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Effects of Poly(L-lysine) Substrates on Attached *Escherichia coli* Bacteria

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Poly(L-lysine) (PLL) is a cationic polymer that is often used for attaching and immobilizing cells to glass substrates for further investigation by, e.g., AFM techniques. Because of their small size, bacterial attachment is most easily done using thick air-dried PLL coatings—though thinner PLL coatings are also used and are commercially available. Nevertheless, the antimicrobial activity of PLL is well-established. Accordingly, we have investigated the physiological effects of suspended PLL and of PLL coatings on individual *Escherichia coli* bacteria through the pole-to-pole oscillations of cytoplasmic MinD-GFP fusion proteins. For planktonic bacteria, suspended PLL concentrations at the micromolar level quenched MinD-GFP oscillations and inhibited bacterial growth. On coverslips with PLL coatings prepared by short exposures of the slides to PLL solutions, followed by rinsing, only a fraction of available bacteria attached after hours of settling time. Min oscillations in the attached bacteria, however, were strong and only moderately slowed. On thick PLL coatings, prepared by drying drops on the slides followed by a brief rinse with deionized water, cells attached well within 15 min. With thick coatings, average oscillation periods for bacteria increased significantly, and considerable cell-to-cell variability was also observed; subsequent replacement of buffer with distilled water led to much larger period increases and/or fading of fluorescence intensity. We demonstrate that Min oscillations are a useful metric for bacteria attached to adhesion layers. We suggest that thick PLL coatings should probably be avoided for bacterial attachment, and that even thin PLL coatings can have significant effects on bacterial physiology.

1. Introduction

Imaging living bacteria with scanning probe methods such as atomic force microscopy (AFM), or even with standard optical microscopic methods, often requires that bacteria be localized to a substrate without compromising their biological viability.[1] Attachment of bacteria to glass surfaces has frequently been accomplished with poly(L-lysine) (PLL).[2−11] The negative surface charges that predominate both on glass or mica and on bacterial surfaces make the positively charged PLL polymer molecules very effective for attachment—so that bacteria can even remain immobilized against the lateral forces exerted by AFM tips during imaging. Since the objective of such studies on live bacteria is to obtain characteristic information of the organism in vivo, it is important that bacterial attachment does not significantly modify bacterial properties.

The antimicrobial action of PLL is well-established. ε-PL, for example, with 25−35 lysine residues shows a wide range of antimicrobial activity with MIC values of 50 μg/mL for *E. coli* and 3 μg/mL for *Pseudomonas aeruginosa*,[12,13] while larger MIC values are reported for these bacteria for α-poly(L-lysine) with molecular weights ranging from 5000 to 14 000.[14] The action of ε-PL involves the electrostatic adsorption of the positively charged polymer to the bacterial surface. In spite of this potential toxicity, PLL is used successfully for attachment of eukaryotic cells, and coverslips with PLL coatings are available commercially. Commercial PLL coatings typically involve a thorough cleaning of the substrate with subsequent brief immersion of the clean slides into a PLL solution. This is then followed with rinsing of the slides with distilled water. (For information on general sample preparation protocols, see also reference 15).

Eukaryotic cells are much larger than bacteria and also have a different and much more flexible envelope. Their contact area with the substrates is therefore much larger, and cell attachment with thin PLL coatings seems to work well for eukaryotic cells. For AFM imaging of bacteria, however, good attachment of bacteria often requires the complete drying of PLL drops on a substrate.[4,5,10,11] Such coatings can be thick and may have an adverse (perhaps bacteriocidal) effect on the attached bacteria.

The effects on bacterial properties of PLL-assisted binding of bacteria to surfaces have not been investigated systematically. We report here the effects of PLL-coated substrates on bacteria as probed by the observation of spatiotemporal density oscillations of MinD-GFP fusion proteins within individual cells of the bacterium *Escherichia coli*.

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1.1. Min Oscillations. During cell growth, two Min proteins (MinD and MinE) undergo pole-to-pole oscillations within individual bacterial cells.[16,17] The subcellular Min oscillations help to determine the cell division site by targeting the protein MinC to alternate cell poles, where it inhibits the assembly of the FtsZ ring and thereby prevents the production of sterile minicells. The biochemistry underlying the Min oscillation is well-understood. MinD:ATP binds to the membrane, where it recruits MinE. The MinE-stimulated hydrolysis of MinD:ATP to MinD:ADP releases both MinD and Min E to the cytoplasm. MinD:ADP then undergoes nucleotide exchange, and the resulting MinD:ATP can rebind. As shown by a variety of theoretical models,[18] the binding-release of MinD and MinE leads to Min oscillations within a cell. As observed by fluorescence microscopy of MinD-GFP fusions, Min oscillations at room temperature have a period of about 40 s,[19,20] which shortens to nearly 10 s at 40 °C.[21] It has recently been shown[22] that extracellular polycations (Mg^{2+}, Ca^{2+}, protamine, and gentamicin) have a strong slowing effect on the cytoplasmic Min oscillation. While the mechanism of this slowing is not yet known, this raises the possibility of using the Min oscillation as a probe of the effect of PLL, another multivalent cation, on individual *E. coli* bacteria.

2. Materials and Methods

2.1. Strains and Growth Conditions. *E. coli* strain PB103-(λDR122) (P_{lac}::*gfp-minDE*) was provided by Piet de Boer.[19] All cells were grown overnight at 37 °C in LB medium. Samples were grown for approximately 14 h at 37 °C. A few drops of this suspension were added to a test tube of new medium along with 50 μM isopropyl-β-D-thiogalactopyranoside (IPTG), and cultures were then grown for an additional 4 h at 37 °C. Approximately 0.5 mL of the new suspension was then injected into a flow cell loaded with pure buffer solution, and the flow cell was subsequently flushed with 3 to 10 mL of pure buffer to obtain an acceptable density of attached bacteria. At times, inoculant was also prepared by centrifugation of the new suspension, which was then followed by replacement of the supernatant with fresh buffer. To avoid possible interference on the Min oscillation from ions in LB or M9 minimum medium,[22] flow cell studies were performed on bacteria under starvation conditions and in an environment that contained only buffer solution and/or PLL.

2.2. Fluorescence Measurement. Cells were viewed on a Leica DMIRE2 inverted optical microscope outfitted with a Hamamatsu ORCA 285 digital charge-coupled-device camera and a 63× objective (numerical aperture 0.9). A mercury arc lamp provided fluorescence excitation radiation via a 450 to 490 nm excitation filter, and a 500 to 550 nm barrier filter allowed green fluorescent protein fluorescence imaging. To automatically record several cycles of the MinD oscillations, shutters were placed in the path of the condenser light and the mercury excitation radiation. The shutters (MAC 5000) were controlled from a Macintosh iMac 1.8 GHz computer using *Open Lab 4* software. Fluorescence images were captured at 2 s or longer intervals depending on the length of the oscillation period to be recorded. Photobleaching was minimized by controlling the length of time that bacteria were exposed to excitation radiation. Individual exposure times were generally between 30 and 200 ms. Measurement of the oscillation period for a time-lapse series of fluorescence images was done for bacteria that were stationary at the bottom of the sample chamber. The oscillation period was determined from a measurement of the average fluorescence intensity in a circular region near one pole with the help of *Open Lab 4* software, as described previously.[22]

2.3. Sample Chamber. Experiments were carried out in a simple flow cell. The bottom of the flow cell consisted of a microscope coverslip that was supported by a thin metal plate with an opening for viewing and imaging of the bacteria. Bacteria in the cell were distributed over an area of about 3.3 cm^2 and a depth of 0.8 mm. Prior to inoculation with bacteria, the flow cell was filled with buffer solution. This was either 10 mM HEPES or 10 mM phosphate buffer with pH near 7. Possible phototoxic slowing of Min oscillation in HEPES buffer was not significant for the short exposures used in these experiments.[22] After inoculation near the input port, the flow cell was flushed with several milliliters of buffer in order to distribute bacteria throughout the flow cell, as well as to reduce their overall number. The remaining bacteria were then allowed to settle in the flow cell in order to enhance the number that attached to the PLL coated bottom coverslip. Image sequences of MinD-GFP fusion protein oscillations were then recorded.

In some experiments, a plain, uncoated coverslip was used. After inoculating bacteria into the cell and following with a flush of pure buffer, most bacteria remained mobile and with a higher concentration in the immediate vicinity of the bottom surface. Solutions of PLL dissolved in buffer were then passed through the flow cell in order to examine the effects of suspended PLL on planktonic bacteria. This was then followed by fluorescence measurements on bacteria that attached to the (uncoated) bottom surface.

2.4. Substrate Preparation with PLL. We investigated two types of PLL. One was preparation P4707, a 0.01% PLL solution from Sigma Chemicals. This preparation is culture-tested and has a molecular weight in the range 70−150 kD (500 to 1000 monomers). The other PLL was a 0.01% (w/v) solution of PLL hydrobromide (Sigma P1274) with molecular weight of <150 kD that has been used successfully to attach bacteria to glass substrates for AFM imaging.[5] The flow cell bottoms were coverslips that were washed with detergent, thoroughly rinsed in tap water, and further treated in a concentrated HCl solution. This was at times also followed by a soaking in ethanol. The slides were then rinsed in deionized water and subsequently blown dry with high-purity argon gas.

Three types of PLL coating methods, often described in the literature, were investigated. In the "rinse" method, variations of which are used for commercial PLL coating of coverslips, 100 μL of the 0.01% solution was placed on a coverslip and spread out over an area of about 1.5 cm^2. The solution was allowed to sit on the coverslip for 30 to 60 min and was then washed off with deionized water and blown dry with argon gas. In the "drain" method,[6] soon after the PLL drop was placed on the slide, the slide was rotated onto its long edge, which was resting on an adsorbing surface, until the slide surface had dried. In the "dry" method,[2,4,5] to obtain a potentially thicker PLL coating, the 100 μL drop was allowed to completely air-dry on the slide, which was then followed with a brief rinse in deionized water. The PLL layers covered most of the slide area accessible to viewing with the microscope.

2.5. AFM Characterization of Surface Coatings. The topographies of the PLL surface coatings were examined with a molecular imaging AFM. We used oxide sharpened Si_3N_4 V-shaped cantilevers with spring constants of 0.03 N/m. The PLL coated surfaces were imaged both dry and submerged in deionized water. Image analysis and rms surface roughness was determined with *WsXM* software.

2.6. Exposure of Planktonic Bacteria to PLL. To test the direct effects of PLL on MinD oscillations, a clean coverslip was used for the flow cell bottom and the cell was filled with pure buffer solution. After inoculating the cell with bacteria, the

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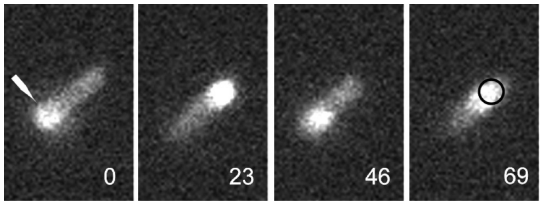

Figure 1. Example of MinD-GFP fusion protein oscillations in one *E. coli* bacterium. For our conditions, the fluorescence intensity oscillates from one pole to the other and back in 46 s. For an automatic recording of the intensity oscillations, a circular region of interest was placed over one pole as shown in the image at far right. Image sequences then allowed a capture of the time dependence of the polar fluorescence intensity and hence the determination of oscillation periods.

bacteria were distributed throughout the cell by circulating a few more milliliters of buffer. At this point, very few bacteria attached themselves to the cell bottom, and bacteria remained essentially planktonic. A few milliliters of PLL solution in buffer was then circulated through the flow cell, and the effects of this were observed with bright field and fluorescence imaging. Cell viability in the presence of PLL was also tested through incubation experiments. Test tubes that contained LB growth medium with different PLL concentrations were inoculated with 5 μL of stock culture and were then incubated at 37 °C for 12 h. Cell growth was estimated by direct visual inspection of the tubes for transparency, with a spectrophotometer at 600 nm (LKB Novaspec), and with bright field microscopy on drops from each tube.

3. Results

3.1. Oscillations of MinD-GFP. A typical fluorescence image contained between 20 and 100 bacteria localized on the PLL coated coverslip of the flow cell. Figure 1 shows four consecutive fluorescence images of such a bacterium, taken at 23 s intervals and cropped from images with many attached bacteria. The high-intensity polar region of the bacterium marked by an arrow in Figure 1 suggests that the bulk of the MinD-GFP fusion proteins reside at that pole at that time. After about 23 s, the fluorescence of the bacterium has transferred to the other pole only to again return to the first pole after another 23 s thus completing one period. All bacteria in the source image for Figure 1 showed MinD oscillations of comparable period.

Although an image may have contained up to 100 attached bacteria, it was generally possible to determine periods of only those cells that had their rod axis oriented parallel to the substrate and that were not obscured by nearby cells. In most cases, that meant that 30% to 50% of visible cells (though in some cases more than 70%) had analyzable periods.

3.2. Effect of Suspended PLL on MinD Oscillations of Planktonic Bacteria. The effects of circulating a few milliliters of 0.01% (w/v) PLL suspended in buffer at pH 7 had dramatic consequences for the planktonic bacteria in the flow cell. The bacteria rapidly settled to the bottom surface within a few minutes and became immobilized. Figure 2A shows a bright field image of a representative bacterium without PLL, while Figure 2B is a bright field image of the same bacterium after a 15 min exposure to 0.01% PLL. No significant change in bacterial morphology was evident upon PLL treatment even 1 h after PLL exposure.

Nevertheless, considerable slowing of Min oscillations was observed upon treatment with suspended PLL. The effects for PLL hydrobromide were generally more rapid than for PLL P4707. For either compound, after about 15 min 95% of attached bacteria no longer showed pole-to-pole Min oscillations. Their fluorescence was either uniform over the bacterium or

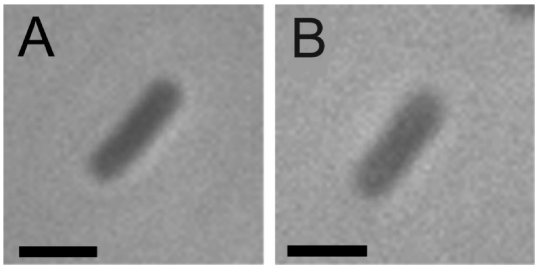

Figure 2. Effect of poly(L-lysine) on bright field images of *E. coli* bacteria. (A) Bright field image of a bacterium on an uncoated coverslip in 10 mM HEPES buffer at pH = 7. (B) Bright field image of the bacterium shown in (A) when the pure buffer was replaced by buffer containing 0.01% (w/v) poly(L-lysine) (Sigma P1274). Image taken 15 min after exposure to PLL. There is no significant change in the size or shape of the bacterium. Scale bar = 2 μm.

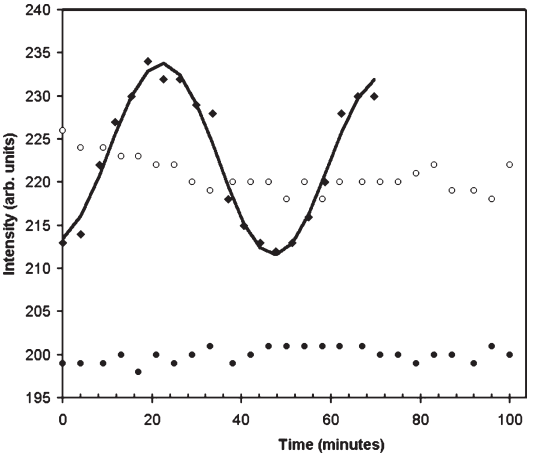

Figure 3. Plot of the time dependence of GFP polar intensity for the bacterium in Figure 2: ◆ Polar intensity variation for the bacterium in pure buffer as for Figure 2A; — Fit of intensity function to the polar intensity variation; ○ Polar intensity variation of the same bacterium in PLL solution as in Figure 2B; ● Background intensity at a place next to the bacterium for either condition.

occasionally concentrated and fixed at one pole. An example of the time dependence of the fluorescence of a bacterium on an uncoated coverslip before and after exposure to PLL is shown in Figure 3. The figure shows strong fluorescence oscillations before PLL addition but only a monotonic decreasing polar intensity (due to photobleaching of the GFP) after PLL exposure. Intensities measured on bacteria are all well above the background as, e.g., indicated by the solid circles in Figure 3.

Essentially the same results were obtained when the concentration of suspended PLL was reduced to 0.001% (w/v). When, after PLL exposure, the flow cell was refilled with PLL free buffer, no further changes in fluorescence images could be observed. The induced quenching of Min oscillations due to suspended PLL was irreversible.

In the flow cell experiments described above, most bacteria settled to the chamber bottom, leaving only few cells in the planktonic state. From bright field images taken at several locations along the flow cell bottom, we estimated the total number of bacteria in the flow cell to range between 10^{14} and 10^{15}. With the PLL concentrations employed and with an assumed average molecular weight of 10^5 Da for PLL, this would imply a PLL concentration of about 3×10^8 PLL molecules per bacterium in the above experiments.

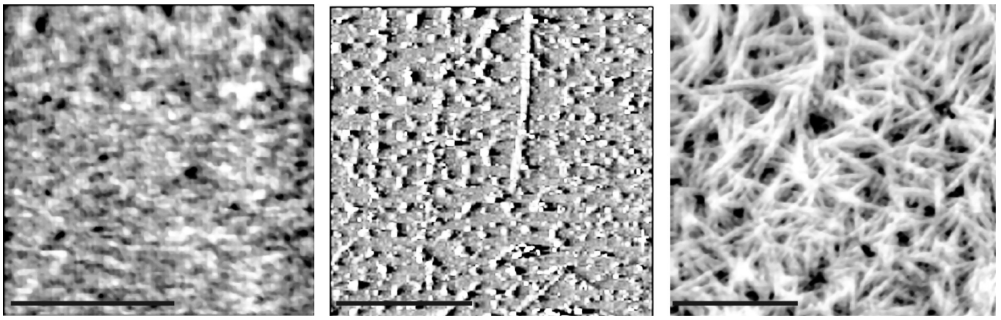

Figure 4. AFM images of PLL coatings on glass coverslips prepared by three different methods. (A) Height image of a coating prepared by depositing a drop of PLL P4707 solution and washing the slide with deionized water after 40 min of drop contact time. The coating is uniform and has a surface roughness of 3.6 nm rms. (B) Error signal image of a PLL coating that was prepared by the vertical drying method. PLL has formed blobs that are separated by nearly PLL-free regions. Analysis of the corresponding height image suggested a blob height of 3−5 nm, leading to an average rms surface roughness of 3.1 nm. (C) Height image of a coating prepared by complete drying of a drop of PLL solution on a coverslip. PLL has formed a network of polymer bundles with an rms roughness of 9.1 nm. With glancing illumination, such films were visible with the naked eye. The scale bar in each image is 10 μm.

To examine the relationship between the disappearance of Min oscillations and cell viability, we added 5 μL of *E. coli* bacterial culture in exponential growth phase to two vials of LB growth media that contained 0.0033% and 0.001% (w/v) of PLL P4707, respectively. After incubation at 37 °C for 12 h, samples from each vial and a control that contained no PLL were examined under the microscope. Except for the control, which showed the expected large density of active bacteria, none of the PLL containing vials showed comparable growth. In the vials that contained the higher PLL concentrations, only the occasional immobile clump of 3 to 4 cells could be observed. Bacterial growth was also determined with absorption measurements at 600 nm. Prior to inoculation with bacteria, the PLL-containing vials showed somewhat reduced transmissions compared to the pure LB control. This we attribute to PLL-induced density variations in the growth medium. After inoculation of the vials and incubation at 37 °C, transmission measurements on the PLL-containing vials showed an insignificant change in transmission, suggesting no significant cell divisions during the subsequent 12 h incubation period. The PLL molecules to cell ratio in the incubation experiments was about 3×10^8 and thus similar to that in the flow cell PLL injection experiments.

3.3. AFM Characterization of PLL Coatings. Figure 4 shows examples of the topography of coverslip coatings of PLL P4707 prepared by three different methods. Figure 4A is an image of a PLL coating prepared by the first "rinse" coating method, where the PLL drop is washed off after a 40 min settling time. This method produced uniform PLL surfaces with surface roughness of 3.6 nm rms. For comparison, the rms height variations on a clean, uncoated slide were 1.1 nm. Some coatings were scratched with a high cantilever force in an attempt to expose the glass substrate. Subsequent comparison of the exposed substrate with the PLL coating suggested a film thickness of 20−30 nm for this type of coating. The topography of an edge-dried slide is shown in Figure 4B. The coverage of the surface appears nonuniform, and PLL polymers appear to form a distribution of isolated 3−5-nm-high blobs. We could not determine whether the regions between the blobs were bare or PLL-coated glass substrate. These coatings had average rms surface roughness of 3.2 nm. Both of these coatings, in Figure 4A and B, were invisible to the eye. The topography of a coating obtained by air-drying of a PLL drop is shown in Figure 4C. Such "thick" coatings were visible to the eye as a gray deposit. Imaged with AFM, the surface of these coatings was strongly textured and even at times showed what appeared to be entangled polymer bundles. These films had an average thickness of 40−50 nm and had an rms roughness of 9.1 nm, which was the highest surface roughness of all coating methods. We also found that the thickness and detailed surface morphology of coatings can depend on the extent to which coatings have been rinsed.

3.4. Min Oscillations with PLL Coatings. The results on the effects of PLL coatings on Min oscillations were obtained from 15 experiments where bacteria were exposed to different PLL coatings prepared by the three coating protocols described in the Methods section. For coatings prepared by the "rinse" or "drain" technique, it often took an hour for bacteria to become sufficiently immobilized so that Min oscillation periods could be determined for a sufficiently large number of bacteria. Eventually, however, nearly 100% attachment of bacteria in a field of view (over 100 cells at times) was generally achieved. Occasionally, bacteria showed poor attachment to smooth coatings, and even after 1 h, only about 10−20% of bacteria were immobilized. The reasons for the occasionally poor attachment could not be determined. Coatings of PLL bromide were generally more efficient at cell immobilization than PLL P4707. On PLL coatings prepared with the "dry" technique, bacterial attachment was more rapid, and nearly all cells had attached 30 min after inoculation.

The mean oscillation period at room temperature for bacteria on an uncoated substrate was approximately 40 s. For bacteria on PLL-coated surfaces, oscillation periods always increased, but the amount of increase varied and depended on the preparation technique of the coatings. Average oscillation periods of attached bacteria for PLL coatings prepared by the "wash" or "drain" method were generally 55 s with standard deviations ranging from 6 to 11 s (n = 25). The effects of these PLL coatings on the periods thus appeared minor: bacteria continued to oscillate for hours after attachment to PLL coatings. On some of the higher roughness coatings, obtained by drop drying, much larger period increases were at times observed, and average periods, for those bacteria whose period could still be measured, of 75 s (with a standard deviation of 14 s) was not uncommon. This near-doubling of the average oscillation period suggests that cells on presumably thicker PLL coatings may be under considerable stress—even though many of these bacteria still continued to oscillate for hours.

Period increases with PLL hydrobromide (P1274) were generally higher than with PLL P4707. On some occasions, Min oscillations of bacteria attached to rough P1274 coatings stopped soon after cell inoculation. In general, however, as long as the flow

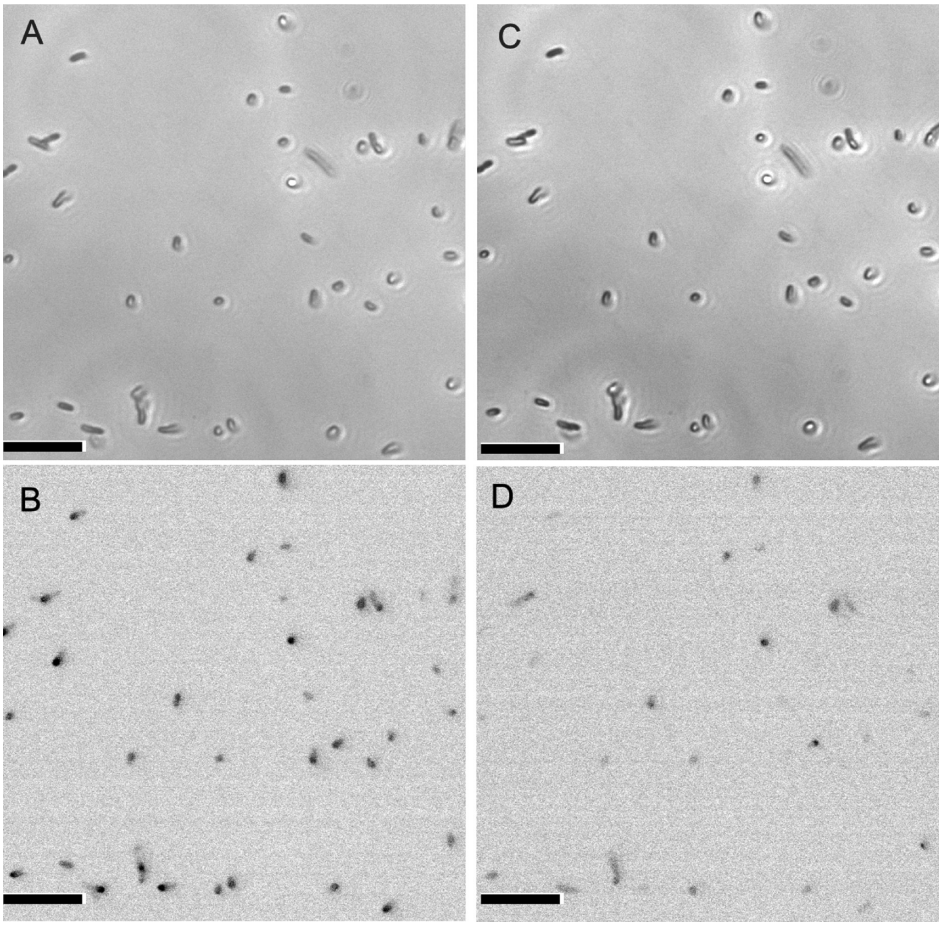

Figure 5. Example of the effect of a thick poly(L-lysine) (P1274) substrate coating, made with the "dry" technique, on bright field and fluorescence images. (A) Bright field image of a group of bacteria that are attached to a poly(L-lysine) coating on a coverslip. All bacteria in 10 mM HEPES buffer at pH 7 were completely immobilized. (B) Fluorescence image of the same group of bacteria as in (A). An average period of 83 s for only 13 bacteria out of the 33 visible in the image could be determined. The remaining bacteria were either not oscillating or were partly oriented away from the surface, thus not permitting a period determination. (C) Bright field image of the same group of bacteria as in (A) 10 min after the buffer was replaced by deionized water. There was no discernible change in the appearance of bacteria in bright field after the fluid exchange. (D) Fluorescence image of the same group of bacteria in deionized water 10 min after fluid exchange. The fluorescence faded for most bacteria, and bacteria progressively disappeared from view. Only two bacteria showed measurable oscillations.

cell remained filled with buffer bacteria continued to oscillate even hours after their attachment to the PLL layer. However, a general feature of Min oscillations was that an increase of the mean oscillation period was always accompanied by a broadening of distribution of oscillation periods taken over a bacterial population, indicating considerable cell-to-cell variability.

A significant change in fluorescence images occurred when the buffer over bacteria attached to PLL coatings was replaced by deionized water. We observed this effect for all the coating methods we used. In deionized water, Min oscillation periods of PLL-attached bacteria increased dramatically and bacteria stopped oscillating as time progressed. Bacterial fluorescence intensity became weak and uniform over the length of bacteria, and bacterial cells became progressively invisible in fluorescence images. Bright field images of the same group of bacteria, on the other hand, did not appear to change after the fluid exchange (indicating lysis did not occur). Parts A and C of Figure 5 are bright field images of bacteria taken 80 min after they attached to a PLL P1274 coating prepared by the drying method and taken, respectively, in 10 mM HEPES buffer or in deionized water. Figure 5C was taken 10 min after fluid exchange. There is no noticeable difference in the bright field images. Figure 5B is a fluorescence image of the same group of bacteria in buffer and illustrates the significant effect that PLL coatings can have on Min oscillations. Of the 33 bacteria shown in the image, 13 were still oscillating with an average period of 83 s. The corresponding fluorescence image in deionized water, 10 min after fluid exchange, is shown in Figure 5D. Two bacteria still show long-period oscillating fluorescence, but for most, the fluorescence intensity is no longer detectable or is very weak, and oscillations are no longer measurable.

4. Discussion

The response of Min oscillations in planktonic bacteria to low concentrations of PLL in the external medium is remarkable. In *E. coli*, Min oscillations select the midcell division site,[9,10] and absence of Min oscillations would prevent orderly cell division and lead to reduced growth. The irreversibility of this effect when cells are returned to a PLL-free buffer suggests that cells are seriously and permanently compromised by suspended PLL and possibly even killed. Low molecular weight ε-PLL (3000−5000 kD) is bactericidal.[12,13] Given the chemical similarity of higher molecular weight PLL, our results on planktonic *E. coli* bacteria suggest that the same is true for the much higher molecular weight PLL used in this study. Although the detailed mechanism of PLL action is not known at this time, PLL quenching of Min oscillations and the observed subsequent decrease in the overall fluorescence intensity is consistent with

PLL penetration of the (outer) cell envelope, followed by effects of PLL on or inside the cytoplasmic membrane.

The effects of PLL coatings on bacterial attachment and on Min oscillations appear influenced by the surface roughness and general surface integrity of the PLL coatings. Variations introduced in the films during the drying and rinsing process of the deposited drops of PLL solution as well as the type of PLL used could lead to variations in the thickness, compactness, and surface structure of coatings. In rough and thus less compact coatings, some PLL molecules could redissolve from the PLL layer more easily than in smooth coatings. These molecules could then affect Min oscillations the way oscillations are affected in planktonic cells. The effect of unadsorbed PLL on the interaction distance of 6 μm glass spheres above a glass plate when both surfaces were saturated with physisorbed PLL was investigated by Pagec et al.[23] They found that as little as 10 ppm of unadsorbed PLL had a measurable effect on the attraction of the sphere. It is therefore not unreasonable to assume that the stronger effect on the Min oscillations of coatings prepared by the "dry" method results from a higher concentration of unadsorbed molecules over such PLL surfaces.

If we assume an average molecular weight for PLL of 10^5 Da, then a 100 μL drop of 0.01% PLL solution spread over an area of 1 cm^2 would produce a coating with about 6×10^{13} polymer molecules/cm^2 (about 2×10^6 molecules/area occupied by a bacterium). We can make a rough estimate of the minimum thickness of the coating. The length of interatomic bonds suggests a monomer length of 450 pm, a monomer area of 0.23 nm^2, and an average polymer length of 317 nm. A PLL polymer molecule with a molecular weight of 10^5 would occupy an area of 163 nm^2 so that a close-packed monolayer of PLL would contain about 6×10^{11} molecules per cm^2. This in turn would imply that the above drop of PLL could form about 100 close-packed layers. Minimum thickness of such coatings would then be 20 nm. The actual arrangement of PLL is no doubt much more entangled, resulting in thicker coatings. This estimate is consistent with our measured thickness of PLL coatings by the "dry" technique. In such thick films, many polymer molecules will not be able to directly bond to the negatively charged glass substrate. These molecules may thus redissolve into the fluid, which could then more strongly affect Min oscillations. Smoother and thinner coatings, on the other hand, would have a lower concentration of free molecules near the surface, and such coatings appeared to leave bacteria much less affected by the PLL layer.

The strong effects of deionized water on the oscillations and on the bacterial fluorescence for thick PLL coatings could in part be due to a higher solubility of PLL in deionized water than in buffer. The progressive extinction of not just Min oscillations but of the whole fluorescence intensity in the presence of deionized water suggests that other factors such as increased turgor pressure and possibly a PLL-induced cell porosity might also play a part. Our cell growth experiments on PLL-containing LB medium showed that, even at concentrations as low as ~70 μM, PLL prevents cell divisions. It thus appears that the irreversible quenching of Min oscillations by a cationic agent, such as PLL, also implies inhibition of cell growth.

The characteristics of deposited PLL coatings should depend on many additional factors such as cleanliness of the substrate, viscosity of the PLL solution, contact time of the PLL solution with the substrate as well as on the ambient humidity, and hence drying time, when PLL drops are dried completely on the substrate. The degree of rinsing of the slide after coatings are deposited is also of importance. We have not investigated the degree to which all these factors might influence coating morphology and what their potential effects on attached bacteria might be. Description of methods of preparation of PLL substrates is often too terse in the literature, sometimes even absent, but considerable variability of PLL substrate thickness and morphology (and consequent impact on bacterial physiology) is likely.

Planktonic cells exposed to millimolar concentrations of suspended PLL cease oscillating and no longer multiply in LB growth medium. In comparison, our results show that oscillation periods on most PLL coatings increased by at least 30−40% compared to periods on uncoated surfaces. The observed broadening of the distributions of periods when bacteria attach to PLL coatings could reflect local density variations of free PLL molecules but may also indicate single cell variability to PLL exposure. Although cells on coatings experienced period lengthening to varying degrees, oscillations for the majority of bacteria on coatings prepared by the "drip" and "drain" method, nevertheless, continued even hours after bacteria attached to the coatings. Even on coatings prepared by the "drying" method, a sizable fraction of bacteria generally continued to oscillate, albeit with much longer periods. This suggests that these bacteria may be stressed but probably are, on the whole, viable as long as they remain immersed in buffer. However, the fact that an important cellular process implicated in cell division is compromised or, for some bacteria, completely halted by PLL suggests that this compound could also have a significant effect on other intracellular processes of interest to an investigator. We anticipate that similar issues may arise with other polycationic surface preparation techniques used for microbial adhesion and suggest that Min oscillations represent a new probe to study the effects of adhesion layers on attached bacteria.

5. Summary

In view of the demonstrated serious effects of PLL on planktonic *E. coli*, the use of PLL as adhesion molecule for bonding bacteria to surfaces for live cell experiments is questionable. High molecular weight poly(L-lysine) in solution was found to quench MinD oscillations of *E. coli* bacteria and prevented cell growth in LB growth medium. In spite of the high toxicity of PLL, coatings of PLL on glass coverslips do enable cell attachment to the coatings without quenching of Min oscillations or eliminating cell viability. However, more subtle phenomenological changes—such as the changed period of Min oscillations observed in this experiment—may be unavoidable. AFM imaging of PLL coatings suggested a possible correlation between Min period lengthening and surface roughness of PLL coatings, with rougher and more textured surfaces giving larger period lengthening but with some period lengthening seen even for the thinnest and smoothest PLL coatings. Even for air-dried PLL drops, the final morphology of the coating will depend strongly on the degree of rinsing of the coating after deposition. We also suspect that integrity of the coating, and hence, bacterial adhesion strength and Min oscillations are influenced by the length and degree of drying of the coating after the rinse. Our results suggest that, to minimize the toxic effects of PLL on cells, PLL coatings should be structures that minimize the number of weakly bonded or unattached molecules near the surface. Air-dried drops of PLL solution with brief rinsing in deionized water do not fall into this category and should probably be avoided. This preparation method seems to result in relatively thick and highly textured coatings that bind bacteria well but can have a strong effect on Min oscillations and possibly on other bacterial phenomenology.

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