Interaction of the antimicrobial peptides caerin 1.1 and aurein 1.2 with intact bacteria by ^{2}H solid-state NMR

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Abstract

Nuclear magnetic resonance (NMR) is commonly used to probe the effect of antimicrobial agents on bacterial membranes using model membrane systems. Ideally, considering the complexity of membranes, the interaction of molecules with membranes should be studied *in vivo*. The interactions of two antimicrobial peptides (AMPs) with intact *Escherichia coli* and *Bacillus subtilis* were investigated using deuterium solid-state NMR. Specifically, we studied caerin 1.1 and aurein 1.2 isolated from the skin of Australian tree frogs. The minimal inhibitory concentration value for *E. coli* and *B. subtilis* was about 100 μg/mL and 30 μg/mL, respectively, for both peptides. A protocol to deuterate the membrane phospholipids of non-mutated *B. subtilis* was established using deuterated palmitic acid. ^{2}H NMR spectra combined with spectral moment analysis support the interaction of the two AMPs with the hydrophobic core of the bacterial membranes. The presence of peptides decreased the order of the lipid acyl chains for both *E. coli* and *B. subtilis*, but at higher peptide concentrations for the Gram(+) bacteria. This may be explained by the presence of other cell wall components, such as the negatively-charged teichoic and lipoteichoic acids in the peptidoglycan, which would interact with the AMPs and decrease their actual concentration on the membrane surface. The mechanism of action of the AMPs thus depends on their local concentration as well as the membrane environment. The differences between the AMPs interaction with *E. coli* and *B. subtilis* reveal the importance of studying intact bacteria.

Keywords

Gram-positive and Gram-negative bacteria, *Escherichia coli*, *Bacillus subtilis*, in-cell NMR, membrane interactions, action mechanism

Highlights

- Interaction of antimicrobial peptides with bacteria was studied *in vivo* by ^{2}H NMR
- Deuteration protocol for *Bacillus subtilis* membranes was established
- Higher concentration of peptides is required to perturb membrane of *B. subtilis*
- Perturbation of bacterial membranes depends on local peptide concentration
- Action mechanism of antimicrobial peptides depends on cell wall composition

Abbreviations

AMP = antimicrobial peptides

C15:0 = pentadecanoic acid

C15:1 = cis-10-pentadecenoic acid

C16:0 = palmitic acid

C17:1 = cis-10-heptadecenoic acid

C18:3 = γ-linolenic acid

d_{31}-PA = deuterated palmitic acid

DMPC = dimyristoyl-phosphatidylcholine

DMPG = dimyristoyl-phosphatidylglycerol

DPC = dodecyl phosphocholine

LPS = lipopolysaccharides

LTA = lipoteichoic acid

NMR = Nuclear Magnetic Resonance

MIC = minimum inhibitory concentration

MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

PG = phosphatidylglycerol

PE = phosphatidylethanolamine

TA = teichoic acid

Introduction

Excessive use of antibiotics, estimated of about 100,000 tons annually, has led to the emergence of pathogen resistance phenomenon [1]. This poses risks to hospitals, with 70% of infections in the USA in 2012 caused by bacteria resistant to at least one common antibiotic [2], and now dominates the hospital scene [3]. Bacteria have the ability to quickly develop multidrug resistance, which continues to emerge due to the low production of new antibiotics by pharmaceutical companies [4]. Therefore, development of new antimicrobial molecules with novel mechanisms of action is very important, and antimicrobial peptides (AMPs) are promising candidates to fight against infectious diseases [5]. These AMPs are of natural source and have been discovered in several organisms such as insects and plants, as well as prokaryotic and eukaryotic cells [6-9].

We focus in this study on caerin 1.1 (GLLSVLGSVAKHVLPHVVPVIAEHL-NH_2) and aurein 1.2 (GLFDIIKKIAESF-NH_2), two natural AMPs isolated from the skin secretions of the *Litoria* genus of Australian tree frog [10, 11]. These two cationic peptides, which exist as random coil in aqueous solution but have α-helical secondary structure in membrane mimetic environments [12, 13], are active against a wide range of Gram-negative and Gram-positive bacteria, especially the latter [11]. The mechanism by which AMPs affect cell viability depends on the peptide sequence [14]. Previous studies of caerin 1.1 and aurein 1.2 suggest that they act via a transmembrane [15] and carpet [16] mechanism, respectively. These studies have exploited phospholipid bilayers, generally composed of DMPC (dimyristoyl-phosphatidylcholine) or a lipid mixture of DMPC/DMPG (dimyristoyl-phosphatidylglycerol), but ideally peptide-membrane interactions should be studied *in vivo*. The structure, dynamics and orientation of peptides depend on the membrane composition [17] and the envelope of bacteria, which is a very complex system [18]. Gram-negative bacteria have an inner phospholipid membrane, a thin peptidoglycan layer, and an outer membrane mainly composed of lipopolysaccharides (LPS) [19, 20]. The LPS are absent in Gram-positive bacteria which do not have an outer membrane, but instead have a thick peptidoglycan layer characterized by the presence of teichoic (TAs) and lipoteichoic acids (LTAs) [21]. On the other hand, *B. subtilis* differ from *E. coli* in phospholipid composition. The membrane of *E. coli* is composed of about 75% phosphatidylethanolamine (PE), 20%

phosphatidylglycerol (PG) and 5% cardiolipin (CL) and three fatty acid (C16, C17, C18) chain lengths [22]. The membrane of *B. subtilis* contains 60% PG, 34% PE and 6% CL and mainly C15, C16 and C17 acyl chains [23, 24].

Our recently published studies of *E. coli* bacteria show an efficient incorporation of perdeuterated exogenous palmitic acid in the membrane phospholipids [25] with a good signal-to-noise ratio for ^{2}H solid-state-NMR spectra. Moreover, this study supports the idea of the importance of using bacteria instead of model membranes because interestingly the effect of fullerenol nanoparticles on *E. coli* bacteria differs to what has been shown for DPPC/DPPG bilayers [26].

In the present work we have expanded our analysis to investigate the effect of caerin 1.1 and aurein 1.2 on intact *E. coli* (Gram-) and *B. subtilis* (Gram+) bacteria. Given that this is the first reported ^{2}H-NMR study of *B. subtilis*, we first characterized the phospholipid profile of the deuterated *B. subtilis* membranes compared to non-labeled and their temperature behavior. Then, the ^{2}H NMR spectral moments were exploited to show the effect of the AMPs, caerin 1.1 and aurein 1.2, on the molecular order and dynamics of the lipid acyl chains.

Materials and methods

Materials

Caerin 1.1 and aurein 1.2 were synthesized by GenScript Corporation (Piscataway Township, NJ, USA) with >95% purity. Dodecyl phosphocholine (DPC) was obtained from Avanti Polar Lipids (Alabaster, AL). Oleic acid (OA), deuterated palmitic acid (d_{31}-PA), deuterium-depleted water and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma Aldrich (Oakville, Canada). *E. coli* BL21 and *B. subtilis* PY79 strains were kindly provided by Prof. S. Jenna (UQAM) and É. Déziel (Institut Armand-Frappier), respectively.

Minimum inhibitory concentration

The activity of caerin 1.1 and aurein 1.2 against *E. coli* and *B. subtilis* was evaluated by measuring of the minimum inhibitory concentrations (MIC) using the serial dilution technique

[27] in a cell suspension containing 10^6 cells/mL, with the peptide solution at different dilutions. Bacteria were incubated at 37°C and the absorbance at 600 nm monitored every 30 minutes over a 24 h period.

Sample preparation

BL21 strain *E. coli* was grown as described [25] with 0.19 mM d_{31}-PA (incorporated into DPC micelles) added in the growth medium with 0.19 mM OA to preserve the fluidity of the membrane [28]. *B. subtilis* (PY79) was also grown with 0.19 mM d_{31}-PA incorporated in the growth medium without oleic acid, but the mid-log growth phase is at an A_{600} of 0.5 compared to 0.4 for *E. coli* [25]. For bacteria exposed to peptide, the pellet was resuspended in 10 mL of Luria broth for 10 min until a homogeneous solution was obtained. Then, the appropriate amount of peptide was added and incubated for 5 min. The samples were centrifuged (1500 g for 10 min at room temperature), and the pellets were then used immediately for ^{2}H NMR studies.

Lipid analysis and viability of Bacillus subtilis

B. subtilis samples were analysed by gas phase chromatography combined with mass spectrometry to determine the fatty acid composition of their membranes. Briefly, lipid extraction was performed in duplicate on approximately 10 mg of lyophilised labeled *B. subtilis* samples using dichloromethane/methanol (2:1 v/v) and 0.88% KCl solution in a Potter glass homogenizer as described in [29]. Polar lipids where then recovered via separation on a silica gel column, with MeOH as eluent. After evaporation at 40°C, transesterification was carried out in 2 mL of H_2SO_4 (2% in methanol) and 0.8 mL of toluene for 10 min at 100°C, then analysed by gas chromatography as described in [25].

B. subtilis samples were also analysed by MTT reduction assays [30] to determine the cellular viability. The cell suspension was diluted in fresh LB medium to an A_{600} of 0.1. A reaction mixture of 20 µL of 5 mg/ml MTT solution and 200 µL of cell suspension was incubated at 37°C for 20 min. After incubation, the formazan crystals were pelleted and dissolved with 1 ml of dimethylsulfoxide at room temperature. After a 15 minute stabilisation at room temperature, the optical density was measured at 550 nm.

NMR experiments and moment analysis

In the case of multi-experiments at different temperatures, ^{2}H NMR analyses were recorded on a solid-state Bruker Avance III-HD wide bore 400 MHz spectrometer (Milton, Canada) operating at a frequency of 61 MHz for ^{2}H nuclei. Others experiments were recorded at 37°C on a 14.1 T hybrid solution/solid-state Bruker Avance III HD (Milton, Canada) operating at a frequency of 92.1 MHz. Static spectra were obtained using the solid-echo pulse sequence [31] with a pulse length of 5 μs, a pulse separation of 60 μs, and a 500 ms recycle delay. Data were collected using 100 k points with a dwell time of 0.5 μs, and a line broadening of 50 Hz was applied. Spectra acquired at 10 kHz MAS frequencies were obtained using a modified Hahn-echo pulse sequence [32, 33], with an initial 45° pulse. A total of 8 k points were collected with a dwell time of 0.5 µs and a repetition delay of 500 ms.

A specific quadrupolar splitting is difficult to determine in ^{2}H solid-state-NMR spectra with a distribution of quadrupolar splittings. Thus, to quantify the intensity distribution of the spectra as a function of quadrupolar splitting, spectral moments M_n [34, 35] were calculated from the symmetric ^{2}H NMR powder patterns using the following equation

$$M_n = \frac{\int_0^\infty \omega^n f(\omega) d\omega}{\int_0^\infty f(\omega) d\omega} \qquad (1)$$

where ω is the frequency with respect to the nuclear Larmor angular frequency ω_0 and f (ω) is the line shape. As shown by Maricq and Waugh [13] and recently reported by Warnet [28], to analyse magic angle spinning (MAS) spectra, spectral moments are more simply extracted according to:

$$M_n = \omega_r^n \frac{\sum_{N=0}^{\infty} N^n A_N}{\sum_{N=0}^{\infty} A_N} \qquad (2)$$

where ω_r is the spinning rate ($\omega_r = 2\pi\nu_r$, where ν_r is expressed in Hz) and A_N is the area of the Nth sideband.

Spectral moments M_1 and M_2 from static spectra were calculated using the MatNMR software [36] and from MAS spectra using MestReNova (Mestrelab Research, Santiago de Compostela, Spain). These spectral moments reveal the changes in gel and fluid phases and were used to calculate the relative mean square width of the distribution of quadrupolar splittings (Δ_2), which informs on membrane heterogeneity:

$$\Delta_2 = \frac{M_2}{1.35M_1^2} - 1 \qquad (3)$$

The values from the static or MAS spectral analysis did not differ significantly and, therefore, MAS spectra, which require a much shorter acquisition time are discussed here and the static spectra are shown in Supplementary Information.

Results and discussion

Characterization of ^{2}H-labelled B. subtilis

Prior to studying *B. subtilis* bacteria by NMR, the efficiency of ^{2}H labelling and its effect on the lipid profile were assessed. The labelling was carried out by growing the bacteria in the presence of d_{31}-PA. Contrary to *E. coli*, OA was not used since oleic acid chains are not naturally found in the lipid composition of *B. subtilis*. As shown in Table S1, deuteration levels were close to 60% for saturated (C16:0) lipids. Note that the labelled palmitic acyl chains are incorporated exclusively in the phospholipids and without modification, i.e., 60% of PG, PE and CL palmitic chains are d_{31}-labelled. As detailed by Tardy-Laporte *et al*. [25], the LPS are not deuterated in *E. coli*. According to Koch *et al*. (addref), LTAs typically account for 6-10 mol% in Gram-positive bacteria, and the biosynthesis of LTAs utilizes a fraction of the bacteria's PG pool (Percy *et al*. 2014 addref). Since PG lipids can have various chain lengths, and assuming that 60% of PG lipids with C16:0 acyl chains are deuterated, the total amount of ^{2}H-labelled LTAs is likely to be negligible and its signal too weak to contribute to the ^{2}H NMR spectra. Interestingly, the functionally important ratio of total saturated-to-unsaturated lipids was only slightly altered from 58:42 in wild type to 64:36 in labelled *B. subtilis* (Table S1 and Figure 1). This minor change, therefore, is not expected to significantly alter the membrane fluidity.

We further assessed whether the labelling protocol, the washing procedure or the NMR experiments affected cell viability. As shown in Figure 1 and Table S2, the changes due to labelling or washing are within the uncertainty of the viability measurement. After 15 hours in the NMR spectrometer, during which the growth medium is unchanged and scant due to rotor packing, 40% of the bacteria survived. This percentage is the same as observed with *E. coli* [25] and compatible with *in vivo* studies in particular using the short MAS experiments which further preserve cell viability [28].

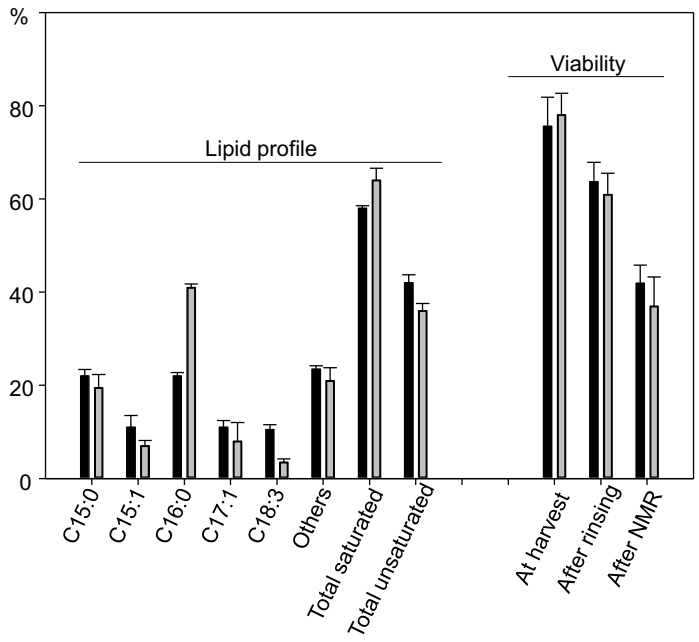

Figure 1. Effect of deuterium labelling protocol on lipid profile (n =2) and cell viability (n = 3) of *B. subtilis*. Black: unlabelled, and gray: ^{2}H-labelled bacteria.

The physical state of the membrane lipids was assessed by following the evolution of spectral moments as a function of temperature. It was previously demonstrated that the ^{2}H solid-state NMR spectra of *E. coli* have "characteristic features" representative of different phases [25] [34]. The ^{2}H spectra obtained at 15°C, 30°C and 52°C are shown in Figure S1. At 15°C, there is a broad spectrum showing the presence of gel/crystalline phase. At 30°C, the appearance of shoulders around ± 20 kHz and with a quadrupolar splitting of 30 kHz at the edges of the plateau indicates the presence of the fluid/liquid phase. This plateau slightly decreases at 52°C with an increase in proportion of the isotropic peak.

The ^{2}H MAS NMR (Figure 2) spectrum at 12°C indicates a higher intensity of spinning sideband manifold, which is characteristic of the gel phase. When the temperature was raised to 52°C, an increase in the intensity of isotropic peak together with a decrease in the spinning sideband manifold was observed. This is due to motional averaging [34] and supported by the spectral moment analysis (Tables 1 and S3). The decrease in both M_1 and M_2 observed with the rise of temperature is indicative of reduced acyl chain order and spectral distribution, respectively. The

value of Δ_2, which informs on the membrane heterogeneity, was low at 12°C and 37°C but increased at 32°C due to the coexistence of gel and liquid-crystalline phases at this temperature. In summary, the results show that the effectiveness of the deuteration protocol was sufficient to enable NMR studies and has only a minor effect on the lipid profile and resulting physical state of *B. subtilis* membranes.

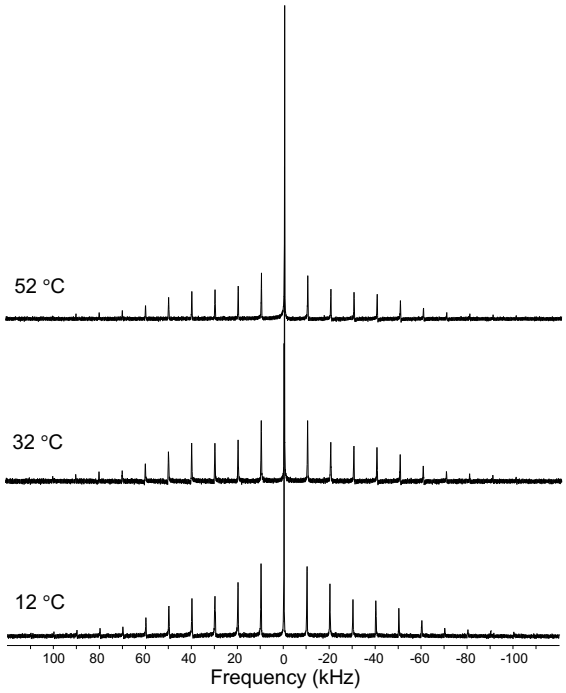

Figure 2. MAS (10 kHz) ^{2}H NMR spectra of intact *B. subtilis* acquired at 12°C, 32°C and 52°C with 8 k scans. The equilibration time in these experiments was 15 min.

Table 1: Spectral moment analysis of the MAS (10 kHz) ^{2}H NMR spectra of labeled *B. subtilis* at different temperatures[a].

T (°C)	M_1 (10^4 s^{-1})	M_2 (10^9 s^{-2})	Δ_2
12	13.7	27.3	0.07
32	11.3	24.5	0.42
37	9.2	14.7	0.12
52	8.2	10.1	0.48

[a]Error bars are estimated as less than +/-5%.

Effect of caerin 1.1 and aurein 1.2 on E. coli and B. subtilis

First, we determined the MIC of caerin 1.1 and aurein 1.2 for *E. coli* and *B. subtilis*. The obtained values show that both peptides had an effect on both types of bacteria with a MIC of 30 μg/mL for *B. subtilis* and 100 μg/mL for *E. coli* (Table S4). These values are consistent with previous studies [37, 38]. The more potent inhibitory effect in the case of *B. subtilis* can be explained by the less complex membrane structure of *B. subtilis*, due to the absence of the outer membrane and the periplasm which facilitate their interaction with phospholipid membranes, and/or the different membrane lipid composition [14, 27, 39].

Further, in regard to the membrane effects of peptides, Figure 3 shows the ^{2}H NMR MAS spectra acquired at 37°C of labeled *E. coli* and *B. subtilis* when exposed to MIC of caerin 1.1 or aurein 1.2. The ^{2}H spectra of *E. coli* demonstrate a slight increase in the isotropic peak intensity with a decrease in that of the spinning sidebands in the presence of either peptide. In parallel, ^{2}H static NMR spectra of *E. coli* (Figure S2) confirm the disordering effect with a slight increase in the isotropic peak intensity and a decrease in the width of the shoulders due to the peptides.

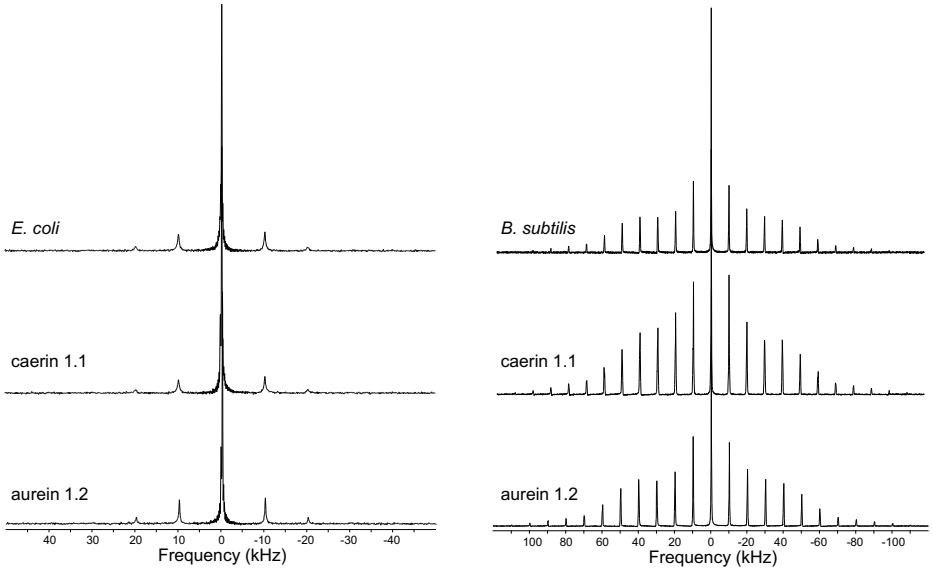

Figure 3. MAS (10 kHz) ^{2}H NMR spectra of intact *E. coli* and *B. subtilis* acquired at 37°C with 8 k scans and with addition of MIC of caerin 1.1 or aurein 1.2.

The corresponding spectral moments' analysis from ^{2}H solid-state and MAS NMR spectra, shown in Table S5 and Table 2, respectively, indicates an increase in lipid chain dynamics with a decrease of M_1 from 3.2 to 2.8 and 2.5 in the presence of caerin 1.1 and aurein 1.2, respectively (Table 2). At 37°C, the membrane phospholipids of *E. coli* are in the fluid phase with a quadrupolar splitting estimated to 40 kHz, and the disorder caused by the presence of the AMPs has increased the proportion of fluid phase. The values of Δ_2 (Table 2) show the coexistence of fluid and gel phases in *E. coli* membranes with an increase from 0.12 to 0.59 and 0.69 in the presence of caerin and aurein, respectively. This increase in the distribution of the quadrupolar splittings in contrast to the decrease in spectral moments is explained by the diminution or the rapid disappearance of CD_3 splittings compared to CD_2 splittings [34].

Table 2. Spectral moment analysis of the MAS (10 kHz) ^{2}H NMR spectra of labelled *E. coli* and *B. subtilis*, without and with the presence of AMPs at different concentrations.

Sample	AMP (μg/mL)	M_1 (10^4 s^{-1})	M_2 (10^9 s^{-2})	Δ_2
B. subtilis[a]	——	9.2 (0.2)[b]	15 (2)	0.16
+ caerin 1.1	MIC	9.4 (0.6)	15 (1)	0.23
	45	6.6 (0.3)	8 (1)	0.37
	60	4.0	3.3	0.51
+ aurein 1.2	MIC	12 (2)	23 (3)	0.07
	45	13 (3)	27 (4)	0.34
	60	3.8 (0.6)	3 (2)	0.78
E. coli[a]	——	3.2 (0.3)	1.6 (0.7)	0.12
+ caerin 1.1	MIC	2.8 (0.3)	1.6 (0.6)	0.59
+ aurein 1.2	MIC	2.5 (0.2)	1.4 (0.2)	0.69

[a] MIC is ~30 μg/mL for *B. subtilis* and ~100 μg/mL for *E. coli*.

[b] Standard deviation based on two experiments.

Table 2 additionally highlights the effect of aurein 1.2 compared to that of caerin 1.1 on *E. coli*, with a higher increase in Δ_2 in the presence of aurein. This result is consistent with the hypotheses by Fernandez *et al*. [15, 37] on the action mechanism of AMPs with model

membranes, which suggest that aurein is able to cause an abrupt destabilization of the membrane and its permeability. Caerin 1.1 rather than aurein 1.2 tends to insert into the phospholipid bilayers by the formation of transmembrane pores with a less destructive effect of the membrane.

In the case of labelled *B. subtilis*, Figure 3 shows that the spectrum of these Gram(+) bacteria have a quadrupolar splitting of 120 kHz, higher than that observed for *E. coli* membranes (~40 kHz). This difference indicates that *B. subtilis* membranes are more rigid than those of *E. coli* at 37°C and is due to the proportion of saturated fatty acids, which is about 64% in *B. subtilis* compared to 51% in *E. coli* [28]. This difference can also be explained by the presence of LTAs in the peptidoglycan of *B. subtilis,* which has a stabilizing effect on lipid membranes. A study by Gutberlet *et al*. [40] demonstrated that the addition of LTA to a dipalmitoylPG matrix causes an ordering effect on the lipid membrane close to the head group region.

Concerning the spectra of labeled *B. subtilis* exposed to AMPs at MIC, the spectral analysis in Table 2 shows an increase in M_1 and M_2. M_1 increased in the presence of caerin 1.1 from 9.2 to 9.4, and to 12 in the case of aurein 1.2. This indicates that at the MIC, the interaction of both peptides with *B. subtilis* membrane reduced the dynamics of the lipid chains. This effect was not observed in *E. coli* when exposed to 75 μg/mL and 50 μg/mL of caerin 1.1 (data not shown), which is below the MIC of 100 µg/mL.

To better understand the interaction of *B. subtilis* with aurein 1.2 and caerin 1.1, bacteria were then exposed to AMP concentrations of 45 and 60 μg/mL, i.e., above the MIC. The spectra are shown in Figure 4 and the corresponding spectral moments in Table 2. Starting with caerin 1.1, the spectra show a decrease in the intensity of the MAS sidebands with intensification of the isotropic peak. At 45 μg/mL, caerin 1.1 exerts its mode of action by disordering the lipid chains. This observation is supported by the decrease of M_1 and M_2 values (Table 2), but a smaller Δ_2 would be expected. As mentioned earlier, this can be explained by the decrease or the rapid disappearance of CD_3 splittings compared to CD_2 splittings [34], but in this case it may also be due to the association of caerin 1.1 and phospholipids causing heterogeneity in the membrane. Disordering was enhanced at 60 μg/mL with the reduction in MAS sidebands from 16 to 4 (Figure 4), and also with the appearance of 'Feature X' circa ± 10 kHz (Figure S3) which reports

on the degradation of the bacterial cells [34]. 'Feature X' is usually present in the spectra but is only visible in the case of intensive degradation which may be due to the caerin 1.1. Our focus was on short term effects and bacteria, therefore, were not incubated for more than 5 min. Greater effects might be expected using a longer incubation time.

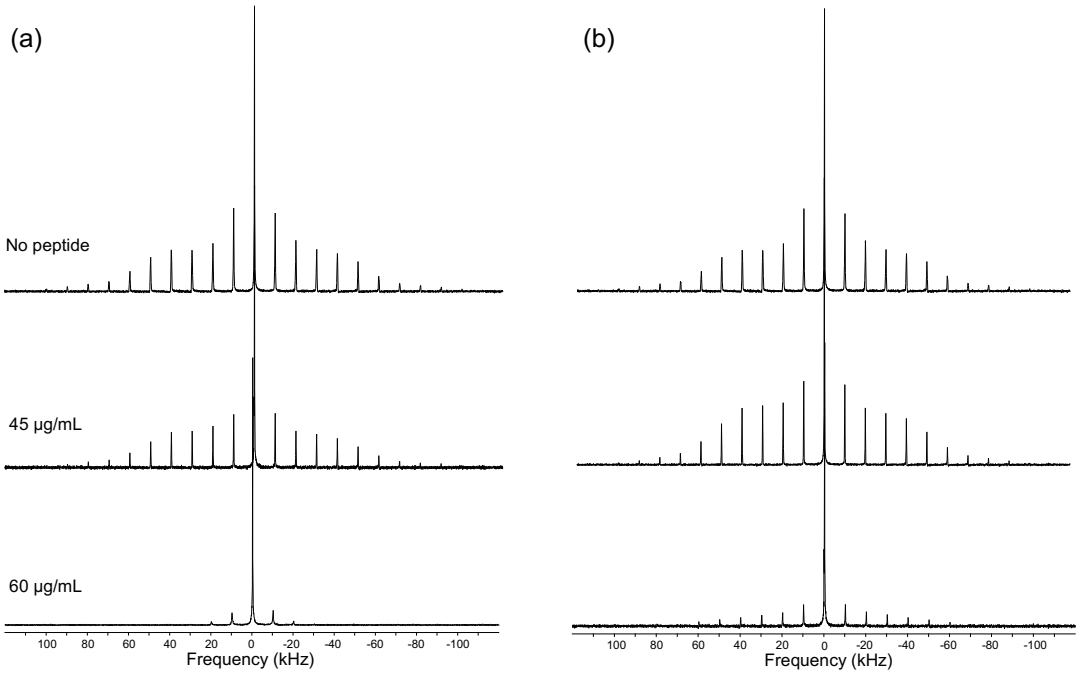

Figure 4. ^{2}H MAS NMR spectra of intact *B. subtilis* acquired at 37°C and with 8 k scans and with the addition of different concentrations of (a) caerin 1.1, and (b) aurein 1.2.

In the case of aurein 1.2, the disorder increase of the lipid chains was observed at 60 μg/mL, which means that a higher concentration of aurein is required to exert its mode of action on the *B. subtilis* (Gram+) phospholipid membrane. This may also be related to the smaller size of aurein with 13 residues compared to caerin 1.1 with 25 residues. A specific interaction of aurein 1.2 with TAs and LTAs or other bacterial components may reduce its interaction with the membrane and the disordering effect.

The difference in peptide concentrations, which caused disorder in membrane lipids of Gram(-) and Gram(+) bacteria, highlight the importance of *in vivo* studies. Gram(+) *B. subtilis* is

characterized by a thick peptidoglycan layer which accounts for about 90% dry weight of the membrane [41]. This rigidity is provided by TAs that are covalently bound to peptidoglycan and by the LTAs anchored to the membrane via diacylglycerol. A negatively charged LTA polymer has been demonstrated to interact with cationic peptides [42] and the interaction of PBP10, LL-37 and melittin with LTA inhibits their antimicrobial activity. The inhibition has been suggested to be due to the decrease of peptide concentration on the membrane as a result of the increase in peptide adsorption to the bacterial surface [41]. Further, that the disordering effect was not observed at the MIC highlights the idea that the inhibition is not via membrane destabilisation but via the interaction of peptides with other bacterial cell membrane components. Further work, however, is necessary to elucidate the relative importance and mechanisms of interaction of aurein 1.2 and caerin 1.1 with TAs and LTAs.

Conclusion

We have investigated the interaction of caerin 1.1 and aurein 1.2 with intact Gram(+) and Gram(-) bacteria by ^{2}H solid-state NMR following deuteration of their membrane lipid acyl chains. To do so, we have proposed a ^{2}H-labelling protocol for the phospholipids of non-mutated *B. subtilis*. Magic-angle spinning combined to moment analysis allowed collecting high-quality spectra with a reduced number of scans. Aurein 1.2, which was reported to act via a carpet mechanism, was shown to be more membrane disruptive on *E. coli* than caerin 1.1 which is known to form transmembrane pores. The disordering effect observed on the membrane lipids at concentrations higher than the MIC on *B. subtilis* seem to indicate electrostatic interactions of caerin 1.1 and aurein 1.2 with other components present in the peptidoglycan, such as the negatively-charged teichoic and lipoteichoic acids, and suggests that the inhibition effect is not only mediated by membrane destabilisation or disruption. Altogether, our results reveal the importance of *in vivo* NMR study, where the action mechanism of AMPs was shown to depend on the membrane environment and on the actual peptide concentration at the lipid membrane surface. Our results have led us to develop strategies for two dimensional ^{13}C-^{15}N solid-state NMR to determine the interaction of AMPs with TA and LTA.

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