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Short note

'Soft-particle' analysis of the electrophoretic mobility of a fibrillated and non-fibrillated oral streptococcal strain: *Streptococcus salivarius*

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Abstract

The electrophoretic mobility of microbial cell surfaces can be analysed in terms of a so-called soft layer model, in which the electrophoretic mobility is described as originating from the potentials over the surface charge layer and the membrane fixed charges. Often, the polyelectrolyte layer deforms under the influence of ionic strength variations. In the soft layer analysis of electrophoretic mobilities this is expressed in the softness $1/\lambda$. Here, we determined the softness of two oral streptococcal strains, *S. salivarius* HB and HBC12 from particulate microelectrophoresis in KCl solutions of varying ionic strength. Electron microscopy of negatively-stained organisms and X-ray photoelectron spectroscopy showed that strain HB had several classes of proteinaceous fibrils with lengths up to 178 nm on its outermost surface, while variant HBC12 had a bald, peptidoglycan-rich outer surface. The fibrillated strain HB appeared as relatively soft ($1/\lambda$ equals 1.4 nm) from analysis of its electrophoretic mobility, while the bald variant HBC12 was hard ($1/\lambda$ equals 0.7 nm) due to its comparatively rigid, peptidoglycan-rich outer surface, characteristic to Gram-positive bacteria. The presence of proteinaceous fibrils on strain HB slightly shielded the membrane fixed charges on HBC12.

Keywords: Electrophoretic mobility; Streptococci; Surface structures; Softness of polymer layer

1. Introduction

Recently, Ohshima [1–3] proposed a new theory to interpret the electrophoretic mobility of soft, polyelectrolyte covered particles as opposed to rigid particles, when measured as a function of ionic strength. This theory describes the electrophoretic mobility of soft particles, of which micro-organisms are typical examples, as originating from a weighted average of potentials over the surface charge layer and that of the volume

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density of membrane fixed charges. The polyelectrolyte layer around microbial cell surfaces collapses upon changing the ionic strength of the suspending fluid, as evidenced by dynamic light scattering [4], with consequent changes in electrophoretic mobility. The theory of Ohshima enables one to determine the volumetric charge density and the softness of this polyelectrolyte layer. The softness parameter $1/\lambda$ of poly (*N*-isopropylacrylamide) hydrogel layers around latex particles, obtained from analysis of electrophoretic mobilities, decreased slightly with temperature from 1.2 nm at 25°C to 0.9 nm at 40°C, due to a phase transition occurring within this temperature range [5]. In comparison, the microbial cell surface of a collection of four *Pseudomonas syringae* strains was much softer and $1/\lambda$ values ranging from 1.50 nm to 3.84 nm, presumably due to the presence of a more or less extensive layer of exopolysaccharides [6]. However, hitherto predictions from a soft particle analysis of microbial electrophoretic mobilities have not been confirmed by electron microscopy studies on structural cell surface components.

Streptococcus salivarius HB is an oral microorganism of which a series of variants have been described that are deficient in adhesive properties [7,8]. Electron microscopy using a negative-staining technique revealed that on *S. salivarius* HB four distinct fibril classes, with lengths of 178, 91, 73, and 63 nm, were detectable. X-ray photoelectron spectroscopy (XPS) on freeze-dried organisms demonstrated that these fibrils were proteinaceous in nature. The consecutive removal of these fibrillar subclasses resulted in a reduction of the water contact angle from 36° for strain HB to 21° for variant HBC12, which is a completely bald variant, devoid of any fibrillar surface appendages [9]. Furthermore, the bald variant HBC12 had a significantly lower isoelectric point (pH 1.3) compared to the parent strain HB (pH 3.0) in a 10 mM potassium phosphate buffer [9], due to a high concentration of surface phosphate groups.

The aim of this study was to determine the softness of *S. salivarius* HB and *S. salivarius* HBC12, the bald variant, from an analysis of electrophoretic mobilities measured in KCl solutions of different ionic strength and to relate the softness measured to the absence or presence of structural features on the microbial cell surfaces.

2. Materials and methods

2.1. Bacterial strains, culture conditions, and harvesting

S. salivarius HB and HBC12 were cultured in Todd Hewitt Broth at 37°C in ambient air. For each experiment the strains were inoculated from blood agar in a batch culture. This culture was used to inoculate a second culture that was grown for 16 h prior to harvesting.

Bacteria were harvested by centrifugation (5 min at 10 000 *g*), washed twice with demineralized water and resuspended in water. To break bacterial chains and aggregates, cells were sonicated for 30 s at 30 W (Vibra Cell model 375, Sonics and Materials Inc., Danbury, CT, USA). Sonication was done intermittently while cooling in an ice/water bath. These conditions were found not to cause cell lysis in any strain.

2.2. Particulate microelectrophoresis

Electrophoretic mobilities were measured at 25°C with a Lazer Zee Meter 501 (PenKem, Bedford Hills, NY, USA) equipped with an image analysis option for tracking and zeta sizing [10]. Measurements were carried out in KCl solutions of various ionic strengths (pH 5). Immediately prior to each measurement, an aliquot of the bacterial suspension was added to the appropriate KCl solution to a density of approx. 1×10^8 cells ml^{-1}. The pH of the solutions did not change upon addition of the bacterial cells. The Lazer Zee Meter was set at 150 V for determination of the electrophoretic mobilities of the bacteria.

The mobilities were fitted to Eq. (1) using a curve-fitting routine kindly provided by Dr Ohshima, Tokyo, Japan:

$$\mu = (\epsilon_r \epsilon_0/\eta)[(\psi_0/\kappa_m + \psi_{DON}/\lambda)/ (1/\kappa_m + 1/\lambda)] + (zeN/\eta\lambda^2) \quad (1)$$

in which μ is the electrophoretic mobility, ϵ_r the relative permittivity, ϵ_0 the permittivity of vacuum, η the viscosity of the solution, κ_m the Debye–Hückel parameter of the polymer layer, $1/\lambda$ a measure for the softness of the polyelectrolyte layer, z the valence of charged groups in the polyelectrolyte, e the electrical unit charge, N the density of charged groups in the polyelectrolyte layer, Ψ_0 the potential at the boundary between the polyelectrolyte layer and the surrounding solution and Ψ_{DON} the Donnan potential within the polyelectrolyte layer [1–3].

All electrophoretic mobilities reported are the

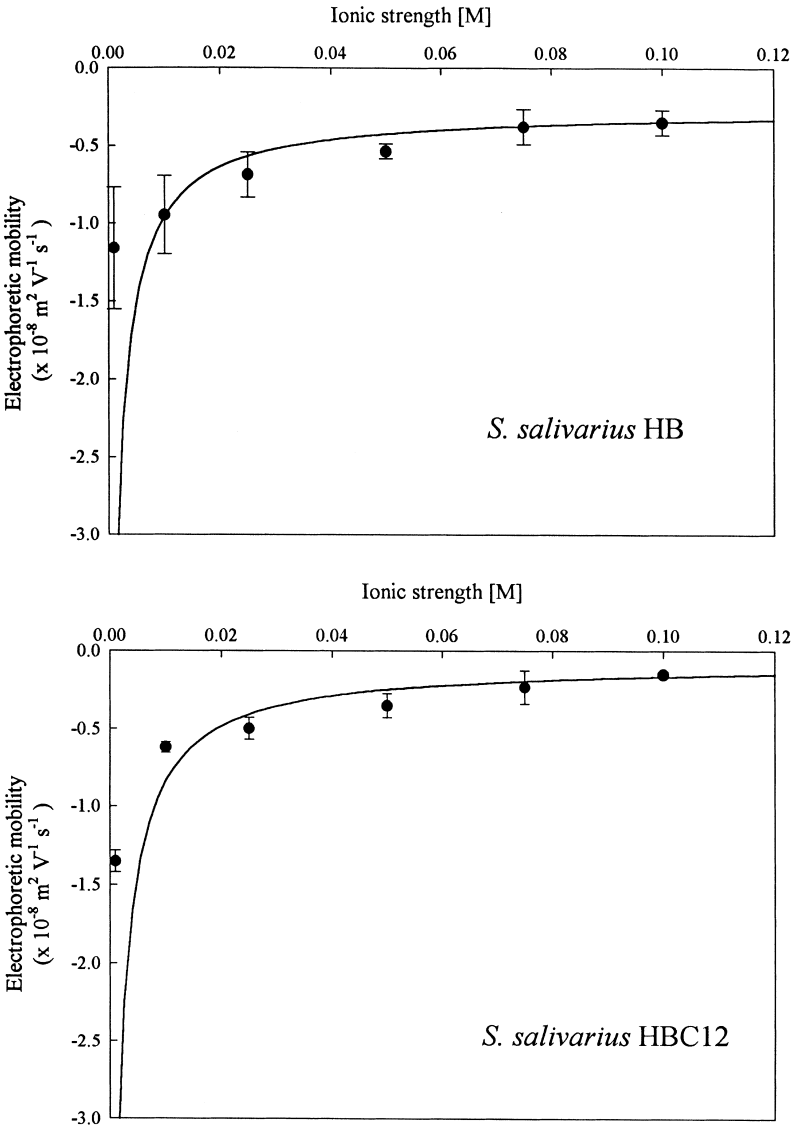

Fig. 1. Electrophoretic mobilities in KCl solutions (pH 5) of *S. salivarius* HB and HBC12 as a function of ionic strength. The solid lines represent the best fit to Eq. (1). Error bars indicate the biological standard deviation over three experiments with separately cultured bacteria.

mean values of three different measurements with separately cultured bacteria.

3. Results

Fig. 1 shows the electrophoretic mobility of *S. salivarius* HB and HBC12 as a function of ionic strength. The electrophoretic mobility of strain HB and variant HBC12 levelled off at high ionic strength to a value of -0.35×10^{-8} and -0.15×10^{-8} $m^2V^{-1}s^{-1}$, respectively. The data points were fitted to Eq. (1) from which the softness $1/\lambda$ and charge density zN were obtained. $1/\lambda$ amounted to 1.4 nm (0.2 for the fibrillar strain HB and 0.7 nm (0.1 for the bald variant HBC12, while zN was -0.013 M $\pm$ 0.003 for strain HB and -0.015 M $\pm$ 0 for HBC12 ($\pm$ denotes the biological SD over three different bacterial cultures).

Fig. 2 presents previously published electron micrographs of negatively-stained microbial cell surfaces [11]. As can be seen, the presence of fibrillar structures on strain HB corresponds with the greater softness calculated from an analysis of electrophoretic mobilities, whereas variant HBC12 does not possess any fibrillar structures and appears completely bald. Correspondingly, analysis of its electrophoretic mobility indicates that the surface of variant HBC12 is a hard surface.

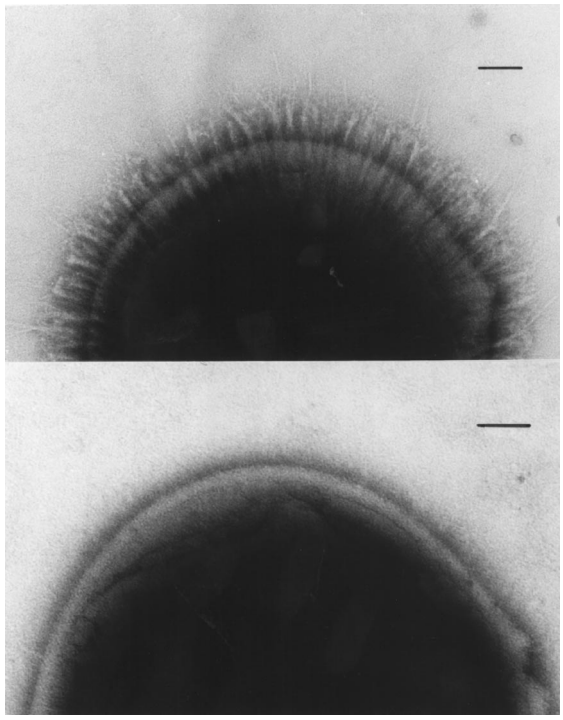

Fig. 2. Electron micrograph of 1% methylamine tungstate-stained *S. salivarius* HB (top), showing different classes of fibrils and of the completely bald, non-fibrillated variant, HBC12 (bottom). Bar denotes 0.1 μm.

4. Discussion

Adhesion of micro-organisms is determined by the physico-chemical (hydrophobicity and electrostatic charge) and structural properties of the interacting surfaces, amongst which the absence or presence of structural surface appendages as fimbriae or fibrils on the microbial cell surface [12–14]. Environmental conditions as pH and ionic strength become influential upon the adhesion process as they modulate both the electrostatic charge properties of microbial cell surfaces as well as the configuration of the structural surface appendages. The behaviour of structural cell surface appendages upon changing the pH or ionic strength not only becomes obvious from dynamic light scattering, demonstrating for instance that microbial diffusion coefficients were generally higher at pH 2 than at pH 7 for strains with structural surface appendages [4], but also from an analysis of the variation of electrophoretic mobilities with ionic strength [6]. The variation of the electrophoretic mobility with ionic strength of the bald and fibrillated oral streptococcal strains measured in this study, demonstrate that fibrillar coatings on microbial cell surfaces must be considered as a soft layer in terms of the Ohshima model, outlined by Eq. (1).

In addition to the structural differences between the fibrillated strain HB and bald variant HBC12 also their surface chemical compositions differ. XPS studies demonstrate a much higher elemental surface concentration ratio N/C for strain HB (0.104) compared to HBC12 (0.053), from which it was concluded that the fibrils are predominantly proteinaceous structures, while on HBC12 XPS probed mostly the comparatively

rigid peptidoglycan layer, characteristic to Gram-positive bacteria [11,15].

In addition to being more rigid, the peptidoglycan layer of HBC12 had a slightly higher volumetric charge density than the fibrillated outer surface of strain HB due to shielding of the peptidoglycan charges by proteinaceous fibrils. Analogously, Takashima and Morisaki [6] suggested that lipopolysaccharides on Gram-negative bacteria may shield cell surface charges.

Summarizing, we have demonstrated that a soft particle analysis of the electrophoretic mobility of fibrillated *S. salivarius* HB and the bald mutant HBC12 as proposed by Ohshima [1–3] corresponds with the electron microscopic observations of their surfaces.

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