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Influence of exopolysaccharides on the electrophoretic properties of the model cyanobacterium *Synechocystis*

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ABSTRACT

The influence of extracellular polymeric substances (EPS) on cell electrokinetics was investigated in the model cyanobacterium *Synechocystis*, in wild-type strains and in ten EPS-depleted mutants. The charge density and the softness of the EPS polyelectrolyte layer were calculated from the dependence of the electrophoretic mobility values of the cells with the ionic strength of the surrounding fluid. Electrophoretic mobility data showed that the eleven *Synechocystis* strains investigated behave as soft particles and cannot be adequately described by classical electrokinetic models of rigid particles. EPS surrounding the cells, especially those released in the growth medium, significantly increased the softness of the cell surface. Furthermore, the anionic nature of EPS resulted in negative surface charge densities, which appeared to be strongly dependent on the composition of the suspending fluid, as documented by a strong reduction of their absolute values in the presence of calcium cations. These finding stresses the importance of the physicochemical properties of EPS and cell surfaces of cyanobacteria, for both cell-to-medium and cell-to-cell communications. In turn, these results emphasize that, whenever possible, natural waters should be used for meaningful ecotoxicological analyses of potential toxics.

1. Introduction

Bacteria constitute the most successful form of life on Earth. They colonize most biotopes and produce a huge biomass (5×10^{30} cells and $35–55 \times 10^{16}$ g of carbon), which represents up to 60–100% of the estimated total carbon in plants [1,2]. The principal reasons for the success of bacteria are their physiological robustness as well as their abilities to rapidly respond and adapt to environmental stimuli [3]. Biofilm is the predominant mode of bacterial growth in most natural, industrial and clinical environments [4]. The biofilm lifestyle is associated with a high tolerance to environmental stresses, including treatments with antibiotics or other biocides. Hence, biofilm formation is a major concern in environmental and biotechnological applications. Biofilms typically consist of densely packed, multispecies populations, encased in a cell-synthesized polymeric matrix attached to a surface [5]. These microbial extracellular polymeric substances (EPS) are heterogeneous in complexity and composition, which varies depending on the microorganisms [6–8]. EPS contain soluble ions and colloidal insoluble matters and are mainly composed of polysaccharides (neutral and acidic), proteins, nucleic acids, lipids and other biological macromolecules [9]. They provide a highly hydrated gel matrix structure that mediates most of the cell-to-cell and cell-to-surface interactions, which are required for the formation and stabilization of biofilms. In EPS, charged groups may associate or dissociate upon changes in pH or ionic strength of the suspending fluid, or upon the approach of a new charged surface of another cell. Regarding their physicochemical properties, EPS can be assimilated to polyelectrolytes since this matrix is composed of long polymers whose gelation is promoted by multivalent cations [10].

The electrophoretic mobility of an object in a liquid is a macroscopic signature of its surface state in interaction with the surrounding fluid and it can be measured by different means. So, electrophoretic mobility is both a rapid and effective technique to assess the stability and behavior of particles in suspension. This analysis was initially developed for objects having a hard surface and has been very successful in hard colloids science. However, the classical electric double layer model is not appropriate for bacterial cells because their surfaces display complex structures. In hard colloids, the electric charges on the outer surface determine the electrophoretic mobility. However, the permeability of the bacterial surface layer to ions and molecules from the

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surrounding fluid strongly influences the mobility. In addition the mobility of bacteria is affected by the non homogeneous spatial distribution of the charged groups present at the cell surface. This is why Ohshima and co-workers [11] developed an alternative model, called the "soft particle model", which is better suited to the analysis of bacterial cells, i.e. biological core particles surrounded by a ion-penetrable EPS layer that strongly influences electrophoretic mobility. This "soft particle electrophoresis theory" was successfully used in several studies of the cell surface of the enterobacterium *Escherichia coli* [12,33] and of other non-photosynthetic bacteria [13–18,34]. However, soft particle electrophoresis has not yet been employed to study the cell surface of cyanobacteria, the cosmopolitan photosynthetic prokaryotes that produce a large part of the terrestrial di-oxygen [19] and biomass for the food chain [20], and which have the potentials for the sustainable production of biofuels [7,21] and bioplastics [22], and for the bioremediation of polluted soils and waters [23]. Many cyanobacteria possess extracellular polymeric substances, mainly of polysaccharidic nature (EPS) [24], which protect cells against environmental stresses (UV, salt, desiccation, heavy metals) and enable them to form biofilms [13]. The cyanobacterial EPS, which are complex [23], can be divided into two main groups: the RPS (released polysaccharides), which are secreted by cells into the surrounding environment, and the CPS (capsular polysaccharides), which are attached to the cell surface [24]. In this study, we investigated the influence of EPS on the electrophoretic mobility of the widely used unicellular model strain *Synechocystis* PCC6803 (hereafter *Synechocystis*), which makes up complex anionic EPS that contain 13 different monosaccharides and various uronic acids [9,25]. For this purpose we used, and compared, the wild type strain (WT) and 10 EPS-depleted mutants that were recently constructed [26]. The electrophoretic mobilities of these 11 strains were analyzed in the framework of the Ohshima's soft particle theory. We also studied the influence on electrophoretic mobility and cell surface parameters of the counter-ions nature in the bulk suspension, emphasizing the strong role of calcium.

2. Materials and methods

2.1. Bacterial strains and culture conditions

The round unicellular cyanobacterium *Synechocystis* PCC6803 strains wild-type (WT) is considered in this work together with and 10 of its EPS-depleted mutants that were recently constructed the details of which are fully reported in [26]. In the present work, the bacteria were grown aerobically under shaking (180 rpm, Infors Multitron II) and white light (2500 lux; 31.25 $\mu E\,m^{-2}\,s^{-1}$ Mazda TF 16 W lamps) at 30 °C on BG11 medium [27] enriched with 3.78 mM Na_2CO_3 [28] thereafter referred to as MM for standard mineral medium (see composition on Supporting information S1). The main composition of *Synechocystis* PCC6803 EPS is detailed in Supporting Information S2. The EPS-depleted mutants were generated after the single or double deletion of the genes sll0923, sll1581, slr1875 and sll5052 that share sequence homology with EPS-production genes in non-photosynthetic bacteria [29,30]. For commodity, all four single deletion mutants are designated as X^- (for instance 0923^- stands for the deletion of sll0923) while all six double deletion mutants are noted X^-Y^- (for instance 0923^-1581^- stands for the deletion of both sll0923 and sll1581). All EPS-depleted mutants were grown in the presence of selective antibiotics, which were added at the following concentrations: Streptomycin (Sm, 2.5 μg/mL), Spectinomycin (Sp, 2.5 μg/mL) and Kanamycin (Km, 50 μg/mL) [28]. The growth of the various strains was followed as the timely increase in absorbance (Beckman DU 640 spectrophotometer) at 580 nm (1 OD_{580} unit corresponding to 2.5×10^7 cells/mL).

2.2. EPS characterization

In order to disclose correlations between the content in EPS and the physicochemical properties, the EPS mass quantification data, extracted from a previous study [26] are expressed in mg EPS/m^2 of cell surface instead of mg EPS/mg of cell protein mass. This unit, classical in physical chemistry, is more relevant for surface studies than a biological material normalization. The cell being almost spherical with a size of 1 μm, the surface per cell is $\pi\ \mu m^2$. Moreover, we made the distinction between CPS and RPS mass quantities to better assess the modification of EPS layer structure and nature among the mutants. Indeed, the gene deletion can influence differently the production of CPS or RPS, which cannot be reflected in the total EPS mass quantification. The method of CPS/RPS titration is given in our former paper [26].

Scanning electron microscopy (SEM) was performed to obtain qualitative imaging of the EPS in the WT strain and in the different mutants. 72 h grown cultures of WT and mutant strains (OD_{580} = 0.7) were chemically fixed overnight with glutaraldehyde (1%) washed twice with UPW (milliQ water) and gradually dehydrated using a CO_2 critical point dryer (BAL-TEC CPD030), as described in [31]. Dried samples were mounted on aluminum stubs using double-sided carbon tape and carbon-coated (Leica EM SCD 500). Cells were observed with a Zeiss Ultra 55 FEG SEM microscope operated at 2.0 kV at a working distance of 2.7 mm. Images were acquired in secondary electron mode using an Everhart Thornley detector.

2.3. Measurement of the electrophoretic mobility of the cells

Following centrifugation, cells resuspended in the studied media at OD_{580} = 0.5 (2.5×10^7 cells/mL) were transferred into disposable folded capillary cells with gold covered electrodes (Malvern). The electrophoretic mobility (EPM) of the cells was measured at 25 °C at a 17° fixed scattering angle with a Zetasizer Nano ZS instrument (Malvern) equipped with a 633 nm laser, using runs performed at a voltage of 150 V and a frequency of 285 Hz. To investigate the influence of the ionic strength of the medium, EPM measurements were performed with cells suspensions in various dilutions of MM in ultrapure water (100%, 80%, 60%, 40%, 20% and 0%) which lead to an ionic strength range of 1–26.6 mM. One can note that the absence of washing, in order to preserve the EPS structure around cells, leaded to a non-zero ionic strength when cells were resuspended in ultrapure water. All measurements were repeated five times and experiments were carried out in triplicate.

2.4. Modeling using soft-particle theory

Electrophoresis is an effective technique to assess the electrical properties of microparticle surfaces but the Smoluchowski mobility formula ($\mu = \zeta \cdot \eta / \varepsilon_0 \cdot \varepsilon_r$) is suitable only for hard particles (where electric charges are located only at the ion-impenetrable particle surface of zero thickness) that are large in comparison to the Debye length. In this formula, μ is the electrophoretic mobility, ζ the zeta potential, ε_0 the permittivity of vacuum, ε_r the relative permittivity of medium and η the viscosity of the medium. The zeta potential represents the potential at the stagnant and slip plane which separates the movable part of the ionic double layer and the particle surface (see Supporting Information S3-a). Bacterial cell surfaces cannot be modeled as so-called "rigid particles" because they are often surrounded by a layer of charged polymers (EPS), as occurs in *Synechocystis*. As described in Supporting Information 3-b, the zeta potential ζ of a soft particle is much more negative than the potential Φ_0 at the outside of the soft layer because of the fixed charge density of surface polymers; thus zeta potential cannot reflect the surface potential of bacterial cells.