Chemotaxis of *Escherichia coli* to *L*-serine

This article has been downloaded from IOPscience. Please scroll down to see the full text article.

2010 Phys. Biol. 7 026007

(http://iopscience.iop.org/1478-3975/7/2/026007)

View the table of contents for this issue, or go to the journal homepage for more

Download details:
IP Address: 59.162.23.19
The article was downloaded on 01/07/2010 at 08:33

Please note that terms and conditions apply.
Chemotaxis of *Escherichia coli* to L-serine

Rajitha R Vuppula, Mahesh S Tirumkudulu and K V Venkatesh

Department of Chemical Engineering, Indian Institute of Technology-Bombay, Mumbai 400076, India
E-mail: mahesh@che.iitb.ac.in and venks@che.iitb.ac.in

Received 12 March 2010
Accepted for publication 13 April 2010
Published 26 May 2010
Online at stacks.iop.org/PhysBio/7/026007

Abstract

A novel experimental technique was used to quantify the motion of *E. coli* to varying serine concentrations and gradients so as to capture the spatial and temporal variation of the chemotactic response. The average run speed and the cell diffusivity are found to be dependent on the serine concentration. The measured diffusivities were in the range of $1.2 \times 10^{-10}$ m$^2$ s$^{-1}$. The study revealed that the rotational diffusivity of the cells, induced by the extracellular environment, also varies with the serine concentration. The drift velocity increased with serine gradients reaching a maximum value of $\sim 5.5 \mu$m s$^{-1}$ at 1.6 $\mu$M $\mu$m$^{-1}$ after which it decreased. Experimental analysis demonstrated the interdependence of run speed, rotational diffusivity and drift velocity that characterizes the motion. Further, the motion was found to critically depend on the oxygen concentration and energy level of the cells.

Online supplementary data available from stacks.iop.org/PhysBio/7/026007/mmedia

1. Introduction

Micro-organisms respond to different chemicals found in their environment by migrating either toward or away from them. This migration could be either toward favorable chemicals called chemoattractants or away from unfavorable chemicals called chemorepellents. The phenomenon of migration in response to a chemical stimulus is important in many biological processes including immune response, embryogenesis, wound healing, etc [13, 33]. It is also important in bio-film formation and bio-remediation of subsurface contaminants [15]. Although chemotaxis has been observed in many bacterial species, the two best examples studied are those of *Escherichia coli* and *Bacillus subtilis*. *E. coli* respond to the chemical stimulus by alternating the rotational direction of their flagella. Here, the counterclockwise (CCW) rotation results in a motion called run, whereas clockwise (CW) rotation leads to tumbling of the bacteria. By modulating the duration of runs and tumbles, the bacteria achieves a net motion toward chemoattractants, or away from chemorepellents. This phenomenon of chemotaxis was first discovered by Pfeffer and Englemann in the year 1880 [1]. Thereafter, many experimental techniques [16] have been employed to quantify the chemotaxis of *E. coli* in the presence of various attractants such as aspartate, serine, glucose, galactose, fucose, etc [2, 9, 19, 20].

In this study, the chemotaxis of *E. coli* to L-serine has been investigated. As is well known the chemotactic response of *E. coli* to serine is mediated by the Tsr receptor (methyl-accepting chemotaxis protein) [3, 6]. Once *E. coli* senses the serine via Tsr, it forms a complex with the ligands. This changes the activity of the histidine kinase CheA, which in turn changes the swimming response of the bacteria independently via the phosphorylated response regulators CheY-P and CheZ. Further, the change in CheY-P concentration decides the probability of tumbles and runs [6]. The chemotactic behavior of *E. coli* can be quantified using various parameters such as average run speed, CW bias, drift velocity, cell diffusivity and rotational diffusivity. The run speed gives the average speed of the cells between consequent tumbles. The CW bias gives the fraction of time spent by the cells in tumble mode. The drift velocity measures the mean speed of cells while the diffusivity characterizes its random motion. Note that the random motion is induced by the collisions of cells with the solvent molecules as well as due to their own tumbling and is calculated as the mean-square distance traversed by the cells upon time. The final parameter, namely rotational diffusivity, is calculated as the mean-square angular deviation during a run upon time.

Adler [1, 2] was the first to quantify the motion of *E. coli* toward serine using agar and capillary assays. It was observed that *E. coli* placed in the center of an agar plate containing uniform concentration of tryptone started migrating toward...
the edge in three distinct populations in the form of rings [1]. The study concluded that the first ring consumed all of the serine and 90% of the oxygen, whereas the second ring consumed aspartic acid and remaining oxygen aerobically. The third ring which was formed at the bottom of agar plate consumed threonine anaerobically. Adler [2] used the capillary assay to study the effect of serine on chemotaxis in *E. coli*. It was noted that *E. coli* moved in two distinct populations (or bands) in a capillary containing a mixture of 20 amino acids. The first population uses all of the oxygen and a part of the serine aerobically. The second one uses the remaining serine anaerobically as it travels along the capillary. Adler thus concluded that the chemotaxis allowed bacteria to find the environment which supplied highest levels of energy. It was further reported that the minimum serine concentration required for chemotaxis was $2 \times 10^{-7}$ M and the maximum chemotactic response, where the maximum number of cells were attracted into the capillary occurred at $10^{-5}$ M [3].

Berg and Brown [9] built a three-dimensional tracking microscope to study the chemotaxis of *E. coli* that automatically followed the individual cell motion and a number of cell parameters could be deduced from these measurements. A capillary assay similar to that used by Adler [2] was adapted in this study to measure the chemotactic behavior of cells with varying chemoattractant concentrations. The average run speed, mean run length, mean tumble time, mean tumble angle, mean change in direction from run to run, etc were measured. It was observed that, with serine concentration, the average run speed increased by 40% whereas the mean tumble time, the mean change in direction from run to run and the mean angular speed while running decreased by 40%. They concluded that this might be because of inhibition of serine on chemotaxis at higher concentrations. However, the drift velocity and diffusivity as a function of serine concentrations were not reported.

Later, Berg and Turner [10] constructed an assay consisting of two stirred chambers separated by a micro-channel plate consisting of a fused array of capillary tubes. Here, the cells were added to one chamber and the attractant to the other, and a linear gradient was assumed along the micro-channel. The cells migrate to the second chamber via the micro-channel plate and the density of the cells was determined from the intensity of the scattered light in the second chamber. It was found that the flux of bacteria increased on addition of an attractant into the second chamber compared to when there was no attractant. The diffusivity was computed from the flux and found to be independent of the attractant gradients. The drift velocity was calculated from the knowledge of the total flux for varying attractant gradients. However in this study, the serine concentration as a function of space and time was not measured. Further, the motility medium consisted of sodium lactate which is a carbon source for *E. coli* and its effect on chemotaxis was neglected. Liu and Papadopoulos [25] used a micro-capillary assay to measure the chemotactic single cell parameters such as average run speed, run length, turn angles and cell diffusivity. The experimental setup in this study consists of two reservoirs communicating through a long capillary. The linear concentration profile for the chemoattractant was achieved by filling one of the reservoirs with the motility buffer while the other one was filled with a solution containing serine. They observed that the average run speed depends on the medium used for growth, the chemoattractant and the bacterial strain.

Recently, Kalinin et al [20] have used microfluidic assays to quantify the chemotaxis. In this study, three parallel channels were patterned in an agarose gel. A fluorescent solution initially flowed through the source channel which was then replaced with the chemoattractant, while a blank buffer flowed through the sink channel. This established a linear chemical gradient in the central channel because of the diffusion that takes place through the agarose gel from the source channel to the sink channel. The cells were then introduced into the central channel and their trajectories were recorded. Using the tracked positions, the cell diffusivity and the chemotactic migration coefficient (CMC), i.e. the average vertical position of all the cells tracked with respect to the central position of the channel, were calculated. They concluded that the cells respond to the spatial gradient of the logarithmic attractant concentration. However, it is important to note that the motility buffer used in this study contained lactic acid (a carbon source) whose influence on chemotaxis was ignored.

In parallel with the experimental work, a number of mathematical models have been developed to describe different aspects of chemotaxis at both the level of a single cell [7, 23, 24, 27] and that of a population [5, 21, 22, 26, 28]. While many of them have focused on the signaling pathway, there exist very few models such as AgentCell [18], *Es o l o* [12] and RapidCell [32] which integrate the cell motion along with the intracellular signaling pathways. We refer the reader to a recent review by Armitage et al [30, 31] for comparison of these models. More recently, Kalinin et al [20] used the Monod–Wyman–Changeux (MWC) model for mixed receptor clusters, and compared the motion predictions with the experimental measurements.

In this study, a novel experimental technique is described for measuring the *L*-serine gradients with respect to space and time in the absence of fluid flow. The motion of *E. coli* is tracked and the drift velocity is measured as a function of time and space over five orders of magnitude of concentration and gradient of serine. The effect of serine concentration on the average run speed, the rotational diffusivity and the cell diffusivity is studied. The experiments show that at a fixed spatial location, the drift velocity increases with the gradient and then decreases at much higher gradients. Further, for a fixed gradient, the drift velocity decreases with increasing spatial distance indicating saturation of receptors and partial adaptation. Experiments also suggest that oxygen plays a key role in the chemotaxis response and the response to a ligand cannot be analyzed in isolation to oxygen.

2. Materials and methods

The motion of *E. coli* K-12 (MTCC 1302) having the Tsr receptor, which respond to serine, was observed against serine
gradients in rectangular micro-capillaries (5 cm (L) × 1000 μm (W) × 100 μm (H)). The media used for the bacterial growth and the experimental methodology are detailed in the supplementary information available at stacks.iop.org/PhysBio/7/026007/mmedia.

2.1. Chemotaxis toward serine concentration and gradients

To observe the movement of E. coli in the absence of gradient, a plug of chemotaxis medium of 5 cm length containing serine at a fixed concentration was drawn into the capillary. Cells were taken into the capillary by contacting the pellet of cells (see supplementary information for details available at stacks.iop.org/PhysBio/7/026007/mmedia) with the mouth of the capillary. The capillary ends were sealed with wax and the E. coli movements were recorded. The duration of the experiments varied from 15 to 20 min. Further, to observe the response of E. coli to different serine gradients, 4.5 cm long liquid plugs of different concentrations of serine in motility buffer were drawn into the capillary followed by a 0.5 cm plug of motility buffer. E. coli movements were recorded at various distances from the pellet along the capillary, where the linear concentration profile is established (figure S1 available at stacks.iop.org/PhysBio/7/026007/mmedia). A schematic diagram of the micro-capillary experimental setup is shown in supplementary information, figure S2 available at stacks.iop.org/PhysBio/7/026007/mmedia. Each experiment was repeated six times on different days to capture the variability, if any. Details of the image analysis are reported in the supplementary information. Unless specified otherwise, experiments were conducted with the buffer saturated with air.

3. Results

Gradients of fluorescent glucose (2-(N-(7-nitro-benz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxy-glucose, 2-NBDG) were established in the capillary and their variation with time was recorded. It was found that a linear gradient was established in the capillary and their variation with time was less than 5% and 12%, respectively. These measurements show that the variation in the absolute concentration and gradient at any location during the course of the experiment was not more than 5% and 12%, respectively. This implies that a pseudo-steady state approximation of a constant absolute concentration and linear gradient is valid during the course of the experiment. Further, to characterize the chemotactic response, experiments were conducted in a capillary by varying both serine concentrations and gradients. In all the experiments, the cells moved out of the plug and into the capillary forming a band of concentrated cells of width 1600 μm (approximate). This band traversed from one end to the other end of the capillary. Note that all measurements were made in the front end of the band, where the local cell concentration was low ∼10^7 cells ml^-1. This was done to ensure that the motion of one cell was not affected by the neighboring cells [4].

The mean square displacements (MSD) of over 1000 cells from experiments performed with uniform serine concentrations are plotted as a function of time and shown in figure 1(a). The MSD increased quadratically for shorter times and showed a linear response after that. Interestingly, the slope of the linear response, i.e., at long times, increased with serine concentration up to 24000 μM, beyond which the slope decreased. The diffusivity, D, of the cells was determined by fitting the MSD to that obtained for a colloidal particle undergoing the Brownian motion [29]:

$$\frac{\langle x^2 \rangle}{2} = D \left[ \frac{D_m T}{kT} \left( \exp \left( \frac{-kT}{D_m} \right) - 1 \right) + t \right],$$

where, m is the mass of the cell, T is the temperature in kelvin and k is the Boltzmann constant. The fit also yields the mass of the cell to be, approximately, 10^{-15} kg, close to the reported value [17]. The diffusivity for the range of experiments initially increases and then decreases with serine concentration (figure 1(b)), consistent with the trend observed for the linear slope at long times. On increasing the serine concentration from 0 to 250 μM, there is a negligible change in the diffusivity. A further increase in serine concentration from 250 to 24000 μM resulted in a significant increase in diffusivity. At even higher serine concentration (240 000 μM), the diffusivity dropped to the pre-stimulus value. A maximum diffusivity of 318 ± 65 μm^2 s^-1 (mean ± standard deviation from the fit) was observed at a serine concentration of 24 000 μM.

Figure 1(c) plots the measured average run speed (v) at five different locations along the capillary for 0 and 5000 μM serine concentrations. A decrease in run speed was observed with increasing distance for both cases with the run speeds being higher in the presence of serine. The measured average run speed as a function of concentration at 500 μM is presented in figure 1(d). It can be observed that the initial increase in serine concentration resulted in an increase in the average run speed. This might be due to the influence of higher energy which is supplied by increasing concentrations of serine. The maximum average run speed of 21.9 ± 0.34 μm s^-1 (±standard error) was observed for a serine concentration of 5000 μM after which it decreased. The average run speed at high serine concentrations (240 000 μM) was similar to that in the absence of serine (i.e., only with the motility buffer), about 18.3 ± 0.69 μm s^-1. This decrease in run speed might be due to the inhibition by serine at high concentrations on cell motion. The observed trend of the initial increase in run speed followed by a decrease at high serine concentrations correlates with the trend observed for the measured diffusivity.

The measured angular displacement during a run is fitted to a Gaussian distribution and used to calculate the rotational diffusivity, which is given by $D_r = \langle \theta^2 \rangle / 2t$, where $\theta$ is
Figure 1. (a) Mean-square displacements about the mean value as a function of time for varying serine concentrations: 0 μM (‘•’), 250 μM (‘♦’), 1000 μM (‘▲’), 5000 μM (‘◦’), 24 000 μM (‘♦’) and 240 000 μM (‘△’). (b) The translational diffusivity as a function of time for various serine concentrations: 0 μM (‘•’), 250 μM (‘♦’), 1000 μM (‘▲’), 5000 μM (‘◦’), 24 000 μM (‘♦’) and 240 000 μM (‘△’). (c) Average run speeds (± standard error) as a function of distance in the absence of serine (‘◦’), and in the presence of serine 5000 μM (‘▲’), when the buffer is saturated with air. Average run speeds in the absence of serine (‘•’), and in the presence of serine 5000 μM (‘△’), when the buffer is saturated with oxygen. (d) Average run speeds (± standard error) as a function of serine concentration at 500 μm. Filled ‘△’ indicates the average run speed of 18.65 ± 0.62 μm s⁻¹ obtained in the absence of serine, when saturated with air.

Figure 2. (a) Rotational diffusivity as a function of serine concentration at 500 μm. Filled ‘△’ indicates the rotational diffusivity 0.38 ± 0.05 rad² s⁻¹ obtained in the absence of serine when saturated with air. (b) Measured drift velocity (± standard error) as a function of distance in the absence of serine (‘◦’), and in the presence of serine 5000 μM (‘△’), when the buffer is saturated with air. Measured drift velocity in the absence of serine (‘•’), and in the presence of serine 5000 μM (‘▲’), when the buffer is saturated with oxygen. (c) Measured drift velocity (± standard error) as a function of distance for varying serine concentration when saturated with air: 0 μM (‘◦’), 50 μM (‘♦’), 250 μM (‘▲’), and 5000 μM (‘●’). The drift velocity of the AER mutant was zero irrespective of serine concentration. The same result was obtained in the presence of saturated air or oxygen (‘★’).

The angular displacement and the angular brackets represent the ensemble average. Figure 2(a) presents the rotational diffusivity as a function of the serine concentration. The rotational diffusivity for the motility buffer (in the absence of serine) was measured as 0.38 ± 0.05 rad² s⁻¹ (‘△’). However, with the increase in serine concentration, the rotational
diffusivity decreased initially, reaching a minimum value 0.21 ± 0.04 rad² s⁻¹ after which it increased at 240 000 μM close to the original value of 0.37 ± 0.05 rad² s⁻¹ observed in the absence of serine. Note that this trend is opposite to that observed for both run speeds and diffusivity.

The run speed and the rotational diffusivity which are independently obtained through experiments can also be used to determine the cell diffusivity [8]:

\[
D = \frac{v^2}{6D_r}.
\]

The values of the diffusivity thus obtained for varying concentrations of serine are also plotted in figure 1(b) (○·), and compared with those obtained using MSD('•'). The two diffusivities are close to each other validating the experimental measurements.

Experiments were performed to determine the drift velocity at various locations along the capillary in the absence and presence of serine but without any gradient (figure 2(b)). A finite drift velocity (~2 μm s⁻¹) was observed in the absence of serine suggesting that the presence of dissolved oxygen in the buffer may be contributing to cell motion. Further, the drift velocity decreased along the length of the capillary. On introducing serine (5000 μM), a similar trend was observed with higher values of the drift velocity (by about 40%). To establish the role of oxygen, we also performed experiments with the motility buffer saturated with oxygen. The measured drift velocity in the absence of serine was significantly higher (by about 50%) for all locations along the capillary. On introducing serine, the velocity increased further (by about 100%) suggesting that both serine and oxygen play a role in eliciting a response. The increase in the drift velocity in the presence of serine and saturated oxygen can be attributed to the higher run speeds (about 24 μm s⁻¹) as shown in figure 1(c) (▲•). These results indicate that the cells consume oxygen and thus respond to the induced oxygen gradient. Further, the cumulative effect of serine and increased oxygen levels significantly increases the run speed of the cells indicating higher energy levels of the cell. The measured drift velocities obtained for uniform serine concentrations of 0 (○), 50 (△), 250 (□) and 5000 μM (●) in the absence of gradients at five different locations are presented in figure 2(c). A significant increase in the drift velocity (from 2 to 3 μm s⁻¹) was observed with the increase in serine concentration (0–5000 μM) at all locations. The observed increase in the drift velocity with serine concentration can be attributed to the concomitant increase in the run speed (see figure 1(d)).

The significant decrease in the drift velocity with distance in the absence of gradients (figures 2(b) and (c)) cannot be attributed entirely to the reduction in run speed as the decrease in the latter is not more than 10–15% at 2500 μM (see figure 1(c)). Instead, the observed trend suggests adaptation of the cell to the established oxygen gradient and thus lower response with distance. Experiments were also performed using a mutant isogenic strain of *E. coli* K-12 lacking AER, a gene responsible for sensing oxygen, both in the presence and absence of serine, to ascertain the influence of oxygen on chemotaxis to serine. The drift velocities were found to be zero irrespective of serine concentrations (figures 2(b) and (c)). As expected, the mutant strain did not respond to varying concentrations of oxygen.

The measured drift velocities obtained at various serine gradients are plotted in figure 3(a)–(f). For the lowest gradient \( G = 0.0016 \text{ μM} \text{ μm}^{-1} \) (figure 3(a)), the observed response was the same as that in the absence of serine (figure 2(d)) indicating that cells could not sense the low serine gradient. With increasing gradient a drift velocity of 3.5 μm s⁻¹ was observed at 500 μm for \( G = 0.016 \text{ μM} \text{ μm}^{-1} \) which monotonically decreased to 1.9 μm s⁻¹ at 2500 μm (figure 3(b)) demonstrating possible adaptation/saturation to both serine and oxygen gradients. A further increase in the gradient (up to \( G = 1.6 \text{ μM} \text{ μm}^{-1} \)) showed a similar trend but with a higher drift velocity. A maximum drift velocity of 5.5 μm s⁻¹ was observed for \( G = 1.6 \text{ μM} \text{ μm}^{-1} \) at 500 μm (figure 3(d)). Beyond \( G = 1.6 \text{ μM} \text{ μm}^{-1} \) (figure 3(e)), the drift velocity decreased at all locations with the lowest velocities observed for the highest gradient (\( G = 160 \text{ μM} \text{ μm}^{-1} \), figure 3(f)). The observed drift velocity at this gradient was similar to that in the absence of serine. The low values of drift velocity at all locations is caused both by adaptation and by the reduced run speeds at high serine concentrations (figure 1(d)).

Next, we present the measured drift velocity, at different spatial locations as a function of varying gradients (figure 4(a)). The drift velocity, at a fixed spatial location, increases with the gradient reaching a maximum around 1.6 μM μm⁻¹ and decreases further at higher gradients. Also, at a fixed gradient, the drift velocity decreases along the length of the capillary. It should be noted that these measurements include contributions from both serine and oxygen. Recall that the latter resulted in significant drift velocities (2–3 μm s⁻¹). In order to obtain the drift velocity in response to only serine, we assume that the observed drift velocity is simply the algebraic sum of the response to serine and oxygen separately. We can then subtract the measured drift velocity in the absence of serine gradients (~2 μm s⁻¹) at that specific location with those obtained in the presence of serine (figure 4(b)). The maximum drift velocity measured due to serine alone is about 3 μm s⁻¹ at around 1.6 μM μm⁻¹.

The experimental drift velocities were also compared with those of Berg and Turner [10] who measured the drift velocity for serine gradients in 50 μm diameter capillaries of length 500 μm. The results are reproduced in figure 5(a) (dotted line with ‘○’). In their experiments, the drift velocity increased with the serine gradient up to 1.2 μM μm⁻¹ reaching a maximum value of 4 μm s⁻¹ after which it decreased. We also compare our measured drift velocities at 500 ± 80 μm (solid line with ‘●’) as shown in figure 5(a). The observed trend matches the reported result in that the drift velocity increased monotonically reaching a maximum value of 5 μm s⁻¹ at \( G = 1.6 \text{ μM} \text{ μm}^{-1} \) and decreased thereafter. The higher drift velocities in our experiment might be due to the difference in *E. coli* strain.

Kalinin et al [20] have recently reported the drift velocity for varying methyl-aspartate gradients as a function of the average logarithmic gradient and shown a linear relationship.
Figure 3. The measured drift velocity (± standard error) as a function of distance for various gradients of serine (G) and initial ligand concentration (L₀): (a) G = 0.0016 μM μm⁻¹ and L₀ = 1.6 μM (‘○’); (b) G = 0.016 μM μm⁻¹ and L₀ = 16 μM (‘△’); (c) G = 0.16 μM μm⁻¹ and L₀ = 160 μM (‘□’); (d) G = 1.6 μM μm⁻¹ and L₀ = 1600 μM (‘•’); (e) G = 16 μM μm⁻¹ and L₀ = 16000 μM (‘▲’); and (f) G = 160 μM μm⁻¹ and L₀ = 160 000 μM (‘■’).

Figure 4. (a) Drift velocity obtained from experiments as a function of serine gradients at three different spatial locations: ‘△’ at 500 μm, ‘□’ at 1000 μm, ‘○’ at 1500 μm, respectively. (b) Modified drift velocity obtained from experiments as a function of serine gradients at three different spatial locations: ‘△’ at 500 μm, ‘□’ at 1000 μm, ‘○’ at 1500 μm, respectively. Here, the modified value was obtained by subtracting the local drift velocity due to oxygen gradient alone (figure 2(c)) from the measured value in the presence of both serine and oxygen (figure 4(a)).

between the two. In order to verify the above for serine gradients, the experimentally obtained local drift velocity was plotted as a function of the local logarithmic gradient (d(ln L)/dx) for all the gradients at various locations (see figure 5(b)). The drift velocities varied from 1.5 μm s⁻¹ to a maximum value of 5.5 μm s⁻¹ at a fixed value of d(ln L)/dx. This variability shows that the drift velocity at a location is dependent not only on the ratio of the ligand gradient and the concentration at a location but also on the past history of the concentrations experienced by the bacteria. Surprisingly, the average value of the velocity at each d(ln L)/dx shows a linear increase (solid line in figure 5(c)), a trend that was also observed by Kalinin et al [20] for their experiments with α-methyl-aspartate gradients. Our study suggests that the response of E. coli to external gradients can be termed logarithmic only in an average sense.
Drift velocity (experimental data while the dotted line with ‘◦’ represents that observed by Berg and Turner [10].)

Figure 5. (a) Drift velocity obtained through experiments as a function of serine gradients at 500 μm: solid line with ‘•’ represent our experimental data while the dotted line with ‘◦’ represents that observed by Berg and Turner [10]. (b) Measured drift velocity (± standard error) as a function of the spatial gradient of the logarithmic serine concentration: \( G = 0.0016 \text{μM} \text{μm}^{-1} \) (‘◦’), \( G = 0.16 \text{μM} \text{μm}^{-1} \) (‘△’), \( G = 1.6 \text{μM} \text{μm}^{-1} \) (‘●’), \( G = 16 \text{μM} \text{μm}^{-1} \) (‘△’) and \( G = 160 \text{μM} \text{μm}^{-1} \) (‘▲’). (c) Average value of the drift velocity (± standard error) at each \( d(\ln L)/dx \) with location. The solid line represents a linear curve fit.

4. Discussion

The earliest methods used to quantify the chemotactic response of *E. coli* were agar assays and capillary assays. In both these methods, *E. coli* moves up a gradient set by the consumption of the attractant and there is no control over the induced gradients. However, more recent experiments have been able to establish controlled gradients using microfluidic techniques [20] that allow the measurements of drift velocities over a wide range of gradients. However, these techniques fail to capture the chemotactic response of *E. coli* to simultaneous temporal and spatial variations. The novel and yet simple experimental method reported here was used to establish controlled gradients and to quantify the chemotactic parameters, namely drift velocity, cell diffusivity, average run speed and rotational diffusivity, as a function of time and space. Our study takes the work of Berg and Turner [10] a step forward in quantifying the chemotactic response of *E. coli* in space and time from single cell measurements.

The variation of MSD with serine concentration and gradients suggest that the cell diffusivity depends on serine concentration. The cell diffusivity increased initially with serine concentration reaching a maximum after which it reduced at high serine concentrations. A similar trend was also observed in the case of run speeds. This trend suggests that the cell responds positively to serine at low concentrations, but the motion is inhibited at high concentrations. This is similar to the phenomenon of substrate inhibition observed in the growth of microorganisms [11]. The diffusivity measured for our strain is close to that for other reported strains, 100–300 μm² s⁻¹ [10, 25].

Our experiments have demonstrated that the measured average run speed decreases as the cell moves from one end to the other end of the capillary. In addition, *E. coli* population moves in a band while consuming both oxygen and stored energy. Similar observations were made by Adler [1]i na capillary containing a mixture of 20 amino acids except that in his case two distinct bands were formed. While the first band consumed all of oxygen and part of serine, the second band anaerobically consumed the remaining serine. Anaerobic experiments with our strain of *E. coli* suggested that it could not consume serine in the absence of oxygen. Further, the aerobic growth rate of *E. coli* in the presence of only serine was less compared to that in the presence of a mixture of 20 amino acids (results not shown). Based on this, we assumed that the consumption of serine by *E. coli* is negligible during the first 15 min which is equal to the duration of the experiments. We also noted that, in the absence of energy source, the cells could not traverse to the center of the capillary (~25 000 μm). This is also in agreement with the recent experiments where the average run speeds of the cells in the absence of any carbon source fell significantly after 30 min from the start of the experiment [14].

The experimentally measured value of rotational diffusivity can be compared to the theoretical value by assuming the cells to be ellipsoids rotating about their semi-minor axis [8]:

\[
D_r = \frac{kT}{\ln(\eta a^3/\eta_n b^3)} \tag{3}
\]

where \( a \) is the semi-major axis, \( b \) is the semi-minor axis, \( k \) is Boltzmann’s constant, \( T \) is the temperature and \( \eta \) is the fluid viscosity. For \( T = 301 \text{K}, a = 1 \text{μm}, b = \)
0.5 μm and η = 10⁻³ Pa s, the rotational diffusivity is equal to 0.44 rad² s⁻¹ which is close to the experimentally determined value of 0.38 ± 0.05 rad² s⁻¹ in the absence of serine. We also observed a variation in the size of the cells with varying serine concentration in the growth medium. The length of the cell increased by about 40% with the increase in serine concentration. However, at very high concentrations, the size decreased to that observed in the absence of serine (results not shown). Assuming a 40% increase in the length of the cell, (a = 1.4 μm), the rotational diffusivity is calculated to be 0.22 rad² s⁻¹ which is in close agreement with the measured rotational diffusivity of 0.21 ± 0.03 rad² s⁻¹ at a serine concentration of 24 000 μM. With a = 1 μm, the measured rotational diffusivity at 240 000 μM is 0.37 ± 0.05 rad² s⁻¹ which is again close to the observed value. The study suggests that the rotational diffusivity plays a significant role in E. coli chemotaxis to serine and is correlated with the size of the cell.

The measured drift velocities show that oxygen levels play a crucial role in the chemotactic response since a significant drift velocity is observed even in the absence of serine. These results suggest that the absence of an energy source, the cells consume oxygen and the existing endogenous energy source. They move in response to the induced oxygen gradient till they run out of stored energy source. Experiments were also performed using an AER mutant, both in the presence and absence of serine (figures 2(b) and (c)). Such a mutant strain responded neither to oxygen nor to serine gradients, implying that the Aer receptor is essential for chemotaxis to serine. The measured drift velocity for the normal cells decreased along the length of the capillary both in the absence and presence of serine, which might be caused by adaptation to oxygen. Further, the increase in local drift velocity (30–40%) with the increase in serine concentration up to 5000 μM (and in the absence of gradients) is due to the increase in the average run speed and the decrease in the rotational diffusivity. The increase in the average run speed is due to higher energy levels caused by the aerobic metabolism of serine. Further, experiments were also conducted to determine the variation of drift velocity against varying serine gradients. The drift velocity is negligible at low gradients and the observed drift is only due to oxygen gradient (figure 3(a)). However, the increase in the serine gradient resulted in an increase in the initial drift velocity and a monotonic decrease of drift velocity with distance. This decrease in the drift velocity with distance demonstrates the adaptation of cells to the regions of higher attractant concentrations. Further the increase in the gradient decreases considerably the drift velocity due to the combined effect of adaptation, insensitivity and inhibition of cells (figure 3(f)).

The existing knowledge of the signaling pathway in E. coli can be used to get a better insight into the observed trends. Recall that the concentration of CheY-P decides the CW bias which in turn effects the drift velocity. For very low serine gradients, the CheY-P concentration decreases marginally for a very short time after which it quickly regains the steady state value. Consequently, the drift velocity is negligible at all locations for low gradients (figure 4(a)). However, with the increase in gradient, the CheY-P drops significantly resulting in large initial drift velocities, which expose the cells to the regions of higher attractant concentrations. The drop in CheY-P is caused by a momentary decrease in the concentration of active receptors (TA), which in turn increases the demethylation rate and decreases the methylation rate. Consequently, during adaptation, the average methylation level of the receptors increases while the simultaneous action of methylation and demethylation returns T_A (and CW bias) to a new steady state value due to imperfect adaptation to serine [9]. Further, the magnitude of the response to subsequent increases in ligand concentration is also reduced since the change in the fraction of the active receptors in each of the methylation state is small. Thus, the combined effect of imperfect adaptation and insensitivity to increasing local ligand concentrations reduces the run lengths and therefore the drift velocity with distance. The recent work of Kalinin et al [20] has suggested a linear relationship between the drift velocity and the logarithmic gradient of ligand (α-methyl-aspartate) concentration. Our experiments suggest that the motion of the cell is highly dependent on the past trajectory since the state of the intracellular pathway characterized by the methylation level of the receptors is a function of the past concentrations that the cell has experienced. The study suggests that the observed linearity captures at best an average behavior of the cells.

A number of issues need to be addressed to get a better quantitative understanding of the chemotaxis to serine. First, the influence of oxygen on the intracellular pathway has to be considered along with that of other chemoattractants/repellers. Further, metabolism of a chemoattractant appears to change the energy level of the cell leading to variations in the run speed. Consequently, the metabolism and the energy status of the cell also need to be integrated with the signaling pathway. It was also observed that the size of the cells varied depending on the concentration of serine which would directly influence the rotational diffusivity (figure 2(a)) and therefore the drift velocity. Finally, the imperfect adaptation of the signaling pathway to serine also needs to be considered as previous studies have shown that the steady state CW bias of the cell changes with serine concentration.

5. Conclusions

E. coli responds to changes in ligand concentration by utilizing a complex intracellular signaling pathway to move toward favorable conditions. Our study captures the spatial and temporal variation of such a response to controlled gradients of serine using a simple yet novel experimental technique. The run speed, rotational diffusivity, translational diffusivity and drift velocity were measured independently for varying serine concentration and gradients using this technique. Further analysis demonstrated the interdependence of these parameters that characterize the motion of the cell. Experiments clearly reveal both the increased response with the gradient and subsequent adaptation/saturation, in addition to inhibition to serine. The study shows that oxygen plays a key role in
the chemotactic response and hence the response to a ligand in the presence of oxygen cannot be analyzed in isolation. Future studies for *E. coli* chemotaxis need to integrate the effect of oxygen, average run speed and rotational diffusivity as a function of chemotactractant concentration to successfully predict the chemotactic behavior.

Acknowledgments

The authors acknowledge the financial support from the Department of Science and Technology and Ministry of Human Resources and Development, New Delhi, India.

References