Sensory Rhodopsin II Transducer HtrII Is Also Responsible for Serine Chemotaxis in the Archaeon Halobacterium salinarum

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Previously, we demonstrated that the methyl-accepting protein HtrII is the transducer for photoreceptor sensory rhodopsin II. Here, we provide experimental evidence that HtrII is also a chemotransducer. Using an agarose-in-plug bridge method, we show that an HtrII overexpression strain has a quicker response to serine than does an HtrII deletion strain. Furthermore, an in vivo flow assay demonstrates that the deletion strain is unable to modulate methylenesterase activity after serine addition or photostimulation, while the overexpression strain shows distinct methanol peaks following both types of stimuli.

The archaeon Halobacterium salinarum exhibits phototactic responses to changes in light intensity and color, aero-
tactic responses to depletion and abundance of oxygen, and chemotactic behavior due to addition or removal of specific chemical reagents (3, 5, 9). In the absence of stimuli, halobacterial cells swim in straight lines characterized by three possible activities: swimming with one pole forward, pausing, and swimming with the other pole forward (reversal). We have shown that, unlike in eubacteria, clockwise rotation of the right-handed helical flagellar bundle in halobacteria exerts a pushing force on the cell body and counterclockwise rotation pulls the cell (1). The flagellar bundle never flies apart as it does in most enteric bacteria (6). Sensory input changes the frequency of reversals to optimize movements towards attractants and away from repelle-
ts. Thus, the cells make net progress in spatial gradi-
ents of these attractants and repellents. Specific chemore-
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fers. Glucose, histidine, and leucine are among the effective attractants, while phenol is a repel-

Isolation of HtrII deletion and overexpression strains.
The phototaxis-defective mutant Pho81 contains a 552-bp IS2 insertion element in the region upstream from the htrII-sopII cluster (13). In order to eliminate the potential insta-
bility of the Pho81 mutant due to the presence of the mobile genomic element, a gene knockout technique was used to delete most of the downstream portion of the htrII (nucleo-
tides 98 to 2298) coding sequence and the adjacent up-
stream portion of the sopII (nucleotides 1 to 231) coding sequence. Southern hybridization analysis with a 27-mer oligonucleotide probe (highly conserved among all trans-
der genes) indicated that the 6.5-kbp PstI fragment is

HtrII is involved in the chemotactic response to serine.
To test the hypothesis that the large periplasmic domain of HtrII has a ligand-binding function, we studied chemotactic responses in strains expressing different levels of the HtrII

![Image]

**A** Western blot analysis with HC23 antibody. **B** Electrophoretic analysis.

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protein. We analyzed chemotactic responses of the deletion and overexpression strains to amino acids with our recently developed agarose-in-plug bridge method (10). In this method, agar plugs containing specific amino acids are placed under coverslips which are then filled with a motile halobacterial cell suspension. The deletion and overexpression strains were screened for responses to all essential amino acids. Among the amino acids, serine and alanine showed different kinetics in chemotactic ring formation around the agarose plug. Unlike those of the overexpression strain, \( \Delta \text{htrII} \) cells did not form a dense chemotactic ring within 5 to 10 min (Fig. 2A, panel I). During this period, overexpression strain cells did form a distinct visible chemotactic ring in response to serine, i.e., a white ring against a dark background (Fig. 2A, panel II). The difference in chemotactic response to alanine between these two strains was not as distinct as that to serine (data not shown). Both strains formed comparable chemotactic rings within 5 to 10 min with a growth medium agarose plug (Fig. 2B).

The \( \Delta \text{htrII} \) deletion mutant is defective in methylesterase responses to serine and 450-nm light \( (\lambda_{\text{max}} = 487 \text{ [for SRII]}) \). The physiological data above clearly indicate that strain \( \Delta \text{htrII} \) is defective in chemotactic responses to serine. To demonstrate that HtrII is involved in methylation-demethylation during photostimulation and chemostimulation, we have studied the methylesterase activities of the deletion and overexpression strains in response to serine and light. Deletion mutant \( \Delta \text{htrII} \) does not exhibit a transient increase in methanol production upon chemostimulation with serine, while responses to Asp and Glu are comparable for strains \( \Delta \text{htrII} \) and \( \text{htrII}^{+}/\Delta \text{htrII} \) (Fig. 3). The same result was obtained after light stimulation (450 nm) followed by the addition of serine (Fig. 4). These results show that the halobacterial strain lacking HtrII does not modulate methylesterase activity upon stimulation with both light and serine. The low level of methanol evolution and the delayed chemotactic ring formation in agarose-in-plug bridge assays observed for strain \( \Delta \text{htrII} \) reflect the possibility of organizational and functional sharing among the 13 known transducers of \( H. \text{salinarum} \). We cannot exclude the possibility that serine might be sensed by transducers other than HtrII, including the four soluble transducers (3, 11). Indeed, we have demonstrated that stimulation by Asp and Glu causes demethylation of two different transducers (the soluble transducer HtrXI and the putative membrane-bound transducer HtrVII) at the same time (4). The amplitudes of the transient increase in methanol evolution after serine stimulation and photostimulation are comparable. These

FIG. 2. Chemotactic response (white ring indicated by two arrowheads) of an \( H. \text{salinarum htrII} \) deletion mutant (\( \Delta \text{htrII} \)) (I) and an \( \text{htrII} \) overexpression mutant (\( \text{htrII}^{+}/\Delta \text{htrII} \)) (II) by the agarose-in-plug bridge method. (A) Serine. (B) Growth medium. Micrographs were taken by a Nikon automatic camera during dark-field microscopy with a 10× objective.
results indicate that the methyl group turnover rates in the putative methylation sites of HtrII by methylesterase induced by chemostimulation and photostimulation should be similar.

We have established that HtrII is a phototransducer and a chemotransducer. Thus, it is reasonable to postulate that there must be a mechanistic dissection of chemotactic and phototactic signaling in HtrII or the presence of specific regions or amino acid sequences in HtrII that are crucial for photostimulation and chemostimulation. Further studies are under way to trace residues to identify their effects in phototactic and chemotactic signaling and adaptation.

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REFERENCES