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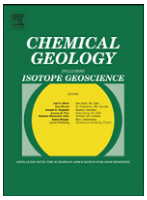

Surface complexation of the phototrophic anoxygenic non-sulfur bacterium *Rhodopseudomonas palustris*

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ABSTRACT

This work extends our knowledge of anoxygenic phototrophic bacteria (APB) interactions and contributes to the database of aquatic microorganism trace element adsorption parameters, such as heavy metal pollutants and limiting micronutrients. The surface properties of a phototrophic anaerobic non-sulfur bacterium (*Rhodopseudomonas palustris* KR-95p), which is often considered a model microorganism for Precambrian anoxygenic primary producers, were studied using potentiometric acid-base titration at various ionic strengths. The electrophoretic mobility of viable cells was measured as a function of pH and ionic strength, as well as cation (Zn, Cd, and Pb) and anion (Mo, W, and Cr) concentration. Zn, Cd, Sr, Pb, Cu, Cs, Al, Ga, Sb, As, Ge, Tl, W, and Mo adsorption was studied as a function of pH at a constant initial element concentration, yielding the pH-dependent adsorption edge. Zn, Cd, Sr, Cu and Pb adsorption was also studied as a function of metal concentration in solution at a constant pH, producing a "Langmuirian" adsorption isotherm. The non-electrostatic linear programming method (LPM) was used to fit the experimental data and assess the number of surface sites and adsorption reaction constants involved in heavy metal and anion binding to the cyanobacteria surface.

The total H/OH binding site number (80–480 μmol/g_{wet}) for *Rps. palustris* encompasses the values reported for other APB (60–120 μmol/g_{wet}) and cyanobacteria (50–200 μmol/g_{wet}) examined using the same technique. For the cations, the adsorption edge follows the order $Pb^{2+} > Cu^{2+} \gg Cd^{2+} \approx Zn^{2+} > Sr^{2+} > Tl^{+} > Cs^{+}$, which is similar to the element hydrolysis order. Trivalent hydrolysates (Al^{3+} and Ga^{3+}) demonstrated increased adsorption with a pH increase at $2 \leq pH \leq 4$ followed by desorption from the bacterial surface at pH > 5–6 as negatively charged $Al(OH)_4^-$ and $Ga(OH)_4^-$, similar to anions, such as MoO_4^{2-} and WO_4^{2-}. This behavior directly reflects the level of trace element complexation with two primary binding sites at the surface, carboxylate and phosphorylate, and certain electrostatic-like interactions between negatively charged anions and positive amine groups in acidic solutions. The main results of the present study are that the *Rps. palustris* exhibit trace element binding properties through their pH-dependence and overall adsorption capacity, which are similar to properties described earlier for other phototrophic bacteria, both anoxygenic bacteria and cyanobacteria. The absolute adsorption capacities for metal cations follow the order *Rps. palustris* < *APB Rdb. blasticus* < cyanobacteria *Gloeocapsa* sp, which presumably reflects a greater concentration of free metal cations in the external milieu for this species. Correspondingly, the adsorbed anion concentration is greatest for *Rps. palustris*, which therefore yields a higher proportion of positively charged amine groups or fewer negative charges overall due to a lower fraction of carboxylates and phosphorylates.

1. Introduction

Among the primary producers of the Earth's hydrosphere, cyanobacteria and anoxygenic phototrophic bacteria (APB) have witnessed the most crucial changes in the ocean chemical composition and redox state (Canfield, 2005; Scott et al., 2008; Johnston et al., 2009; Raiswell and Canfield, 2012). These microorganisms have been preserved since early Precambrian; therefore, they have encountered a range of concentrations and bioavailabilities of dissolved metals, notably micronutrients (e.g., Zn, Cu, Cd, Ni, Co, Mn, and Mo), that is likely much broader than other bacteria and algae. Although anoxygenic photosynthesis was especially important in shallow marine environments before oxygenic photosynthesis and widespread atmospheric oxygenation (Olson and Blankenship, 2004; Canfield, 2005; Johnston et al., 2009), it remains dominant in numerous ecological niches, from

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oxyclines in stratified lakes and humid soils to bacterial biomats in thermal settings (Van Gemerden and Beeftink, 1983; Imhoff, 1992; Madigan and Jung, 2009). Thus, anoxygenic phototrophic bacteria will likely encounter a broad range of trace element concentrations. Among various APB, *Rhodopseudomonas palustris* is one of the most metabolically versatile bacteria (Frank et al., 2004). It can use sunlight, inorganic and organic compounds for energy. In contrast to other investigated APB, such as *Rdb. blasticus*, that can only grow autotrophically on hydrogen, *Rdp. palustrus* can grow autotrophically through sulfide and thiosulfate oxidation as well as photoautotrophically through Fe(II) oxidation (Jiao et al., 2005). It acquires carbon from many types of green plant-derived compounds or via carbon dioxide fixation, and it can fix nitrogen (Larimer et al., 2004). It is generally accepted that, during Earth's biosphere evolution, anoxygenic phototrophs preceded cyanobacteria for the essential portion of the Achaean and Early Proterozoic periods (Johnston et al., 2009; Lyons and Reinhard, 2009). Therefore, comparing protons and metal cations as well as anion adsorption parameters for cyanobacteria and various APB may shed new light on the potential affinity of micronutrients for cell surfaces of the main bacterial taxons and may aid in reconstructing the past trace element availability for aquatic bacteria.

Experimental investigations into trace elements (TE), such as micronutrients and metal toxicants, adsorption on anoxygenic phototrophic bacteria may have important environmental applications. However, compared with the detailed information on surface chemistry and metal adsorption affinities of heterotrophic aerobic and anaerobic bacteria (Fein et al., 1997; Langley and Beveridge, 1999; Small et al., 2001; Sokolov et al., 2001; Borrok et al., 2004; Haas, 2004; Borrok and Fein, 2005; Claessens et al., 2006; Claessens and Van Cappellen, 2007) as well as of cyanobacteria (Phoenix et al., 2002; Dittrich and Sibler, 2005, 2006; Pokrovsky et al., 2008), anoxygenic phototrophic non-sulfur bacteria remain poorly characterized, except for several studies focused on Fe redox chemistry and isotope fractionation (Croal et al., 2004; Kappler and Newman, 2004; Kappler et al., 2005; Posth et al., 2010) and a recent study on adsorption of several micronutrients on another APB, *Rdb. blasticus* (Pokrovsky et al., 2013). *Rps. palustris* was used in the present investigation due to its unique and complex properties. *Rps. palustris* is the most eurybionte and metabolically versatile purple nonsulfur bacteria, ubiquitous in all types of fresh water and humid soil habitats (Imhoff and Trüper, 1989; Oda et al., 2003; Imhoff, 2006). Compared with the earlier studied APB *Rdb. Blasticus, Rps. palustris* can use not only organic matter but also reduced sulfur compounds during photosynthesis. In addition, the *Rps. palustris* cells are approximately two-fold larger in terms of size than *Rdb. blasticus* cells, which exhibit a two-fold smaller specific surface square (~4.3 and ~8.3 μm^2/cell).

To extend the thermodynamic database on bacteria–micronutrient interactions for a large variety of trace elements, from micronutrients (Cu, Zn, and Mo) to indifferent (Ga, Cs, Sr, and W) and toxic (Al, Cd, and Pb) metals, we used a combination of acid–base titration, electrophoretic measurements with and without trace elements (TE), and TE adsorption as a function of solution pH and metal concentration. In particular, we aimed to 1) compare the cell-binding parameters among cations with various hydrolysis affinities; 2) investigate the degree of cation and anion complexation with a bacterial surface using electrostatic (electrophoresis) technique; and 3) apply thermodynamic modeling for metal, anion and proton interactions with non-sulfur anoxygenic phototrophic bacteria to quantitatively compare our data with data available for other phototrophic bacteria. We anticipate that reaching these goals should aid in constraining the TE interaction with APB surfaces in both the paleo and contemporary environments as well as provide a quantitative basis for distinguishing trace element adsorption trends among various groups of the first primary producers on the Earth.

2. Materials and Methods

2.1. Anoxygenic phototrophic bacteria culture processes and characterization

The anoxygenic non-sulfur phototrophic bacteria (APB) *Rhodopseudomonas palustris* KR-95p used in this study were obtained from the collection of the Laboratory of Ecology and Geochemical Activity of the Microorganisms (Winogradsky Institute of Microbiology, Moscow, Russia) and isolated from a layered cyanobacterial mat developed in a thermal sulfur spring (Neskuchenskiye springs, Kunashir, Kuril islands). The APB were cultured in Pfenning's growth medium with the following composition (Pfenning and Trüper, 1989; Kompantseva et al., 2010): KH_2PO_4 (330 mg/L), $MgCl_2*6H_2O$ (330 mg/L), NH_4Cl (330 mg/L), KCl (330 mg/L), Na_2SO_4 (330 mg/L), $CaCl_2$ (50 mg/L), $NaHCO_3$ (0.5 g/L), sodium acetate (1 g/L), casamino acids (0.1 g/L), yeast extract (0.1 g/L), B_{12} (20 μg/L), and a trace elements solution (1 ml/L). Stock bacterial cultures were maintained in air-free glass bottles at the light intensity 30 μmol photon m^{-2} s^{-1} and temperature 28–30 °C. Under such conditions, the bacteria reached the stationary growth phase after 1–2 weeks. All adsorption and titration experiments were performed using intact cells at the end the exponential-beginning of the stationary phase. Before each experiment described below, the bacteria were rinsed in the inert electrolyte solutions (0.01–1 M NaCl for the surface titration and 0.01 M $NaNO_3$ for the adsorption experiments). Using $NaNO_3$ as a substitute for NaCl during adsorption was necessary to avoid metal complexation with Cl^- at the relatively low TE concentration in solution.

The bacterial cell concentration was quantified by *1)* measuring the suspension optical density (O.D.) using a spectrophotometer at the wavelength 650 nm; the calibration curve O.D.-wet weight was linear up to 0.9 a.u.; *2)* weighing the wet centrifuged pellets (20 min at 5000 g); and *3)* freeze-drying the centrifuged pellets. The wet/freeze-dried weight conversion factor for *Rps. palustris* KR-95p was 10.0 ± 0.5.

2.2. Electrophoretic measurements

Microelectrophoresis is widely used to characterize the microbial cell surface electric double layer (EDL) (Richmond and Fisher (1973) and Van der Wal et al. (1997a) for principles and discussions). The viable cell electrophoretic mobilities were recorded using a "Zetaphoremeter IV" Z 4000 (CAD Instrumentation, France). The measurements were performed in quartz cells equipped with two Pd electrode chambers and illuminated by a 2 mW He/Ne laser. During the measurements, the electric field 80 $V \cdot cm^{-1}$ was applied in each direction, and moving cell images were transmitted to a computer via a CCD camera. The experiments were performed in 0.01, 0.01, 0.1 and 0.1 M NaCl solutions at pH values ranging from 1 to 10. Three replicates were performed, each with a new bacteria suspension. The uncertainty for the electrophoretic mobilities ranged from 5 to 20%, being the highest near the isoelectric point, as determined by repetitive analyses of standard TiO_2 and Al_2O_3 suspensions.

For consistency with previous studies on phototrophic bacteria, the electrophoretic mobilities measured in this study were converted to zeta potentials using the Smoluchowski-Helmholtz equation (Wilson et al., 2001):

$$\zeta = \frac{(\varepsilon \times \mu_E)}{(4\pi \times \eta)} \tag{1}$$

where ζ is the zeta potential (mV), and ε, η and μ_E represent the dielectric constant of the solution, viscosity and electrophoretic mobility, respectively ($\mu_E = V_E/E$ with V_E = electrophoretic rate (s^{-1}) and E = electric field ($V \cdot m^{-1}$)).