Antimicrobial peptides with varied chemical compositions trigger hyperpolarization of the bacterial membrane

Anasztázia Hetényi,[a] Kaushik Nath Bhaumik,[a] Réka Spohn,[b] Anett Dunai,[b] Lejla Daruka,[b] Csaba Pál,[b] Tamás A. Martinek[a,c]

[a]Department of Medical Chemistry, University of Szeged, Dóm tér 8, Szeged HU-6720, Hungary

[b]Synthetic and Systems Biology Unit, Institute of Biochemistry, Biological Research Centre, National Laboratory of Biotechnology, Hungarian Research Network; Szeged, HU-6726, Hungary [c]HUN-REN–SZTE Biomimetic Systems Research Group, Dóm tér 8, Szeged HU-6720, Hungary

https://doi.org/10.17952/37EPS.2024.P2094

Introduction

Alleviating the impact of antimicrobial resistance is an ongoing medical necessity and one of the biggest hurdles. Striking is directed towards the introduction of expedited methodologies for antibiotic development, but new compounds rarely manifest a diminished predisposition to resistance. Consequently, adjuvant strategies to maintain or improve the efficacy of current antibiotic treatments are attractive approaches, and new complementary mechanisms are in demand. Several previous observations point to hyperpolarization as a potentially useful adjuvant approach.

Results

In search of the bacterial hyperpolarization effects, we chose 17 AMP sequences (Table 1). The selection was based on the secondary structure, including α-helix, polyproline helix, disulfide-stabilized β-sheet, and disordered sequences.

Table 1

Peptides	Abbreviation	Sequence
Apidaecin 1B	AP	GNNRPVYIPQPRPPHPRL-OH
Protegrin-1	PROT1	RGGRLCYCRRRFCVCVGR-NH_2
Tachyplesin II – Lys	TP2K	KWCFKVCYKGICYKKCK-NH_2
Tachyplesin II	TP2	RWCFRVCYRGICYRKCR-NH_2
Indolicidin-OH	IND	ILPWKWPWWPWRR-OH
LL37	LL37	LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES-OH
Indolicidin	INDa	ILPWKWPWWPWRR-NH_2
R8	R8	FLGKVFKLASKVFKAVFGKV-OH
PGLa – Arg	PGRLa	GMASRAGAIAGRIARVALRAL-NH_2
PGLa	PGLa	GMASKAGAIAGKIAKVALKAL-NH_2
PGLa-OH	PGL	GMASKAGAIAGKIAKVALKAL-OH
Guavanin 2	GUA2	RQYMRQIEQALRYGYRISRR-NH_2
Cecropin P1	CP1	SWLSKTAKKLENSAKKRISEGIAIAIQGGPR-OH
Buforin II	BUF2	TRSSRAGLQFPVGRVHRLLRK-OH
Bactenecin 5	BAC5	RFRPPIRRPPIRPPFYPPFRPPIRPPIFPPIRPPFRPPLGPFP-OH
Magainin II-OH	MAG2	GIGKFLHSAKKFGKAFVGEIMNS-OH
Magainin II	MAG2a	GIGKFLHSAKKFGKAFVGEIMNS-NH_2

We assessed alterations in the polarization of the cytosolic membrane potential of E. coli cells in reaction to antimicrobial peptides (AMPs) in Table 1. To prevent complete membrane disruption, we administered subinhibitory concentrations, consistently at 0.5 MIC. Polarization was tracked through flow cytometric analysis utilizing the membrane potential-sensitive dye $DiOC_2(3)$ (Figure 1a-c). All measurements was performed after 15 minutes of incubation.

Figure 1

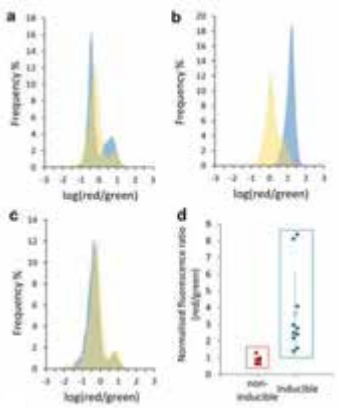


Figure 1. Bacterial hyperpolarization effects of AMPs at subinhibitory concentrations. Representative histograms of the log ratio calculated from the red and green channels for LL37 (**a**) MAG2 (**b**) and AP (**c**) (yellow untreated, blue AMP treated). (**d**) Population means of the red/green fluorescence ratios normalised to the values obtained for the untreated cells. Ratios below one correspond to depolarisation, and values above one indicate hyperpolarization. Values are displayed against the inducibility of the secondary structure.

Our results demonstrated that several AMPs could induce hyperpolarization in a sequence-dependent manner. We calculated the average hyperpolarization effect across biological replicates and compared the results with the structural characteristics of the sequences (Figure 1d). AMPs with membrane-induced helical structures typically generate hyperpolarization at subinhibitory concentrations without causing bacterial depolarization. Conversely, sequences with membrane-insensitive conformations fail to shift the bacterial population to a predominantly hyperpolarized state. A specific side chain pattern is required, which creates distinct hydrophobic and ionic faces on the helices, allowing for sufficient residual flexibility without self-aggregation. We found no correlation with known non-membrane lytic targets.

We hypothesized that the disruption of polarization in bacteria is also driven by direct interactions between the membrane and AMPs. To investigate this, we developed a cytosolic bacterial membrane mimetic system using DOPG:DOPC large unilamellar vesicles (LUVs), where AMP-induced changes in membrane potential could be monitored with the voltage-sensitive fluorescent dye oxonol VI. LUV samples were prepared with varying ion gradients at a constant pH of 7.2 to enhance the detection of any dynamic polarization effects. Specifically, we used 100 mM NaCl inside and 100 mM KCl outside for the cation gradient, and 100 mM NaCl inside with either 100 mM NaH_2PO_4 or $NaNO_3$ outside for the anion gradient. We recorded the membrane potential changes induced by AMPs at 0.5 MIC in the presence of the cation gradient.

The ion gradient-dependent fluorescence intensity curves showed that most AMPs generated an inside-positive diffusion potential. This indicates that AMPs enhance cross-membrane permeability for K^+ more than Na^+, suggesting that AMPs act as K^+-selective ionophores at sublethal concentrations. However, AP and CP1 did not induce diffusion potential in the cation gradient LUV model. Measurements using an inside Cl^- gradient against $H_2PO_4^-$ also generally produced an inside-positive diffusion potential, pointing to a higher cross-membrane permeability for Cl^- over $H_2PO_4^-$, indicating Cl^- selective anion transport, with the exception of AP.

Figure 2

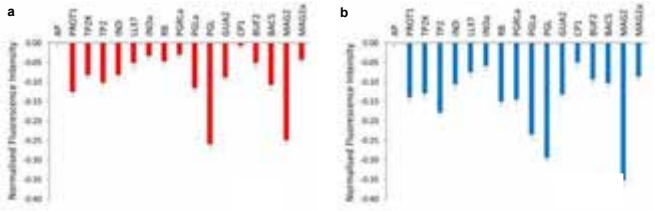


Figure 2. Membrane polarisation measurements in large unilamellar vesicles. AMP-induced fluorescence intensity decreases, implying the diffusion potential for the K^+/Na^+ gradient model (red) (**a**). AMP-induced fluorescence intensity decreases, indicating the diffusion potential for the $Cl^-/H_2PO_4^-$ gradient model (blue) (**b**).

We concluded that the AMPs induced diffusion potential in the LUV model in a sequence-dependent manner (Figure 2). The observed changes in fluorescence intensity demonstrated that AMPs create diffusion potential through selective cross-membrane ion transport for K^+ or Cl^- (over Na^+ and $H_2PO_4^-$, respectively), or both. These findings suggest that direct membrane–AMP interactions at subinhibitory concentrations can influence diffusion potential across a lipid bilayer.

We hypothesized that AMPs enable voltage generation through subtle electrogenic transport without fully disrupting the ion concentration gradients across the membrane. To examine the extent of macroscopic ion exchange across the bilayer, we directly measured time-dependent ion concentration levels in LUVs using NMR spectroscopy. We monitored Na^+ and $H_2PO_4^-$ by detecting ^{23}Na and ^{31}P NMR signals in LUVs, $Dy(PPP)_2^{7-}$ was employed as an NMR shift reagent to distinguish between internal and external Na^+, while external and internal phosphate signals were discernible without a shift reagent. After 20 minutes, ion exchange either halted or slowed significantly, resulting in a stable, steady-state ion gradient or a slow drift. This finding confirms that AMPs do not rapidly dissipate the ion gradient, thereby sustaining a quasi-steady-state membrane potential. The rate-limiting step in this exchange is the slow transport of the less preferred ion.

Figure 3

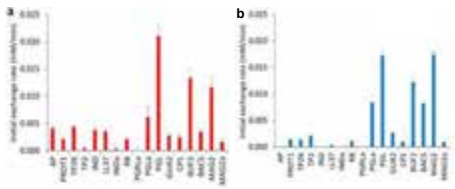
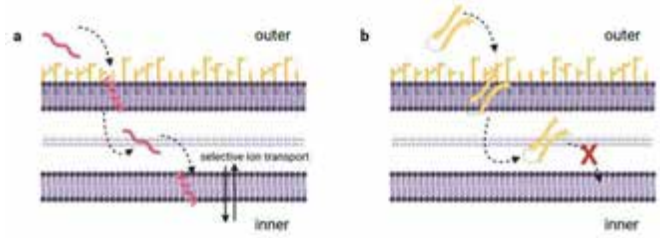


Figure 3. Ion transport measurements in large unilamellar vesicles. Initial exchange rates observed for AMPs in the Na^+/K^+ gradient model (**a**). Initial exchange rates observed for AMPs in the $Cl^-/H_2PO_4^-$ gradient model (**b**).

Conclusion

Our findings underscore the potential of AMPs as a valuable tool for chemically hyperpolarising bacteria, with significant implications for antimicrobial research and bacterial electrophysiology.



Acknowledgement

Supported by the Ministry of Innovation and Technology of Hungary from the National Research, Development and Innovation Fund (NKFIH K134754). Project no. TKP2021-EGA-32 has been implemented with the support provided by the Ministry of Culture and Innovation of Hungary from the National Research, Development and Innovation Fund, financed under the TKP2021-EGA funding scheme.