Variation of swimming speed enhances the chemotactic migration of *Escherichia coli*

R. V. S. Uday Bhaskar¹ · Richa Karmakar¹ · Deepthi Deepika¹ · Mahesh S. Tirumkudulu¹ · K. V. Venkatesh¹

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Abstract Studies on chemotaxis of *Escherichia coli* have shown that modulation of tumble frequency causes a net drift up the gradient of attractants. Recently, it has been demonstrated that the bacteria is also capable of varying its runs speed in uniform concentration of attractant. In this study, we investigate the role of swimming speed on the chemotactic migration of bacteria. To this end, cells are exposed to gradients of a non-metabolizable analogue of glucose which are sensed via the Trg sensor. When exposed to a gradient, the cells modulate their tumble duration, which is accompanied with variation in swimming speed leading to drift velocities that are much higher than those achieved through the modulation of the tumble duration alone. We use an existing intra-cellular model developed for the Tar receptor and incorporate the variation of the swimming speed along with modulation of tumble frequency to predict drift velocities close to the measured values. The main implication of our study is that *E. coli* not only modulates the tumble frequency, but may also vary the swimming speed to affect chemotaxis and thereby efficiently sample its nutritionally rich environment.

Keywords Chemotaxis · Adaptation · *Escherichia coli* · Trg sensor

Introduction

Chemotaxis is the biased migratory response of a microorganism or a group of cells to chemical cues in the environment. The chemotaxis phenomenon has been studied extensively in *Escherichia coli* which achieves the biased motion by altering the rotational direction of its flagella (Adler 1966; Berg and Brown 1972; Macnab and Koshland 1972; Eisenbach 2004; Berg 2004; Wadhams and Armitage 2004). Counterclockwise (CCW) rotation as viewed from the flagella end of the cell results in a motion called run, whereas clockwise (CW) rotation leads to tumbling of the bacteria. By modulating the duration of motor bias, and therefore the duration of runs, the bacteria achieves a net motion towards favorable chemicals or away from repellents. The regulation of the motor bias is achieved through a well characterized signaling pathway which involves sensing of the ligand through a membrane sensor, response through a signaling pathway and adaptation to the new environment. The current understanding of the molecular basis for chemotaxis comes from characterization of the high-abundance receptors, namely Tar and Tsr, which are responsible for sensing the amino acids, aspartate (and its non-metabolizable analogue, Methyl-D-aspartate, henceforth referred to as MeAsp) and serine, respectively. Detailed enumeration of the components of the signaling pathway and the phenotypic response in terms of the swimming speed and bias, has shown that the cells achieve chemotaxis by modulating the CW bias of the flagellar motor while keeping the swimming speed constant during the CCW mode (Berg 2004).

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R. V. S. Uday Bhaskar and Richa Karmakar have contributed equally to this work.

✉ K. V. Venkatesh
venks@iitb.ac.in

Mahesh S. Tirumkudulu
mahesh@che.iitb.ac.in

¹ Department of Chemical Engineering, IIT Bombay, Mumbai 400076, India

Apart from the Tar and Tsr receptors, *E. coli* senses its environment through three low-abundance receptors, of which the Trg receptor is used to detect glucose (Adler et al. 1973). Since glucose is the preferred carbon source for its growth, it is important to characterize the phenotypic response of the cells when the Trg receptor is activated. While detailed studies have focused on the molecular mechanism of sensing and adaptation via the Trg receptor (Hazelbauer and Engstrom 1980; Feng et al. 1999; Li and Hazelbauer 2005), the latter is yet to be characterized in terms of the phenotypic response, namely, swimming speed and motor bias. In addition, glucose is also sensed through a PhosphoTransferase System (PTS) sugar uptake mechanism which operates in conjunction with the receptor mediated response through Trg (Adler and Epstein 1974; Lux et al. 1995; Neumann et al. 2012). Thus to study the response exclusively through the Trg receptor, we expose the two strains of cells, namely, wild type K12 and a mutant Δtrg , to varying concentrations and gradients of a non-metabolizable analogue of glucose, namely, 2-Deoxy-D-glucose (2Dg) (Adler and Epstein 1974). While it is well known that the modulation of tumble frequency causes a net drift, recent experiments by Saragosti et al. (2011) have shown that the cells also exhibit directional persistence wherein the tumble angle is lower for cells moving up the gradient. Further, we have shown recently that the swimming speed (or run speed) of *E. coli* varies with varying uniform concentrations of glucose and 2Dg (Deepika et al. 2015). For a fixed run duration, cells exposed to the attractants achieved a higher peak-swimming speed after a tumble compared with that in plain motility buffer. Experiments with Δtrg mutant show no change in swimming speed with varying concentrations of the non-metabolizable analogue, suggesting that sensing plays a role in the observed variation of swimming speed. These results indicate that variation in swimming speed will play a role in the chemotactic response of the cells when exposed to gradients since the drift velocity is known to be a linear function of the swimming speed (Rivero et al. 1989). Thus, the focus of the current study is to investigate the role of swimming speed on the drift velocity of bacteria in gradients of 2Dg and quantify the contribution of swimming speed variation towards the enhancement of net drift.

We show for the first time, that while cells achieve chemotaxis by modulating the tumble duration, the magnitude of the drift velocity increases with variation of swimming speed via the Trg sensor. This results in a biased motion whose magnitude is far higher than that achieved through the modulation of bias alone. The two-state chemotaxis model of Barkai and Leibler (1997) was modified to include variation of swimming speed and was able to capture the observed drift velocities. In addition, the measured drift velocity followed the Weber law with a

slope higher than that obtained in response to MeAsp where there is no swimming speed variation indicating higher chemotactic sensitivity. It is of significance to note that the modulation of swimming speed and tumble duration occurs through sensing alone (and not metabolism) thereby providing an advantage to migrate efficiently via Trg sensing towards sugar-rich environments.

Materials and methods

A wild type strain *E. coli* K12 (MTCC 1302 equivalent to CGSC#4401, also referred to as EMG2) was used in the experiments. Note that the commonly used strain MG1655 (CGSC#6300) is closely related to ancestral *E. coli* K12 (EMG2 or WG1) (Hayashi et al. 2006). The strain was obtained from IMTECH, Chandigarh, India. The wild type strain is chemotactic to glucose/2Dg and this was confirmed in our recent study (Deepika et al. 2015). Experiments were also performed with Δtrg mutant strain of K12, which is an isogenic strain of K12, lacking the Trg receptor. The motion of the two strains was observed in varying concentrations and concentration gradients of 2Dg in rectangular micro-capillaries [5 cm (L) $\times$ 1000 μ m (W) $\times$ 100 μ m (H)]. The smallest dimension of the rectangular microchannel is 100 μ m which is much larger than 10 μ m at which motion becomes restricted by confinement (Liu and Papadopoulos 1995, 1996). The medium preparation for the bacterial growth and relevant experimental details are given in the Supplementary Information.

Two different sets of experiments were performed in the rectangular cross-section micro-capillary. In the first set, the cells were mixed uniformly with a constant

Fig. 1 **a** Experimental set-up for measurement of cell response in uniform ligand concentration. Cells and 2Dg are uniformly present all over the micro-capillary (Experiment E1). **b** Experimental set-up to quantify cell response in steady linear gradients of ligand. Here, cells are placed as a pellet at $x = 0$ from where the cells migrate towards higher concentration of 2Dg. A steady linear gradient of 2Dg is established by contacting two liquid plugs containing a lower and a higher concentration of 2Dg (Experiment E2)

concentration of 2Dg (Experiment E1, Fig. 1a) and introduced into the micro-capillary after about 1 min. The motion was recorded at several locations along the micro-capillary. The number density of the cells in the micro-capillary was about 10^6 /ml. In the second set of experiments, a pellet of cells was contacted at one end of the micro-capillary containing buffer with a linear gradient of 2Dg (Experiment E2, Fig. 1b). The linear gradients were established by drawing 4.5 cm long liquid plugs of different concentrations of 2Dg into the micro-capillary followed by 0.5 cm long liquid plug of motility buffer. The rapid mixing at the interface of the two liquid plugs results in a linear gradient that changes negligibly for the duration of the experiment (10–12 min). Four different gradients were obtained by using four different concentrations of 2Dg liquid plugs, namely, 100, 500, 1000 and 10000 μ M. The experimental system is very robust (Vuppala et al. 2010a, b; Saragosti et al. 2011; Masson et al. 2012) and does not require the use of pumps or complex microfluidic channels to establish a steady gradient. The existence of a stable linear gradient was confirmed by performing a similar experiment with fluorescent glucose [2-(*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxy-glucose, 2-NBDG]. The values of the linear gradients were obtained by performing identical experiments with 2-NBDG. The cells emerge from the pellet as soon as they come in contact with the liquid, and start to move towards the opposite end of the micro-capillary. We record the motion of the population front as it approaches 500, 1000 and 1500 μ m mark from the cell pellet. The concentration and the concentration gradient at the population front was determined from concentration profiles by performing identical experiments using 2-NBDG. Note that the local concentration and its gradient can be used to determine the local logarithmic concentration gradient. The estimated cell concentration at the population front was about 10^6 /ml. In both sets of experiments (E1 and E2), the micro-capillary ends were sealed with wax and movement of *E. coli* were recorded via a microscope equipped with a CCD camera. Each experiment lasted for about 10–12 min and was repeated three times on different days to capture the variability. *E. coli* movements were recorded under dark-field mode and the images were taken at frame rates of 20 fps along the micro-capillary. The trajectories of the cells were obtained using a commercial software, Image Pro Plus, and this data was further processed to obtain the swimming speed and distribution of tumble duration for about 1000 cells for each strain and condition. The software parameters were set such that only those cells within ± 1 μ m of the focal plane were considered for motion analysis. Further, only those tracks were considered where the cell spent at least 0.5 s in the focal plane thereby ensuring that tracks

with large out-of-plane motions were ignored by the analysis. A tumble event was identified when the swimming speed of the cell was below half of the average swimming speed and the change in the turn angle was $>4^\circ$ between successive frames. These conditions were obtained by visual inspection of run and tumble events, and are close to those reported by Alon et al. (1998). The values obtained for average swimming speed of 21 μ m/s in motility buffer for our K12 strain matches closely with that obtained for the wild-type K12 (MG1655) strain (20 μ m/s) by Masson et al. (2012). These results confirm the correctness of the tracking algorithm and the subsequent calculations used to calculate the swimming speed, fractional tumble time, average turn angle etc. The images captured at various locations along the micro-capillary with and without gradients of 2Dg were further analyzed to extract drift velocity (V_d). The image analysis is similar to that reported in our earlier study (Deepika et al. 2015) and further details are provided in the Supplementary Information.

Model description

The two-state model proposed by Barkai and Leibler (1997) is used to describe the receptor dynamics. The model considers the receptors to be in two functional states, namely, active and inactive. The details of the model and its implementation has been described in Vuppala et al. (2010b) for the motion of *E. coli* in gradients of MeAsp. Here, we adopted the same model to capture the motion of the bacteria in gradients of 2Dg with the following modifications. The dissociation constant, K_d , was used as a fitting parameter to obtain drift velocities close to the measured value. The model incorporates the variation of swimming speed, since the measured swimming speed varied with gradient. Note that in case of MeAsp gradients, the swimming speed had remained unchanged (Vuppala et al. 2010b). The rotational diffusivity (D_r) calculated by Berg (2000) for a sphere, 0.062 rad²/s, was used in the calculation. The angular distribution of tumble angles is observed to follow a gamma distribution in agreement with previous studies (Berg and Brown 1972). The model also incorporates directional persistence wherein the tumble angle was higher for cells moving down the gradient compared to cells moving in the opposite direction (Saragosti et al. 2011). The measured tumble angle distribution of cells moving up and down the gradient were used in the model. The details of the swimming speed variation, tumble angle distribution along with model equations and numerical implementation are presented in the supplementary information.

Results

The two different sets of experiments in a micro-capillary (E1 and E2) yielded swimming speeds, tumble duration, and drift velocity. Figure 2a presents the distribution of the swimming speeds in terms of fraction of run events from about 1000 cells measured in motility buffer (E1), a constant concentration of 1000 μM of 2Dg (E1), and in a gradient of 0.16 $\mu\text{M}/\mu\text{m}$ of 2Dg (E2). A run event occurs when a cell is in run mode between two subsequent frames (about 0.047 s apart). The plot is obtained from about 10,000 run events for each condition. The distribution in the presence of the buffer is narrow and peaks at about 15 $\mu\text{m}/\text{s}$ with a mean value of 19.9 $\mu\text{m}/\text{s}$ while that in the presence of a constant concentration of 2Dg is broader with a mean value of 24.4 $\mu\text{m}/\text{s}$. The standard deviation in all cases was between 7 and 10 $\mu\text{m}/\text{s}$. Further, in the presence

of a 2Dg gradient, the distribution broadens and shifts to even higher swimming speeds with a peak value of 40 $\mu\text{m}/\text{s}$ and a mean of about 38 $\mu\text{m}/\text{s}$. Clearly, there are a significant number of cells with swimming speeds $>45 \mu\text{m}/\text{s}$ compared to the earlier two cases. Figure 2b presents angular distribution of the swimming speed for the above three conditions and shows that the swimming speed is invariant with respect to orientation even in case of gradient. The averaged swimming speeds for the 2Dg gradient experiment (E2) exhibit speeds as high as 36 $\mu\text{m}/\text{s}$ (in Fig. 2c) which is 60 % higher than the swimming speeds observed in uniform concentrations of 2Dg (24.4 $\mu\text{m}/\text{s}$ at 1000 μM).

To relate the swimming speeds to the run duration (t_{run}), we obtained the peak swimming speed for fixed run durations (i.e., in between tumbles) for the above three cases (see Fig. 2d). These data also include incomplete

Fig. 2 Measured average swimming speeds and swimming (or run) speed distribution in the presence of 2Dg for wild type K12. **a** Distribution of swimming speeds in the presence of plain motility buffer (filled square), in uniform concentration (1000 μM) of 2Dg (open circle), and 0.16 $\mu\text{M}/\mu\text{m}$ gradient of 2Dg (filled triangle). **b** Angular distribution of the swimming speed. The numbers along the circumference are angles in degrees while the numbers 10, 20, 30 and 40 in the radial direction are the swimming speeds in $\mu\text{m}/\text{s}$. Note that the swimming speed is invariant of orientation. The legend is the same as that for (a). **c** Swimming speeds measured in the presence (filled triangle, E2, Fig. 1b) and absence (filled circle, E1, Fig. 1a) of

2Dg gradients. Clearly, the swimming speeds vary both in uniform and gradients of 2Dg. Here, C represents the concentration of 2Dg and the error bars represent standard errors. **d** Peak swimming speed achieved for a fixed duration of run in case of 2Dg gradient (0.16 $\mu\text{M}/\mu\text{m}$, open circle). The solid lines are fit to the lowest peak swimming speeds for three cases (solid line for gradient, dashed line for uniform concentration, 1000 μM , and dotted line in motility buffer) with the fitting equation being, $A(1 - \exp(-t_{\text{run}}/\alpha))$, where t_{run} is the run duration and α is a fitting parameter. The values of A were 24, 30 and 36 $\mu\text{m}/\text{s}$ for motility buffer, uniform and 2Dg gradient, respectively, while α was 0.35 s for all cases

runs wherein the cells either tumbled only once or did not tumble at all for the recorded duration of the cell trajectory. A curve was fit to the data such that it represented the lowest peak swimming speeds observed for a given run duration. It is clear from the figure that cells reach a higher minimum peak swimming speed in a gradient compared to either a uniform concentration or motility buffer. The minimum peak swimming speed in the presence of gradient was at least $6 \mu\text{m/s}$ greater than that in uniform concentration of 2Dg and about $12 \mu\text{m/s}$ higher than that observed in motility buffer. These results clearly imply that the bacteria achieves higher swimming speeds for the same duration of run in gradients of 2Dg through Trg sensing mechanism. It has been recently shown that the mean squared angular displacement during a run reduced in the presence of uniform concentration of 2Dg (Deepika et al. (2015)) indicating smoother runs in the presence of an attractant. Figure 3a plots the distribution of the angular displacements between two consecutive frames during a run, in terms of fraction of run events. The distribution is a Gaussian with mean, $\langle\theta\rangle$, being close to zero. In the presence of uniform concentration of 2Dg, the distribution

becomes narrower compared to that in motility buffer with the spread of the distribution being the lowest in case of the gradient. This suggests that the runs are wobbly in motility buffer but become increasingly smooth as cells are exposed to 2Dg with the smoothest runs achieved in case of gradient. Next, we plot the distribution of fractional tumble duration for the three cases (Fig. 3b). As is well known, the fractional tumble duration decreases when the cells move up a gradient of an attractant. The measured distribution marginally shifts to lower fractional tumble duration in uniform concentrations of 2Dg but shifts significantly in the presence of the gradient. The distribution shows that there are almost no cells for fractional durations greater than 0.2 in the gradient though a significant fractional of cells are present for both motility buffer and uniform concentrations of 2Dg. This clearly demonstrates the modulation of tumble frequency in response to gradients of 2Dg as has been reported in the past. Figure 3c plots the fractional tumble duration as a function of 2Dg concentration. In case of uniform concentration of 2Dg, the fractional tumble duration was invariant with respect to concentration suggesting perfect adaptation. However, in

Fig. 3 **a** Distribution of run events (in fraction) as a function of run angles. The *filled square* is for $0.16 \mu\text{M}/\mu\text{m}$ 2Dg gradient and the *solid line* is the corresponding Gaussian fit. The *dashed line* and the *dotted line* are Gaussian fits for $1000 \mu\text{M}$ 2Dg and motility buffer, respectively. **b** Distribution of fractional tumble duration. Here, the *solid line* is for $0.16 \mu\text{M}/\mu\text{m}$ 2Dg gradient, the *dashed line* is for $1000 \mu\text{M}$ and the *dotted line* is for motility buffer. **c** Measured

fractional tumble duration as a function of 2Dg concentration in the presence (*open triangle*) and absence (*filled square*) of gradient. Note that the fractional tumble duration does not change in the absence of gradient suggesting perfect adaptation. The fractional tumble duration drops sharply in the presence of gradient. The fractional tumble duration is found to be higher for Δtrg strain (*filled circle*) compared to WT strain (*filled square*)

the presence of a gradient, the fractional tumble duration is modulated and drops to as low as 0.1 in our experiments. As the concentration of 2Dg in a gradient increases the fractional tumble duration decreases and reaches a minimum value around 1000 μM , and increases there after. Increase of fractional duration is due to partial saturation of receptors and lower adaptation time at those concentrations. Plug experiments (E2) performed with Δtrg strain in both motility buffer and gradient of 2Dg are presented in Fig. 4. These results clearly show that both the run speed distribution and the fractional tumble time distribution are nearly the same. This clearly indicates that the Trg receptor plays an important role in the observed run speed variation. In contrast to WT, the Δtrg strain does not respond to the 2Dg gradient leading to an invariant fractional tumble duration (as shown in Fig. 4b). It can be noted that a fractional tumble duration of 0.35 in case of Δtrg strain was higher than that observed for the wild type K12 strain (0.25) in uniform concentration of 2Dg. Trg receptor might play a role in the observed change of tumble duration through the mechanism responsible for the change is unclear.

The above experiments performed in the presence of gradient also yield drift velocities at various locations along the micro-capillary. Figure 5a shows the measured drift velocity at different positions along the micro-capillary for four different 2Dg gradients. For all the four gradients, the drift velocity decreases with distance from the cell plug (located at $x = 0$). As the cells move along the capillary, fractional tumble duration gradually increases due to adaptation thus lowering its drift. For the lowest gradient, the drift velocities measured beyond 500 μm from the plug were equal to that observed in the absence of 2Dg (i.e., motility buffer). The small drift in the presence of only motility buffer is caused by a combination of cell diffusion and oxygen gradients created by the cells

migrating from the cell plug. The migration of the cells can also be analyzed in terms of the orientation distribution, as shown in Fig. 5b. In case of uniform concentration of 2Dg, the distribution is isotropic but is biased towards the high concentration end (towards the right side in the plot) in case of the gradient. This clearly shows that majority of the cells move up the gradient resulting in a net drift. To explain the observed chemotactic response, we use an existing two-state model (Barkai and Leibler 1997) for the intracellular pathway and incorporate the extra-cellular influence such as ligand concentration and Brownian motion to predict the drift velocity for different 2Dg gradients (see Supplementary Information for details). Although, the swimming speed is a function of 2Dg concentration, the swimming speed variation at the three locations (500, 1000, and 1500 μm) for a fixed gradient was $<10\%$. Thus the measured average swimming speed of 28.7, 34.8, 36.2 and 31.3 $\mu\text{m/s}$ was used to predict the drift velocity for gradients of 0.016, 0.059, 0.16 and 1.6 $\mu\text{M}/\mu\text{m}$. Further, the measured drift velocity in motility buffer which is attributed to cell diffusion and oxygen gradient was subtracted from the measured values in gradients for comparison with the model predictions since the model only accounts for the response to 2Dg. The predicted values compare satisfactorily with measurements for all positions along the micro-capillary and for various gradients used in this study (see Fig. 5). At short distances from the plug end ($x = 0$), the drift velocities are high due to very low tumble frequency for cells moving up the gradient. As the cells adapt, the response to the increasing concentration reduces the tumble frequency thereby reducing the drift velocity. The existing experimental protocol allowed measurement only beyond 500 μm from the plug thereby missing the initial steep drop in the drift velocity (Fig. 6).

Fig. 4 **a** Distribution of swimming speeds in the presence of plain motility buffer (open circle) and 0.16 $\mu\text{M}/\mu\text{m}$ gradient of 2Dg (filled square) for Δtrg strain. Data for WT cells in MB is also included (open triangle). **b** Distribution of fractional tumble duration. Here, the

solid line is for 0.16 $\mu\text{M}/\mu\text{m}$ 2Dg gradient and the dotted line is for motility buffer for Δtrg strain. Fractional tumble duration for WT cells in motility buffer is also included (dashed line)

Fig. 5 **a** Drift velocity of *E. coli* measured at different positions of micro-capillary for 2Dg gradients—0.16 (filled circle), 0.0589 (open triangle), 1.6 (asterik) and 0.016 $\mu\text{M}/\mu\text{m}$ (filled square). **b** Cell

orientation distribution in uniform concentration (1000 μM , left panel) of 2Dg and 0.16 $\mu\text{M}/\mu\text{m}$ (right panel) of 2Dg gradient

Figure 7a compares the predicted drift velocity with the measurements at 1000 μm for various 2Dg gradients. The measurements show an initial increase with concentration that is attributed to the high sensitivity of the receptor at low concentrations. Further increase leads to a peak drift velocity followed by a decrease which is caused by saturation of the receptors and adaptation. The predicted drift velocity compares well with the measurements except at the lowest concentration, and captures both the increase and the subsequent decrease. Figure 7b presents the

predicted drift velocity for a fixed gradient and for three different locations as a function of dissociation constant (K_d). At low values of K_d , the attractant binds strongly to the receptor leading to a faster adaptation, while at very high values the low affinity of the ligand to the receptor leads to a weaker sensing and therefore a low drift velocity. A maximum is observed at $K_d = 160 \mu\text{M}$ [close to that observed for MeAsp (Vuppala et al. 2010b)], and was chosen to match the experimentally measured drift velocities for all gradients. In the past, experiments with varying

Fig. 6 Drift velocity as a function of position in 2Dg gradient of **a** 0.016 $\mu\text{M}/\mu\text{m}$, **b** 0.059 $\mu\text{M}/\mu\text{m}$, **c** 0.16 $\mu\text{M}/\mu\text{m}$, and **d** 1.6 $\mu\text{M}/\mu\text{m}$. Points are experimental measurements and solid line is model prediction. In all cases, the model predictions are close to measurements. Note that our experiments are unable to access the drift velocity close to the plug ($x < 500 \mu\text{m}$) since the initial 60–120s are spent in positioning the micro-capillary on the microscope, during which time the cells travel about 300–400 μm

Fig. 7 **a** Comparison of the measured drift velocity (filled square) with theoretical prediction at a distance of 1000 μm from the plug for the K12 strain as a function of the 2Dg gradient. The solid line is the model prediction. **b** Variation of drift velocity as a function of the dissociation constant, K_d , for a gradient of $0.16 \mu\text{M}/\mu\text{m}$ at different spatial locations—500 μm (solid line), 1000 μm (dotted line) and 1500 μm (dashed line) respectively. The arrow indicates $K_d = 160 \mu\text{M}$ used in simulations. **c** Drift velocity as a function of

logarithmic concentration gradient of 2Dg—0.16 (filled circle), 0.0589 (open triangle), 1.6 (asterisk) and $0.016 \mu\text{M}/\mu\text{m}$ (filled square). Note that the drift velocity in motility buffer is subtracted from the measured values. **d** Average value of the drift velocity as a function of logarithmic concentration gradient of 2Dg (filled square). For comparison, drift velocity measured in MeAsp gradient (open triangle) is also shown. Lines are linear fits to demonstrate Weber law

MeAsp/serine gradients have shown that the drift velocity varies linearly with the average logarithmic gradient. In order to verify the same for the 2Dg gradients, the experimentally obtained local drift velocity is plotted as a function of the local logarithmic gradient ($d(\ln C)/dx$) for the four gradients separately at the four locations (see Fig. 7c). Our experiments were designed such that the value of $d(\ln C)/dx$ at each spatial location was the same irrespective of the gradient. The four points at each value of $d(\ln C)/dx$ corresponds to the four gradients with increasing drift velocities for increasing gradients. Note that the measured drift velocity excludes the base contributions from effect of cell diffusivity and oxygen gradient. For a fixed gradient, the drift velocity increases with logarithmic gradient but the measured drift velocities for a fixed logarithmic gradient and across gradients do not collapse. These results confirm the earlier findings of Vuppala et al. (2010b) that the drift velocity at a location is dependent not only on the ratio of the ligand gradient and the local concentration but also depends on the past history of the concentrations experienced by the bacteria. Consequently, the measured drift velocities follow the Weber law

only in an average sense (see Fig. 7d). For comparison, Fig. 7d also includes data for MeAsp (Vuppala et al. 2010b) along with its linear fit. The slope for 2Dg is almost 70 % higher than that for MeAsp and is attributed to the effect of increased swimming speed since the average increase in the swimming speed over that observed for MeAsp is of the same magnitude. The mathematical model incorporates both mechanisms which lead to net drift velocity, namely, modulation of tumble frequency (TF), and swimming speed (SS) while accounting for directional persistence during tumbling. While modulation of tumble frequency is essential in achieving a net drift, Saragosti et al. (2011) have shown that the turn angles for cells moving up the gradient of attractants are lower than for those moving in the opposite direction leading to a directional persistence and an enhanced drift. A further increase in swimming speed (irrespective of direction) would also contribute to increased drift velocities. Figure 8 presents the predicted drift velocity along with measured value at $x = 500, 1000$ and $1500 \mu\text{m}$ for the four gradients while highlighting the enhancement due to run speed variation in the theoretical value. The contribution to the enhanced drift

Fig. 8 Influence of tumble frequency (TF) and swimming speed (SS) on drift velocity for varying gradients of 2Dg measured at three different locations, **a** 500 μm , **b** 1000 μm , and **c** 1500 μm . The empty bar represents experimental measurements, the black bar represents drift velocity predicted by considering only variation in tumbling frequency (TF) while the shaded bar represents the enhancement in drift velocity by accounting for the variation in swimming speed (TF + SS)

velocity due to increased swimming speed is between 35 and 45 % thereby indicating that swimming speeds plays an important role in enhancing chemotactic response via the Trg receptor.

Discussion

The phenomenon of chemotaxis in *E. coli* has been mainly understood via detailed studies of single cell motion in response to MeAsp, a non-metabolizable substrate, and serine which are sensed by the Tar and the Tsr receptors, respectively (Eisenbach 2004; Berg 2004). While most studies have reported constant swimming speeds in gradients of MeAsp (Berg and Brown 1972; Vuppala et al. 2010b), recent experiments by Ahmed and Stocker (2008) have shown that the swimming speeds can increase by as much as 30 % in steep gradients of MeAsp. Such a behavior has also been observed in gradients of serine (Berg and Brown 1972; Vuppala et al. 2010a). Recently, we have demonstrated that the Trg receptor, which senses glucose, plays a role in the variation of swimming speed even in uniform concentration (zero gradient) of 2Dg, a non-metabolizable analogue of glucose (Deepika et al. 2015). While the variation of swimming speed with different concentrations of 2Dg was demonstrated in two different wild type strains of *E. coli*, namely K12 and RP437, the influence of gradient on the swimming speed is

yet to be characterized. Further, the role of variation in swimming speed on the net chemotactic drift has not been quantified.

One of the key findings of the study has been the increase of swimming speed in gradients of 2Dg. The measured swimming speeds were not only greater than that observed in motility buffer but also higher than in uniform concentrations (zero gradient) of 2Dg suggesting that the increase is not merely due to the ligand concentration but also due to a response to the gradient. However, the swimming speed was independent of the orientation of the cell leading to equal speeds both up and down the gradients. This is in contrast to modulation of tumble frequency wherein the frequency is lower while migrating up the gradient of attractants. In addition to the above two effects, recent studies have shown that the cells also exhibit directional persistence wherein the tumble angles for cells moving up the gradient is smaller compared to those moving down the gradient. While modulation of tumble frequency is essential for achieving a chemotactic drift, the remaining two effects enhance the drift velocities.

When the cell is exposed to 2Dg gradients, the tumble duration was not only lower than the steady state value at the same local concentration but the swimming speeds were also much higher than the steady state values at the same local concentration. This suggests that in response to a step increase in 2Dg, the swimming speeds may transiently increase and subsequently drop to a new steady

state value that is higher than the pre-stimulus value. This is accompanied with a drop and a subsequent recovery of the tumble duration to a fixed steady state value. Our results clearly suggest that the cell modulates both tumble duration and swimming speed in response to changing concentrations of 2Dg. In the gradient experiments, we observe swimming speeds close to 40 $\mu\text{m/s}$ which is almost two times that observed in plain motility buffer. Further, for a fixed run duration, the observed peak swimming speeds were higher in gradients compared to both motility buffer and uniform concentration of 2Dg. One of the consequences of the increased swimming speeds along with a decreased tumble duration is smoother swimming resulting in a reduced angular displacement of the cells during the run. None of these effects were observed in the mutant strain lacking Trg receptor, strongly suggesting a key role of the receptor in the variation of swimming speed. The increased swimming speeds may be attributed either to the absence of imperfections in the flagella bundle (Subramanian and Koch 2009; Deepika et al. 2015) and/or higher motor speed (Eisenbach et al. 1990). An interesting question that follows from these observations relates to the mechanism by which a receptor may modulate the motor speed. The motor speed is dependent on the membrane potential and the flow of excess protons (H^+ ions) from outside the inner membrane to the cytoplasm. Recently, Demir and Salman (2012) have shown that the chemotactic receptor plays a key role in modulating the intra-cellular pH leading to a variation in swimming speed. It is possible that a change in the intra-cellular pH and/or the membrane potential may also occur in response to 2Dg through the Trg receptor to bring about the observed change in swimming speeds though more work is required to confirm this. Alternatively, Boehm et al. (2010) have demonstrated that *E. coli* can modulate flagellar motor output in response to nutrient concentration. They show that the motor speed is controlled by a molecular brake, YcgR, which when activated upon by the binding of the nucleotide second messenger cyclic di-GMP, interacts with the motor protein MotA to decrease the motor speed. Mutants lacking YcgR exhibited swimming speeds of 29 $\mu\text{m/s}$ which was 20 % higher than that for the wild type cells. These results point to the possibility of enhanced swimming speeds via the modulation of the activity of YcgR in response to ligands.

The experiments presented here along with those performed in uniform concentration of 2Dg (Deepika et al. 2015) have demonstrated that the net drift in a gradient of attractant is a result of modulation of tumble frequency and variation in swimming speed. A modified two-state model of Barkai and Leibler (1997) was used to estimate the relative contributions of these mechanism towards the net drift. Our analysis indicates that swimming speed can

contribute significantly (up to 45 %) to the net drift. The chemotactic response to gradients of attractants is typically quantified in terms of the Weber law (Mesibov et al. 1973) wherein the drift velocity increases linearly with the logarithmic gradient, with the slope representing the sensitivity of the chemotaxis response. The increased drift velocities observed in our experiments with 2Dg exhibited a slope that was higher than that observed in gradients of MeAsp. The latter experiments showed no swimming speed variation indicating that a variation in swimming speed brings about higher chemotactic sensitivity.

Conclusion

The main implication of our study is that *E. coli* modulates the tumble frequency which may be accompanied with variation in swimming speed to affect chemotaxis and thereby efficiently sample its nutritionally rich environment. Further, while it is well known that the sensing mechanism controls the motor bias, our experiments demonstrate that the same sensing mechanism can bring about a variation in swimming speed. Thus, when a cell moves up a gradient, not only are the tumbles suppressed but an increase in swimming speed will result in significantly higher drift velocities.

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