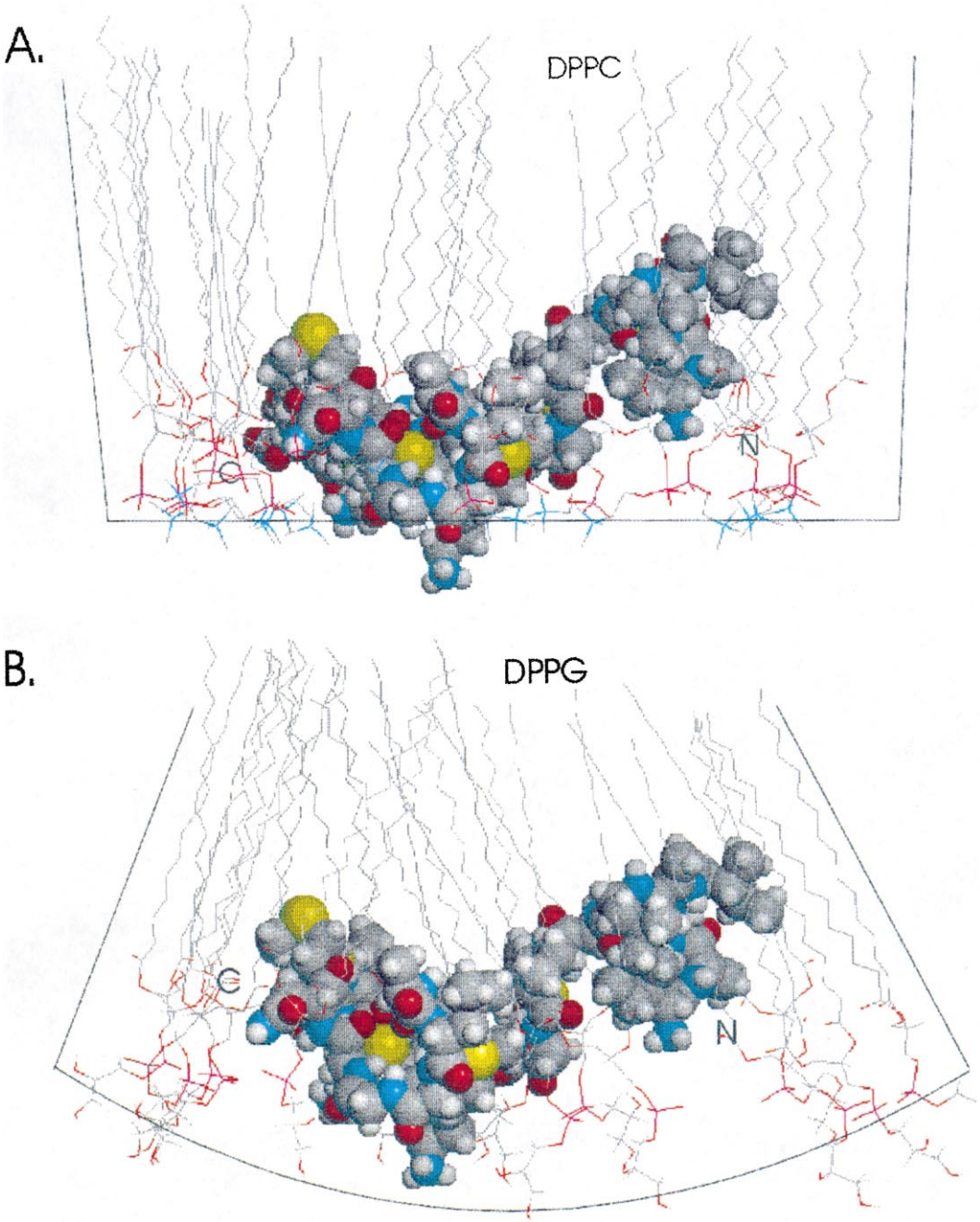

Fig. 4. (A) Assembly of nisin with DPPC. The lines around the assembly represent the cylindrical arrangement of the lipids around nisin that is characteristic of the lipid organisation within a bilayer (no interface curvature). (B) Assembly of nisin with DPPG. The lines around the assembly represent the cone-shaped arrangement (interface curvature) of the lipids around nisin that is characteristic of a micelle organisation.

thermore, the area occupied by one molecule of lipid is increased from 20 to 35% with most lipids when nisin is present and goes up to 50% for PG. The effect of nisin on the PG matrix is clearly shown in Fig. 4B: nisin tilts the PG acyl chains, resulting in a curved lipid interface that corresponds to micelle formation (cone-shaped organisation). This effect is neither observed for the other lipids, as shown for dipalmitoylphosphatidylcholine (DPPC) on Fig. 4A, nor for a pure PG monolayer (data not shown).

Table 2
Characteristics of the assembly between nisin and different lipids[a]

Lipid	Nb lipids (1)	Energy (2) (kcal/mol)	(2)/(1)	Energy/lipid (pure lipids; kcal/mol)	Area (Å^2)	Area/lipid (Å^2)
Phosphatidic acid	17	−107	−6	−23	66	54
CL	12	−163	−14	−34	200	169
PI	18	−108	−6	−25	78	61
PS	17	−97	−6	−18	82	62
PE	19	−56	−3	−21	68	50
PG	16	−132	−8	−33	91	58
DPPC	18	−90	−5	−37	65	57
POPC	17	−78	−5	−21	69	54
DOPC	14	−78	−6	−18	103	75

[a]Assemblies are characterised in terms of number of lipids around the nisin molecule (column 2); the total energy of the assembly (column 3) and the energy per lipid molecule (column 4) that is compared to the energy per lipid in a pure lipid monolayer (column 5). The area occupied by one lipid molecule in the assembly (column 6) is also compared to the same area in a pure lipid monolayer (column 7). The energies are given in kcal/mol and are determined with a variation of maximum 10%.

This curvature could have different consequences. First, since the area of one PG molecule increases, the lipid matrix is less compact and could accommodate a higher number of nisin molecules adsorbed and/or inserted in the membrane. Second, nisin, by inducing an interface curvature, could initialise the formation of a pore. In that aspect, our data are in agreement with the 'wedge model' proposed in the literature [4,8], in which nisin first aggregates on the membrane surface and then adopts a transmembrane configuration without changing its orientation towards the phospholipid headgroups. In this situation, nisin is positioned as on a micelle-like surface and thus should be forming the pore. Thus the presence of PG could facilitate the formation of pore and could explain why membranes leak in the absence of a *trans*-negative potential, but in the presence of increasing amounts of PG [33]. The other anionic phospholipids (PS, phosphatidic acid and CL) shown to have an effect on nisin activity, do not induce such an interface curvature (data not shown) but they could increase nisin binding due to electrostatic attractions.

The translocation of the C-terminal domain of nisin from one side of the bilayer to the other [37] and the pore formation suggest an, at least transient, transbilayer configuration for nisin. However, the stable orientation of nisin at the interface determined here and by fluorescence experiments [34] is parallel to the lipid interface. The wedge model, supported by the effect of nisin on PG, could reconcile all data.

In conclusion, our calculations support that: (1) nisin is globally positioned at the lipid/water interface of membranes, with its N-terminal domain inserted more deeply in the membrane in agreement with fluorescence studies [34]; (2) this N-terminal moiety is responsible for the insertion of nisin in lipids, in agreement with the experimental results obtained with the Glu^{32}–nisin Z mutant [33] and the His-tag mutant [37]; and (3) the presence of PG in the membrane could have a direct action on pore formation by inducing a deeper insertion of nisin in the lipids and concomitantly, a layer interface curvature. This curvature is in agreement with the wedge model proposed in the literature [4,8].

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