Bacterial chemoreceptor signaling probed by flash photorelease of a caged serine

Shahid Khan,* Kwame Amoyaw,* John L. Spudich,‡ Gordon P. Reid§ and David R. Trentham.§
*Departments of Anatomy and Structural Biology, and Physiology and Biophysics, Albert Einstein College of Medicine. Bronx, New York 10461; ‡Department of Microbiology and Molecular Genetics, University of Texas Medical School, Health Science Center, Houston, Texas 77030; and §Division of Physical Biochemistry, National Institute for Medical Research, Mill Hill, London NW7 1AA United Kingdom

ABSTRACT A caged serine, a photolabile compound that liberates serine upon photolysis, has been synthesized. Smooth-swimming responses of the bacterium *Escherichia coli* to caged serine photorelease were videotaped. The mean latency was measured from the videorecords using computerized motion analysis. This time was ~0.2 s. Caged photorelease of a photolabile but nonchemotactic serine analogue had no effect on the swimming behavior of the bacteria. A tumbly mutant strain lacking *tsr*, the serine chemoreceptor, did not respond to caged serine photorelease.

INTRODUCTION

Serine chemotaxis in *Escherichia coli* is one of the best understood examples of chemotaxis in micro-organisms (1). The serine chemoreceptor, *tsr*, is an integral membrane protein. Cytoplasm based signaling between *tsr* and the flagellar motor involves formation of a signalling complex between *tsr* and the cytoplasmic proteins *che*W and *che*A; followed by subsequent transfer of phosphate from *che*A to *che*Y, which binds to the motor, and *che*B. *Che*B phosphorylation modulates its methylesterase activity, thereby switching off the *tsr* signalling state.

Flagellar motors respond to serine stimuli by rotating counter-clockwise (CCW). The motile behavior of the cell reflects the integrated behavior of the six or so flagella on the cell body, which switch rotation sense independently of one another. CCW rotating flagella can bundle and rotate together leading to a smooth-swimming response. Bundle breakup is thought to occur when an as yet undetermined number of flagella switch to clockwise (CW) rotation and the cell tumbles. In this report we study the kinetics of the response of bacteria to rapid exposure to *L*-serine through photolysis of a caged serine. This photochemical approach overcomes diffusional delays that otherwise may inhibit analysis of biological systems (2).

RESULTS AND DISCUSSION

Fig. 1 shows five sample paths of swimming *E. coli* digitized using commercially available EV motion analysis software (Motion Analysis Inc., Santa Rosa, CA) run on a SPARC IPC (Sun Microsystems, Mountain View, CA). The paths were traced by thresholding out dark-field images. Centroids were calculated for each thresholded object and the successive displacements of centroids from frame-to-frame linked together to form paths. The circles mark the frame at which the flash occurred. Following the flash, the swimming paths straightened out. In addition to the occasional sharp changes of direction marking tumbling events, the paths deviated more frame-to-frame before as compared to after the flash. It is probable that the translation of a cell when powered by a bundle in which some flagella are rotating CW is not as well oriented as when all the flagella are rotating CCW.

For analysis of the data, we modified the algorithm previously developed by Sager et al. (3). Our algorithim enabled digitization at higher frame rates and restricted the analysis to only those cells present in the field of view at the time of flash. Parameters such as *lvl* (mean speed) and *ngdr* (net to gross displacement) (4) were used to exclude dirt and immotile cells from the analysis. The *rcdi* (rate of change of direction) was found to be the most sensitive monitor of the tactic response.

The caged serine was an *N*-substituted 1-(2-nitrophenyl)ethyloxycarbonyl derivative of *L*-serine. The quantum yield with respect to photolysis is ~0.7 and the release of serine following a flash was exponential with a half time of 40 ms at pH 7.0 and 21°C. This class of photosensitive protecting group for amino acids was introduced by Patchornik et al. (5). The serine was photolyzed on the microscope slide by focusing obliquely onto the sample the output from a flash lamp (6) with an mirror housing. Fig. 2 shows the change in *rcdi* of a pooled population of 500 cells subjected to flash photolysis of 1 mM caged serine releasing an estimated 100 μM serine. The half time of the smooth-swimming response is ~200 ms. The response is specific for serine. Photolysis of the same derivative of *L*-alpha-amino-*n*-butyric acid, a photolabile analogue of caged serine, has

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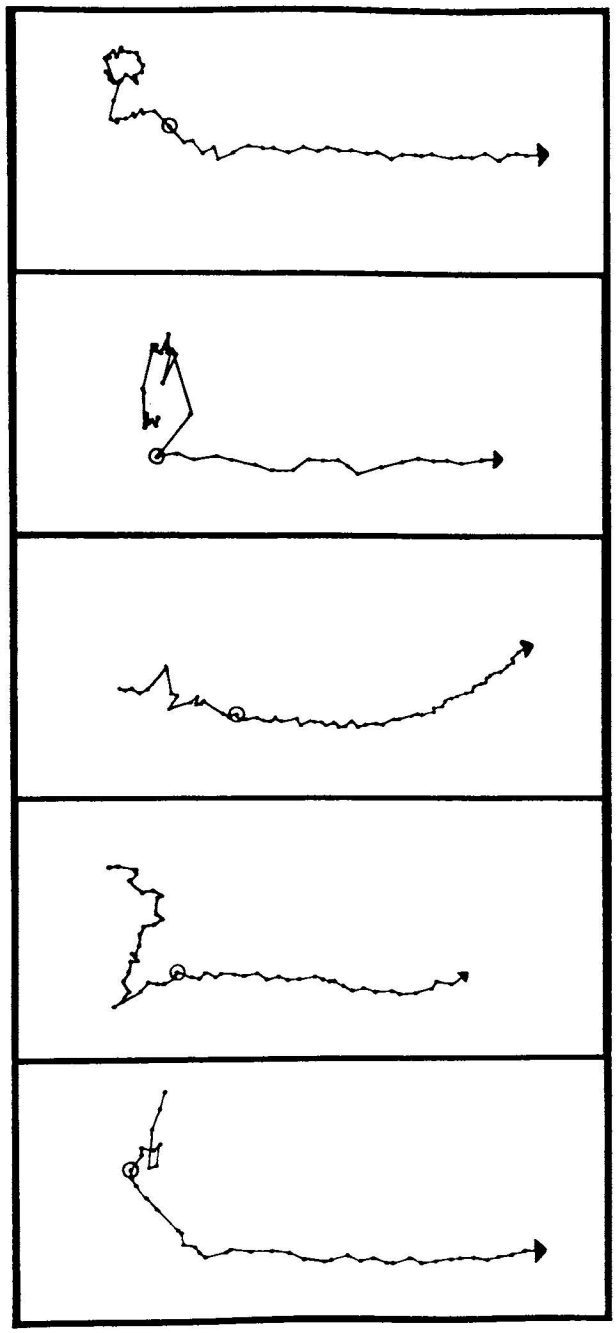

FIGURE 1 Paths of swimming *E. coli* tracked by computer upon playback of videorecords. The paths were digitized at 30 frames per second. Arrows denote direction of travel. For each path the frame in which the flash occurred is circled. 1 mM caged serine; 5 mM dithiothreitol; 20 mM phosphate, pH 7.0 buffer and 22°C.

no effect on the motile behavior of the bacteria. Photorelease of caged serine is not sensed by the mutant strain AW518, deleted for *tsr*.

These experiments document both the successful synthesis of a caged serine and measurement of a

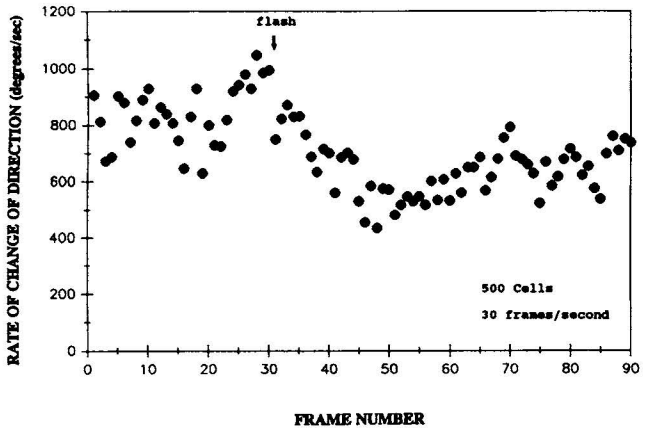

FIGURE 2 Automated analysis of *E. coli* swimming response to caged serine photorelease. A 3 s videorecord was digitized at 30 frames per second. An event marker triggered the flash one second after a tone signal initiated digitization. The intensity of the flash was empirically set to the level such that the excitation phase as well as part of the adaptation phase of the response was captured in the first two seconds. Conditions as in Fig. 1.

response latency for serine chemotaxis in *E. coli*. The only previously recorded measurements of chemotactic response times in bacteria were made by ionophoretic stimulation of *E. coli* cells tethered by a single flagellum onto glass coverslips. Response latencies were obtained for the attractant aspartate and certain metal ion repellents. These ranged around 200 ms, comparable to the measured serine response time in free-swimming cells. Taken together with the availability for overexpression systems of serine chemotactic signaling components (1), the flash photolytic approach provides a means for correlating behavioral responses with biochemical reaction kinetics of phosphate and methyl group transfer.

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