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The bio-physics of condensation of divalent cations into the bacterial wall has implications for growth of Gram-positive bacteria

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ABSTRACT

Background: The anionic-polyelectrolyte nature of the wall of Gram-positive bacteria has long been suspected to be involved in homeostasis of essential cations and bacterial growth. A better understanding of the coupling between the biophysics and the biology of the wall is essential to understand some key features at play in ion-homeostasis in this living system.
Methods: We consider the wall as a polyelectrolyte gel and balance the long-range electrostatic repulsion within this structure against the penalty entropy required to condense cations around wall polyelectrolytes. The resulting equations define how cations interact physically with the wall and the characteristic time required for a cation to leave the wall and enter into the bacterium to enable its usage for bacterial metabolism and growth.
Results: The model was challenged against experimental data regarding growth of Gram-positive bacteria in the presence of varying concentration of divalent ions. The model explains qualitatively and quantitatively how divalent cations interact with the wall as well as how the biophysical properties of the wall impact on bacterial growth (in particular the initiation of bacterial growth).
Conclusion: The interplay between polymer biophysics and the biology of Gram positive bacteria is defined for the first time as a new set of variables that contribute to the kinetics of bacterial growth.
General significance: Providing an understanding of how bacteria capture essential metal cations in way that does not follow usual binding laws has implications when considering the control of such organisms and their ability to survive and grow in extreme environments.

1. Introduction

The bacterial cell wall is formed by a rigid network of carbohydrates (peptidoglycan) and amino acids that are responsible for many cellular functions and protect bacteria against external physical stresses [1]. In Gram positive bacteria, in addition to peptidoglycan, the cell wall contains the highly charged anionic polyelectrolytes, teichoic acid (TA), which may constitute up to 60% of the wall's mass [2]. At physiological pH, the chemical groups (phosphoryl, hydroxyl and amino) composing the wall are deprotonated [3] and the bacterial wall can be considered as a negatively charged polyelectrolyte gel.

Two types of TAs have been described depending on their attachment to the bilayer membrane or the cell wall [2]: The lipo-TAs (LTAs), anchored to the cytoplasmic membrane, extend into the peptidoglycan layer whereas the wall TAs (WTAs) are attached directly to peptidoglycan and extend through the cell wall. Given that TAs are anionic polyelectrolytes embedded in the peptidoglycan, the repulsion between TAs can only be balanced by binding cationic groups. The electrostatic interactions involved in the wall play a fundamental role as they define the wall volume and rigidity [4–7]. Beside their involvement in the physical electro-mechanics of the wall, LTAs/WTAs are thought to be important for cation homeostasis, which is essential to the physiology of Gram positive bacteria [8]. The morphology of strains lacking LTAs, is altered, and results in swelling and aggregation of bacterial cells [9] with strains lacking both LTAs and WTAs not viable [10].

The importance of cation homeostasis is well documented. For example, calcium (Ca^{2+}) participates in synergistic interactions with enzymes to facilitate the anchoring of surface proteins involved in bacterial adhesion [11,12], whereas magnesium (Mg^{2+}) plays a fundamental role in peptidoglycan biosynthesis, wall strength, prevention of cell lysis and growth [13–15]. The impact of the deletion of WTAs on growth can only be partially rescued by increasing the magnesium in the growth medium [9], demonstrating how central wall polyelectrolytes are for growth. The importance of the wall composition is also highlighted when phosphate availability is

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limited or when external Mg^{2+} is reduced as in this case, the phosphate content of WTA is exchanged with uronic acids and the WTA is transformed to teichuronic acid, thus trying to maintain the overall anionic properties of the wall [16,17]. In this situation, bacteria synthesize more WTAs and in doing so increase the probability of attracting divalent cations, such as Mg^{2+} [18]. While the retention of divalent cations by the cell wall is essential, specific transporter proteins embedded in the underlying cytoplasmic membrane are required for their uptake by the bacterial cell [19,20]. This indicates that there must be transport of divalent cations across the bacterial wall; from the outside world to the membrane bound receptor. The flux of cationic substances through the wall most likely accounts for the ability of cationic antimicrobial peptides (CAMPs) to access the cell membrane; intrinsic sensitivity to CAMPs is dependent on the amount of negatively charged groups in the cell wall [21,22].

A biochemical model concerning divalent cations transport has recently been suggested [23]. In this model, when metals cations are sparse chelation appears but weakens when their concentrations increase. It is proposed that this permits the ability of divalent cations to be released and slide along the molecules of the wall before finally being absorbed by the bacterium [24]. This model suggests/requires the presence of an undefined cooperativity to explain the switch between these two behaviours (cation attraction vs. cation release). What is probably more intriguing in this study is that at low concentration of divalent cations, chelation is total [23,24]; which seems to contradict usual laws of thermodynamics and statistical physics upon which cooperativity phenomena are usually based. In classical thermodynamics, regarding binding sites and involving bulk concentrations of cations, the entropy should dominate at low concentration of divalent cations always leaving free cations in solution that should be detected experimentally meaning that total chelation should not be an option [23,24]. It does seem, therefore, that another explanation of the mechanism needs to be invoked to explain the binding behaviour the cell wall toward divalent cations.

In another field seemingly distant from bacteriology, soft matter physics (also known as, condensed matter physics), neutral polymers and charged polymers (polyelectrolytes) have been studied along with their interactions with counterions. From the point of view of physics, the presence of both short range entropic interaction and long range electrostatic interaction (coulomb force) define the physical mesoscopic properties of polyelectrolytes [25]. Those interactions have an impact on polyelectrolyte structures. In addition, unique physical properties emerge due to the quasi-linear structure of polyelectrolytes within ionic solutions that are not apparent when only binding affinities, and related cooperativity, are considered. The physical behaviour of solutions containing a mixture of gel polyelectrolytes immersed in electrolyte solutions was first highlighted using physics by Gerald S. Manning in 1969 [26–29]. It is the aim of this manuscript to underline how the understanding of the physical biology or biophysics of a system composed of polyelectrolyte gels and electrolytes mixed together can provide insight into the attraction and movement of across the bacterial wall.

In order to introduce how we envisage the mechanisms underlying this process, the paper is divided in several parts. In the first part, we underline the main/critical physical parameters of the Gram positive bacterial cell wall. The second part, provides a synopsis of Manning's theory with particular reference to the notion of the condensation of ions on polyelectrolytes. In the third part, we suggest a condensation theory for the bacterial wall that will, in turn, be directly compared to: (i) recent data produced by Thomas III and Rice [23] regarding calcium binding to bacterial wall material (part four) and (ii), to data produced by Webb [30] regarding the role of magnesium in bacterial growth (part five).

1.1. Part 1 – sketch and notation of the bacterial cell wall

The wall of Gram positive bacteria is composed of a gel of polyelectrolytes (Fig. 1). The typical mesh size of length L defines the spatial location of a single polyelectrolyte that can be treated independently of its junction with other polyelectrolytes. We shall assume that the length L is constant across the wall. As the single polyelectrolyte is composed of N monovalent charges, q, the line density of charge of a single polyelectrolyte is simply Nq/L. The monovalent charges are surrounded by cations that are restricted within a volume V_{poly} around the polyelectrolyte. This restriction is the result of charge condensation derived from Manning's theory (see below). Considering single divalent cations the bulk concentration shall be noted C_0.

1.2. Part 2 - manning theory in the case of polyelectrolytes

The essential ingredients regarding Manning theory [26–29] with particular reference to the notion of the condensation of ions on polyelectrolytes are given below.

Let us assume that a single polyelectrolyte can be treated as a rod carrying N negative charges each noted, q, over an average length, L; and that no extra salt is added to the solution or equivalently that, Debye length is larger than Manning length [31]. These assumptions provide the charge line density, Nq/L (Fig. 1A). Neglecting the extremities of a single polyelectrolyte for simplicity (or equivalently concentrating on the determination of electrical properties within the bacterial wall) and considering Gauss theorem, the radial electric field, E, can be deduced as: $E = Nq/2\pi\varepsilon_r\varepsilon_0 Lr$. Where ε_r and ε_0 are the relative permittivity and the vacuum permittivity, respectively. Still using the radial symmetry, as the electric potential V is linked to the electric field under the form, $E = -\vec{\nabla}V$, one finds: $V = A - Nq\ln(r)/2\pi\varepsilon\varepsilon_0 L$where, A, is an integration constant. Next to the polyelectrolyte, the potential energy, E_p, of a counterion of valence Z and hence total charge, Zq is: $E_p = ZqV$. Using Boltzmann theory, the probability to find a counterion at a distance r from the polyelectrolyte is thus: $\sim\exp(-E_p/k_BT)$; where k_BT is the thermal energy. As a result, using the electric potential one finds: $\exp(-E_p/k_BT) \sim 1/r^{2\xi}$, where $\xi = ZNl_B/L$ and $l_B = q^2/4\pi\varepsilon\varepsilon_0 k_BT$ is the Bjerrum length, i.e. the distance at which the electrostatic energy is comparable to the thermal energy. If one determines the amount of counterions located within a distance r_0 from the polyelectrolyte, result given by the integral $\int_0^{r_0} \exp(-E_p/k_BT)2\pi r dr \sim [r^{2(1-\xi)}]_{r=0}^{r=r_0}$, one sees that the integral diverges at $r = 0$ if $\xi = ZNl_B/L > 1$. This is unrealistic physically and therefore Manning suggested that a certain amount of counterions would necessarily condense onto the polyelectrolyte to drop the value of N, and therefore brings ξ toward unity. As a conclusion, within a solution containing polyelectrolytes there are always two populations of ions, namely free and condensed counterions. Note that the condensed ions are not fixed onto the polyelectrolyte but can move between charges.

1.3. Part 3 - fraction of charges condensed onto bacterial cell wall polyelectrolytes

To determine the fraction of charges on the polyelectrolytes being compensated by condensed counterions, one needs to determine the energy required to partially discharge the polyelectrolyte and compare this energy to the entropy penalty linked to concentrating counterions within a volume similar to the polyelectrolyte volume, V_{poly} (Fig. 1B). To do so, we use a mean field theory. Let us assume that each monovalent charge on the polyelectrolyte is partially compensated by an average factor θZ (Z being the valence of the condensed counterion and θ the probability that a counterion is present) leading to a new charge value: $q' = q(1-\theta Z)$. Let us assume also that those charges aligned onto the polyelectrolyte are indexed by the letter "i", where i varies between 0 and N, and interact together via a Debye-Huckel potential, $V_{DH}(r_i)$. So the electric potential felt by a given charge on the polyelectrolyte is a function of all the other charges on the same polyelectrolyte. In this case, the energy du_i required to change the charge indexed by the