Effect of Antibiotics and Antimicrobial Peptides on Single Protein Motility

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Abstract: Following the movement of individual molecules of a bacterial surface protein *in vivo* we investigated the effects of antibiotics and antimicrobial peptides on protein motility and membrane structure. In previous work we engineered the λ-receptor of *Escherichia coli* such that less than one receptor per cell is *in vivo* biotinylated and can bind to a streptavidin coated bead. Such a bead served as a handle for the optical tweezers to follow the motion of an individual receptor. In an un-perturbed living cell the λ-receptor performs a confined diffusive motion. The λ-receptor links to the peptidoglycan layer, and indeed, a perturbation of the peptidoglycan layer had a pronounced effect on the motility of the receptor: The motility significantly decreases upon treatment with vancomycin or ampicillin, to study the effect of vancomycin we used strains with increased membrane permeability. As the motility of an individual receptor was monitored over an extended amount of time we were able to observe a temporal evolution of the action of vancomycin. Antimicrobial peptides (AMPs) are alternatives to conventional antibiotics in the treatment of bacterial infections. Therefore, we also investigated the effect of the toxic AMP polymyxin B (PMB) which targets both the outer and inner membranes and kills the organism. PMB significantly decreased the motility of the λ-receptor. On the basis of these findings we confirm that the λ-receptor is firmly attached to the peptidoglycan layer, and that an antibiotic or AMP mediated destruction of the dynamic peptidoglycan synthesis decreases the receptor motion.

Keywords: Antibiotics, ampicillin, vancomycin, antimicrobial peptides, single molecule biophysics, λ-receptor, *E. coli*, peptidoglycan.

INTRODUCTION

The vast majority of previous investigations regarding the action of antibiotics and antimicrobial peptides have been performed by ensemble experiments, such yielding average information regarding the influence of a particular potential drug. Ensemble studies have been and are by far the most common way to investigate biological phenomenon, and they are the standard against which single molecule experiments are compared. The rapid evolution of single molecule biophysics has proven, however, that studies on the single molecule level can reveal spatial and temporal inhomogeneities, which are hidden in ensemble studies. Such inhomegeneities can be important and carry fundamental information regarding the basic function of a given bio-molecule. One beautiful example is that of polymerase, where single molecule studies have revealed information regarding the working mechanism, energy turn-over, typical speeds and forces, this type of information being hidden in ensemble studies [1, 2].

The bacterial outer membrane structure is a typical target both for antibiotics and antimicrobial peptides and the motility of outer membrane proteins can therefore be used as an indirect measure of their action. The diffusion of individual proteins has been studied mainly in eucaryotic cells, for a review see [3]. Only very few experimental investigations have reported on the diffusion of single proteins in the outer membrane of metabolically competent procaryotic cells [4-6]. These investigations focused on the motility of the λ-receptor in the outer membrane of *E. coli*. The λ-receptor is an 18 stranded β-barrel with the purpose of transporting maltodextrins across the outer membrane and is also the receptor of bacteriophage lambda. The λ-receptor is an integral outer membrane protein attaching firmly to the peptidoglycan layer [7]. Though this layer is strong enough to maintain a significant osmotic pressure difference across it, it constantly undergoes a dynamic disassembly and reassembly giving rise to local dislocations. Hence, any protein firmly attached to this layer would undergo the same local dislocations. Here we used an engineered λ-receptor [4], where we inserted a biotin acceptor site into an external loop of the protein. In energetically competent cells, the λ-receptor performs a confined diffusional motion, a 'wiggling' motion, within a range of approximately 50 nm [4, 5, 8]. This motility of the λ-receptor has recently been shown to be energy dependent and to decrease significantly upon energy depletion of the cell [8]. It was also proposed that the observed motility of the λ-receptor was a result of its close linking to the peptidoglycan layer, which undergoes a constant, dynamic, and energy consuming re-construction, and that the purpose of linking the λ-receptor to the peptidoglycan layer was to 'wiggle' the protein, which then, in turn, could facilitate transport of maltodextrins through the porin.

In the present investigation we used the λ-receptor as a tracer to monitor the action of vancomycin and ampicillin, two common antibiotics hindering peptidoglycan synthesis, on the outer membrane of *E. coli*. Also, we investigated the effect of the antimicrobial peptides, polymyxin B (PMB) and polymyxin B-nonapeptide (PMBN), on λ-receptor motility. PMB is a common antimicrobial peptide, which is known to increase inner and outer membrane permeability and kill the

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microorganism. PMBN is a truncated derivate of PMB and only increases outer membrane permeability. Both these drugs are known to act rapidly and in very small doses [9]. The paper is organized such that the methods and results of applying each drug is presented individually. The discussion unifies all results into a model of how antibiotics and antimicrobial peptides act on a single protein in the outer bacterial and which implications this has for our understanding of the attachment of the λ-receptor in the outer bacterial membrane.

POSITION MEASUREMENTS

In order to find the positions visited by the λ-receptor a streptavidin coated polystyrene bead was attached to the receptor and used as a handle for position-measuring optical tweezers. The λ-receptor was *in vivo* biotinylated, the efficiency of the biotinylation being very low; on average less than one λ-receptor pr bacterium had biotin attached to an extra cellular site. This ensures that each streptavidin coated bead was only attached to a single biotinylated λ-receptor. The strains were derived from S2188 [10] and the biotinylation procedure and controls are described in Ref. [4]. The motion of the protein can be deduced from the motion of the bead [4], and the motion of the bead was monitored by optical tweezers based on a Nd:YVO_4, laser, wavelength 1064 nm. The trap was implemented in a Leica inverted microscope DM IRBE and the forward scattered light collected by a quadrant photodiode (Hamamatsu S5981). For a detailed description of the settings and how the laser signal is converted to position measurements please see Refs. [4, 8]. The laser power delivered at the sample was around 10 mW and the total exposure time a couple of seconds. At such low exposure doses there is no detectable physiological damage on bacterial species, even if they are in the most intense region of the trap [11]. The first step in the position measurement procedure was to trap a bead free in solution and perform a force calibration at the same height as a bead attached to a λ-receptor [4]. Secondly, we measured a time-series of the positions of a bead attached to a λ-receptor, this attachment being characterized by a 'wiggling' motion of the bead [4]. Unspecifically attached beads did not 'wiggle' and if an unspecifically attached bead is pulled away from the bacterium using the optical tweezers, a viscoelastic tether composed of membrane material will be extracted [12].

VANCOMYCIN

Vancomycin blocks peptidoglycan synthesis and is normally used against gram positive bacteria lacking the outer membrane part. Vancomycin is too large to pass the outer membrane of normal gram negative bacteria. However, gram negative bacteria with the mutation of increased outer membrane permeability (*imp*) are susceptible to larger molecules as vancomycin or bile salts. Increased membrane permeability in *E. coli* was found in 1989 [13], and it was recently established that this was due to the absence of one of two outer membrane proteins [14, 15]. To test the action of vancomycin we made *imp* strains as described below.

Strains

To conduct these experiments we needed a strain with increased outer membrane permeability, without pili, and without λ-receptors. The reasoning behind the latter two requirements is that pili would disturb the measurement procedure and we only wished to have the biotinylated λ-receptors present. Thomas Silhavy and Natividad Ruiz kindly provided strain NR701, which is MC4100 *imp*4213 *yfhS*::Tn10. We used a P1 transduction to transfer *imp4213* in the strain S1964 [16]. To follow the transfer of *imp4213*, the gene of arabinose was chosen as a marker. NR701 is *ara*-, so first we made it *ara*+. To complete the P1 transduction a single phage colony was obtained by growing P1vir on the bacteria S971 [17]. The single phage colony was used on MG1655 to transfer *ara*+ into NR701. Then *ara*+ *imp4213* was co-transduced into S1964 [16] which is without pili, is *ara*::Tn10 and has no λ-receptors. This strain was termed TM14. Then the plasmid pLO16 [4] coding for the modified λ-receptors was inserted. A successful insertion of *imp*4213 was controlled by sensitivity to bile salts and no growth in the presence of an SDS disk.

Methods

Bacterial colonies were grown over night at 37 ºC on YT agar [18] supplemented with 25 μg/ml chloramphenicol. A single colony was picked and suspended into M63 media [19] containing 1 μg/ml B1, 25 μg/ml Chloramphenicol, 0.1 % casein hydrolysate, and 0.2 % glycerol. The bacteria were grown in shaking water bath over night at 37 ºC. 0.1 ml of the overnight culture was then diluted into 3 ml of fresh M63 media and grown at 37 ºC until log-phase. To induce the expression of the λ-receptor 3 ml of fresh M63 media supplemented with IPTG was added to a final IPTG concentration of 0.5mM. The bacteria were grown for an additional ½ hour with IPTG. 1 ml cell culture was spun down at 1673 g for 5 minutes and the cells resuspended in 100 μl buffer. The buffer used throughout the experiments was 10 mM potassium phosphate, 0.1 M KCl, pH 7. The procedure until this point was carried out at 37 ^{o}C, after this point at room temperature unless otherwise stated.

The beads used were streptavidin-coated polystyrene beads with a diameter of ~ 0.5 μm (Bangs Laboratories, Inc). The beads were suspended in millipore water and centrifuged at 1673 x g for 10 min. The supernatant was discarded and the beads resuspended in buffer and sonicated for at least 15 min to remove aggregates. To immobilize the bacteria a droplet of 10 mM poly-l-lysin was spread on a 0.17 mm coverslip and left to dry. This coverslip formed the bottom of a perfusion chamber. 10 μl of the bacterial solution was perfused into the chamber, this step was repeated. The bacteria were left for 15-25 minutes to adhere to the surface. Ten microliters of 12.5 mg/ml heparin was flushed into the samples and left for 15 minutes. Heparin decreases the attraction between the surface and the streptavidin-coated beads. The samples were rinsed four times with buffer. 10 μl washed streptavidin coated polystyrene beads was then perfused into the chamber and left for 15 minutes to attach to the λ-receptors. Finally, the chamber was flushed 3-5 times with M63 glucose media. Glucose was used as a carbon source inside the perfusion chambers to support anaerobic growth. The chambers were stored at 4^{o}C until use.

The positions of beads attached to λ-receptors in untreated cells were measured as described above. Thereafter

the cells were treated with vancomycin by flushing 20 µl of 0.08 mg/ml vancomycin in M63 glucose through the chamber 5 times. This concentration is 100 × MIC [20]. MIC is the minimal inhibitory concentration, i.e., the smallest amount of anti-microbial agent that is required to inhibit the growth of the organism. After a delay of roughly 3 min the chamber was flushed 5 times again with vancomycin solution and finally, the mobility of the exact same λ-receptor was measured again.

We also performed a control to ensure that the flushing procedure itself was not the cause of the observed decrease of motility. For this 'flow control' experiment the entire procedure was repeated but without poison in the last flushings.

Results

Fig. (**1**) shows a timeseries of positions visited by a single λ-receptor before and after treatment with vancomycin, note that it is exactly the same receptor which is monitored both before and after treatment. The gap in the timescale denotes the time where the vancomycin treatment takes place. The right inset shows the distribution of the visited positions before (broad histogram) and after (narrow histogram) vancomycin treatment, full lines are Gaussian fits. The left inset shows a scatter plot of the positions visited before (black) and after (grey) vancomycin treatment.

From Fig. (**1**) it is clear that the motility of the λ-receptor decreases upon treatment with vancomycin. We have chosen to quantify the motility of the λ-receptor by the width of the position histograms (inset of Fig. **1**), the larger the standard deviation of the histogram, σ, the more motile the λ-receptor. Before vancomycin treatment the standard deviation of the receptor bead complex was σ_{before}= (9.6 ± 0.97) nm (mean ± SEM), and after treatment σ_{after} = (3.4 ± 0.44) nm, n = 10. To test whether these two numbers are significantly different a Students T-test was made. The resulting p-value gives the probability that the observed difference is accidental. In other words, a low p-value supports the hypothesis that the numbers are significantly different. A T-test on the σ values with and without vancomycin gave p = 0.0002. Hence, we conclude that the motility of a λ-receptor in an untreated cell is significantly larger than in a cell treated with vancomycin.

The scatter plot shown in Fig. (**1**) appears asymmetrical. However, a detailed investigation of the ellipticity of the positions visited by the λ-receptor [4] shows that the principal axes of the distribution are randomly aligned with respect to the bacterial axes. In some cases, as shown in the inset of Fig. (**5**), the distribution appears nearly symmetric. As the maximum travel of the receptor is ~50 nm, a distance much smaller than the cell diameter (500 nm), and as only beads located in the center of a bacterium were chosen, it is fair to assume that the motion is quasi two dimensional.

The result of the 'flow control' experiments was that the motility of the λ-receptor was completely unaffected by the flushing procedure (n=12).

The cells were grown at 37 °C. The measurements shown in Fig. (**1**) and the numbers given above were from experiments performed at room temperature (22 °C). Using a heated sample holder we also performed similar measurements at 37 °C. The results obtained at 37 °C (n=10) were indistinguishable from those performed at 22 °C (n=10), however, they were more noisy which is probably because the experiments were technically more difficult and the temperature was higher.

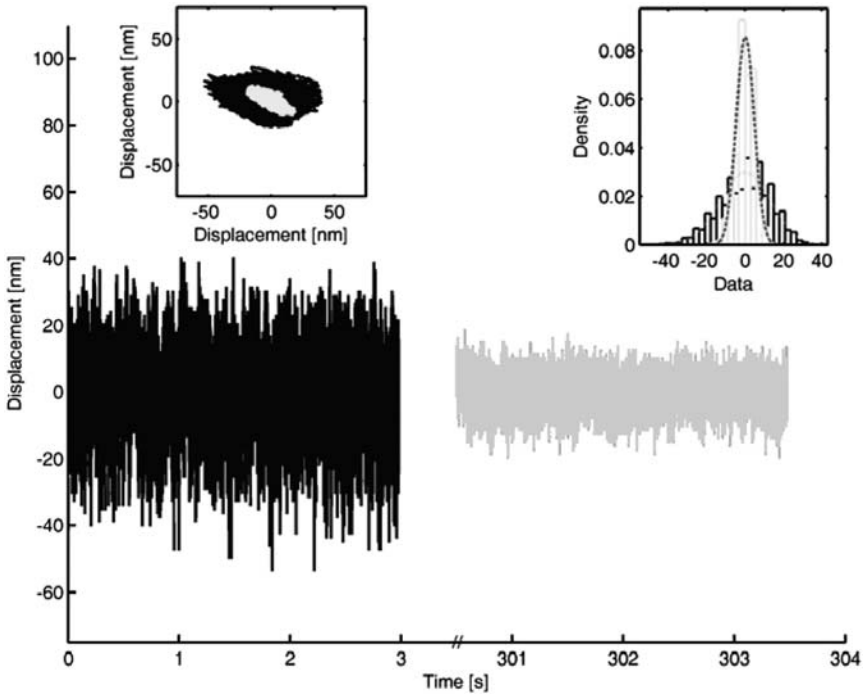

Fig. (1). Effect of vancomycin treatment. Time series of positions visited by an individual λ-receptor before (black) and after (grey) vancomycin treatment. The right inset shows a histogram of positions visited before (broad histogram) and after (narrow) vancomycin treatment. The left inset shows a scatter plot of positions visited before (black) and after (grey) vancomycin treatment.

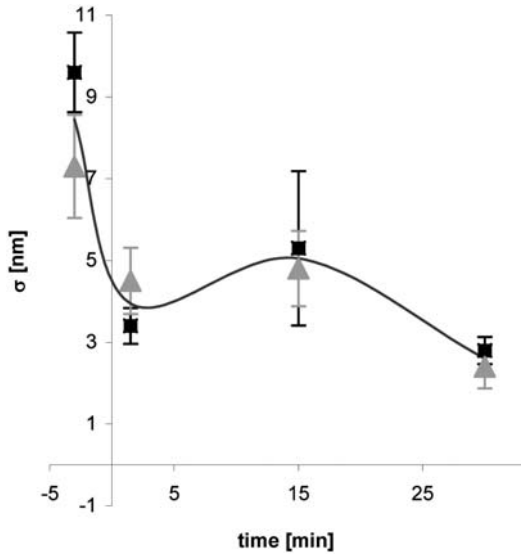

Fig. (2). Temporal evolution of the action of vancomycin. The graph shows the motility of the λ-receptor, quantified by σ, as a function of time. Vancomycin was added at -3 minutes, just after the first symbol of the graph. Black squares: 22 °C, grey triangles: 37 °C. Errorbars show SEM.

We also monitored the time evolution of the λ-receptor motility pattern upon vancomycin treatment, this is visualized in Fig. (**2**). The experiment was done both at 22 °C and 37 °C. Immediately after vancomycin treatment the motility decreased, hence, vancomycin seemed to have a rapid effect. The motility of the receptor was a bit higher after 15 minutes than immediately after treatment. After 30 minutes the motility was smaller than after 0 minutes, and significantly smaller than after 15 minutes (on a 5 pct. significance level). Hence, both at 22 °C and 37 °C there seems to be an evolution of λ-receptor motility as a function of time after beginning of vancomycin treatment: There is a rapid effect (after 0-3 minutes), then the receptor regains some motility, and finally (after 30 minutes), motility is down to a minimum, possibly corresponding only to thermal fluctuations. Possible reasons for this behavior are proposed in the discussion section.

AMPICILLIN

Ampicillin is a common antibiotic, efficient towards gram negative bacteria and hindering peptidoglycan synthesis. The effect on λ-receptor motility of ampicillin treatment with was first reported in [8] but for sake of completeness the results are briefly reviewed here.

Strains

The strains used are *E. coli* K12 S2188 [10] harboring the pLO16 plasmid [4].

Methods

In the experiments with ampicillin the procedure was largely as described under 'Vancomycin methods'. However, one important difference is that all solutions (starting from the 3 ml fresh M63 into which the overnight culture was diluted) were supplemented with 12 % sucrose. Sucrose was supplemented in order to osmotically stabilize cells with comprised cell walls. After reaching log-phase the M63-IPTG solution was supplemented with 100 μg/ml ampicillin. Due to the length of time needed for the action of ampicillin and to regenerate the cell wall after ampicillin treatment we did not examine the same λ-receptor before and after treatment, different populations were observed. Three types of experiments were conducted: 1) With ampicillin present both during growth and during the measurement procedure. 2) With ampicillin present during growth but not during the measurement procedure. 3) A control where ampicillin was not present neither during growth nor during the measurement procedure.

Results

Fig. (**3**) shows the motility of a λ-receptor in a metabolically competent cell (population 3) and in a cell treated with ampicillin (population 1). Insets show corresponding histograms and scatterplots. Treatment with ampicillin significantly decreases λ-receptor motility. The numbers resulting from the analysis are summarized in Table **1**.

The standard deviation of populations 1 and 3 characterizing the motility of the λ-receptor significantly decreases upon treatment with ampicillin (Students T-test gave $p = 2.1 \times 10^{-14}$). Also, a comparison of populations 1 and 2 showed a significant difference ($p = 8 \times 10^{-15}$). But populations 2 and 3 are indistinguishable. Hence, the λ-receptor mobility significantly decreases upon ampicillin treatment, but if the cells are transferred to ampicillin free growth media, the original λ-receptor motility can be restored [8]. The standard deviation characterizing the motility of the ampicillin control is slightly higher than that of the vancomycin controls. This could be due to the change in index of refraction caused by the presence of sucrose in the measurement chamber (which, in turn, changes the properties of the optical trap).

Table 1. Results of Ampicillin Treatment

	Population 1	**Population 2**	**Population 3 (Control)**
Growth	+ Ampicillin	+ Ampicillin	No Ampicillin
Experiment	+ Ampicillin	No Ampicillin	No Ampicillin
n (# datasets)	38	28	24
σ ± SEM [nm]	3.43 ± 0.32	10.25 ± 0.69	11.32 ± 0.85

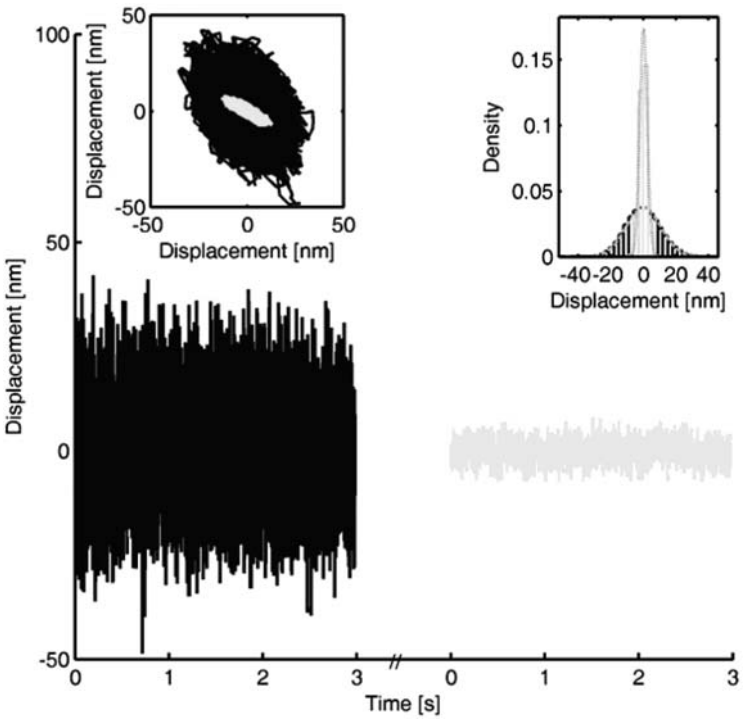

Fig. (3). Effect of ampicillin treatment. The left part of the time series (black) shows the positions visited of a λ-receptor in a metabolically competent cell (population 3), the right part (grey) in a cell treated with ampicillin (population 1). The right inset shows a histogram of positions visited without (broad histogram) and with (narrow) ampicillin treatment. The left inset shows a scatterplot of positions visited without (black) and with (grey) ampicillin treatment.

ANTIMICROBIAL PEPTIDES

The two antimicrobial peptides investigated in the present study are polymyxin B (PMB) and its truncated derivative polymyxin B-nonapeptide (PMBN). Fig. (**4**) shows the chemical construction of PMB, and if a cut is made at the arrow the left part of the structure is PMBN. PMB increases the permeability of both the inner and outer membranes of gram negative bacteria [9]. This renders the bacterium unable to maintain an electrochemical gradient across its cell membrane and causes cell death. PMBN, on the other hand, only makes the outer cell wall more permeable (does not cause cell death).

Strains

The bacterial strain used is the same as in the ampicillin experiments.

Methods

As for vancomycin experiments. In the experiments with PMB first the motion of an individual λ-receptor was first measured in an untreated cell. Then the chamber was flushed with 5 x 10 µl buffer containing 100 µg/ml PMB and the motility of the same λ-receptor was measured after treatment with PMB. In the experiments with PMBN, 125 µM PMBN was flushed into the chamber before flushing with heparin and the chamber was left for 30 minutes with PMBN. The reason for this particular point of the procedure is that PMBN is known to bind electrostatically to the membrane, and heparin might thus shield this binding [21]. This concentration of PMBN is well above the concentrations which are reported to have an effect [9]. As a control for the PMBN experiments all steps were repeated but without addition of PMBN.

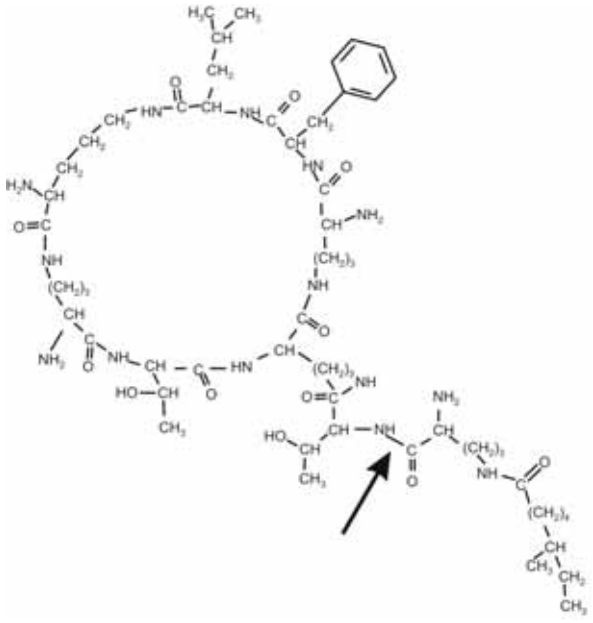

Fig. (4). Schematic drawing of the chemical structure of PMB. If a cut is made at the arrow the left part of the structure is the truncated derivative PMBN.

Results

Treatment of cells with the toxic PMB significantly decreases λ-receptor motility. This is shown in Fig. (**5**) which shows a time series of the motility of the same λ-receptor before (black) and after (grey) treatment with PMB. The histograms of positions visited were well fitted by Gaussian functions as shown in the right insert. The left inset shows a scatter plot of positions visited.

Again, λ-receptor motility was quantified by the standard deviation of the position histograms, and the results were σ_{before} = (9.13 ± 0.59) nm, and σ_{after} = (3.32 ± 0.67) nm, n = 12. These numbers are significantly different (p = 0.00003).

Treatment with PMBN did not give rise to a change in λ-receptor motility upon comparison with a control: σ_{PMBN} = (9.53 ± 0.41) nm, n = 34, and $\sigma_{control}$ = (9.68 ± 0.53) nm, n = 18.

DISCUSSION

Our results show that the motility of individual λ-receptor in the outer membrane of *E. coli* is strongly influenced by antibiotics and antimicrobial peptides because treatment with vancomycin, ampicillin, and PMB significantly reduced λ-receptor motility. To investigate the effect of vancomycin treatment we cloned a strain with increased outer membrane permeability (*imp*). A comparison of the vancomycin control to the PMB, PMBN, and antibiotics controls show that λ-receptor motility is unaffected by the *imp* modification.

A strength of single molecule investigations is the ability to follow temporal behaviors masked in ensemble experiments. This, we utilized here to follow the temporal evolution of the action of vancomycin on λ-receptor motility. Though vancomycin rapidly (within 0-3 minutes) decreased λ-receptor motility, the motility decreased significantly further after 30 minutes. This could give clues about the detailed action of vancomycin [22]: Vancomycin binds to intermediates in peptidoglycan synthesis. Therefore, rapid action of vancomycin may be simply steric rather than compromised structural integrity. Probably the bacterium can, to some extend, resist this first steric action of vancomycin, and this is the reason for the temporarily increased receptor motility. On longer time scales, vancomycin compromises structural integrity by hindering peptidoglycan synthesis, this causing a total decrease in receptor motility.

A physical model of the attachment of the λ-receptor in the outer membrane of *E. coli* was put forward in Ref. [4]. Later this model was expanded to include possible effects of energy depletion, which is also known to cause a significant decrease in λ-receptor motility [8]. Basically, the expanded model assumes the λ-receptor to perform a diffusive motion within the cell wall structure, the diffusive motion being mediated by thermal fluctuations and possibly also by active (ATP consuming) movement. Alternatively, the observed decrease in motility upon energy depletion could be caused by a change in the membrane structure, e.g., by an aggregation of proteins which could mediate a stiffening of the effective potential felt by the protein in the membrane [8]. The λ-receptor is firmly attached to the peptidoglycan layer [7], and in [8] it was proposed that the observed motility of the λ-receptor was a consequence of the local, dynamic, and energy consuming dislocations of the peptidoglycan layer.

Fig. (**6**) shows a schematic drawing of the effect of the antibiotics and antimicrobial peptides used in the present

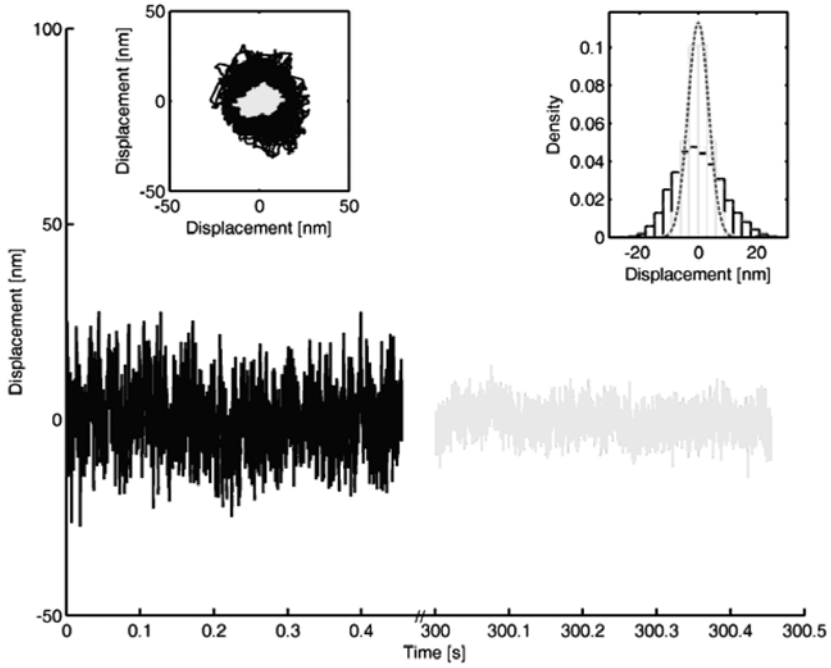

Fig. (5). Effect of PMB treatment. Time series of positions visited by an individual λ-receptor before (black) and after (grey) PMB treatment. The right inset shows a histogram of positions visited before (broad histogram) and after (narrow) PMB treatment. The left inset shows a scatterplot of positions visited before (black) and after (grey) PMB treatment.

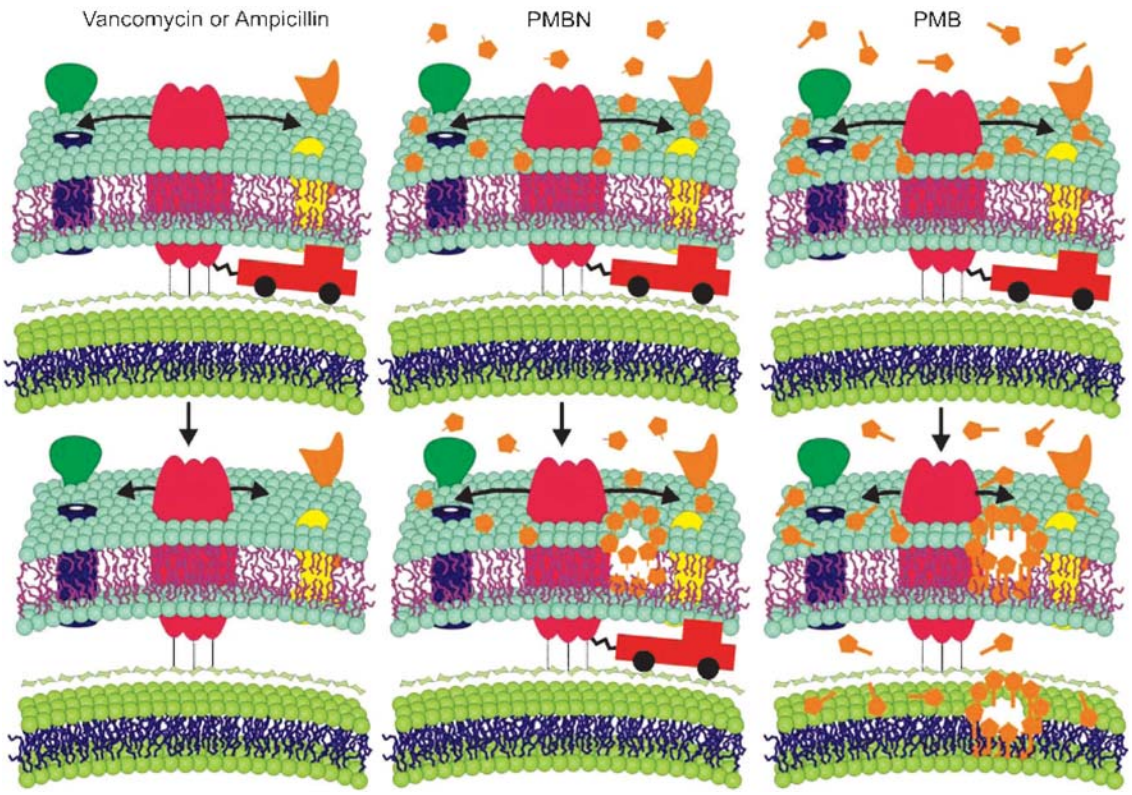

Fig. (6). Schematic drawing of the action of vancomycin, ampicillin, PMBN, and PMB on the membrane structure, and hence on the motility of the λ-receptor. The upper row shows the situation before treatment, the lower row after treatment. An active motion of the λ-receptor is illustrated by the truck and the larger side-wards arrows. The λ-receptor λ-receptor is the pink tri-mer, the outer membrane has light blue head groups, the peptidoglycan is the thin greenish structure to which the λ-receptor is attached, the inner membrane had green head groups, and both PMB and PMBN are colored orange.

investigation. The upper part shows the situations before treatment, the lower part the situations after. In an untreated cell, the λ–receptor performs an 'active' motion (visualized by the truck in Fig. **6**). Vancomycin and ampicillin both hinder peptidoglycan synthesis and are observed to decrease motility. PMB increases permeability of both the inner and outer membranes, destroys the electrochemical gradient across the inner cell wall and kills the cell. This also decreases λ-receptor motility. As PMB destroys the electrochemical gradient across the inner cell wall its biological action is somewhat similar to that of azide and arsenate, as reported in [8]. Hence, the results of PMB support the results and hypothesis put forward in [8].

We did not see an effect of PMBN treatment. This is probably because PMBN does not effect λ-receptor motility. PMBN was added during preparations but not in the final chamber. As its interaction is known to be strong and electrostatic PMBN is assumed to be firmly attached to the membranes also during the position measurements. However, there is a small risk that the bacteria resumed 'normal' protein motility when the drug was not abundantly present.

Altogether, the results of the present investigation show that a hindering of peptidoglycan synthesis, either by vancomycin or by ampicillin, decreases λ-receptor motility. The same effect is seen by treatment with PMB, which targets both the outer and inner membranes and kills the cell. No effect was seen with PMBN which only targets the outer membrane. Also, the *imp* modification of the bacteria which affected the outer membrane permeability had no effect. These observations strongly support the hypothesis put forward in Ref. [8] that the motility of the λ-receptor is mediated through its firm connection to the peptidoglycan layer. In other words, the constant local dislocations of the peptidoglycan layer give rise to the observed motility of the λ-receptor. Perturbations of the outer membrane had no effect on λ-receptor motility.

CONCLUSION

We investigated the effect of antibiotic treatment on the motility of a single protein, the λ-receptor, in the bacterial outer membrane. An *E. coli* strain with increased outer membrane permeability thus allowing for transport of vancomycin was cloned. Treatment of this strain with vancomycin significantly decreased λ-receptor motility, this effect being present both at 22°C and at 37°C. A strength of the measurements was that the motility of an individual λ-receptor was observed for an extended period of time, both before and after exposure. This allowed us to observe a temporal evolution in the action of vancomycin: Vancomycin rapidly decreased the motility of the receptor, after 15 minutes the receptor regained motility, but after 30 minutes there was a significant further decrease of motility. Treatment with ampicillin, which is known to hinder peptidoglycan synthe-

sis, also decreased λ-receptor motility and hence confirmed that the observed motility is closely linked to the dynamic dislocations of the peptidoglycan layer. Also treatment of the cells with PMB decreased receptor motility. PMB is an antimicrobial peptide which increases permeability of both the inner and outer membranes and kills the cells. PMBN, which only targets the outer membrane, had no effect on λ-receptor motility. Altogether, we observed that if the re-construction of the peptidoglycan layer is hindered by vancomycin or ampicillin, or if the cell is killed by PMB, the motility of the λ-receptor decreases. This supports the hypothesis put forward in Ref. [8] that the λ-receptor is firmly attached to the peptidoglycan layer, and that its motility originates from the energy consuming motion of the peptidoglycan layer. Apart from providing detailed information on the biological causes of bacterial membrane protein motility, this investigation also paves the way for future investigations on the temporal evolution of the action of antibiotics and antimicrobial peptides on a single molecule level.

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