Computerized Analysis of Chemotaxis at Different Stages of Bacterial Growth

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ABSTRACT Chemotactic behavior as a function of growth stage in an *Escherichia coli* strain commonly used for chemotaxis studies was characterized using computerized image analysis. The response and adaptation to saturating, step-like additions of the attractant L-aspartate were measured. Steady-state average tumbling frequency and adaptation time increased nearly twofold during logarithmic phase. In contrast, precision of adaptation, P, defined as the ratio between steady-state tumbling frequencies in the presence and absence of attractant, appeared to be constant throughout growth ($P = 1.0 \pm 0.2$). The variation of tumble duration over growth was consistent with a hydrodynamic mechanism for tumble termination.

INTRODUCTION

The chemotaxis network of *Escherichia coli* guides the organism toward specific chemical attractants and away from repellents. It is an excellent model system for studying the response and adaptation of cells to their environment. The constituent proteins are members of a large family of two-component sensory systems in which stimuli modulate the activity of a specific protein kinase, which phosphorylates effector protein (Amsler and Matsumura, 1995). In *E. coli*, chemotaxis is mediated by transmembrane receptors (methyl-accepting chemotaxis proteins, or MCPs) and the cytoplasmic proteins CheA, CheB, CheR, CheW, CheY, and CheZ (Stock and Surette, 1996).

In an isotropic medium, swimming bacteria alternate between tumbles, which randomize the direction of motion, and smooth-swimming runs (Berg and Brown, 1972). Chemotaxis is achieved by control of the tumbling frequency in response to temporal changes in chemoeffector concentration (Macnab and Koshland, 1972; Brown and Berg, 1974; Berg and Tedesco, 1975; Block et al., 1982). When the concentration of an attractant increases, tumbling is temporarily suppressed. The average tumbling frequency eventually returns to prestimulus behavior, thereby restoring sensitivity to new chemical stimuli (Macnab and Koshland, 1972; Berg and Tedesco, 1975; Alon et al., 1999).

It is of interest to characterize further the strains used for studying chemotaxis and to determine which properties of chemotaxis are invariant or modulated under the global changes in the cell that occur in a growing bacterial culture (Bremer and Dennis, 1996). The pioneering studies of Adler defined conditions for optimal chemotaxis of an *E. coli* K-12 strain. It was observed that bacteria are more motile and more strongly chemotactic in logarithmic phase than in stationary phase (Adler and Templeton, 1967; Adler, 1972). Most subsequent studies of bacterial chemotaxis were performed at a single stage of growth, usually mid-logarithmic phase. A quantitative study showed that mean run speed and flagellar synthesis increase during logarithmic phase, peak at mid-log phase, and decrease thereafter (Amsler et al., 1993).

We extend these studies by studying physiological aspects of chemotaxis over the course of growth in RP437, an *E. coli* strain commonly used for chemotaxis studies (Parkinson and Houts, 1982). Response and adaptation to a saturating stimulus of the attractant L-aspartate was measured by video microscopy and computerized image analysis (Alon et al., 1998). A transient smooth-swimming response of unstimulated cells placed in a thin fluid layer between a glass slide and a coverslip was avoided by using plastic slides and coverslips. Adaptation was precise to within experimental error, whereas adaptation time and steady-state tumbling frequency increased nearly twofold during logarithmic growth. The mean tumble duration varied inversely with swimming speed, consistent with a hydrodynamic mechanism for reassembly of the flagellar bundle.

MATERIALS AND METHODS

Bacterial culture

Overnight cultures of *E. coli* K-12 strain RP437 (Parkinson and Houts, 1982) grown at 30°C in tryptone broth (1.3% Bacto Tryptone, 0.7% NaCl) were diluted 1:50 into 20 ml Tryptone broth in 125-ml flasks and shaken at 200 rpm at 30°C. Optical density (OD; 1 cm^{-1}) was measured at 600 nm with a DU-65 Beckman (Fullerton, CA) spectrophotometer. Culture density was measured in triplicate with a Petroff-Hausser counting chamber. Cells with a visible septum were counted as 2 cells.

Measurement of relative cell volume

RP437 cells were harvested by centrifugation at 4°C. Relative total cellular protein mass was measured by lysing known quantities of wild-type cells in a solution of 0.75% sodium dodecyl sulfate and 0.9% NaCl, sonicating

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briefly, boiling for 10 min, and diluting appropriately in bicinchoninic acid (Pierce, Rockford, IL). The OD_{562} of samples was measured in triplicate relative to bovine serum albumin (BSA) standards. A cytoplasmic volume of 0.6 fl at 0.6 OD (Scharf et al., 1998) was assumed. The cytoplasmic volume of cells at other growth stages was calculated using relative total cellular protein mass and assuming a constant ratio of protein mass to cell volume throughout growth (Bremer and Dennis, 1996).

Video analysis of cell behavior

One ml of culture was harvested by centrifugation at room temperature for 10 min at 800 × *g*. Cells were washed and gently resuspended in a volume of chemotaxis buffer (100 μM L-methionine, 7.6 mM $(NH_4)_2SO_4$, 2 mM $MgSO_4$, 20 μM $FeSO_4$, 0.1 mM EDTA, 60 mM potassium phosphate buffer, pH 6.8) that yielded 150 to 200 bacteria in a microscopic field of view at 40×. Samples were incubated at room temperature for 15 min before analysis. For each chemotaxis experiment, 5 μl of cells were mixed into 5 μl of 2 mM L-aspartate (in chemotaxis buffer) for a resulting stimulus of 1 mM L-aspartate. Controls were prepared by mixing 5 μl of cells with 5 μl chemotaxis buffer.

Samples were loaded on a plastic micro slide (Cat. No. 705–301, PGC Scientific, Frederick, MD) in the center of a 1 × 1 cm square inscribed with a China marker and were gently spread out with a plastic coverslip (Cat. No. 12–547, Fisher, Pittsburgh, PA) to create a layer of liquid of less than 10 μm thick, as judged by the area of the drop. To prevent cells from adhering to these surfaces, the slides and coverslips were first dipped in 0.5% Tween-20 (Sigma, St. Louis, MO) in chemotaxis buffer, drained, allowed to dry, and dipped in 0.1% ultra-pure agarose (Life Technologies, Gaithersburg, MD) in chemotaxis buffer and drained thoroughly. An alternative treatment by dipping the slides and coverslips in 0.1% BSA (>99% purity) in chemotaxis buffer (Alon et al., 1999) yielded indistinguishable results. Bacterial motion was analyzed by video microscopy and computerized image analysis as described (Alon et al., 1998).

The steady-state average tumbling frequency for a single unstimulated sample, $TF_{st,0}$, was calculated as the mean ± SE of the average tumbling frequency of 20 to 30 10-sec movies recorded at 1-min intervals after sample preparation (Fig. 4, top curve). The time to 50% recovery from the attractant stimulus, $\tau_{1/2}$, for a single sample was defined as the time after stimulation at which tumbling frequency is $(TF_i + TF_{st,1})/2$, where TF_i is initial tumbling frequency after stimulation, and $TF_{st,1}$ is the tumbling frequency after full recovery from a stimulus of 1 mM L-aspartate (Fig. 4). Adaptation time, $\tau_{1/2}$, and steady-state tumbling frequency, $TF_{st,0}$, were reported as the mean ± SE of at least three independent measurements. Precision of adaptation is $P = TF_{st,1}/TF_{st,0}$. Run speed and tumble duration were calculated as described (Alon et al., 1998). Control experiments using 10-fold lower cell concentrations yielded essentially the same behavior. Furthermore, the mean tumbling frequency and run speed of cell populations were found to be nearly constant over at least 20 min of observation. This suggests that factors such as consumption of the oxygen in the medium by the cells have negligible effects on the present measurements.

RESULTS

Bacteria exhibit a transient smooth-swimming response on glass slides

The present video microscopy technique relies on observing the cells in a thin fluid layer (several microns thick), so that they do not swim out of the focal plane of the microscope. Preliminary experiments revealed a transient smooth-swimming response in unstimulated cells after being placed in a thin fluid layer between a glass slide and a coverslip (VWR, South Plainfield, NJ). A period of low tumbling frequency ($0.2 \pm 0.1\ s^{-1}$) was observed after sample loading; the mean tumbling frequency then increased and approached a steady-state value. The initial response lasted an average of 2 to 3 min, though it varied from sample to sample in the range of 1 to 5 min. The run speed varied similarly and was about 50% of its steady-state value after contact with glass slides. Coating the slides by dipping them in an agarose solution or cleaning them with chromic acid did not eliminate these responses. Similarly, this effect was observed when the cells were washed into different media before the measurement (their own growth medium or the motility medium of Berg and Brown, 1972). The smooth swimming response was not observed when the cells were placed in a thick fluid layer (of order 0.1 mm, between bridged coverslips) and the cells were viewed swimming near the glass surface. This suggests that the confined geometry used in the present study plays a role in the smooth swimming response, perhaps by preventing the chemical or thermal perturbations induced by the glass surfaces to diffuse away. For example, chemical perturbations that caused a reduction of proton motive force induced a similar counterclockwise bias in flagellar rotation and reduction in run speed in previous studies (Khan and Macnab, 1980). Contact with glass surfaces has been reported to induce other changes in other cells, such as the conversion of normally discoid erythrocytes to crenated forms (Farnsworth et al., 1993).

The transient period of low tumbling frequency was not observed in the present study when plastic slides were used; furthermore, steady-state tumbling frequency and mean run speed were similar on glass and plastic slides (data not shown). Data are reported from samples loaded on plastic slides.

Run speed peaks in mid-logarithmic phase

Bacterial density under the defined growth conditions was measured using a Petroff-Hausser counting chamber (Fig. 1). Relative cell size, as estimated by relative total protein concentration per cell, decreased over growth (Fig. 2). The mean run speed (Fig. 3) rose to a peak of 19.0 ± 0.6 μm/s at mid-log phase (~0.6 OD). The growth phase at which speed is maximal is in agreement with previous studies (Adler and Templeton, 1967; Amsler et al., 1993). Note that variations in buffer and growth condition used in various studies appear to affect bacterial swimming speed (e.g., compare Berg and Brown, 1972 and Lowe et al., 1987).

Tumbling frequency and adaptation time increase over the course of growth

Steady-state tumbling frequency and response to a saturating, step-like stimulus of L-aspartate were measured at different stages of growth between 0.3 and 0.9 OD. At earlier and later growth stages, bacterial motion was too slow to permit data collection. The average tumbling fre-

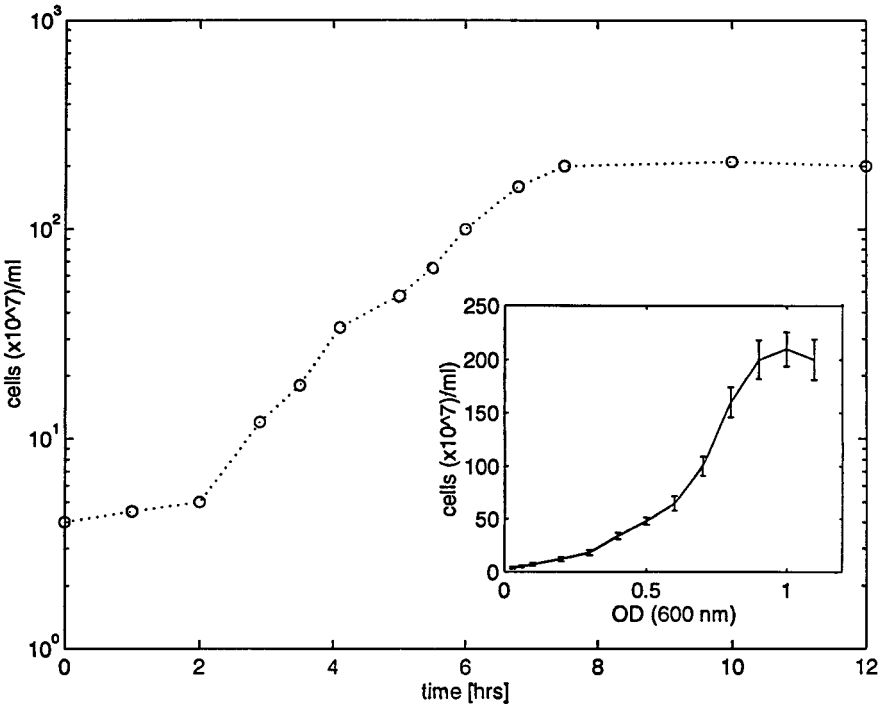

FIGURE 1 Growth of RP437 at 30°C in Tryptone broth. Twenty-milliliter cultures were initiated by addition of 0.4 ml of stationary-phase cells from overnight cultures. Direct cell counts were made with a Petroff-Hausser chamber. *Inset*: OD_{600} vs. cell density.

quency of a population of unstimulated cells was constant to within experimental error over at least 20 min (Fig. 4, *top curve*). Steady-state tumbling frequency, $TF_{st,0}$, increased over growth from $0.35 \pm 0.03\ s^{-1}$ at 0.3 OD to 0.50 ± 0.04 at 0.9 OD (Fig. 5 *A*). With a step addition of 1 mM L-aspartate, tumbling frequency dropped to less than $0.05\ s^{-1}$ at all stages of growth studied (data not shown) and gradually recovered to prestimulus levels (Fig. 4, *bottom curve*). The adaptation time, $\tau_{1/2}$, defined as the time for 50% recovery from the smooth swimming response, increased from 8 ± 1 min at 0.3 OD to 15 ± 1.5 min at 0.9 OD (Fig. 5 *B*). The adaptation times of populations stimulated with 10 mM L-aspartate were the same to within 1 min (data not shown). Precision of adaptation, P, defined as the

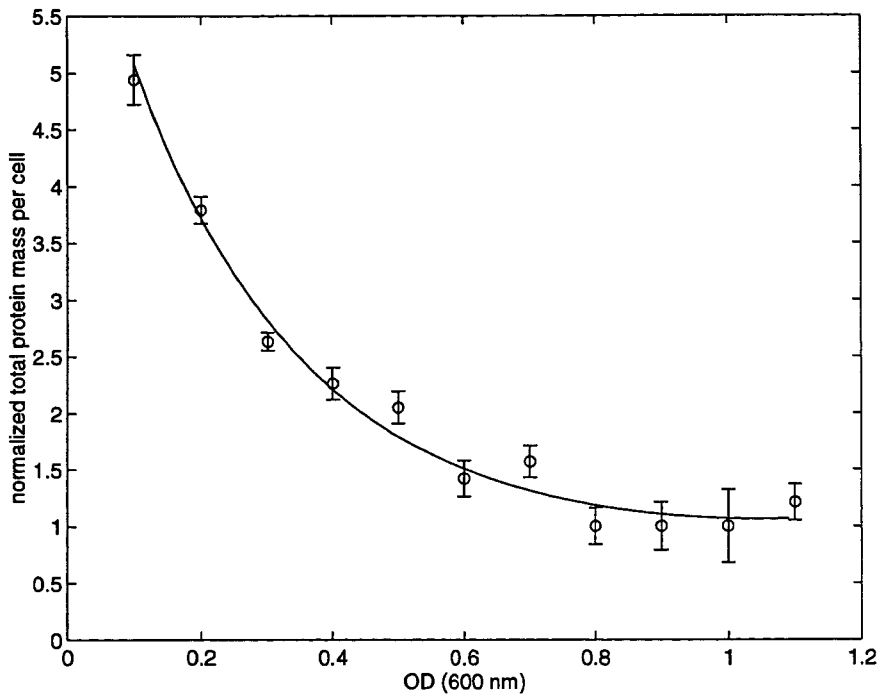

FIGURE 2 Total protein mass per cell measured relative to BSA standards. Cell density at each stage of growth (Fig. 1) was used to determine relative total protein mass/cell, which was then normalized to that calculated for cells at 1.0 OD. The curve is a smooth fit.

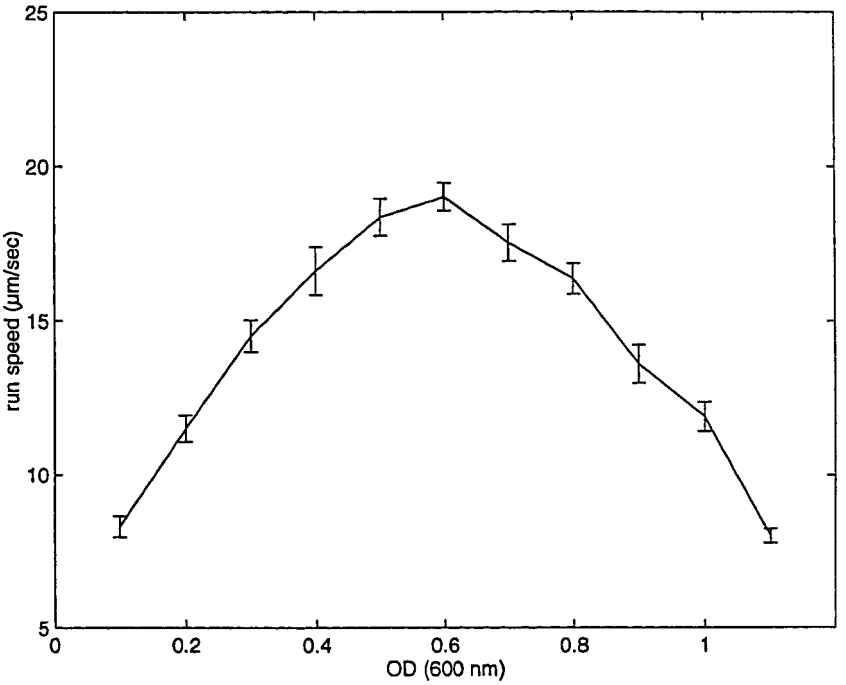

FIGURE 3 Variation in cell run speed over growth. The run speed for each trail was defined as the average of the top 10% of the trail speed (Alon et al., 1998). Each point corresponds to data from three independent measurements, each consisting of 20 10-s recordings at 1-min intervals.

ratio between steady-state tumbling frequencies in the presence and absence of a saturating stimulus, was 1.0 ± 0.2 at all stages of growth studied (Fig. 5 *C*). Precise adaptation to L-aspartate was reported in several studies (Macnab and Koshland, 1972; Berg and Brown, 1972; Berg and Tedesco, 1975; Alon et al., 1999). Tumbling frequency was similar when the cells were resuspended in the motility medium of Berg and Brown (1972), which consists of 10 mM potassium phosphate buffer at pH 7.0 and 0.1 mM EDTA. The cells also responded and adapted precisely to L-aspartate in this medium.

Tumble duration varies approximately inversely with run speed

Tumble duration showed a minimum of 0.15 ± 0.01 s at 0.6 OD (Fig. 6), in agreement with the value of 0.14 s measured

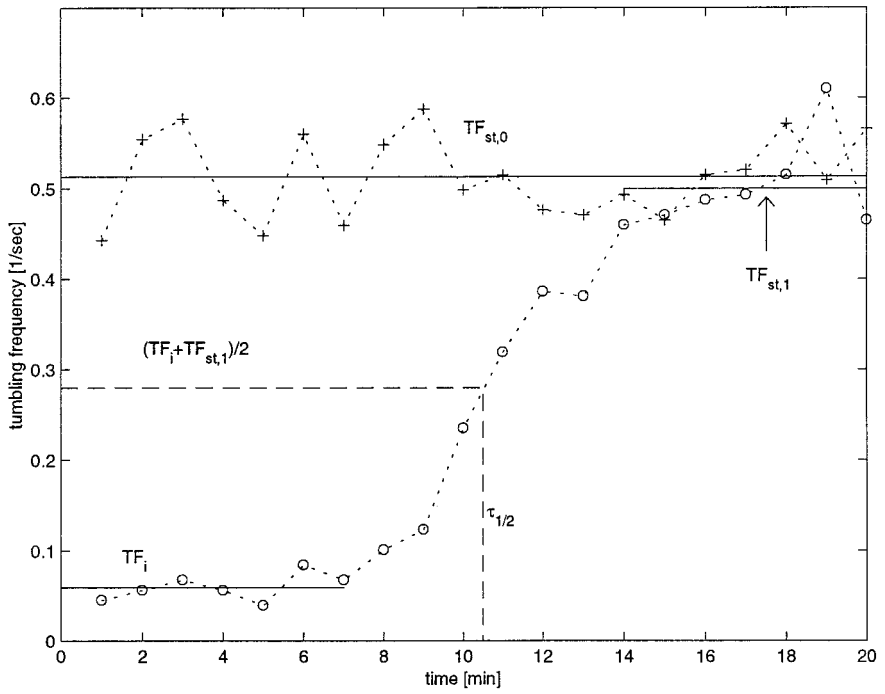

FIGURE 4 Measurement of average tumbling frequency of stimulated and unstimulated cells. Average tumbling frequency of a cell population during a 10-s video segment was measured at 1-min intervals for 20 min after sample preparation. Cells at a given stage of growth were unstimulated (+) or stimulated with 1 mM L-aspartate (○). The vertical dotted line corresponds to the time for 50% adaptation to a saturating stimulus of L-aspartate, $\tau_{1/2}$. Other parameters described in the text are shown ($TF_{st,0}$, TF_i, and $TF_{st,1}$).

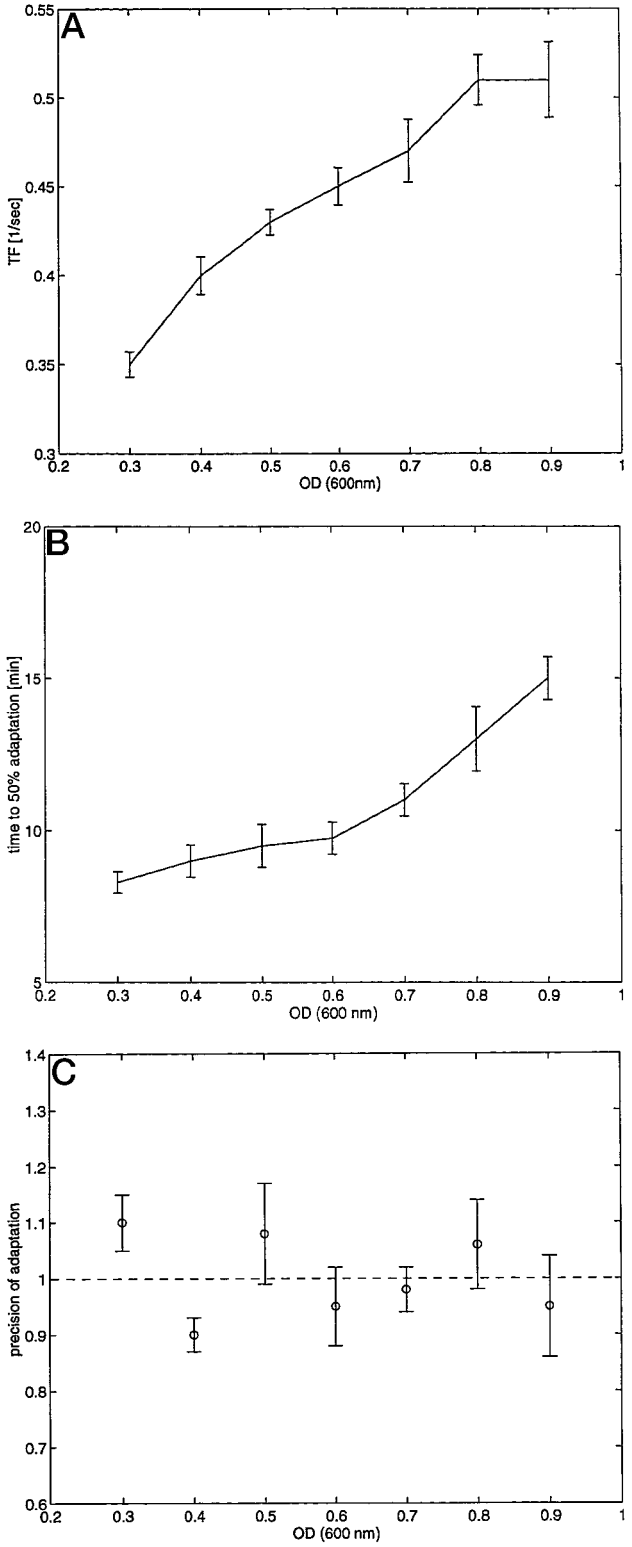

FIGURE 5 Variation as a function of growth stage in (*A*) steady-state tumbling frequency, $TF_{st,0}$, and (*B*) time to 50% adaptation to a saturating stimulus of L-aspartate. (*C*) Precision of adaptation, P, to a saturating stimulus of L-aspartate as a function of growth stage. The dotted line at $P = 1$ corresponds to precise adaptation. In all cases, data are reported as the mean ± SE of at least three independent measurements. Note that the axes do not reach zero.

previously using three-dimensional tracking microscopy (Berg and Brown, 1972). Tumble duration showed a dependence on growth stage that was inversely related to that of mean run speed (Fig. 6, *solid line*).

The flagella fly apart during a tumble and reassemble into a counterclockwise-rotating bundle when the tumble is terminated. The duration of a tumble has been suggested to be determined by a hydrodynamic mechanism for tumble termination (Berg and Tedesco, 1975; Anderson 1975; Alon et al., 1998). For instance, the net action of the unbundled flagella or partially formed bundle could propel the cell in a certain direction, and the resulting fluid flow could push back the remaining flagella to help form the complete bundle. This assumes that most of the flagella spin counterclockwise during a tumble, or that the clockwise motor intervals are short. The typical time scale for such a hydrodynamic process can be estimated as $C(L/V)$, where L is the length of the bacterium, V is its velocity, and C is a dimensionless constant. This expression appears to describe variations in the observed tumble duration reasonably well (Fig. 6, *dotted line*). Here, V is mean run speed (Fig. 3), L is the diameter of a sphere with a volume equal to the average cell volume at the corresponding growth stage (0.6 fl at 0.6 OD and volumes at other growth phases determined according to relative total protein mass/cell, Fig. 2), and $C = 2.66$, the best-fitting constant of proportionality to the measured tumble duration.

DISCUSSION

This work probed several aspects of bacterial chemotaxis with growth and further characterized the behavior of the strain most commonly used for studies of this signaling network. Under the present conditions, the average tumbling frequency of a cell population increased as growth progressed, as did the adaptation time for saturating L-aspartate stimuli. At all growth stages, the steady-state tumbling frequency after adaptation to the stimulus was equal to the prestimulus tumbling frequency. This feature, known as precise adaptation, thus seems to be invariant over growth. It is interesting to consider this in light of recent observations that precise adaptation is preserved despite wide variations in the concentrations of the various chemotaxis proteins, whereas average tumbling frequency and adaptation time vary with protein concentrations (Barkai and Leibler, 1997; Alon et al., 1999).

In a previous study, the steady-state tumbling frequency in the presence of high concentrations of the attractant L-serine was found to be lower than in its absence (Berg and Brown, 1972). In contrast, precise adaptation to L-aspartate was observed. That study employed different conditions, namely growth on a chemically defined medium and measurement in a low salt chemotaxis buffer at 32°C. Under the present conditions, we find precise adaptation to L-serine (not shown). One possible explanation is that under certain

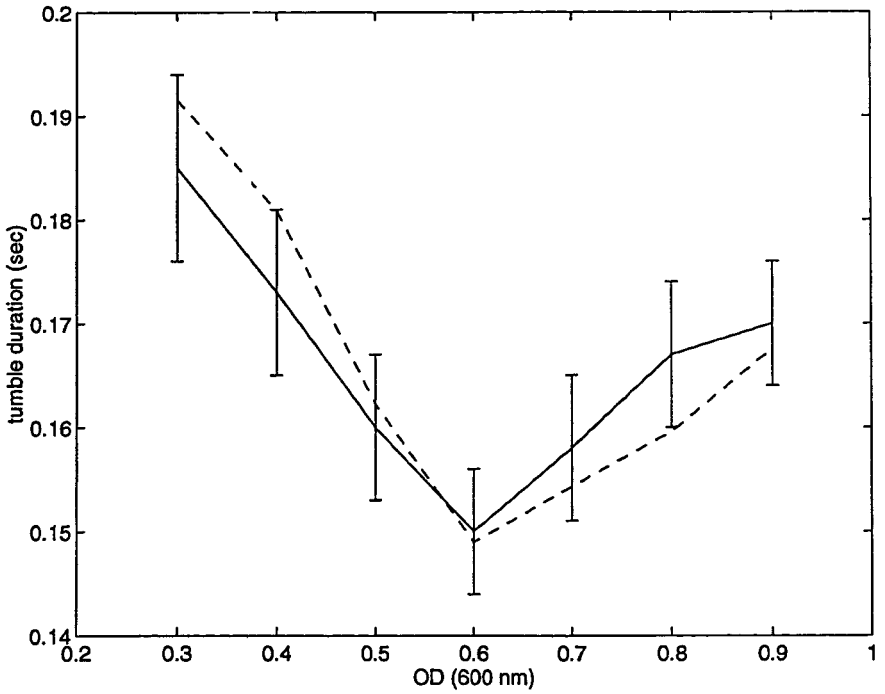

FIGURE 6 *Solid line*: measured variation in average tumble duration over growth. *Dotted line*: theoretical variation in average tumble duration (see text). In all cases, data are reported as the mean ± SE of at least three independent measurements.

conditions, the serine receptor Tsr assumes a conformation in which full methylation can no longer compensate completely for the effect of saturated serine binding. This might be connected to the sensitivity of Tsr to a variety of environmental factors such as temperature (Maeda and Imae, 1979) and salt (Qi and Adler, 1989). Further work is needed to uncover the reason for the lack of exact adaptation to L-serine under certain conditions and to further characterize chemotaxis physiology as a function of growth and measurement conditions.

Tumbles occur in *E. coli* when the flagellar bundle flies apart; they are terminated when the bundle is reconstituted (Macnab, 1996). A roughly inverse relationship between run speed and tumble duration over bacterial growth (Figs. 3 and 6) is observed. This relation is consistent with the argument that tumble duration is determined largely by hydrodynamic interactions that lead to reassembly of the flagellar bundle rather than biochemical regulation of the flagellar motor. For instance, tumble duration was found to be the same in wild-type cells and tumbly mutants (Berg and Brown, 1972) and to be independent of P-CheY concentration (Alon et al., 1998). The time scale for such hydrodynamic interactions can be estimated as the ratio of bacterial length to velocity in agreement with observed variations in tumble duration over growth.

Bacterial chemotaxis is emerging as a model system for understanding how collective network properties arise from interactions of individual components. A full understanding of this network will require accurate quantitative analysis of cell behavior. This study helped characterize the *E. coli* strain most commonly used to investigate the physiology of the chemotaxis response. In particular, it demonstrated that different network functions can change or remain invariant under the global changes that occur in the cell over growth.

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