Water–Rock Interaction, Wanty & Seal II (eds)

Molecular simulations of metal-bacteria systems

K.J. Johnson & J.B. Fein
University of Notre Dame, Notre Dame, IN, USA

R.T. Cygan
Sandia National Laboratories, Albuquerque, NM, USA

ABSTRACT: We apply molecular simulation techniques to examine the atomic-scale interactions that occur between cations and the cell-wall components of bacteria. We examine the adsorption of Cd^{2+} and Pb^{2+} onto peptidoglycan and teichoic acid components of the bacterial cell wall using classical energy force-field methods. Within the framework of molecular mechanics and the Cerius^2 modeling program, we use energy minimization, conformational analysis, and molecular dynamics to examine the different components of the cell wall and determine binding energies and configurations of the cell-wall components, both with and without the metals present. This approach allows us to compare the adsorptive properties of individual functional groups in terms of bond distance and binding energies.

1 INTRODUCTION

Bacteria have been found in most near-surface fluid-rock systems. Studies have shown that bacteria exhibit a strong affinity for the adsorption of metals and thus bacterial adsorption can affect contaminant transport, mineral formation, bioavailability, and mineral solubility. They are also of great interest due to their applications in bioremediation.

In recent years, the adsorption interactions of aqueous metal cations onto the surfaces of bacteria have been extensively studied using primarily laboratory and field techniques. For the most part, adsorption of metal onto the bacterial surface has been modeled as a bulk partitioning process, with the major concern being the amount of metal adsorbed to the bacteria cell wall, not the specific site of adsorption. Surface complexation models (SCMs) are more flexible than the bulk partitioning approaches, but the application of SCMs to bacterial systems requires a detailed understanding of the chemistry of the cell wall and of the specific adsorption reactions that occur. Bulk adsorption measurements and studies using techniques such as surface complexation modeling (SCM) and X-ray adsorption spectroscopy (XAS) offer constraints on the adsorption chemistry, but each approach has limitations. Bulk adsorption measurements provide only circumstantial evidence for the mechanism of metal adsorption onto bacterial surfaces. The XAS technique allows for a detailed study of the local environment and coordination of atoms on the cell wall, but it provides relatively loose constraints on the steric configuration of the adsorption sites and does not yield an overall understanding of the chemistry of the cell wall molecules. The objective of the present study is to provide a third, and complementary, approach for constraining the binding mechanisms involved in cation adsorption onto bacterial surfaces. Using molecular mechanics, we are able to model metal binding to a range of functional groups on the cell wall polymers and identify the specific types of carboxylate groups and phosphoryl groups, and the individual binding energies and distances for monodentate and bidentate complexes involving those sites.

2 CELL-WALL CHARACTERISTICS

The species modeled in this study is *Bacillus subtilis*, a common gram-positive soil bacterium. The cell wall of *B. subtilis* is composed of two major components, peptidoglycan and teichoic acid that contain carboxyl and phosphoryl functional groups, respectively (e.g. Beveridge & Murray 1980). These two functional groups are believed to be the dominant active sites for metal adsorption on the cell wall of *B. subtilis* at near-neutral pH. The peptidoglycan structure consists of two sugars, N-acetyl glucosamine

(NAG) and N-acetyl muramic acid (NAM), with a side peptide chain attached to the NAM. The peptide chain includes four amino acid groups with the *D*-glutamic acid and the *meso*-diaminopimelic acid (DAP) containing the two carboxyl groups of interest. Peptidoglycan comprises up to 50% of the cell wall by mass (e.g. Beveridge & Murray 1980). Teichoic acids comprise the other major portion of the cell. Teichoic acid is a polymer of glycerol linked by phosphate groups, the active adsorption site; *d*-alanine may also be present. There are generally 20–30 residues present in a chain and teichoic acid can represent up to 70% of the dry mass of the cell wall (Ellwood & Tempest 1969). The teichoic acid is linked covalently to the peptidoglycan sugars by a linkage unit containing two sugars and a phosphoryl group. The phosphoryl group in the linkage unit is also active in adsorption.

Potentiometric titration experiments yield site concentrations and acidity constants for the dominant proton-active functional groups on the cell wall. It has been inferred that functional groups with pK_a values of 4.6 and 6.9 represent carboxyl and phosphoryl sites, respectively, but the titration data provide only circumstantial evidence for such identification (Fein et al. 2003). Due to the presence of these functional groups, and the distribution of their acidity constants, the surface charge, and hence metal adsorption properties, of the bacterial surface is highly pH dependent. In this molecular simulation study, we consider all functional groups to be fully deprotonated to represent the pH dependence of the bacteria surface for metal adsorption. The valence electron of the deprotonated carboxylate group is considered to be delocalized between the two oxygen atoms and becomes the major site of metal adsorption.

3 SIMULATION METHODS

Molecular simulations were performed to obtain the energy optimized configuration for each structure. All atomic positions were allowed to freely translate during each simulation. The Consistent Valence Force Field (CVFF) was used to describe the interatomic potentials among the various atoms of the system. Through this force field, each atom has an assigned partial charge and a set of parameterized analytical functions to describe bonded and non-bonded interactions. The CVFF was originally parameterized for applications involving peptide and protein structures.

Molecular simulations were used to graphically develop three-dimensional models of the peptidoglycan and teichoic acid. To model the critical intra-molecular interactions of peptidoglycan and

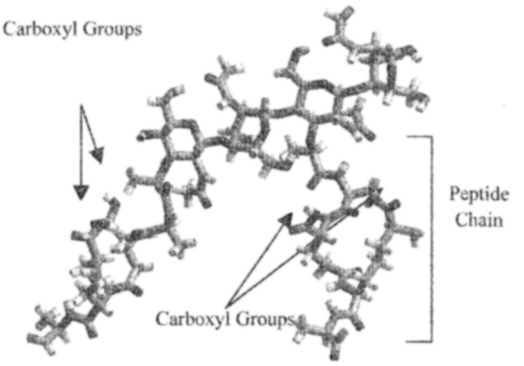

Figure 1. Optimized configuration of a protonated peptidoglycan dimer.

teichoic acids within the cell wall, the potential energy of the system must be defined. The total potential energy for the simulation is obtained through the summation of the following energy components:

$$E_{Total} = E_{coul} + E_{VDW} + E_{Bond\ Stretch} + E_{Torsion} + E_{Angle\ Bend} \quad (1)$$

The Coulombic and van der Waals energies represent the non-bonded terms, and the bond stretch, angle bend, and torsion correspond to the bonded interactions. The non-bonded terms control the binding and sorption of the metal cation to the organic molecules, whereas the bonded terms generally describe the atomic configuration within the organic framework. The E_{Coul} accounts for the long-range electrostatics interactions and the van der Waals energy, E_{VDW}, represents the short-range interactions that prevent the overlap of atomic electronic clouds. In this investigation, we recognize that E_{Coul} and E_{VDW} are the dominant components of the total potential energy that control sorption of the metals to the cell wall.

3.1 *Model development*

The CVFF force field was applied to the simple monomer representations of peptidoglycan and teichoic acid, and the structures were optimized until a global minimum energy was reached. This procedure was facilitated through the graphical interface within the Cerius^2 modeling software. Once the monomers models were developed, we were able to link them and create dimers (Fig. 1), peptidoglycan-teichoic acid structures, and a larger peptidoglycan strand. The optimized potential energies from these various structures were recorded and used to evaluate the metal-organic interactions.

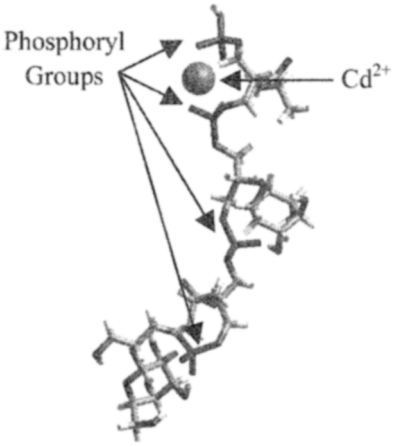

Figure 2. A teichoic acid dimer with two phosphoryl groups interacting with Cd^{2+}. Using molecular mechanics we are able to measure both the binding energies and the binding distance of the metal-ligand complex.

3.2 *Metal interactions*

We were able to successfully simulate the interactions of metal cations with our model cell wall components of *B. subtilis*. Following optimization of the simple structures, the functional groups of interest were deprotonated to represent a circumneutral pH. The structures were further energy optimized and examined to ensure that a global energy minimum was attained. Once fully optimized, a cadmium or lead cation was placed at an arbitrary distance from each functional group of interest. The system was minimized again resulting in a metal ion coordinated or sorbed to the functional group (Fig. 2). The binding energies for the individual cations were then derived by comparison of the potential energy of the cell wall models with those having associated metals.

4 RESULTS AND DISCUSSION

4.1 *Structural optimization*

Through the use of molecular modeling we successfully developed models of the major metal-binding components of the *B. subtilis* cell wall. The overall structure of the peptidoglycan and teichoic acid remain relatively stable with most conformational changes occurring near the deprotonated functional group to obtain the most stable metal-ligand configuration. These atomistic models allow for a better understanding of the response of the cell wall to both pH changes and cation interaction.

4.2 *Metal binding*

The peptidoglycan and teichoic acid structures were optimized in the presence of Cd^{2+} and Pb^{2+}. No solvating water molecules were incorporated in these simulations. Studying the individual binding energies of peptidoglycan monomer linked to the teichoic acid dimer, we were able to compare the binding energies of individual functional groups. As expected, bidentate adsorption of the metal provides a greater binding energy for both Cd^{2+} and Pb^{2+}, with the bidentate Cd-phosphoryl group structure having the highest binding energy of 2186 kJ/mol. For monodentate pairings, the glutamic acid carboxylate group exhibited the highest binding energy for both cations, 2047 kJ/mol for the Cd-carboxylate adsorption. Only a relative comparison of these values should be made due to the inability of the CVFF parameters to accurately model sorption energy that experimentally is about ten times smaller. Hydration effects, which are ignored, would also significantly lower these binding energies.

The binding energies for Cd^{2+} and Pb^{2+} are similar when bound to individual functional groups; however, Cd^{2+} displayed a higher binding energy than Pb^{2+} for nearly all adsorption sites. This is opposite of what we observe in bulk adsorption experiments (Borrock & Fein 2003) and indicates that our simple sorption models are not sufficient in representing the bacteria-metal interactions. Hydration effects and possibly covalent binding at the sorption site are processes that cannot be ignored in the simulations.

5 CONCLUSIONS

Molecular simulations provide a complementary approach to SCM and XAS in examining the detailed chemistry of the bacterial cell wall and the specific adsorption reactions that may occur. With this technique we are able to model and compare specific carboxylate and phosphoryl groups and their individual binding energies and distances from cations. Molecular dynamics simulations were also developed to examine the structures derived from the energy optimizations, and to model the influence of water solvation of the cell wall components in the presence of solvated Cd and Pb cations. Future modeling efforts will expand the complexity of the molecular dynamics simulations and will investigate covalent binding effects.

REFERENCES

Beveridge, T.J. & Murray, R.G.E. 1976. Uptake and retention of metals by cell walls of *Bacillus subtilis*. *J. Bacteriol.* 127: 1502–1518.

Beveridge, T.J. & Murray, R.G.E. 1980. Sites of metal deposition in the cell wall of *Bacillus subtilis*. *J. Bacteriol.* 141: 876–887.

Cygan, R.T. 2001. Molecular modeling in mineralogy and geochemistry. In R.T. Cygan & J.D. Kubicki (eds.), *Reviews in Mineralogy and Geochemistry: Molecular Modeling Theory: Applications in the Geosciences* 1–35. Washington D.C.: The Geochemical Society.

Ellwood, D.C. & Tempest, D.W. 1969. Control of teichoic acid and teichuronic acid biosynthesis in chemostat cultures of *Bacillus subtilis* var. niger. *Biochem. J.* 111: 1–5.

Fein, J.B., Boily, J.-F. & Yee, N. 2003, submitted. Potentiometric titrations of *Bacillus subtilis* cells and a comparison of modelling approaches. *Geochim. Cosmochim. Acta.*

Fein, J.B., Daugherty, C.J., Yee N. & Davis, T.A. 1997. A chemical equilibrium model for metal adsorption onto bacterial surfaces. *Geochim. Cosmochim. Acta.* 16: 3319–3328.

Graham, L.L. & Beveridge, T.J. 1994. Structural differentiation of the *Bacillus subtilis* 168 cell wall. *J. Bacteriol.* 176: 1413–1421.