A complementary study of hydrophobicity and surface charge of *Thiobacillus ferrooxidans.* The effect of ionic surfactants

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Komplementárne štúdium hydrofóbnosti a plošného náboja baktérie* Thiobacillus ferrooxidans*. Efekt iónogénnych surfaktantov

Hydrofóbnosť a náboj bunečnej steny sú rozhodujúce faktory počiatočného štádia adhézie baktérií na tuhé povrchy. Hydrofóbnosť bakteriálnych buniek sa posudzuje najmä dvoma spôsobmi. Ako prvý spôsob, umožňujúci kvalitatívne určenie hydrofóbnosti, sa využíva sledovanie adsorpcie iónogénnych surfaktantov, resp. elektroforetickej pohyblivosti ktorá ju sprevádza. Druhým spôsobom semikvantitatívneho stanovenia hydrofóbnosti buniek je testovanie ich interakcie s kvapalnými uhľovodíkmi v tzv. BATH teste (Bacterial Adhesion To Hydrocarbons). Stanovenie hydrofóbnosti použitím BATH testu je však komplikované z dôvodu spolupôsobenia elektrostatickej interakcie, ktorú môžme opäť odhadnúť z elektroforetickej pohyblivosti buniek.

Cieľom práce bolo posúdiť hydrofóbnosť buniek Thiobacillus ferrooxidans oboma uvedenými spôsobmi zvlášť a v kombinácii, tj. BATH test bol uskutočnený v prítomnosti surfaktantov a súčasne bola meraná elektroforetická pohyblivosť buniek metódou ELS (Electrophoretic Light Scattering). V neprítomnosti surfaktantov bola zistená záporná elektroforetická pohyblivosť (náboj) a silná hydrofilnosť povrchu baktérií. V prítomnosti katiónaktívneho surfaktantu (CTAB) sa v dôsledku jeho postupnej adsorpcie elektroforetická pohyblivosť znižovala (dokonca pri vysokých koncentráciách prechádzala do kladných hodnôt). Súčasne sa indukovala hydrofóbnosť buniek. Po prekročení určitej CTAB koncentrácie sa však hydrofóbnosť začala znižovať, hoci elektroforetická pohyblivosť bola stále na zostupe (čo svedčí o neustávajúcom raste adsorpcie CTAB). To svedčí o adsorpcii CTAB katiónov s polárnymi skupinami orientovanými smerom k povrchu buniek a hydrofóbnymi reťazcami smerom do roztoku pri nízkych koncentráciách CTAB a o opačnej orientácii pri vyššej koncentrácii CTAB. Po pridaní aniónaktívneho surfaktantu (SDS) k adsorpcii nedošlo, o čom svedčí iba mierne zvýšenie zápornej elektroforetickej pohyblivosti a skoro vôbec neovplyvnená pôvodná hydrofilnosť buniek.

Na základe toho môžme predpokladať, že elektrostatické interakcie ovplyvňujú hydrofilnosť/hydrofóbnosť buniek T. ferrooxidans BATH testom iba v malej miere.

Key words: *Thiobacillus ferrooxidans, hydrophobicity, CTAB, BATH, electrophoretic, zeta potential*

Introduction

Studies of physico-chemical factors influencing initial attachment of bacteria onto solid (mineral) substrates are often based on complementary surface charge and hydrophobicity measurements (see e.g. Gilbert et al. 1991). Similarly, in physico-chemical models of bacterial adhesion, surface charge (zeta potential) and hydrophobicity (surface energy) of bacterial cells and/or mineral particles are considered to be important (but independent, i.e. additive) parameters (Škvarla, 1993).

The BATH hydrophobicity test is frequently used to evaluate the bacterial cell wall hydrophobicity (van der Mei et al., 1991). In this test, aqueous suspension of bacterial cells is mixed with a test liquid hydrocarbon under well controlled conditions. After mixing, the two phases are allowed to separate. If the cells are hydrophobic, they adhere to apolar hydrocarbon phase and rise with it to form an upper hydrocarbon „cream". If the cells are hydrophilic, they remain in the bulk polar aqueous phase. The measure of the (relative) hydrophobicity of the bacterial cell wall is the proportion of cells bound to the hydrocarbon phase, determined by measuring the decrease in light absorbance of the aqueous phase. However, apart from a theoretical uncertainty of the additivity assumption for the surface hydrophobicity and charge in the microbial adhesion models in general, it is the BATH test itself, which is difficult to interprete. The argument is that during the mixing and separation stage of the BATH test, besides the hydrophobic/hydrophilic interactions (which is the test based on), interfering electrostatic double layer interactions also operate between droplets and cells that can in principle (if not suppressed) overestimate (when attractive) or underestimate (when repulsive) the resultant proportion of hydrocarbon-bound cells, i.e. their hydrophobicity parameter. Really, it has been experimentally verified that an increase in ionic strength of the aqueous medium may result in an „enhanced" hydrophobicity of bacteria, due to the elimination of the repulsive electrostatic interactions. But how one can distinguish between a „true" and „electrostatically mediated" BATH cell wall hydrophobicity ? One possibility is to compress the double layer of both bacterial cells and hydrocarbon droplets in the BATH test by increasing the ionic strength

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(Recenzované, revidovaná verzia dodaná do 8.3.2002)

of the aqueous solution with an indifferent salt. Simultaneously, the true surface charge of the bacterial cell wall with the „decompressed“ double layer can be measured microelectrophoretically at the initial (lower) ionic strength.. Alternatively, both BATH hydrophobicity and surface charge measurements can be applied using an identical solution of a low ionic strength. In this case, however, when interpreting the measured BATH hydrophobicity of cells, their (and hydrocarbon droplets´) surface charge should be taken into account.

Another possibility of evaluating the bacterial cell wall hydrophobicity (only qualitatively) is to measure the surface charge of cells in the presence of various ionic surfactants (James, 1991). Organic molecules of anionic surfactants (anions) are preferentially adsorbed on the (obviously negatively charged) lipophilic cellular surface via their nonpolar moieties, with the negative polar groups oriented towards the aqueous medium. Hence, an increase in the negative surface charge of cells in the presence of anionic surfactants may reveal a hydrophobic character of the cell wall. In contrast, cationic surfactant organic molecules (cations) adsorb especially via their positive polar groups, lowering the negative surface charge of cells followed by a charge reversal. In the presence of lipids in the cell wall the charge reversal concentration of the surfactant is lower than that for a nonlipid cell wall.

The aim of this paper was to compare both mentioned methods of monitoring the cell wall hydrophobicity of *Thiobacillus ferrooxidans*. Also we tried to combine them, i.e. to follow the surfactant adsorption effect in the BATH test. The problem is that, when studying the effect of a surfactant adsorption on the BATH hydrophobicity of cells in the aqueous medium, the situation is more complicated in that a variation in the hydrophobicity may occur not only due to the surfactant partitioning to the cells´ surface but also to the hydrocarbon droplets´ surface, unless the surfactant-treated cells are washed and resuspended to remove unadsorbed surfactant molecules from the aqueous phase before mixing it with the hydrocarbon phase. Goldberg et al. (1990), studying the influence of cetylpyridinium chloride (CPC) on the microbial adhesion to hexadecane, showed that the adhesion-promoting effect of CPC was due to the partitioning of the cationic surfactant to the cell wall/water but not to the hexadecane/water interface.

This paper is a continuation of the research on the surface properties of *Thiobacillus ferrooxidans* (Škvarla and Kupka, 1996a,b).

Materials and methods

Bacterial cells preparation

The used *Thiobacillus ferrooxidans* strain Tf [CCM 3973] was originally isolated from the Smolnik (Slovakia) mine acid water. For the experiments, cells were grown aerobically in the 9 K liquid medium with $FeSO_4$ as a main energy source at pH = 1.6 and 30^o C. Cells were harvested at the early stationary growth stage (surfactant adsorption experiments) or at various phases (growth experiments) by membrane filtration and washed with 0.1 M HCl and distilled water to remove Fe-compounds. Cells were finally suspended in distilled water and diluted as needed.

Surface charge

0.7 ml of a surfactant [cationic cetyltrimethylammonium bromide (CTAB) or anionic sodium dodecyl sulfate (SDS)] solution with varying concentrations was added to rectangular (1cm^2) disposable acrylic microcuvettes containing 0.7 ml of the cell suspension (1.4 ml of the cell suspension was added to the microcuvette without the surfactant). The electrophoretic mobility, μ_e, was determined as a measure of the surface charge of cells by the electrophoretic light scattering (ELS) technique in the Zeta Plus apparatus (Brookhaven Instruments Corp., USA). The maximal standard error was typically 0.05×10^{-8} m^2 s^{-1}V^{-1}.

Hydrophobicity

The adhesion of cells to hydrocarbon droplets was adopted to evaluate their relative hydrophobicity (BATH test). 0.14 ml of hexane was added to the microcuvettes containing 1.4 ml of the bacterial cell suspension (with or without the surfactant) immediatelly after completing the ELS measurements. The cell suspensions were vortexed for 60 s. After phase separation for the duration of 10 minutes, the absorbance of the lower aqueous phase was measured at 546 nm in the Spectronic 401 spectrophotometer (Milton Roy Com., USA) with a precision of 0.001. The relative hydrophobicity of cells, H, was calculated as the percent decrease in the absorbance of the aqueous cell suspension after 10 minutes (A_{10}) as compared to the absorbance of the original suspension (A_0), i.e. $H = 100(1-A_{10}/A_0)$.

Results

To qualitatively evaluate the native hydrophilicity of the *Thiobacillus ferrooxidans* cell wall, surfactants were added to the cell suspension. The influence of the cationic CTAB on the electrophoretic mobility is shown in Fig.1 for three different cell concentrations (characterized by A_0 = 0.018, 0.021 and 0.043). It can be seen that initially negative cells are recharged at c_{CTAB} of ca. 7 μM (A_0 = 0.018), 13 μM (A_0 = 0.021) and >30 μM (A_0 = 0.043). Apparently, the CTAB organic cations are adsorbed on hydrophilic cells in proportion to their total surface area available through polar heads with the hydrocarbon chains protruding to the solution. It is expected that this makes the outer bacterial cell wall increasibly hydrophobic. To confirm the natively hydrophilicity of the cell wall, the anionic SDS was also added to the cell suspension. Since the negative electrophoretic mobility of cells is barely increased in the presence of c_{SDS} up to 30 μM or even 125 μM (lytic concentration for SDS), the outer cell wall of *T. ferrooxidans* is really proven hydrophilic with lipopolysaccharides (and not with lypophilic lipids) as a dominant component.

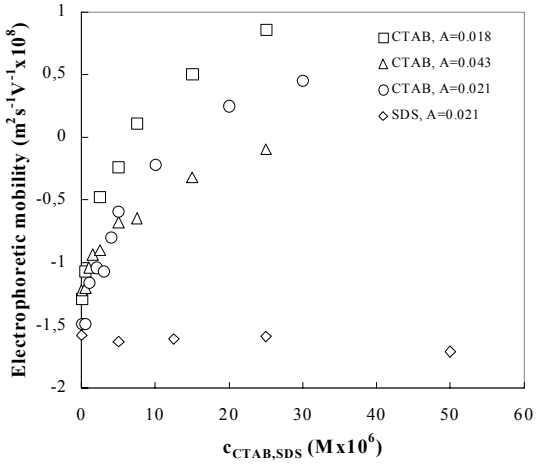

Fig.1 Electrophoretic mobility of Thiobacillus ferrooxidans *as a function of CTAB and SDS concentration*

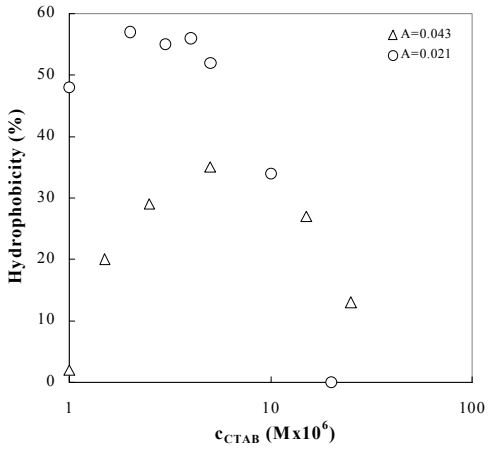

Fig.2 Hydrophobicity of Thiobacillus ferrooxidans *as a function of CTAB concentration*

To evaluate the native hydrophilicity and CTAB-induced hydrophobicity of *T. ferrooxidans* quantitatively, the BATH test was used. We assume that CTAB is completely dissociated into organic cations. That is, no neutral undissociated molecules, that might be transferred to the hexane phase, are considered to be present in CTAB solutions used. Fig.2 shows the dependence of the relative hydrophobicity parameter H on c_{CTAB}. One can notice that at c_{CTAB} = 0 the hydrophilic and negatively charged cells *of T. ferrooxidans* are not adhered to hexane drops (H = 0).

At low c_{CTAB} we can see a continuous increase in H with increasing c_{CTAB}. The CTAB-mediated decrease in the negative surface charge of cells (see Fig.1) may favour their electrostatic attraction to the (probably slightly negatively charged) hexane drops. But, we believe the increase of H with c_{CTAB} is a result of the increasing hydrophobicity of cells due to the formation of adsorption layer of CTAB cations bound to negatively charged groups (and with hydrocarbon tails oriented toward solution) at the cell surface. A break in the above

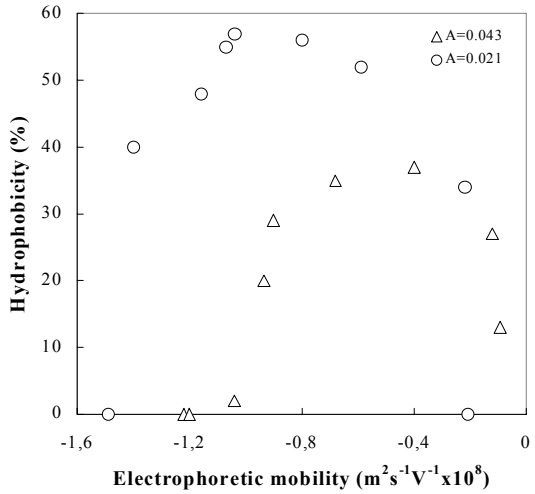

Fig.3 Correlation between hydrophobicity and electrophoretic mobility of Thiobacillus ferrooxidans

tendency is observed at c_{CTAB} ca. 3 μM (A_0 = 0.021) and 7.5 μM (A_0 = 0.043) when a maximal hydrophobicity is observed (H ≅ 50 %). At higher c_{CTAB}, H starts to decrease despite of the less negative electrophoretic

mobility. Probably, CTAB cations are continuosly adsorbed on the cell surface but with the hydrocarbon tails being oriented toward the cell surface. Free CTAB cations can also be present in the solution. This leads to a decrease in the surface hydrophobicity of the cells. Note that the „optimal" CTAB concentration for adhesion to hexane depends on the (generally low) initial cell concentration as is also seen for the isoelectric point (Fig.1). Fig.3 shows a correlation between H and the electrophoretic mobility of cells.

Conclusions

Two methods have been applied (individually and combined) to evaluate hydrophobicity and surface charge of *Thiobacillus ferrooxidans* in the presence of ionogenic surfactants, namely the electrophoretic mobility and the BATH test. It has been found that the cell wall of the bacterium is negatively charged and strongly hydrophilic. The electrostatic interaction between the hydrocarbon phase and the bacterium cell wall (due to its surface charge), although interfering with the hydrophobic interaction in the BATH test, influences the resultant hydrophobicity parameter marginally.

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