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Surface charge and hydrodynamic coefficient measurements of *Bacillus subtilis* spore by optical tweezers

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ABSTRACT

In this work we report on the simultaneous measurement of the hydrodynamic coefficient and the electric charge of single *Bacillus subtilis* spores. The latter has great importance in protein binding to spores and in the adhesion of spores onto surfaces. The charge and the hydrodynamic coefficient were measured by an accurate procedure based on the analysis of the motion of single spores confined by an optical trap. The technique has been validated using charged spherical polystyrene beads. The excellent agreement of our results with the expected values demonstrates the quality of our procedure. We measured the charge of spores of *B. subtilis* purified from a wild type strain and from two isogenic mutants characterized by an altered spore surface. Our technique is able to discriminate the three spore types used, by their charge and by their hydrodynamic coefficient which is related to the hydrophobic properties of the spore surface.

1. Introduction

The electrostatic interaction among charged colloids or among colloids and surfaces is one of the fundamental issues in condensed matter physics. For instance, the electrostatic interactions between charged microparticles determine the stability of colloidal dispersions and particle aggregation. In biology, these electrostatic forces are crucial for the interpretation of ion adsorption and ion permeation processes as well as in adhesion processes of bacteria and bacterial spores onto surfaces. These processes are the base of a variety of phenomena ranging from the development of new biomaterials for biomedical application to the prevention of food contamination by bacterial biofilms and to the delivery of drugs and antigens to human mucosal surfaces [1]. In this context the adhesion of bacterial spores to surfaces is of particular interest for at least two reasons: (i) decontamination of spores during the industrial food chain (from food preparation to packaging) and (ii) use of spores as a platform to deliver drugs and antigens to the human mucosal surfaces.

Spores are metabolically quiescent cells, extremely resistant to harsh environmental conditions. Spore resistance to extreme conditions is due to their structure characterized by a dehydrated cytoplasm surrounded by several protective layers: the thick cortex formed of peptidoglycan, a multilayered coat mostly formed by proteins and the recently identified crust, formed of proteins and glycoproteins [2]. Because of their resistance to thermal and chemical treatments treatments spores cannot be efficiently removed by standard procedures. In the presence of water and organic molecules spores germinate originating cells able to grow and compromise the quality and safety of food products. Understanding the physicochemical nature of the interaction between spores and surface could probably lead to the use of more appropriate decontamination procedures. The resistance properties of spores, together with the safety record of several spore-formers species, is also the base for considering them as a mucosal delivery system [1]. Also in this case, a detailed knowledge of the surface properties of spores would help to rationalize the display of drugs and antigens on the spore surface [3–5].

The interaction potential between colloids and between colloids and surfaces is intensively discussed in literature and remains a challenge for both experiment and theory. Despite its limitations, the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory [6] describes very well the interaction between charged colloids in aqueous salt solutions.

Therefore the charge carried by a single colloid or biological object is a very relevant parameter to be measured. Usually this can be done by measuring the electrophoretic mobility μ, that is defined as the ratio of the velocity v of a charged particle over

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the applied electric field strength E i.e. $\mu = v/E$. The zeta potential (ζ) is the electrokinetic potential measured at the slipping plane in the double layer and is related to the electrophoretic mobility by the relation: $\mu = \epsilon_r\epsilon_0\zeta/\eta$, being η the viscosity of the fluid and ϵ_r and ϵ_0 the dielectric constant and the absolute permittivity of vacuum, respectively. Therefore the knowledge of these associated parameters is of fundamental importance to characterize a colloidal system. Commercial instruments, *Zetasizers*, are today available to measure the electrophoretic mobility of microscopic particles and, consequently, the zeta potential. These instruments are based on Laser Doppler Velocimetry (LDV) and Phase Analysis Light Scattering (PALS) techniques. Nevertheless these instruments give ensemble averaged results which have been reported to be dependent on the particle concentration [7] and are not adequate when the shape of particles differs significantly from that of a sphere as in the case of the bacterial spores investigated in this work.

In the last three decades, optical tweezers [8,9] (OT), have revealed as a formidable tool in many areas of science. In particular, they have been used to shed light in colloidal physics for their ability to manipulate single particles. They are based on a strongly focussed laser beam that exerts a restoring force on a microscopic dielectric particle close to the focal point. In such a way the trapped particle results to be confined in an optical potential that can be considered with a good approximation harmonic. So far, the restoring force is elastic: $F_{opt} = -\kappa x$, being κ the stiffness of the optical trap and x the displacement from the laser focus. Optical tweezers in combination with a nanometre resolution position detection technique [10] represent a very sensitive force transducer allowing the measurement of forces from hundredths of pN down to few fN [11,12]. In particular, recent experiments have demonstrated the possibility to measure electrophoretic mobility of single colloids in polar and non-polar fluids [13–15].

Here we show how to extend these techniques to the case of single bacterial spore dispersed in water. In particular we demonstrate that our technique is able to measure, simultaneously, the hydrodynamic coefficient and the electric charge of single particles with arbitrary shape. In this experiment we used bacterial spores that, at first approximation, can be considered of ellipsoidal shape and with a complex surface. The only requirement of our technique is that the confined thermal motion of the trapped object is purely translational. When this condition is fulfilled the hydrodynamic coefficient and the electric charge can be measured in a fast, reliable and precise way. In addition we demonstrate the possibility to discriminate genetically modified bacillus spores from their surface charge. Our method is also able to estimate the hydrodynamic factor of wild type and mutants spores. This could open the possibility to measure the degree of hydrophobicity of spores in different environmental conditions.

2. Theory

The motion of a charged particle of diameter d and mass m subjected to a restoring force with stiffness κ and to an external electric field is described by the Langevin equation (here for one coordinate only):

$$m\ddot{x}(t) + \gamma\dot{x}(t) + \kappa x = F_T(t) + F_E \tag{1}$$

where γ is the hydrodynamic coefficient which, for a spherical particle, is related to the medium viscosity by the relation: $\gamma = 3\pi\eta d$. $F_T(t)$ is the random thermal force that drives the Brownian motion, it has the statistical properties of white noise and is defined as:

$$F_T(t) = \sqrt{2\gamma k_B T}\xi(t) \tag{2}$$

where k_B is the Boltzmann constant, T the absolute temperature and $\xi(t)$ is the zero-mean, δ-correlated Gaussian white noise.

In a fluid with low Reynolds number, like water, the inertial term can be neglected since the decay time due to viscous losses is of the order of m/γ, i.e. 10^{-7} s which is much smaller than the time resolution used in this experiment (of the order of 10^{-5} s, see below).

The electric force is $F_E = Q_{eff} \cdot E$ where Q_{eff} is the effective charge of the particle, resulting from the screening of free ions, and E the electric field strength. In this experiment we used a sinusoidal electric field $E(t) = E_0 \sin(2\pi f_p t)$, being f_p the modulation frequency, thus Eq. (1) becomes:

$$\gamma\dot{x}(t) + \kappa x = F_T(t) + Q_{eff}E_0 \sin(2\pi f_p t) \tag{3}$$

Since this equation is linear, its solution is simply the sum of two terms $x(t) = x_T(t) + x_E(t)$: the first represents the motion of the particle in presence of the thermal motion only, while the second is due to the electric field only [14]. In particular the latter is the well-known deterministic motion of a driven oscillator:

$$x_E(t) = \frac{Q_{eff}E_0}{\kappa[1 + (f_p/f_C)^2]^{1/2}} \sin(2\pi f_p t - \phi) \tag{4}$$

where $f_C = \kappa/(2\pi\gamma)$ is the corner frequency of the optical trap and ϕ is a constant phase lag between the particle motion and the driving field: $\phi = arctg(f_p/f_C)$.

It is very convenient to study this dynamics using the autocorrelation function (ACF) defined as $ac(t) = \langle x(t')x(t'+t)\rangle$. Since the periodic electric field and the thermal motion are uncorrelated, the total autocorrelation function is, again, the sum of two autocorrelation functions: one for the thermal and another for the periodic motion induced by the electric field:

$$\begin{aligned} ac(t) &= ac_T(t) + ac_E(t) \\ &= \frac{k_B T}{\kappa} e^{-t/\tau} + \frac{Q_{eff}E_0}{2\kappa^2[1 + (f_p/f_C)^2]^{1/2}} \cos(2\pi f_p t) \end{aligned} \tag{5}$$

where $\tau = \gamma/\kappa$ is the characteristic time of the optical trap which is related to the *corner frequency* $f_C = 1/(2\pi\tau)$. Eq. (5) can be written as the normalized function $acn(t) = ac(t)/ac(0)$:

$$acn(t) = \frac{1}{1+\Gamma^2} e^{-t/\tau} + \frac{\Gamma^2}{1+\Gamma^2} \cos(2\pi f_p t) \tag{6}$$

The particle motion can be also described in the frequency domain through its power spectral density (PSD). The Wiener–Khinchin theorem states that the spectral decomposition of the autocorrelation function is given by the power spectrum of that process, therefore the PSD is given by:

$$\begin{aligned} S(f) &= \frac{A_{psd}}{f_C^2 + f^2} + B_{psd} \cdot \delta(f - f_p) \\ &= \frac{k_B T}{\pi^2\gamma} \frac{1}{f_C^2 + f^2} + \frac{k_B T}{\kappa} \pi\Gamma^2 \delta(f - f_p) \end{aligned} \tag{7}$$

which is the superposition of a lorentzian function related to the thermal motion and a peak at the driving frequency of the forcing electric field.

The parameter Γ that appears in Eqs. (6) and (7) is the ratio of the periodic force and the thermal force scaled by the ratio of the frequency modulation of the external field and the corner frequency of the optical trap:

$$\Gamma^2 = \frac{\langle F_E^2\rangle / \langle F_T^2\rangle}{1 + (f_p/f_C)^2} \tag{8}$$