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Theoretical evaluation of wall teichoic acids in the cavitation-mediated pores formation in Gram-positive bacteria subjected to an electric field

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ABSTRACT

Background: Electroporation is a method of choice to transform living cells. The ability of electroporation to transfer small or large chemicals across the lipid bilayer membrane of eukaryotic cells or Gram-negative bacteria relies on the formation of transient pores across the membrane. To exist, these pores rely on an insulator (the bilayer membrane) and the presence of a potential difference on either side of the membrane mediated by an external electric field. In Gram-positive bacteria, however, the wall is not an insulator but pores can still form when an electric field is applied. Past works have shown that the electrostatic charge of teichoic acids, a major wall component; sensitizes the wall to pore formation when an external electric field is applied. These results suggest that teichoic acids mediate the formation of defects in the wall of Gram-positive bacteria.

Methods: We model the electrostatic repulsion between teichoic acids embedded in the bacterial wall composed of peptidoglycan when an electric field is applied. The repulsion between teichoic acids gives rise to a stress pressure that is able to rupture the wall when a threshold value has been reached. The size of such small defects can diverge leading to the formation of pores.

Results: It is demonstrated herein that for a bonding energy of about ~ 1 − 10 k_BT between peptidoglycan monomers an intra-wall pressure of about ~ 5 − 120 k_BT/nm^3 generates spherical defects of radius ~ 0.1 − 1 nm diverging in size to create pores.

Conclusion: The electrostatic cavitation of the bacterial wall theory has the potential to highlight the role of teichoic acids in the formation pores, providing a new step in the understanding of electroporation in Gram-positive bacteria without requiring the use of an insulator.

1. Introduction

As a tool, the electroporation technique has been used over the last two decades to deliver gene to cells [1] or in animal or plant tissues [2–5], to promote drug uptake by cells [6] and to implement food safety measures via electroporation-related sterilization mechanisms that are independent of temperature [7].

The ability of electroporation to transfer small chemicals (e.g., drugs) or large protein complexes (e.g., genes) across the bilayer membrane of cells rely on the formation of transient pores [8]. The mechanism of transient pores formation in eukaryotic cells and Gram-negative bacteria is now well understood and has been modeled in depth using physics [9,10]. However, as the structure of Gram-positive and Gram-negative bacteria differ significantly, it is difficult to transfer and apply the set of results obtained from Gram-negative to Gram-positive bacteria. Consequently, how an electric field can create transient pores is still incomplete in the case of Gram-positive bacteria and electroporation protocols are usually developed through lengthy trial and error procedures. Moreover, it is important to point out that a single and uniform electroporation protocol for all classes of bacteria and cells has not yet been found and that the different methods and tools used to enhance electroporation in Gram-positive bacteria, reviewed in [11], create a natural precedent in underlying the lack of general understanding concerning electroporation in Gram-positive bacteria.

Recent works have demonstrated nonetheless that the formation of pores in Gram-positive bacteria relies on the electrostatic charge carried by the teichoic acids that are major constituents of the wall of Gram-positive bacteria [12] and that the bacterial lipid membrane located underneath the wall can stabilize the pore once the later is formed [13].

The central role of teichoic acids for bacteria has been underlined in (i) the regulation of the bacteria morphology and division, (ii) bacteria ion homeostasis, (iii) the protection from host defense and antibiotics, (iv) the adhesion to the host, (v) the colonization of the host and (vi) the horizontal transfer of genes [14,15]. Unsurprisingly, teichoic acid is now a target of choice for new antibiotics [16]. Finally, the negative charges carried by teichoic acids [14] make them an essential component of the bacterial wall to interact with an external electric field [12].

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Theories describing pore formation in eukaryotic cells or Gram-negative bacteria consider that the increase in conductivity across the outer bilayer membrane is associated with pores arising from a competition between an interfacial energy mediated by the external electric field and a tension line once the pore is formed [17]. Naturally, these theories have to consider the bilayer membrane as an insulator initially, so that an interfacial energy can be defined.

In Gram-positive bacteria, however, this stance regarding an interfacial energy cannot hold as the bacterial wall is permeable to ions and is therefore not an insulator. This suggests therefore that pores arise from a change in energy defined inside the volume of the wall that may expand to form pores at the wall surfaces.

The bacterial wall is a polymeric gel made of peptidoglycan units interacting via covalent bonds. Inside the wall, a number of molecules exist among which the teichoic acid composing 60% of the cell wall and whose charge is inherently negative due to phosphate groups composing the polyelectrolyte [14]. Binding of free cations to teichoic acids is also thought to minimize repulsion between nearby phosphate groups, which can affect polymer structure and therefore cell wall integrity [18,19]. The best example of such an interaction is when the bacterium wall is incubated at low tonicity provoking its swelling and a concomitant reduction in the zwitterions (i.e., cations) interacting with the wall surface (see Table 1). It seems therefore that swelling results from an excess of negative wall charge, very probably driven by wall teichoic acids repulsion. Augmenting the repulsion between teichoic acids is well known to weaken the wall. Indeed, the bacteria electro-competency step that consists of incubating bacteria at low tonicity to drop the medium conductivity also makes their wall more susceptible to electric fields. Taken together, these observations suggest therefore that when the electrostatic equilibrium between the wall and the surrounding medium is altered, the wall is affected. Finally, the overall teichoic acid charge can also be modulated via addition of positive D-alanyl residues reducing its binding capacity for cations [20]. In particular, the inactivation of the *dlta* gene that has been shown to inhibit the addition of D-alanyl residues makes Gram-positive bacteria more susceptible to the external electric field [12]. This biological observation is in line with a role of wall teichoic acids in electroporation.

It is therefore not unreasonable to think that the negative charge of teichoic acids may be involved in generating pores when an external field is applied.

This can be explained as follows: consider a negatively charged teichoic acid embedded in the wall and surrounded by free counterions. Under an external electric field, provided that the later is strong enough, it would not be surprising to see most of the free ions interacting with the teichoic acids to leave the bacterial wall, thereby unmasking the negative charges of teichoic acids. If the wall density of teichoic acids is enough, this could, in turn, increase the repulsion between them. As teichoic acids are embedded in a peptidoglycan gel their repulsion should result in the creation of a very high wall mechanical tension that could rupture the peptidoglycan gel locally once the tension has reached a threshold level. This mechanism is similar to cavitation and it is this mechanism that the present work aims to model.

Table 1
Example of surface charge density of Gram-positive bacteria as a function of the external concentration of electrolytes (data from [23,24]).

Strain	Wall thickness (nm)	Surface charge density (C/m^2)	Electrolyte concentration (M)
Corynebacterium sp. Strain DSM 44016	66	0.61	0.1
	78	0.51	0.01
	108	0.35	0.001

2. Renormalization of the electrostatic charge of teichoic acids in a peptidoglycan matrix.

Let us consider a free teichoic acid in solution. These polyelectrolytes are negatively charged, and as a result, if the solution contains also free electrolytes, the cations from the solution should gather around the teichoic acid to balance its negative charge. This "screening" will happen over a certain length scale, λ, that is defined in part by the concentration of free electrolytes in solution (Fig. 1A). In particular, if the concentration of electrolytes is low, the length scale λ should increase. This means that two free and identical teichoic acids will not repulse each other if their separation distance is larger than: 2λ, even so they have the same negative charge (Fig. 1B).

Consider now a set of teichoic acids that are not free in solution but fixed because embedded in a peptidoglycan matrix, i.e., the bacterial wall. Assuming spatially fixed teichoic acids is correct so long that the time scale considered here and needed to change the value of λ are much shorter that the time scale required for an acid to diffuse out of the matrix.[1] Their separation distance is now fixed by their density in the wall. Let us note ρ, the density of teichoic acid in this case. The average distance that separates two teichoic acids in the bacterial wall is now ~ $2/\rho^{1/3}$. This means that the two teichoic acids will start to repulse each other if their bacterial wall concentration is such that $\rho \geq \rho_c \sim 1/\lambda^3$ (Fig. 1C). As a result, the screening can be imperfect in cases where the density of teichoic acids is too high or the concentration of electrolytes is too low, or both. In these conditions, it is possible to redefine an effective charge Q for teichoic acids: $Q \sim Q_0 \exp(-c\rho^{-1/3}/\lambda)$ (Appendix A), where c is a constant that refers to the shape of the teichoic acid (e.g., $c = (4\pi/3)^{-1/3}$ for a spheric shape) and Q_0 is the true charge of teichoic acids when no counter-ions are present. Using $\rho_c \sim 1/\lambda^3$, the charge can be rewritten simply as $Q \sim Q_0 \times e^{-(\rho_c/\rho)^{1/3}}$. With this new renormalized charge, it is now possible to deduce the new physical properties of the wall.

3. Repulsive electrostatic energy in the bacterial wall

If we assume that $\rho \geq \rho_c$, the teichoic acids will repulse each other and an energy can be defined (Fig. 1C). Let us further assume that a teichoic acid will only be affected by its closest neighborhoods; the repulsive energy between two teichoic acids separated by an average distance, $2c\rho^{-1/3}$, is as follows: $Q_0^2 \times e^{-2(\rho_c/\rho)^{1/3}}/4\pi\varepsilon_0\varepsilon_r 2c\rho^{-1/3}$.

As each teichoic acid feels a repulsion from close neighborhoods only and that the number of neighborhoods that are electrostatically "visible" per teichoic acid is ρ/ρ_c; the total repulsive energy felt by one teichoic acid is $\left(Q_0^2/4\pi\varepsilon_0\varepsilon_r 2c\rho^{-1/3}\right) \times (\rho/\rho_c) \times e^{-2(\rho_c/\rho)^{1/3}}$.

To determine the repulsive energy that is present in the entire bacterial wall, the energy of a single teichoic acid needs to be summed up over all the teichoic acids present in the wall. As the number of teichoic acids present in the wall is ρV_{wall}, where V_{wall} is the volume of the wall, the repulsive energy inside the bacterial wall at the lowest order (i.e., for pair interaction only) is

$$E_{\text{elec}} \sim \alpha_0 \rho^{7/3} \times e^{-2(\rho_c/\rho)^{1/3}}/\rho_c \quad (1)$$

with $\alpha_0 = Q_0^2 V_{\text{wall}}/16\pi\varepsilon_0\varepsilon_r c$. A factor 1/2 is introduced in Eq. (1) to avoid counting twice the same pair interaction between teichoic acids. Eq. (1) assumes that the two surfaces of the wall have a negligible impact in the repulsive energy as otherwise a surface term should be introduced. This means that the validity of Eq. (1) is likely to be optimal for thick bacterial walls, namely, when the ratio surface to volume of the wall tends toward zero ($S_{\text{wall}}/V_{\text{wall}} \to 0$).

[1] Imposing an electrical field over a very short period of time should warrant this.