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Controlling of Bacterial Elongation Growth by Studying the Factors Effecting of It

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ABSTRACT

Inhibition of PGN elongation caused by activation of PGN autolysin by lysis of the bacteria PGN cell wall by electrolyte ions. Another electrolyte ion-mediated lysis of the bacteria cell wall is thought to be due to disruption of the outer membrane structure by N- and C-terminal lipoproteinases, but is mitigated by the activity of PGN hydrolases and autolysins. We investigated bacterial cell wall lysis by electrolyte ions by using minimum inhibitory concentration value for the solution against gram positive bacteria .Halo antibacterial tests also found that Cu , Zn ,Mg,Li ions have strong antibacterial activity against bacteria. Lysis of bacteria peptidoglycan (PGN) cell walls by electrolyte ions is thought to result from damage to PGN biosynthesis by TG and TP and PGN inhibition by PGN autolysin activation. The other is caused by lysis of the outer membrane cell wall of bacteria by electrolyte ions and is thought to result from disruption of the outer membrane structure and inhibition of PGN elongation through disruption of PGN biosynthetic TPs. and activation of PGN autolysin.

INTRODUCTION

In Gram-positive bacteria, wall teichoic acid (WTA), lipoteichoic acid (LTA), and peptidoglycan are important bacterial components for binding metal ions from the environment. Peptidoglycan is a sugar chain (polysaccharide) backbone composed of N-acetylmuramic acid and N-acetylglucosamine, and peptide side chains (amino acids and diaminopimelic acid) are crosslinked by peptide bonds to form a three-dimensional structure. increase. Teichoic acids are

polysaccharides of polyglycerol phosphate or polyribitol phosphate (depending on the bacterial strain) that are either anchored to the cytoplasmic membrane (LTA) or covalently attached to the cell wall (WTA) (Bhavsar, Erdman et al. (2004). The carboxyl group on peptidoglycan is the anionic site for metal binding and the phosphodiester group is the primary metal binding site for teichoic acid. (1) Previous studies on the metal-binding behavior of *B. subtilis* have focused on metal-binding capacity and affinity. Analysis suggested the possible presence of negative cooperativity. Most Gram-positive cell walls have similar functional groups that contribute to metal binding. Current studies using solid-state NMR show that Mg2+ preferentially binds to phosphate groups and displaces D-alanine from phosphate groups. Solid-state NMR experiments were also performed to estimate the wall teichoic acid binding constant using the 31P chemical shift based on the magnesium concentration used in the experiment.(2, 3, 4)

As the cell wall approaches saturation with metal ions, the slope of the Scatchard plot gradually decreases, with a concomitant decrease in apparent binding affinity. This decrease becomes more pronounced as the steady-state concentration increases . As the equilibrium concentration increases, the bound/unbound ratio decreases correspondingly. Data from the lower/unbound ratio show a line and slope indicating weaker binding affinity. This can also be interpreted as a decrease in the cell's electrostatic potential and consequently in its affinity for metal ions. Data points were selected in region I based on the limits of quantification and the point where the curvature begins.(5)

The data show the variation of the bound/unbound ratio range for both region I and region II in each experiment. In the standard addition process metal ions are added and bind to the cell wall. After adding the first group of ions, the concentration of unbound metal ions is too low to be detected. However, the equilibrium concentration required to generate a response depends on the cell wall mass and varies from experiment to experiment. The difference in bound/unbound ratio after each standard addition can be seen from the two highest values . In Region II, the variation in bound/unbound range is less dramatic and is a result of variations in equilibrium concentrations at the end of each experiment. A high proportion of unbound metal ions in solution reduces the bound/unbound area as the cell wall sample reaches the saturation point for metal binding. Ideally, a Scatchard plot analysis provides a concise view of this equilibrium behavior regardless of bound/unbound regions. The numbers representing the relationship between bound and unbound metal ions must be very accurate.(6)

However, equilibrium constant cannot be defined as it depends on the particle size and the electrostatic properties of individual particles. Measurements in *B. subtilis* sacculus (whole cell wall) showed a similar binding capacity, but a higher binding affinity was observed. This sample yielded a binding affinity for region I and a binding affinity of region II. The bound/unbound area was much higher than the fragment samples. Nevertheless, the equilibrium remains perturbed by the inhibition of diffusion. (7)

Binding affinities are attributed to a combination of diffusion effects and the concentration dependence of the affinity constant, because the process of disrupting the cells in the French press

produces a distribution of particle sizes, it is expected that each experiment will yield a different particle size distribution. Peptidoglycan forms a three-dimensional network and the diffusion of metal ions through large fragments is very different from that through small fragments. Within the fragment distribution, each particle exhibits a unique balance of metal ions between the interior and exterior of the cell wall fragment. The negative cooperativity of metal binding to the cell wall also affects on affinity constant, causing the calculated values to be dependent on the equilibrium concentration range.. The larger bonded/unbonded region examined, the higher the binding constant for region II. We conclude that the value of affinity constant for region I does not depend on the bound/unbound ratio. (8,9)

Revision of the metal binding model requires comparison with previous literature. The bound/unbound area dependence of region II may be due to electrostatic interactions between the cell wall, metallic cations and counter ions. The cell wall is polyelectrolyte in terms of the peptidoglycan and teichoic acid components, both of which have a strong negative charge due to deprotonated carboxyl and phosphoryl functionalities around pH 7. The metal binding behavior of other polyelectrolytes has been (10). Studies using two polyelectrolytes, RNA and humic acid, showed similar binding behavior compared to cell wall samples. Humic acids are natural organic polyelectrolytes containing multiple carboxyl and phenolate groups. Using voltammetry, it was shown that the stability constants of metal ion complexes decreased with increasing metal ion concentrations and decreased with increasing ionic strength values . Increasing the Zn2+ concentration decreased the stability constant .Gradually increasing the ionic strength of the solution at a constant Zn2+ concentration decreased the observed stability constant. .(11,12)

The most extreme MgSO4 concentration ever measured was used to demonstrate bacterial growth. Growth studies in media containing up to about 1 M (25%) MgSO4 have been performed in previous studies . The study also describes an increase in experimental media containing the highest concentration of magnesium ever used. Previous studies with Dead Sea water used up to 1.5 MgCl2. There are not many studies on growth at high lithium concentrations, and this work appears to be the first to document growth at high concentrations of LiSO4. One report describes a *Micrococcus* strain adapted to the following conditions: Growth was poor in 2 M LiCl and not in 1 M LiSO4, but growth was observed in 1.5 M LiCl. We have shown that salt-tolerant bacteria can thrive in soils containing much higher concentrations of her MgSO4 than are typical on Earth. It should be noted that MgSO4 salts may also be abundant . The finding that isolates can grow under conditions of 2M MgSO4, alkaline pH, and low temperature suggests that microbes may also survive in near-surface condition .However, there is no reason to believe that the anaerobic isolates are less cold-tolerant than the aerobes studied here. Further research is needed to isolate and study hardy anaerobic bacteria under conditions. At these locations, organisms have been found that are psychotolerant, anaerobic, and able to grow on 2 MgSO4 in the laboratory. It seems possible that microbes could survive and thrive. (13,14,15).

The importance of magnesium ions in cell viability is well documented). Magnesium ions play a role in peptidoglycan synthesis, cell wall strength, and prevention of cell lysis . Gram-positive bacterial surfaces contain carboxyl, phosphoryl, hydroxyl, and amino functional (16). At physiological pH, these groups are deprotonated and contribute to metal binding .(17)

Magnesium ions are important biologically active metal ions and are among the most abundant divalent cations in nature. We found that electrostatic effects are responsible for the strong bonding between metal ions. This binding strength is reduced when the cell's negatively charged functional groups are neutralized by divalent metal ions. Purified cell wall fragments of *B. subtilis* (including peptidoglycan and WTA) However, binding still occurs in region II Fragments. As explained below, these values change as pH changes and WTA is also chemically removed from the cell wall. We therefore envision a cell wall binding model in which peptidoglycan provides the metal binding sites required for structural integrity and WTA provides the metal sites required for bacterial cytoplasmic biochemistry. (3 16)

The observation of two distinct binding regions has not been demonstrated in previous studies of metal binding to cell walls. Metal binding affinities have always been expressed as a single value using experimental protocols that mixed different samples containing a single concentration of metal ion . Comparison with literature reports the binding affinity of region I for Ca2+ with peptidoglycan, and WTA also contains region II, binding 1.8-fold greater from previous study . Observation of region I is important because if the binding data are limited to region II only, it can be misleading that the binding is weak. A similar trend is observed for Mg2+ binding to cell wall fragments. The binding affinity of region I for Mg2+ was previously reported for a fragment of a cell wall sample containing both peptidoglycan and WTA . Region-binding II is 3.1 times larger than the previous report, whereas it is 28 times larger than the previous report.(18)

Furthermore, there was little difference in the metal-binding properties of Mg2+ and Ca2+ in our cell wall metal-binding data, and that of Zn2+ and Cd2+ (10). RNA has a poly(ribose phosphate) backbone similar to the poly(glycerol phosphate) backbone of teichoic acid. Mg2+ ions bound to RNA have also been found to exhibit curvature in Scatchard plots (19). This behavior has been explained as a result of electrostatic changes leading to negative cooperativity or as two classes of binding sites . Class 1 is the group of strongly bound ions and class 2 is the group of weakly bound ions that cause tails in the Scatchard plot . As a result, diffuse ionic binding is based on long-range electrostatic interactions, which do not obey the laws of mass action, and thus conventional equilibrium constants cannot be defined (20). Other studies on RNA have shown that ionic strength plays a major role in determining binding affinities with RNA and metal ions, thus providing evidence for the classification of two classes of binding sites. . Application of this electrostatic model at low sodium concentrations has been shown to fit experimental data well . It has been shown that as the concentration of competing monovalent cations increases, electrostatic effects reduce the curvature of the Scatchard plot and subsequently decrease the affinity .(21)

As a result, the reported stability constants for metal binding to polyelectrolytes are directly dependent on many variables such as ionic strength, temperature and divalent metal ion . The dependence on ionic strength appears to have a greater impact on region I of the Scatchard plot (often referred to as class 1 binding sites in other publications) . Individual experiments with different ionic strengths were not performed, but based on observations with other polyelectrolytes, one can expect the affinity within region I to decrease with increasing ionic strength . If the cell wall electrostatic potential is already neutralized by the binding of cationic metal ions, the electrostatic effect is less pronounced. concentration (10).

By comparing the binding of both cell wall fragments (including WTA covalently attached to peptidoglycan) to the binding of peptidoglycan alone, it has been reported that peptidoglycan contributes half of the cell's metal-binding capacity . These results indicate that calcium has a binding constant and a binding capacity for cell walls containing both peptidoglycan and WTA Peptidoglycan alone was found to have a binding constant and a binding capacity . Although our region I binding constants are much larger, the binding capacity data are similar to those reported by Matthews et al. . For *B subtilis*, peptidoglycan is responsible for 47% of Ca2+ binding, but 34% of Mg2+ binding. The apparent difference in binding capacity between Ca2+ and Mg2+ and peptidoglycan is not statistically different with 95% confidence intervals. The observation that calcium binds to peptidoglycan at levels similar to Mg2+ contrasts with previous reports that teichoic acid alone is responsible for Ca2+ binding to the cell wall . Since metal binding strongly depends on the electrostatic properties of cells, we cannot expect perfect specificity for any particular divalent cation. (22,23)

Based on statistical t-test analysis, no obvious differences are observed between the affinities or binding capacities of specific metal ions for the cell wall. In subsequent studies by Doyle et al. For Ca2+, Mn2+, Ni2+, Sr2+, Zn2+, and Mg2+ . Similarly, the binding capacities for all divalent metal ions are excellent with values . It turns out that Similar to Ca2+, Mn2+, Ni2+, Sr2+, Zn2+, Mg2+ (3,24)

First, experiments were performed at a measured pH of 5.65, with dissolved carbon dioxide . Additional experiments were performed with a low concentration of 0.001 M HEPES buffer adjusted to pH 7.25 to investigate the effect of pH on the binding constant and binding capacity of Mg2+. Increased binding capacity due to more binding sites. This makes sense for both regions I and II, as they bind to the carboxyl groups of diaminopimelic acid. Increasing the pH with NaOH causes binding site competition between Na+ and Mg2+ . However, due to its high charge density, Mg2+ overwhelms Na+ binding and preferentially displaces Na+, resulting in higher binding capacity. There appears to be a change in regions I and II when the pH is increased from 5.65 to a buffered pH of 7.25. The decrease in region I affinity constant is likely due to the trace addition of NaOH to adjust the pH to 7.25. The slightly higher ionic strength is expected to reduce the electrostatic potential of the cell wall (25). However, although an increase in KA is observed in region II, statistically this increase makes no difference between pH 5.65 and 7.25. (26)

The two regions of binding affinity on the Scatchard plot are sometimes described as two classes of binding sites. The washed cell wall contains two types of binding sites (carboxyl and phosphoryl groups). However, we also observe two regions of binding affinity for peptidoglycan purified . A two-site model predicts a similar volume of region I between the two samples, but this is not observed. Rather, the binding capacities of regions I and II are best interpreted in terms of electrostatic effects, although these two regions cannot be assigned to specific functional groups. (27,28)

Conclusion

In these experiments, the binding constants and capacities of metal ions (Mg2+) were determined using purified *Bacillus subtilis* cell wall fragments containing either peptidoglycan or peptidoglycan with covalently attached WTA. We found much higher metal ion stability constants than those previously reported. It has similar metal binding capacity he tested no significant difference between the two ions (Mg2+). This is in contrast to the binding capacity results published by Beverridge. for Mg2+, as well as showing that teichoic acid alone is responsible to the cell wall binding capacity. Metal binding affinity values have been found to depend on the amount of metal bound to the sample due to electrostatic effects. Under the conditions used in these experiments, we obtained much higher binding constants than previously reported.

Metallic bonding is believed to be primarily an electrostatic phenomenon at low ionic strengths. This model should be able to explain how bacteria grow under different conditions, including the amount of divalent metal ions present. We show that the cell wall has a strong affinity for metal ions in solution when the ionic strength is low. This property may be part of a cell survival mechanism that traps and binds important bioactive divalent metal ions to the cell wall. Metal ions are required for cell wall integrity, and binding to peptidoglycan provides this stability. However, cell wall-associated metals also serve as reservoirs from which the cytoplasm draws metals when extracellular metals are scarce. The initial metal binding event is very strong, but eventually transitions to weaker binding when the metal is abundant in the cell wall. Weak binding indicates that metals may return to the extracellular fluid, but these metals may also penetrate the cell wall and enter the cytoplasm . When metal-rich, these weakly interacting cations are available for biochemical processes without the need to remove the peptidoglycan-bound metal.

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