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Interaction of Nisin with Planar Lipid Bilayers Monitored by Fluorescence Recovery After Photobleaching

C.J. Giffard[1], **S. Ladha**[2], **A.R. Mackie**[2], **D.C. Clark**[2], **D. Sanders**[1]
[1]Department of Biology, University of York, Box 373, York Y01 5YW, UK
[2]Institute of Food Research, Norwich Laboratory, Norwich Research Park, Colney, Norwich, NR4 7UA, UK

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Abstract. Nisin, a prominent member of the lantibiotic family of antimicrobial agents, has wide application as a food preservative despite poor understanding of its mode of action. Fluorescence recovery after photobleaching has been used with planar lipid bilayers as a model membrane system to examine how nisin might interact with the surface of bacterial cells. Nisin associates with planar lipid bilayers in the absence of an applied membrane potential causing an array of effects consistent with adsorption of nisin onto the membrane surface which involves inhibition of the lateral diffusion and fluorescence of the lipid probe N-(7--1,2,3-benzoxadiazol-4-yl) phosphatidylethanolamine (NBD-PE) and a reduction of the capacitance of the bilayer. Nisin adsorption is dependent on phospholipid composition. In the presence of dioleoylphosphatidylcholine (PC): cardiolipin (CL) 4:1, the rate of lateral mobility of phospholipid is reduced to 61% of the control level which decreases to a value of 46% when CL is replaced by 1-palmitoyl-2-oleoylphosphatidylserine (PS). These effects on bilayer parameters are transient, and with time the values return to near original levels. High electrical conductivity is observed on application of a voltage ramp suggesting that insertion into the membrane follows surface association. Results have been interpreted in terms of a model in which nisin initially binds to the surface of the membrane causing a modulation of bilayer properties.

Key words: Nisin — Lantibiotic — Planar lipid bilayer — FRAP — Mode of action

Present address: School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, UK

Correspondence to: C.J. Giffard

Introduction

Nisin, a 34-residue (3353 Da) post-translationally modified lantibiotic, is produced by various strains of *Lactococcus lactis* subsp *lactis.* This peptide has antimicrobial activity against a broad range of Gram-positive bacteria and spores and has therefore gained wide use in the food industry as a natural preservative (Hurst, 1981; Delves-Broughton, 1990; Molitor & Sahl, 1991). As a member of a class of bacteriocins termed the lantibiotics, the structure of nisin is defined by the unusual amino acids dehydroalanine and dehydrobutyrine (Jung, 1991). The thioether groups of these amino acids form the intrachain sulfide-bridged rings, lanthionine and β-methyllanthionine, which hinder the formation of regular secondary structure (Fig. 1). Apart from these defined rings, nisin appears to have an unstructured conformation with a central flexible hinge (van de Ven et al., 1991). Although the conformation changes in the N-terminus when nisin enters a membrane-mimicking environment (sodium dodecyl sulfate, dodecylphosphocholine micelles), this does not seem to affect the general fold of the molecule (van de Hooven et al., 1993). Nisin contains three lysines and two histidines, but no acidic residues, and therefore possesses a net positive charge.

Despite its widespread use, the mode of action of nisin at the molecular level is not clear. The majority of the evidence indicates that the primary target for nisin action is the cytoplasmic membrane. This was first suggested by Ramseier (1960) who concluded that nisin was acting as a surfactant. More recent evidence suggests that the mechanism of action involves insertion of nisin into the cytoplasmic membrane leading to loss of low M_r compounds such as ions, amino acids and ATP. This results in breakdown of the membrane potential and complete cessation of biosynthetic activity (Ruhr & Sahl, 1985; Kordel & Sahl, 1986; Abee, 1995; Sahl, Jack &

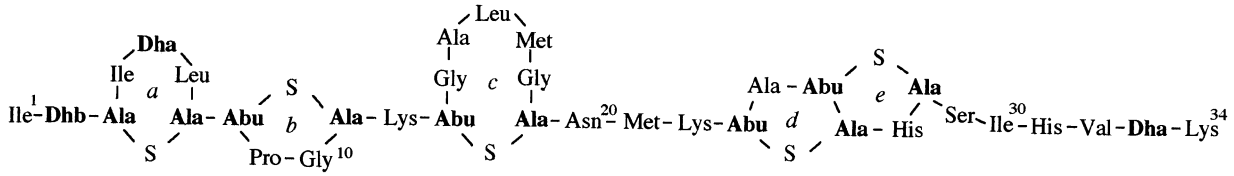

Fig. 1. Schematic representation of the structure of nisin. Unusual residues are highlighted in bold. Dha, dehydroalanine: Dhb, dehydrobutyrine: Ala-S-Ala, lanthionine; Ala-S-Abu, β-methyllanthionine.

Bierbaum, 1995). Studies with membrane vesicles and intact cells have demonstrated a requirement for an energized membrane for nisin activity (Sahl, Kordel & Benz, 1987; Sahl, 1991). This conclusion has been reinforced by studies with bacteria and liposomes which imply that the components of the proton motive force are necessary for nisin action (Gao, Abee & Konings, 1991; Bruno, Kaiser & Montville, 1992; Okereke & Montville, 1992; Garcerá et al., 1993).

Planar lipid bilayers (PLBs) comprise a powerful approach for analysis of permeability modifiers (Miller, 1983). Nisin activity in PLBs appears to be dependent on a transmembrane electrical potential with a threshold conditional on the amount of anionic phospholipid present (Sahl et al., 1987; Benz, Jung & Sahl, 1991). The use of PLBs to study nisin action has led to the hypothesis that depolarization of the membrane is due to the formation of voltage-dependent channels. It appears likely that an electrostatic interaction between the cationic groups of nisin and the negatively charged phosphate groups of phospholipid is involved in the initial stages of interaction between nisin and the membrane, as an antagonistic effect of isolated phospholipid components was observed on the biological activity of nisin (Henning, Metz & Hammes, 1986). The proposal for an electrostatic interaction is supported by the effect of nisin on liposome fluidity. The greatest reduction in fluidity, determined by anisotropy with liposomes containing anionic lipids, was consistent with an interaction of nisin with charged phospholipid head groups (Kordel, Schuller & Sahl, 1989). This electrostatic interaction has been suggested to cause a local perturbation of the phospholipid dynamics of the liposomal bilayer structure (Driessen et al., 1995).

To explore in more depth the effect of nisin on the biophysical properties of membranes, fluorescence recovery after photobleaching (FRAP) measurements were performed on ''solvent-free'' PLBs (Ladha et al., 1996), in the presence and absence of nisin. This technique enables measurement of the lateral diffusion of phospholipids within the bilayer while simultaneously gaining an estimate of the effect on membrane structure by monitoring capacitance. It has therefore been possible to observe the overall effect of nisin association with, and insertion in, the membrane on a range of bilayer parameters thereby providing alternative insights into its mechanism of action to those offered by other artificial membrane systems.

Materials and Methods

Materials

Cardiolipin (CL), dioleoylphosphatidylcholine (PC) and 1-palmitoyl-2-oleoylphosphatidylserine (PS) were purchased from Avanti Polar Lipids (Alabaster, AL). The fluorescent phospholipid analogues, N-(7-nitro-1,2,3-benzoxadiazol-4-yl) phosphatidylethanolamine (NBD-PE) and fluorescein-phosphatidylethanolamine (Fluorescein-PE), were purchased from Molecular Probes (Eugene, OR).

Nisin (50,000 U/mg) was obtained from Aplin and Barrett (Trowbridge, Wilts, UK) and was used without further purification. A 10 mg/ml stock of nisin in 0.02 M HCl was prepared fresh each day. Other chemicals used were of high purity and were purchased from Sigma (St. Louis, MO).

Planar Lipid Bilayer (PLB) Formation

Virtually ''solvent-free'' PLBs were formed according to the method of Montal & Mueller (1972), using apparatus as described by Ladha et al. (1996). A 25 μm thick Teflon film containing a small circular hole of area 200–400 μm^2 formed by electrical discharge, was clamped between the two halves of a chamber machined from Teflon. Each half cell was fitted with a silica glass window. Thus, once assembled the cell contained a compartment divided by the Teflon septum containing a single hole which could be observed through the optical windows. Prior to bilayer formation the area around the hole was precoated with 1% (v/v) hexadecane in hexane. After evaporation of hexane, the compartment was filled with filtered, unbuffered 1 M KCl (pH 5.2–5.6) to a level above the hole. Lipid, 6.5 mM in hexane, incorporating NBD-PE at 1% molar ratio, was layered onto the buffer surface in each compartment and the hexane was allowed to evaporate. The formation of a bilayer was monitored optically through a microscope, and electrically via capacitance measurements, as the level of solution in the *trans*-compartment was lowered to below the level of the hole and then raised again to original levels.

Temperature was maintained between 22–24°C. Membrane potentials were recorded *cis* with respect to *trans* which was held at ground. Thus a negative potential (indicated as −) means that the charge on the *cis*-side of the bilayer is negative. Nisin was added to the *cis* compartment. Membrane current was measured under voltage clamp conditions using a low noise operational amplifier connected to a pair of Ag/AgCl electrodes in direct contact with aqueous solutions. The amplified signal was simultaneously displayed on an oscilloscope and recorded on a chart recorder or DAT tape. The imposition of a −100 or +100 mV voltage-clamp was continuous throughout each relevant experiment, apart from when capacitance measurements were

taken in parallel with lateral diffusion and fluorescence measurements. For construction of a current-voltage (*I-V*) relationship applied voltage was ramped over the range ±140 mV, for 45 sec in each direction. The membrane RC time constant was of the order of 2 sec thus the measured *I-V* relationship approximated its steady state. Currents generated by nisin were too great to be measured when amplified at potentials over −100 mV.

Fluorescence Recovery After Photobleaching

FRAP was used to measure the lateral diffusion of the fluorescent probe NBD-PE in virtually ''solvent-free" PLBs as described by Ladha, Mackie & Clark (1994) and Ladha et al. (1996). A conventional FRAP setup based on an upright Nikon Optiphor microscope was modified to enable measurements of phospholipid mobility in PLBs. After formation of a bilayer with acceptable capacitance a laser spot, of diameter ($1/e^2$) 3.3 μm, was focused on the center of the bilayer. The spot size was small enough for the bilayer to act as a ''near infinite'' reservoir. At least five FRAP measurements were recorded prior to the addition of nisin. Nisin was added to a final concentration of 10 μM and the resulting medium was neutralized immediately by the addition of an equivalent volume of 0.02 M KOH which had been observed to maintain pH equality on either side of the membrane. Measurements were taken every 5–10 min for the duration of the experiment. FRAP data were analyzed using nonlinear least-squares fitting to an expression defining the time dependence of fluorescence recovery observed with a circular beam of Gaussian cross-sectional intensity as follows (Y guerabide, Schmidt & Yguerabide, 1982):

$$F(t) = \frac{F(0) + F(\infty)(t/\beta\tau_D)}{1 + (t/\beta\tau_D)}, \quad (1)$$

where $F(t)$ is the observed fluorescence as a function of time, $F(0)$ and $F(\infty)$ are respectively the intensities of the fluorescence immediately, and at finite time after the bleach pulse, β is the depth of bleach parameter and τ_D is the diffusion time. The lateral diffusion coefficient, D, is given by $D = \omega^2/4_{\tau D}$, where ω is the half-width at $1/e^2$ height of the laser beam at its point of focus on the membrane. The extent of recovery (%r), i.e., the percentage of mobile fluorophores in the membrane, is given by the expression:

$$\%R = \frac{F(\infty) - F(0)}{F(t < 0) - F(0)} \times 100, \quad (2)$$

where $F(t < 0)$ is the prebleach fluorescence (Wolf, 1989). Ten FRAP curves were collected for every measurement and were averaged before analysis.

Apparent Membrane Thickness Estimation

The decrease in capacitance can be used to calculate the apparent increase in thickness of the hydrophobic region of the membrane, δ_M,

$$\delta_M = \frac{\varepsilon_0 \varepsilon_r A_M}{C_M}, \quad (3)$$

where ϵ_0 is the permittivity of a vacuum (8.854×10^{-12} Fm^{-1}), A_M is the area of the membrane and C_M is the capacitance of the membrane. The relative permittivity of membrane dielectric, ϵ_r, was taken to be 2.2, which corresponds to the hydrophobic core dielectric constant and is relative to the chain length of the appropriate alkane.

Results

Nisin Causes a Reduction in the Rate of Lateral Diffusion of membrane Phospholipid

Figure 2*A* shows that the addition of nisin to the *cis*-side of a representative ''solvent-free'' PLB comprising PC:CL 4:1 molar ratio resulted in a decrease in the lateral diffusion coefficient of NBD-PE from 96×10^{-9} cm^2 sec^{-1} to 56×10^{-9} cm^2 sec^{-1} after 55 min. Equivalent data for several bilayers are summarized in the Table, where the diffusion coefficient minimum is expressed as a percentage of the value before addition of nisin. It is noteworthy that controls in the absence of nisin revealed no consistent or significant change in lateral diffusion coefficient for periods in excess of 90 min (Fig. 2*D*). The nisin-induced decrease in lateral diffusion coefficient was dependent on the lipid composition of the bilayer and on the amount of negative charge present (Table). When bilayers were composed solely of PC, the lateral diffusion coefficient of NBD-PE reached a minimum of 63% of control. The inclusion of anionic phospholipids in the bilayer led to further reductions in the diffusion coefficient. The effect of incorporating a molar ratio of 20% PS led to a minimum value of diffusion coefficient that was 46% of the control value, and this value was reduced to 32% of the control when PS was included to a final ratio of 1:1 with PC. When negative charge was supplied by CL as opposed to PS, the inhibition in diffusion rate appeared more comparable with that of PC alone. However, further experiments indicated that the inclusion of elevated amounts of CL led to an additional reduction the diffusion coefficient compared to control values (*data not shown*).

Some reduction in the diffusion coefficient was observed within 10 min of the addition of nisin probably reflecting the first contact nisin makes with the bilayer surface. A further and more substantial reduction in diffusion coefficient always followed between 35 and 60 min after nisin addition. With time, the constraints imposed by nisin on phospholipid diffusion eased and mobility approached values in the region of the initial or control levels. These data provide strong evidence that nisin, on contacting the surface of the bilayer, adsorbs and thereby hinders the lateral diffusion of phospholipid containing entrained NBD-PE.

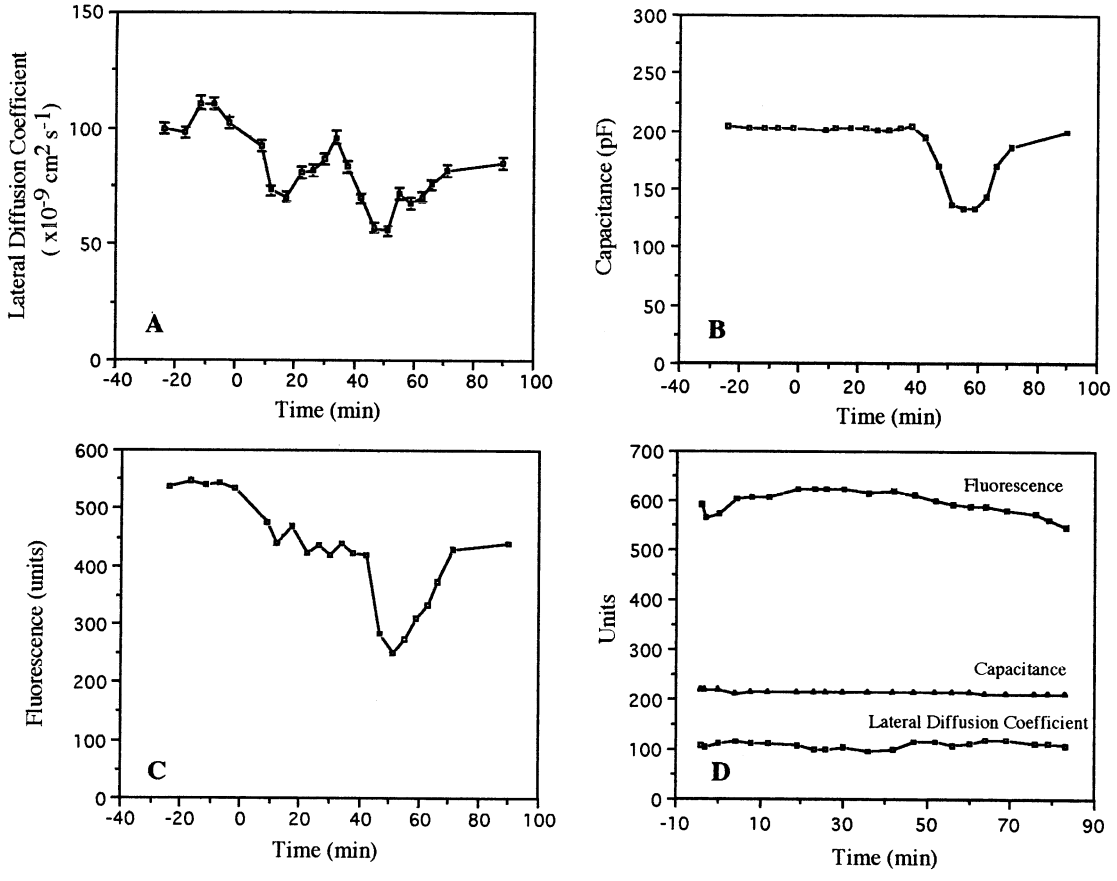

Fig. 2. Time-dependent effect of 10 μM nisin on (*A*) lateral diffusion coefficient, (*B*) capacitance, and (*C*) fluorescence for a bilayer of area 0.035 mm^2 formed from PC:CL 4:1 containing NBD-PE, 1% molar ratio, in the presence of unbuffered 1 M KCl, pH 5.2. The lateral diffusion coefficient and fluorescence were derived from an average of 10 FRAP curves with standard errors of less than 5%. Capacitance was monitored immediately after each set of FRAP readings had been collected. These recordings from a single bilayer are representative examples from a series of six independent determinations. (*D*) Representative control experiment with conditions identical to those in *A-C*, but in the absence of nisin.

Measurements were carried out with Fluorescein-PE as an alternative reporter molecule in the lipid system PC:CL 4:1. The reduction in the lateral diffusion coefficient was 65% of control and hence in good agreement with the value observed with NBD-PE (Table), hence confirming that the observed effect was not a nisin-NBD-PE specific interaction. The percentage recovery of fluorescence after each photobleaching experiment of the membrane was total (i.e., 100%) and varied by no more than an average of 4%. Therefore all phospholipids in the measuring domain were mobile and no immobile fractions were formed.

Effect of nisin on PLB Capacitance

The principal decrease in the lateral diffusion coefficient on addition of nisin coincided with a decrease in capacitance of the bilayer (200 to 133 pF for the representative PLB comprising PC:CL 4:1 in Fig. 2*B*). In six bilayers the decrease in capacitance amounted to 71% (Table). The capacitance decrease implied that nisin was affecting the hydrophobic core of the membrane. Thus, the surface-association of nisin may be followed by partial insertion into the central region of the bilayer. Again, nisin-free controls revealed no discernable change in capacitance for periods in excess of 80 min (Fig. 2*D*). Furthermore, nonspecific interactions of nisin were monitored using an unpunctured Teflon film. The film was pretreated and lipid and nisin were added as usual. Capacitance was observed to decrease by 0.9 pF over 2 hr, a change of 2% in background capacitance levels. This eliminated the possibility of nonselective interactions between nisin and Teflon. In a separate control experiment, with a bilayer formed from PC:CL 4:1, the nisin-induced decrease in capacitance was observed in the absence of NBD-PE. In this case capacitance reduced to 68% of control thereby showing that the reduction in capacitance was independent of the presence of NBD-PE.

The change in capacitance was dependent on the

Table. Time-dependent effect of 10 μM nisin on the maximum percentage change from pre-nisin values of lateral diffusion coefficient, capacitance, and fluorescence on bilayers of various compositions all containing NBD-PE at 1% molar ratio level

Lipid composition (molar ratio)	% Control level[a]			
	Diffusion coefficient	Capacitance	Fluorescence	Apparent change in membrane thickness (nm)
PC:CL 4:1	61 ± 4	71 ± 3	60 ± 0	+10.2
PC:PS 4:1	46 ± 4	77 ± 3	76 ± 1	+6.3
PC:PS 1:1	32 ± 1	87 ± 1	ND[b]	+2.2
PC	63 ± 1	114 ± 4	71 ± 3	−1.0
PC:CL 4:1 +100 mV	54 ± 2	70 ± 5	61 ± 3	+9.3
PC:CL 4:1 −100 mV	51 ± 2	LEAK	51 ± 1	

The values shown are maximal effects recorded between 35 and 60 min after addition of nisin, and are given as the mean ± SE for between 3 and 6 PLBs.
[a] Typical absolute values for controls (*cf.* Fig. 2) were of the order of 100×10^{-9} cm^2 sec^{-1} for the diffusion coefficient; 220 pF for capacitance; 600 units for fluorescence.
[b] ND = not determined.

lipid composition of the bilayer. When PC was the sole phospholipid component of the bilayer, capacitance increased rather than decreased (Table). The increase in capacitance with PC PLBs upon the addition of nisin was permanent and no tendency for a return to a lower value was observed. However, a reduction from control values was facilitated by the presence of negative charge at the bilayer surface. When the head groups are composed of CL or PS, nisin must associate in a manner which involves interaction with the central core of the bilayer. An apparent change in membrane thickness, which has been estimated from the capacitance data (see Materials and Methods) and the values given in Table, is induced by nisin. The greatest increase in apparent thickness was observed with CL, as opposed to PS, a point which might indicate that CL provides a hydrophobic environment which is more easily modified by nisin.

Figure 2 also demonstrates that capacitance, along with the lateral diffusion coefficient, exhibited a tendency to return to near-original values.

Effect of Nisin on the Fluorescence of NBD-PE

Addition of nisin to the bilayer caused a rapid (10 min) decline in NBD-PE fluorescence (Fig. 2*C*). This was followed by a larger decrease (420 to 250 units in the representative PC:CL 4:1 bilayer in Fig. 2*C*) which coincided with the decrease in capacitance and diffusion coefficient. Subsequently, fluorescence spontaneously increased with time, again corresponding with the other parameters, albeit to a lesser degree. With every FRAP measurement performed, the fluorescence of the bilayer decreased slightly. To obtain a true measurement of the decrease in fluorescence, a control was performed with a PC:CL 4:1 PLB during which no measurements were taken until the nisin-induced capacitance drop was observed. The reduction in fluorescence in this case was not due to photobleaching, and was determined to be 61% of the control (*cf.* Table). This confirmed that the nisin-dependent effect was different from the photobleaching that occurs with continued illumination of the bilayer with the laser beam. When the alternative Fluorescein-PE probe was used the measured suppression of fluorescence was 65% of control, thus verifying that the effect of nisin on fluorescence was probe independent.

As with the capacitance and diffusion coefficient, the decrease in fluorescence, when compared to the control, varied with the amount and type of negative charge present. The reduction in fluorescence to 60% of the control in the presence of CL was greater than the nisin-dependent reduction to 76% of control observed when PS provided the negative charge (Table).

Effect of Nisin in the Presence of Transmembrane Potential

Bilayers containing low levels of CL were inherently more stable than those incorporating PS. Therefore the lipid composition PC:CL 4:1 was selected for a comparison of the effects on nisin action of applying transmembrane potentials of +100 or −100 mV. The nisin-induced reduction in the lateral diffusion coefficient of NBD-PE was enhanced in the presence of a potential, but the effect was polarity independent (Table). This enhancement may have been due to electrically induced stress caused by field strength effects of the transbilayer potential. The impact on capacitance in the presence of a

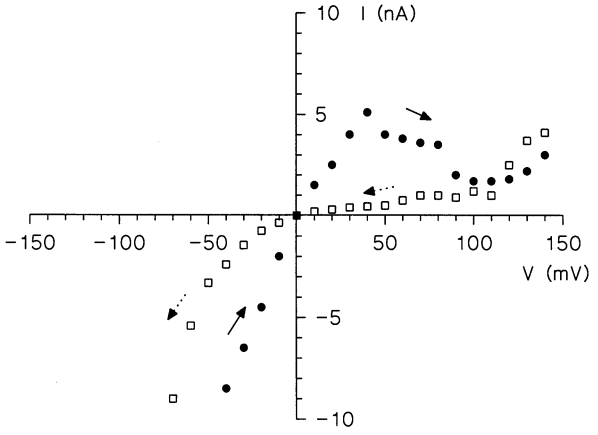

Fig. 3. Current-voltage plot of a bilayer, area 0.035 mm^2 formed from DOPC:CL 4:1 containing NBD-PE at 1% molar ratio. Nisin was added to the *cis*-side of the bilayer at 10 μM final concentration. A voltage ramp (±140 mV) was applied after completion of FRAP measurements and current flow across the membrane was recorded. Saturation of the amplifier was reached at potentials more negative than −70 mV. The aqueous phase was unbuffered 1 M KCl, pH 5.3. This is a typical result from 3 replicates. (→, ●, −140 to +140 mV; ←, □, +140 to −140 mV)

membrane potential was more substantial. When the applied potential was *cis*-negative, nisin altered the stability of the bilayer such that it became leaky to ion flow. This effect on capacitance suggests that negative potentials facilitate insertion of nisin into the membrane, thereby causing leakage which did not occur when the potential at the surface of the bilayer was zero or positive (Table). Fluorescence was also affected as emissions were reduced in presence of an applied negative potential.

Conductivity

Macroscopic measurements of bilayer conductivity were made within 2 min of completing FRAP measurements once parameter values had returned to near-original levels (time = 90 min, Fig. 2). A voltage ramp from zero mV was imposed on the bilayer in positive and negative directions and the current flow was measured. Figure 3 shows that the *I-V* relationship was strongly rectifying and exhibited greater membrane conductance when the potential clamped across the membrane was *cis*-negative. Furthermore, the *I-V* relationship exhibited distinct hysteresis, with larger currents being recorded at positive-going voltages. These results indicate that time- and voltage-dependent events play a critical role in the appearance of nisin-induced membrane conductance.

Discussion

Nisin-Membrane Surface Association and Insertion

The approaches used in the present work yield significant insights into the structure-function relationships of nisin through the combined measurement of nisin effects on PLB conductivity and capacitance. Simultaneous FRAP measurements on the PLBs have enabled nisin effects on the lateral diffusion and fluorescence emission of a fluorescent lipid probe to be deduced. Inspection of combined data, shown in Figs. 2 and 3, reveals a complex sequence of events indicated by changes in fluorescence intensity and lateral diffusion and precede alterations in capacitance, and subsequently membrane conductivity.

The fluorescence intensity and lateral diffusion data show two distinct phases of behavior following addition of nisin. Firstly, a relatively rapid decrease in the fluorescence intensity and lateral diffusion of NBD-PE occurs following addition of nisin to the *cis*-side of the bilayer which is not accompanied by a change in capacitance. The reduction in fluorescence intensity does not recover with time but rather stabilizes approximately 10 min after addition of nisin at a new intermediate level. Such a change in fluorescence is consistent with the transfer of the NBD-PE moiety into a more hydrophilic environment, possibly caused by the proximity of the positive charges present on the nisin molecule resulting in electrostatic interactions with the anionic head groups of phospholipid at the bilayer interface. The surface charge of the membrane will be active over a short distance (Debye length of 3 Å, in the conditions of these experiments) and will affect the homogeneous distribution of surrounding ions such that positively charged ions partially compensate for the negative charge density of phospholipids at the PLB surface. However, nisin molecules that diffuse close enough to the negative charges of the bilayer surface would be drawn closer to the membrane surface, perhaps forming intermolecular nisin-linkages concomitantly. The persistence of the decrease in fluorescence intensity suggests that a constant number of NBD-PE molecules remain in close association with the adsorbed molecule.

A reduction in lateral diffusion coefficient of NBD-PE accompanies the decrease in fluorescence intensity. However, in contrast, the reduction in diffusion coefficient is transient and this parameter recovers almost to pre-addition levels some 30 min after the addition of nisin. This observation is important as it suggests that with time lateral diffusion of a large majority of NBD-PE molecules is no longer affected by the nisin associated with the PLB. Thus, the physical barriers to lateral diffusion imposed by the adsorbed nisin disappear. We are reduced to speculate as to why this may be. One plausible explanation could involve a time-dependent aggregation of adsorbed nisin. If nisin aggregates assemble through random collisional processes, this would reduce the surface area of the PLB coated by nisin and remove the barriers to NBD-PE lateral diffusion, thus forming a vital critical step in pore assembly within the membrane.

If the randomly distributed adsorbed nisin monomers remain associated with their complement of lipid at the PLB surface, this would lead to a maintained reduction in fluorescence intensity. As nisin monomers aggregate, or assemble, nonbound NBD-PE could perhaps diffuse more freely in the PLB. Driessen et al. (1995) demonstrated that nisin electrostatically interacts with anionic charge if present on the bilayer surface and suggested that nisin may then become bound as a partially neutralized complex on the lipid surface, which could comprise an intermediate in pore formation.

The second phase in the association process occurs over 35 to 60 min after the addition of nisin. At this stage, fluorescence intensity, capacitance and lateral diffusion all decrease substantially. This is consistent with a significant reordering of the lipid in the PLB. The large reduction in fluorescence intensity reflects the transfer of NBD moieties to a more polar environment and the increase in capacitance is consistent with a substantial thickening of the hydrophobic core of the PLB. Such a thickening process could result from the insertion of nisin aggregates into the membrane and the subsequent perturbation to the ordering of the lipid acyl chain that this involves. Indeed nisin is capable of altering the phase transition temperature on interaction with liposomes composed of dimyristoylphosphatidylcholine (DMPC): dimyristoylphosphatidylserine (DMPS) 4:1 (Kordel et al., 1989) and a decrease in NBD-PE fluorescence has been shown to occur when phospholipids undergo phase segregation (Hong et al., 1988). The interaction of nisin with negatively charged phospholipids, in particular CL, at the bilayer surface seems to encourage penetration of the hydrophobic region.

It is possible that nisin partially inserts into the membrane while maintaining a residual surface association. This notion is supported by recent studies on a nisin variant in which a tryptophan has been substituted by site-directed mutagenesis for isoleucine at position 30 (Martin et al., 1996). Tryptophan fluorescence quenching by spin-labeled lipids verified that position 30 inserted deeper into the bilayer in the presence of CL, as opposed to PE, and comparison with similar studies on other lantibiotics in which fluorescence quenching was assayed in the N-terminal domain, and the known flexibility in the hinge region of residues 20 to 21, leads to the conclusion that a portion of the nisin molecule may remain surface-associated even after membrane insertion of the C-terminus.

Interestingly, the parameters observed in the present study are transient and once the conductivity signal increases there is a relaxation of the capacitance signal, fluorescence intensity and self-diffusion of the lipid. We conclude that disordering of the lipid acyl chains in the PLB is associated with insertion of the nisin into the membrane and once pores are formed within the membrane the environment of the lipid is restored.

Nisin-induced Membrane Conductivity

The initial interaction of nisin occurs in the absence of a membrane potential. A conducting configuration most likely requires aggregation of nisin monomers and their insertion into the membrane, though the involvement of a membrane potential in these processes is unclear. Application of a voltage ramp to nisin-associated bilayers results in a rapid increase in conductivity with greater current flow at negative potentials. A membrane potential has been shown to enhance the effect of nisin on liposomal interactions (Driessen et al., 1995). Large molecules were not released from liposomes, thus a mode of action of nisin involving membrane solubilization or surfactant activity can be eliminated (Garcerá et al., 1993). A large *cis*-negative conductivity was observed here and has not been reported previously. Unstable openings that fluctuated over a wide range of conductances have been observed in the presence of a *cis*-positive transbilayer potential of sufficient magnitude when negative phospholipid charge was supplied by PS (Sahl et al., 1987; Benz et al., 1991). The increased solvent content of the black lipid bilayer system used by Sahl et al. (1987), compared to the ''solvent-free'' PLBs used here, could affect the structure of the peptide aggregate in the hydrophobic section of the bilayer and perhaps influence its response to applied voltage (Montal & Mueller, 1972). Additionally, the presence of CL, a major component of Gram-positive bacterial membranes, in the artificial membrane may indicate involvement of this lipid in the opposing polarity dependence we observed here.

The model we advance for nisin-membrane interaction is largely consistent with that recently proposed by Driessen et al. (1995). Association of nisin with the membrane phospholipid head groups appears to be followed by aggregation on the membrane surface and the formation of multimers which may partially insert into the hydrophobic core-forming structures that are capable of inducing conductivity in response to applied transmembrane potentials.

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References

Abee, T. 1995. Pore-forming bacteriocins of Gram-positive bacteria and self-protection mechanisms of producer organisms. *FEMS Microbiol. Lett.* **129:**1–10

Benz, R., Jung, G., Sahl, H.-G. 1991. Mechanism of channel-formation by lantibiotics in black lipid membranes. *In:* Nisin and Novel Lan-

tibiotics. G. Jung, H.-G. Sahl, editors, pp. 359–372. ESCOM, Leiden.

Bruno, M.E., Kaiser, A., Montville, T.J. 1992. Depletion of proton motive force by nisin in *Listeria monocytogenes* cells. *Appl. Environ. Microbiol.* **58:**2255–2259

Delves-Broughton, J. 1990. Nisin and its uses as a food preservative. *Food Technol.* **44:**100–117

Driessen, J.M., van den Hooven, H.W., Kuiper, W., van de Kapm, M., Sahl, H.-G., Konings, R.N.H., Konings, W.N. 1995. Mechanistic studies of lantibiotic-induced permeabilization of phospholipid vesicles. *Biochem.* **34:**1606–1614

Gao, F.H., Abee, T. Konings W.N. 1991. Mechanism of action of the peptide antibiotic nisin in liposomes and cytochrome c oxidase-containing proteoliposomes. *Appl. Environ. Microbiol.* **57:**3914–3923

Garcerá, M.J.G., Elferink, M.G.L., Driessen, A.J.M., Konings, W.N. 1993. In vitro pore-forming activity of the lantibiotic nisin. Role of protonmotive force and lipid composition. *Eur. J. Biochem.* **212:**417–422

Henning, S., Metz, R., Hammes, W.P. 1986. Studies on the mode of action of nisin. *Int. J. Food Micro.* **3:**121–134

Hong, K., Baldwin, P.A., Allen, T.M., Papahadjopoulos, D. 1988. Fluorometric detection of the bilayer-to-hexagonal phase transition in liposomes. *Biochem.* **27:**3947–3955

Hurst, A. 1981. Nisin. *Advances in Applied Microbiology* **27:**85–123

Jung, G. 1991. Lantibiotics-Ribosomally synthesised biologically active polypeptides containing sulfide bridges and α,β-didehydro-amino acids. *Angew. Chem. Int. Ed. Engl.* **30:**1051–1068

Kordel, M., Sahl, H.-G. 1986. Susceptibility of bacterial, eukaryotic and artificial membranes to the disruptive action of the cationic peptides Pep5 and nisin. *FEMS Microbiol. Lett.* **34:**139–144

Kordel, M., Schuller, F., Sahl, H.-G. 1989. Interaction of the pore-forming peptide antibiotics Pep5, nisin and subtilin with non-energized liposomes. *FEBS Lett.* **244:**99–102

Ladha, S., Mackie, A.R., Clark, D.C. 1994. Cheek cell membrane fluidity measured by fluorescence recovery after photobleaching and steady-state fluorescence anisotropy. *J. Membrane Biol.* **142:**223–228

Ladha, S., Mackie, A.R., Harvey, L.J., Clark, D.C., Lea, E.J.A., Brullemans, M., Duclohier, H. 1996. Lateral diffusion in planar lipid bilayers. A FRAP investigation of its modulation by lipid composition, cholesterol or alamethicin content and divalent cations. *Biophys J.* (*in press*).

Martin, I., Ruysschaert, J.-M., Sanders, D., Giffard, C.J. 1996. Interaction of the lantibiotic nisin with membranes revealed by fluorescence quenching of an engineered tryptophan. *Eur. J. Biochem.* (*in press*)

Miller, C. 1983. Integral membrane channels: Studies in model membranes. *Physiological Reviews* **63:**1209–1242

Molitor, E., Sahl, H.-G. 1991. Applications of nisin: a literature survey. *In:* Nisin and Novel Lantibiotics. G. Jung, H.-G. Sahl, editors, pp. 435–439. ESCOM, Leiden

Montal, M., Mueller, P. 1972. Formation of bimolecular membranes from lipid monolayers and a study of their electrical properties. *Proc. Natl. Acad. Sci. USA* **69:**3561–3566

Okereke, A., Montville, T.J. 1992. Nisin dissipates the proton motive force of the obligate anaerobe *Clostridium sporogenes* PA 3679. *Appl. Environ. Microbiol.* **58:**24673–2467

Ramseier, H.R. 1960. Die wirkung von nisin auf *Clostridium butyricum* prazm. *Archiv. fur Mikrobiologie* **37:**57–94

Ruhr, E., Sahl, H.-G. 1985. Mode of action of the peptide antibiotics nisin and influence on the membrane potential of whole cells and on cytoplasmic and artificial membrane vesicles. *Antimicrob. Agents Chemother.* **27:**841–845

Sahl, H.-G. 1991. Pore formation in bacterial membranes by cationic lantibiotics. *In:* Nisin and Novel Lantibiotics. G. Jung, H.-G. Sahl, editors. pp. 347–385. ESCOM, Leiden

Sahl, H.-G., Kordel, M., Benz, R. 1987. Voltage-dependent depolarization of bacterial membranes and artificial lipid bilayers by the peptide antibiotic nisin. *Arch. Microbiol.* **149:**120–124

Sahl, H.-G., Jack, R.W., Bierbaum, G. 1995. Lantibiotics: biosynthesis and biological activities of peptides with unique post-translational modifications. *Eur. J. Biochem.* **230:**827–853

van de Ven, F.J.M., van den Hooven, H., Konings, R.N.H., Hilberts, C.W. 1991. NMR studies of lantibiotics. The structure of nisin in aqueous solution. *Eur. J. Biochem.* **202:**1181–1188

van den Hooven, H.W., Fologlari, F., Rollema, H.S., Konings, R.N.H., Hilbers, C.W., van de Ven, F.J.M. 1993. NMR and circular dichroism studies of the lantibiotic nisin in nonaqueous environments. *FEBS Lett.* **319:**189–194.

Wolf, D.E. 1989. Designing, building and using a fluorescence recovery after photobleaching instrument. *Method Cell Biol* **30:**271–306

Yguerabide, J., Schmidt, J.A., Yguerabide, E.E. 1982. Lateral mobility in membranes as detected by fluorescence recovery after photobleaching. *Biophys. J.* **39:**69–75