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Membrane Lipids Determine the Antibiotic Activity of the Lantibiotic Gallidermin

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Abstract Lantibiotics, a group of lanthionine-containing peptides, display their antibiotic activity by combining different killing mechanisms within one molecule. The prototype lantibiotic nisin was shown to possess both inhibition of peptidoglycan synthesis and pore formation in bacterial membranes by interacting with lipid II. Gallidermin, which shares the lipid II binding motif with nisin but has a shorter molecular length, differed from nisin in pore formation in several strains of bacteria. To simulate the mode of action, we applied cyclic voltammetry and quartz crystal microbalance to correlate pore formation with lipid II binding kinetics of gallidermin in model membranes. The inability of gallidermin to form pores in DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine) (C18/1) and DPoPC (1,2-dipalmitoleoyl-sn-glycero-3-phosphocholine) (C16/1) membranes was related to the membrane thickness. For a better simulation of bacterial membrane characteristics, two different phospholipids with branched fatty acids were incorporated into the DPoPC matrix. Phospholipids with methyl branches in the middle of the fatty acid chains favored a lipid II–independent DPoPC permeabilization by gallidermin, while long-branched phospholipids in which the branch is placed near the hydrophilic region induced an identical lipid II–dependent pore formation of gallidermin and nisin. Obviously, the branched lipids altered lipid packing and reduced the membrane thickness. Therefore, the duality of gallidermin activity (pore formation and inhibition of the cell wall synthesis) seems to be balanced by the bacterial membrane composition.

Keywords Biosensor · Cyclic voltammetry (CV) · Gallidermin · Lipid II · Model membrane · Quartz crystal microbalance (QCM)

Introduction

Gallidermin is a peptide antibiotic produced by *Staphylococcus gallinarum* that belongs to the lantibiotics. Its activity against *Propionibacterium acnes* makes gallidermin attractive as a drug candidate for the treatment of acne (Cotter et al. 2005).

Lantibiotics are characterized by the presence of unusual amino acids forming thioether ring systems, e.g., lanthionine (Jung 1991). Nisin, the best-characterized type A lantibiotic (Moll et al. 1999), combines different killing mechanisms when interacting with gram-positive bacteria: disturbance of the membrane barrier function by pore formation and the inhibition of bacterial cell wall biosynthesis. The pore formation process has been analyzed by several model membrane systems (Ruhr and Sahl 1985; Driessen et al. 1995; Breukink et al. 2000). Nisin uses the cell wall precursor lipid II as a membrane target and thus

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inhibits the lipid II carrier function in cell wall synthesis. Subsequently, nisin recruits lipid II as a pore constituent (Brötz et al. 1998; van Heusden et al. 2002; Breukink et al. 2003; Hasper et al. 2004; Hsu et al. 2004).

We recently compared the antibiotic activity of nisin and gallidermin against different strains of bacteria (Bonelli et al. 2006). Compared with nisin, gallidermin displays nearly identical rings A and B as binding moieties (Hsu et al. 2004), but it only consists of 22 amino acids (Kellner et al. 1988). The truncated C terminus results in a molecular length of approximately 30 Å (Fig. 1). Pore formation and inhibition of cell wall biosynthesis differed from strain to strain (Bonelli et al. 2006).

The present report focuses on the molecular mechanisms of gallidermin activity and its influencing factors thereof by biosensor-based model membrane studies. Quartz crystal microbalance (QCM) and cyclic voltammetry (CV) enable the simultaneous detection of the peptide binding (a hint for the role of membrane targets) and a disturbed barrier function.

By means of QCM, we recently obtained kinetic constants for nisin binding to membranes in dependence on the presence of lipid II (Christ et al. 2007). CV can detect permeabilization of model membranes and therefore allows a correlation of pore-forming characteristics of gallidermin

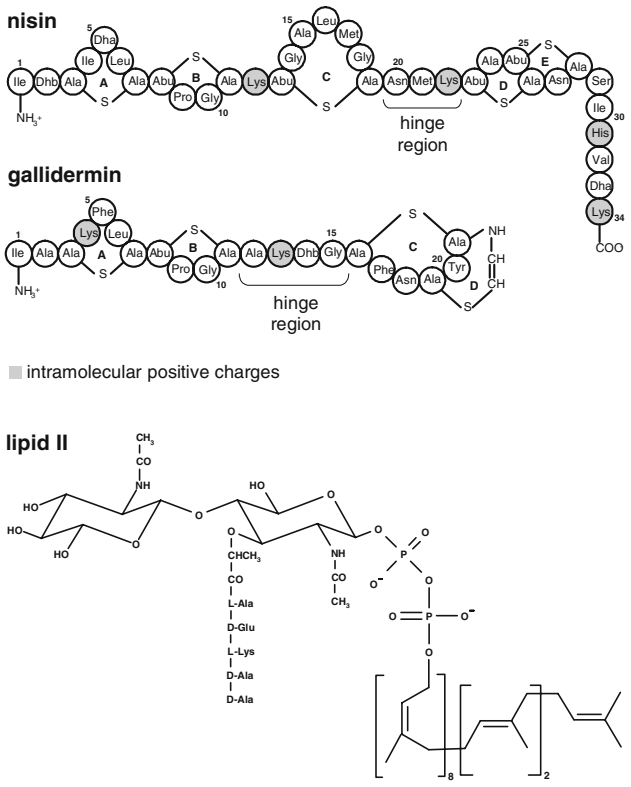

Fig. 1 Structures of the lantibiotics nisin and gallidermin and the cell wall precursor lipid II

with the content of membrane constituents. The membranes of gallidermin-sensitive strains contain certain fractions of branched fatty acids. Anteiso branched fatty acids represent up to 50% of membrane lipids in *Staphylococcus, Streptococcus,* and *Lactococcus* (MacLeod and Brown 1963; Johnsson et al. 1995; Lambert et al. 1998). We drew on this fact and introduced phospholipids with branched fatty acids into model membranes for the first time to correlate their membrane effects with lantibiotic activities.

Binding studies to lipid II confirm the potency of gallidermin to interfere with the cell wall biosynthesis. CV emphasizes the role of membrane thickness and branched lipids for the pore-forming ability of gallidermin. We conclude that the presence of branched fatty acids in bacterial membranes is an important factor for increased gallidermin activity. Gallidermin can use both of these mechanisms for killing bacteria, but membrane constituents ultimately determine whether they operate in a given indicator strain.

Materials and Methods

Chemicals

1-Hexadecanethiol ($C_{16}H_{33}SH$), potassium chloride (KCl), hydrogen peroxide (H_2O_2), sulfuric acid (H_2SO_4), and potassium ferricyanide ($K_3[Fe(CN)_6]$) were purchased from Fluka (Neu-Ulm, Germany). The phospholipids 1,2-dimyristoleoyl-sn-glycero-3-phosphocholine (DMoPC), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), and 1,2-dipalmitoleoyl-sn-glycero-3-phosphocholine (DPoPC) were purchased from Avanti Polar Lipids Inc. (Alabaster, AL). 1-O-hexadecyl-2-[12-methy]-palmitoyl-sn-glycero-3-phosphocholine (brPL1) and 1-O-hexadecyl-2-[α-hexyl]-palmitoyl-sn-glycero-3-phosphocholine (brPL2) were prepared from 1-O-hexadecyl-sn-glycero-3-phosphocholine ("lyso-PAF"; Bachem, Switzerland) and the respective fatty acid as described by Rattay et al. (1995). The syntheses of the 12-methyl-palmitic acid and the α-hexyl-palmitic acid were carried out according to Dobner et al. (1989) and Breusch and Ulusoy (1953). Sodium sulfate (Na_2SO_4) and chloroform ($CHCl_3$) were obtained from Riedel-de Häen (Seelze, Germany).

Purification of Nisin and Lipid II

Nisin and gallidermin were purified from culture supernatants of *Lactococcus lactis* NIZO22186 (Chan et al. 1996) and *Staphylococcus gallinarum* Tü 3928 (Meyer et al. 1994), respectively, by chloroform extraction as previously described (Bonelli et al. 2006). Lipid II was synthesized

with membrane preparations of *Micrococcus luteus* DSM 1790 (Brötz et al. 1997) and purified as previously described (Schneider et al. 2004).

Modification of the Quartz Crystals

The quartz crystals (SiO_2) were cleaned and prepared as previously described (Christ et al. 2005, 2007). Immobilization of the model membranes onto the sensors was performed by the Langmuir-Blodgett technique. Because comparative CV experiments on nisin pore formation provided similarity with those membranes, in which a first monolayer was self-assembled by hexadecanthiol, we continued to use the latter for the present study. The model membranes consisted of DOPC with or without 0.1 mol% lipid II, DPoPC with or without 0.1 mol% lipid II, DPoPC/20 mol% brPL1 with or without 0.1 mol% lipid II, DPoPC/20 mol% brPL2 with or without 0.1 mol% lipid II, and DMoPC with or without 0.1 mol% lipid II.

QCM Measurements

QCM measurements were performed at 25°C with a LiquiLab21 QCM (ifak e.V., Barleben, Germany), which enables the simultaneous detection of frequency and damping changes in real time (Christ et al. 2007). Kinetic binding constants were calculated from the frequency curve, according to the following:

$$\Delta f = -\frac{k_{\text{ass}}\,[A]\,f_{\max}}{k_{\text{ass}}\,[A] + k_{\text{diss}}}\left(1 - e^{-\,[k_{\text{ass}}\,[A] + k_{\text{diss}}\,(t-t_0)]}\right)$$

$$\Delta f = \Delta f_0\, e^{-\,[k_{\text{diss}}\,(t-t_0)]}$$

where k_{ass} is the association rate, k_{diss} is the dissociation rate, $[A]$ is the peptide concentration, Δf is the frequency change at point in time t, Δf_0 is the frequency change at point in time t_0, and $f_{\max}$ is the frequency change of a maximal covered quartz crystal.

The changes in damping frequency were detected within the indicated time range. For a proper evaluation, these data were related to the changes in the resonance frequency. D/f slopes represent time-independent absolute data, which reflect the viscoelasticity on the membrane surface.

CV Experiments

CV experiments were performed with a CHI-430 electrochemical QCM from CH Instruments Inc. (Austin, TX) as previously described (Christ et al. 2007). The working electrode is displayed by a gold-coated quartz crystal (quartz crystal diameter 13.7 mm, electrode diameter 5 mm). An Ag/AgCl electrode filled with 3 M aqueous KCl and a platinum wire were used as reference electrode and counter electrode, respectively. The quartz crystal was inserted at the bottom of a Teflon cell (3 ml cell volume). A total of 5 mM $K_3[Fe(CN)_6]$ was soluted in 0.1 M Na_2SO_4 (background electrolyte) and used as redoxactive substance. Potentials ranging from −0.05 to 0.6 V were applied to the working electrode with a scan rate of 10 mV s^{-1}. Substance solutions were added approximately after 35 scans; overall, 70 scans were recorded for each experiment.

All experiments were performed at 25°C. Stock solutions (300 µM) were diluted to final concentrations of 1.5 µM and 15 nM, respectively, related to the volume of the measurement cells.

Statistical Analysis

Experiments were repeated for at least three times. Data are represented as mean value ± standard deviation. Statistical significance was determined by the unpaired Student's *t*-test. A P value of ≤ 0.05 was considered statistically significant.

Results and Discussion

Simulation of Gallidermin Activity by DOPC Membranes

Gallidermin has been shown to inhibit cell wall biosynthesis similar to nisin in an in vitro assay, but exceeded the activity of nisin kinetically (Bonelli et al. 2006). It remained unclear whether this reflects differences in the kinetics of lipid II binding. Although gallidermin and nisin share the N-terminal lipid II binding motif, subtle structural differences exist and could explain different inhibitory kinetics. Besides the reduced length of gallidermin by approximately 20 Å compared with nisin, the C-terminal part of gallidermin is comparably inflexible because S-aminovinyl-D-cysteine (AviCys) as the fourth ring. Although this should be of less importance for the initial contact to lipid II, a subsequent membrane incorporation of the lantibiotic could be influenced by these peculiarities.

We applied the QCM technique to analyze the binding kinetics of gallidermin onto DOPC membranes in the absence or presence of 0.1 mol% lipid II. Kinetic binding constants, such as k_{ass}, k_{diss}, and k_{D}, were calculated from the QCM frequency curves (Table 1). The peptide rapidly associated to pure DOPC films and exceeded the association rate of nisin (k_{ass} 752 M^{-1} s^{-1}) more than fivefold. Gallidermin seems to be able for a closer contact to or a faster insertion into the membrane than nisin. This seems to

Table 1 Kinetic binding constants of gallidermin binding to DOPC with or without lipid II membranes

Membranes	k_{ass} (M^{-1} s^{-1})	k_{diss} (s^{-1})	k_D (M)
DOPC	4135 ± 276	$9.87 \times 10^{-4} \pm 4.47 \times 10^{-4}$	$2.81 \times 10^{-7} \pm 1.55 \times 10^{-7}$
DOPC/0.1 mol% lipid II	1828 ± 876	4.40×10^{-4}* $\pm 1.32 \times 10^{-4}$	$2.69 \times 10^{-7} \pm 0.84 \times 10^{-7}$

* Statistically significant for DOPC/lipid II compared with pure DOPC

cover a lipid II effect on the association rate in contrast to nisin which displayed a sixfold increased k_{ass} value in the presence of lipid II. However, considering the dissociation rate, gallidermin maintained the binding to lipid II fourfold longer than nisin ($1.77 \cdot 10^{-3}$ s^{-1}), which finally confirmed strong binding of gallidermin to DOPC/lipid II identical to nisin (k_D $2.69 \cdot 10^{-7}$ M).

The data for the absolute frequency drops are not shown here. Like nisin (Christ et al. 2007), no correlation between the absolute frequency drop and the lantibiotic activity was observed.

The QCM technique further allows conclusions on the viscoelastic properties of the membrane-bound peptides. Viscous bound masses induce higher damping frequencies than tightly bound structures. Most valuable information can be obtained by plotting damping frequencies against the mass-dependent frequency changes. The resulting slopes are independent on time and clearly reflect the surface characteristics. The ratio of damping frequencies and the mass-dependent frequency changes (D/f slope) of gallidermin binding was lower than that of nisin (0.538 ± 0.178 vs. 0.594 ± 0.214 onto pure DOPC; and 0.515 ± 0.153 vs. 1.494 ± 0.360 onto DOPC/lipid II), which confirmed a more tight association or stronger membrane insertion of gallidermin.

CV analysis was applied to focus on the pore-forming ability of gallidermin. Recent CV data confirmed increased Faraday current with respect to nisin concentrations and the presence of lipid II (Christ et al. 2007). However, gallidermin was not able to form pores or induce permeability changes in this model membrane (not shown).

Reduced Membrane Thickness Influences Gallidermin Activities

If the molecular length is the main reason for the inability of membrane permeabilization, membranes of a reduced thickness should be permeabilized by gallidermin. To test this hypothesis, we applied DPoPC (C16/1) membranes instead of DOPC (C18/1). The influence of the different phase transition temperatures (DOPC, −21°C; DPoPC, −36°C) is only marginal because both phospholipids exist in the fluid lamellar phase during the experiments at 25°C. For CV investigations, quartz sensors were coated with DPoPC with or without 0.1 mol% lipid II. However, no increase in the Faraday current was evident (not shown). Gallidermin is not able to permeabilize the DPoPC-membrane, which was independent of lipid II. Gallidermin might be too short to span through the membrane. To check whether binding characteristics could be the reason for the CV data, kinetic binding constants of gallidermin to DPoPC with or without lipid II membranes were calculated from QCM data (Table 2).

The overall binding ability of gallidermin onto DPoPC membranes was lower than that of DOPC membranes. This was mainly due to a reduced association. The effect of lipid II is evident by a marginally slower dissociation rate. Lipid II incorporation into DPoPC membranes was also probably different from DOPC, and therefore, its accessibility for gallidermin might have changed.

The D/f slopes of pure DPoPC and DPoPC/lipid II membranes (0.383 ± 0.163 and 0.325 ± 0.056, respectively) were generally lower compared with DOPC films with or without lipid II (0.518 and 0.456, respectively), which confirms a thinner membrane. After addition of gallidermin to DPoPC with or without lipid II membranes, no significant increase was observed for the D/f values (0.364 ± 0.193 and 0.387 ± 0.200, respectively).

In summary, thinner membranes neither promoted pore formation by gallidermin nor substantial lipid II binding, which could explain the inhibition of cell wall synthesis. Further membrane factors, e.g., membrane constituents, had to be considered to explain the in vivo activity of gallidermin against certain bacteria strains.

Table 2 Kinetic binding constants of gallidermin binding to DPoPC with or without lipid II membranes

Membranes	k_{ass} (M^{-1} s^{-1})	k_{diss} (s^{-1})	k_D (M)
DPoPC	1078 ± 774	$1.32 \times 10^{-3} \pm 0.31 \times 10^{-3}$	$1.80 \times 10^{-6} \pm 1.20 \times 10^{-6}$
DPoPC/0.1 mol% lipid II	772 ± 49	6.67×10^{-4}* $\pm 0.92 \times 10^{-4}$	$9.35 \times 10^{-7\dagger} \pm 0.59 \times 10^{-7}$

* Statistically significant for DPoPC/lipid II compared with pure DPoPC

† Statistically significant for gallidermin values compared with nisin data

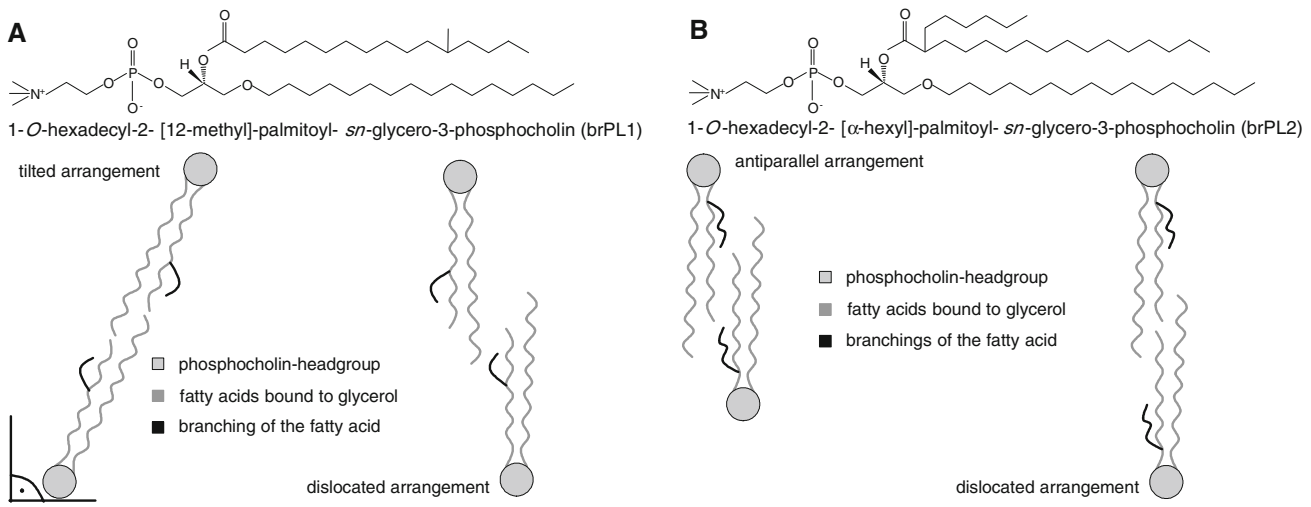

Fig. 2 Structure and possible arrangements of the branched phospholipids. **a** brPL1 and **b** brPL2

Gallidermin Needs Branched Fatty Acids for Membrane Permeabilization

Nearly all bacteria strains with sensitivity to gallidermin, such as *Staphylococcus*, *Streptococcus*, and *Lactococcus*, contain considerable fractions of branched fatty acids in their membranes (MacLeod and Brown 1963; Johnsson et al. 1995; Lambert et al. 1998). Anteiso branches by methyl groups at position 3 of the fatty acid terminus can represent up to 50% of bacterial membrane fatty acids. Therefore, it can be assumed that branched fatty acids influence the membrane structure and the possible molecular mechanisms of peptide activities.

To test this hypothesis, we incorporated two different phospholipids (brPL1 and brPL2) containing branched fatty acids (Fig. 2) into the DPoPC with or without lipid II membranes at a concentration of 20 mol%, which corresponds to the average content of branched fatty acids in bacterial membranes. They represent two extremes of branches; whereas brPL1 with a methyl branch at position 5 of the fatty acid terminus is near an anteiso branching and might induce structural disturbance in the inner membrane core, the brPL2 displays longer branching near the interfacial region.

Gallidermin was able to permeabilize pure and lipid II–containing DPoPC/brPL1 membranes (Fig. 3). The current increase depended on both the concentration of gallidermin and the presence of lipid II. In contrast, the data observed for nisin were different. Independent from lipid II, a current increase was only observed at the higher nisin concentration, which should be related to an unspecific membrane disordering. Rattay et al. (1995) proposed that phospholipids with short chain branched fatty acids are tilted and dislocated against each other compared with unbranched phospholipids, as shown in Fig. 2a. This should lead to

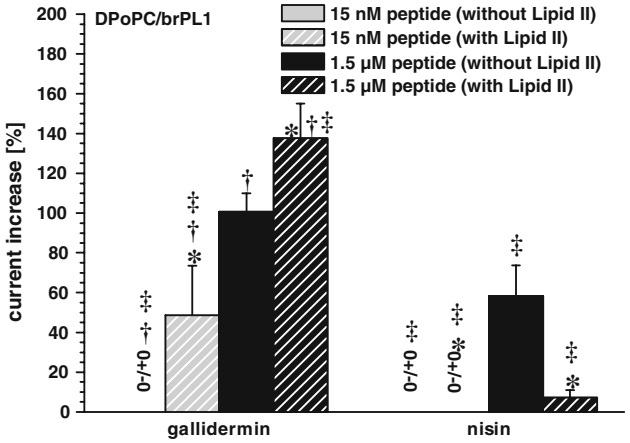

Fig. 3 CV investigations on the permeabilization of DPoPC/brPL1 with or without lipid II membranes by gallidermin and nisin. Methyl branches induced disturbances in membrane packing, and thus the membrane incorporation of gallidermin and the permeabilizing effect were increased. *Statistically significant for DPoPC/brPL1/lipid II compared with pure DPoPC/brPL1. ‡Statistically significant for values compared with DOPC data without branched phospholipids. †Statistically significant for gallidermin values compared with nisin data

reduced interactions in between the phospholipids and to local disturbances of the membrane structure. It could facilitate the incorporation of molecules and thus explain the lipid II–independent effects of gallidermin and nisin. The fact that gallidermin but not nisin induced stronger permeabilization in the presence of lipid II could indicate a stronger membrane incorporation of gallidermin molecules and an influence on lipid II in the membrane core.

Considering gallidermin binding kinetics (Table 3), the association rate of gallidermin onto DPoPC/brPL1 was 2.5-fold higher, and the dissociation was 2-fold slower compared with pure DPoPC-membranes. An improved incorporation of gallidermin into the brPL1-containing

Table 3 Kinetic binding constants of gallidermin and nisin binding to DPoPC/brPL1 with or without lipid II membranes

Membranes	k_{ass} (M^{-1} s^{-1})	k_{diss} (s^{-1})	k_D (M)
Gallidermin			
DPoPC/brPL1	2717 ± 1176	$6.70 \times 10^{-4‡} \pm 1.82 \times 10^{-4}$	$2.38 \times 10^{-7†} \pm 0.28 \times 10^{-7}$
DPoPC/brPL1/0.1 mol% lipid II	1727 ± 626	$1.50 \times 10^{-3} \pm 1.05 \times 10^{-3}$	$8.43 \times 10^{-7*} \pm 3.70 \times 10^{-7}$
Nisin			
DPoPC/brPL1	1063 ± 136	$1.33 \times 10^{-3} \pm 0.32 \times 10^{-3}$	$1.35 \times 10^{-6} \pm 0.35 \times 10^{-6}$
DPoPC/brPL1/0.1 mol% lipid II	1646 ± 1116	$2.61 \times 10^{-3} \pm 1.03 \times 10^{-3}$	$1.46 \times 10^{-6‡} \pm 0.22 \times 10^{-6}$

* Statistically significant for DPoPC/brPL1/lipid II compared with pure DPoPC/brPL1

‡ Statistically significant for values compared with DPoPC data without branched phospholipids

† Statistically significant for gallidermin values compared with nisin data

membrane seems to dominate and covers potential effects of lipid II on binding. The generally low binding ability of nisin correlates with the absence of current increase. The presence of lipid II evidently did not improve nisin binding.

The binding data could partly explain recent findings by Bonelli et al. (2006) like the exceeding activity of gallidermin over nisin in some strains of *Lactococcus lactis*. The presence of branched membrane lipids modulates the membrane properties and induces better access to lipid II for gallidermin, to more efficiently segregate it from bacterial cell wall synthesis.

To investigate whether the postulated membrane effects of brPL1 and the altered lipid II accessibility depend on the nature of lipid branches, 20 mol% of brPL2 instead of brPL1 was incorporated into DPoPC membranes. According to Rattay et al. (1995), longer branched fatty acids display a straight position in the membrane, arranged in an antiparallel (interdigitated) orientation or displaced against each other (Fig. 2b). Obviously, the long branched fatty acids are generally able to keep a tighter lipid arrangement than the short branched fatty acids and might also have a more significant influence on the overall membrane thickness.

Gallidermin and nisin showed nearly identical effects on the permeabilization of brPL2-containing DPoPC membranes (Fig. 4). The permeabilizing intensity strictly depended on the presence of lipid II and on the peptide concentration. Compared with DPoPC/brPL1 membranes, the reduced current in the absence of lipid II indicated a less disordered phospholipid arrangement. Consequently, the much higher membrane permeabilization by gallidermin and nisin in the presence of lipid II can be considered as a lipid II–dependent pore formation. Lipid II is probably differently positioned in DPoPC/brPL2 membranes, which improves interactions with gallidermin and nisin without causing lipid II–independent membrane disturbances.

An improved accessibility of lipid II should be reflected in the binding kinetics of gallidermin and nisin, which are

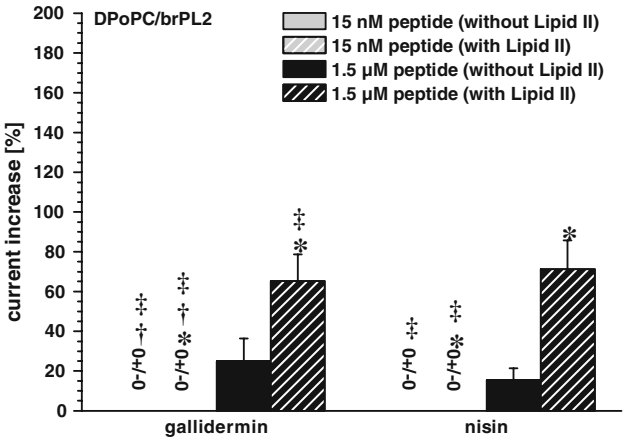

Fig. 4 CV investigations on the permeabilization of DPoPC/brPL2 with or without lipid II membranes by gallidermin and nisin. Gallidermin and nisin displayed identical pore-forming ability dependent on lipid II. *Statistically significant for DPoPC/brPL2/lipid II compared with pure DPoPC/brPL2. ‡Statistically significant for values compared with DOPC data without branched phospholipids. †Statistically significant for gallidermin values compared with nisin data

summarized in Table 4. Nisin exhibit a strong binding onto DPoPC/brPL2 membranes. A lipid II effect is evident and correlated with the pore-forming capacity. In contrast, the overall binding ability of gallidermin to DPoPC/brPL2 membranes was slightly reduced compared with DPoPC/brPL1 membranes. Possibly, the effect of gallidermin membrane insertion on binding data is diminished but not ceased. The targeting of lipid II is not reflected in the binding kinetics.

To clarify, whether brPL2 had an impact on the membrane order and the lipid II accessibility, or whether brPL2 also reduces the overall membrane thickness, gallidermin effects were further investigated on a C14/1 (DMoPC) membrane. In contrast to DPoPC membranes, gallidermin was able to permeabilize DMoPC membranes already in the absence of lipid II, as indicated by a current increase of 47.53 ± 1.20% at a 1.5 µM peptide concentration. This

Table 4 Kinetic binding constants of gallidermin and nisin binding to DPoPC/brPL2 with or without lipid II membranes

Membranes	k_{ass} (M^{-1} s^{-1})	k_{diss} (s^{-1})	k_D (M)
Gallidermin			
DPoPC/brPL2	$1246^{\dagger} \pm 340$	$3.77 \times 10^{-4*,\ddagger} \pm 2.35 \times 10^{-4}$	$2.94 \times 10^{-7*} \pm 1.46 \times 10^{-7}$
DPoPC/brPL2/0.1 mol% lipid II	1121 ± 455	$1.05 \times 10^{-3\dagger,\ddagger} \pm 0.17 \times 10^{-3}$	$9.06 \times 10^{-7} \pm 2.95 \times 10^{-7}$
Nisin			
DPoPC/brPL2	$3512^{\ddagger} \pm 49$	$8.47 \times 10^{-4\ddagger} \pm 2.48 \times 10^{-4}$	$2.36 \times 10^{-7} \pm 1.02 \times 10^{-7}$
DPoPC/brPL2/0.1 mol% lipid II	9041 ± 3266	$2.09 \times 10^{-3} \pm 1.48 \times 10^{-3}$	$2.16 \times 10^{-7\ddagger} \pm 0.87 \times 10^{-7}$

* Statistically significant for DPoPC/brPL2/lipid II compared with pure DPoPC/brPL2

‡ Statistically significant for values compared with DPoPC data without branched phospholipids

† Statistically significant for gallidermin values compared with nisin data

strongly supports the correlation of membrane thickness and gallidermin pore-forming ability.

In consequence, the effects of gallidermin strongly depend on the membrane characteristics, especially the membrane thickness. Phospholipids with branched fatty acids are able to influence the membrane fluidity and the lipid packing in bacterial membranes and, more importantly, to reduce the membranes thickness. One might assume that membrane order and thickness have an impact on lipid II conformation, and thus branched fatty acids indirectly influence the interaction between gallidermin and lipid II recruitment for pore formation.

Conclusion

Our study illustrates that the duality of the antibiotic activities of gallidermin is strongly influenced by the membrane matrix. In general, the smaller molecular size of gallidermin impedes membrane spanning for pore formation. However, gallidermin has a strong tendency to membrane incorporation, and thus binding of lipid II as a target is evidently not represented in the binding kinetics.

The presence of branched fatty acids in the bacterial lipids seems to be the key for gallidermin activity. Depending on the nature of the branch (length and position), those lipids can either induce a membrane lipid disordering with subsequent modified lipid II accessibility, or a change in the lipid arrangement that leads to a general reduction of the membrane thickness.

Our interpretations of the effect of membrane components on gallidermin activity open new ways for considering lantibiotics. Further studies on the membrane constituents of gallidermin-sensitive and -insensitive strains are necessary to explain individual differences in the lantibiotic activity.

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