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Phosphate Taxis in *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa **was shown to be attracted to phosphate. The chemotactic response was induced by phosphate starvation. The specificity of chemoreceptors for phosphate was high so that no other tested phosphorus compounds elicited a chemotactic response as strong as that elicited by phosphate. Competition experiments showed that the chemoreceptors for phosphate appeared to be different from those for the common amino acids. Mutants constitutive for alkaline phosphatase showed the chemotactic response to phosphate regardless of whether the cells were starved for phosphate.**

Phosphorus compounds are essential constituents in organisms. In nature, phosphate is often found to be a growth-limiting factor for organisms. To deal with phosphate limitation, bacteria have evolved complex regulatory systems to assimilate phosphorus very efficiently (14). In microorganisms, phosphate limitation results in the synthesis of several protein species. These include alkaline phosphatase, an outer membrane channel-forming protein, a binding protein for phosphate, and two hemolysins (6, 10, 11).

It is known that motile bacteria exhibit chemotactic responses to a wide range of chemical stimuli, including amino acids, sugars, and organic acids (1, 12). Though many works on bacterial chemotaxis have been reported, no paper seems to report about the chemotactic response to phosphate in bacteria. A majority of the previous workers used media containing potassium phosphate buffer for chemotaxis assay, and this may be a reason for the absence of information on phosphate taxis. In this report, we describe chemotaxis to phosphate in *Pseudomonas aeruginosa*.

P. aeruginosa PAO1 was used throughout the study. Cells were grown in T_{10} minimal medium (7) containing 10 mM potassium phosphate at 37°C with shaking. For phosphate limitation, cells overnight grown were inoculated to T_0 medium, which was prepared by omitting potassium phosphate from T_{10} medium, and incubated with shaking at 37°C.

The chemotactic response to phosphate was determined by a rapid capillary assay technique which is a modification of Adler's procedure (2). In this technique, digital image processing was used to count the number of bacteria accumulating toward the mouth of a capillary containing attractant gel. This obviated the necessity of doing plate counts of capillary contents to determine the number of cells attracted and made it possible to assess the bacterial response to phosphate within a few minutes.

A small chamber similar to that described by Adler (2) was used for microscopic observation of bacterial motility. The chemotaxis buffer used in the present study was 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer (pH 7.0). Cells were washed twice with chemotaxis buffer and resuspended in the same buffer at a concentration of ca. 6×10^8 cells per ml. More than 90% of the bacteria were very motile, as judged by microscopic observation. Glass capillary tubes with an internal diameter of ca. 10 μm were prepared from microinjection glass tubings (1-mm diameter; type G-1; Narishige Co., Tokyo, Japan) by using a microcapillary-producing unit (PB-7; Narishige Co.). The capillary was filled with chemotaxis buffer containing known concentrations of potassium phosphate plus 1.5% agarose and then was inserted into the chamber by using a joystick micromanipulator (NM-151; Narishige Co.).

Cells in a 20-μl suspension were placed on a coverslip, and the coverslip was placed upside down on a U-shaped spacer to fill the chamber with the cell suspension immediately preceding the videotaping. The depth of the bacterial suspension was about 80 μm, and the assays were performed at 37°C. Cells were videotaped through a phase-contrast microscope (IMT-2; Olympus Co., Tokyo, Japan; magnification, ×300; bright field) fitted with a 20× objective (LWDCDPlan; Olympus Co.) and a video camera (FCD-725; Ikegami Co., Tokyo, Japan). The focus of the microscope was maintained at the mouth of the capillary.

Videotapes were played back and digitized with an image processor (LA525X; Pias Co., Osaka, Japan). The image processor detected areas of dark bacterial cells on a light background in a grid of 480 vertical by 512 horizontal pixels. The scanned area in the field of microscopic view was ca. 100 by 80 μm. Only those cells within the focus were recorded and processed. The number of cells per each video frame was counted by the software program of LA525X and stored in a personal computer.

Phosphate elicited a positive tactic response from *P. aeruginosa* cells grown in the phosphate-limiting medium (Fig. 1). The response was observed soon after the start of microscopic observation, and the number of cells per frame increased linearly with time. No positive response to phosphate was detected with cells grown in phosphate-rich T_{10} medium.

The response of bacteria was dependent on the concentration of phosphate in the capillary (Fig. 2). The concentration-response curve showed a peak at 5×10^{-3} M phosphate. The lowest concentration needed to elicit an observable response (the threshold concentration) was 5×10^{-5} M. At concentrations below this level, the number of cells per frame gradually decreased with time. This movement away from the capillary, which was also observed with phosphate-sufficient cells in Fig. 1, was probably due to the aerotactic reactions.

To study the specificity of chemoreceptors for phosphate, various other phosphorus compounds were surveyed for their ability to attract *P. aeruginosa*. *P. aeruginosa* was attracted to PP_i only when the concentration was higher than

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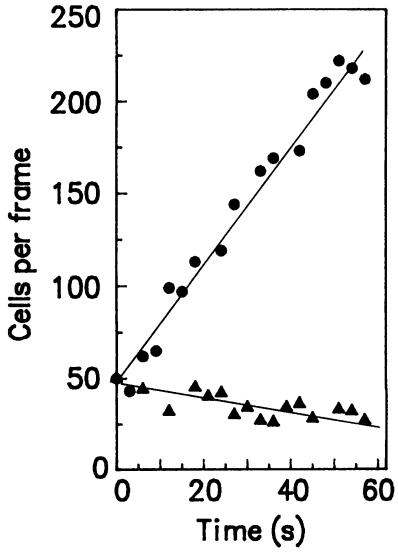

FIG. 1. Chemotactic responses of phosphate-starved cells (●) and phosphate-sufficient cells (▲) of *P. aeruginosa* toward 10^{-2} M phosphate. For cell counting, one videotape frame was analyzed for each given time point.

10^{-3} M. No chemotactic response was observed with 1-glycerophosphate, 2-glycerophosphate, polyphosphates, or ATP at concentrations from 10^{-6} to 10^{-3} M (data not shown). The data for higher concentrations could not be collected because of the contamination of the phosphate. *P. aeruginosa* is known to be attracted to a majority of the 20 common amino acids (9). Phosphate did not inhibit the taxis for the amino acids, and none of the 20 amino acids inhibited the chemotactic response to phosphate in phosphate-starved cells (data not shown). This result suggests that the chemoreceptors for phosphate are different from those for the common amino acids.

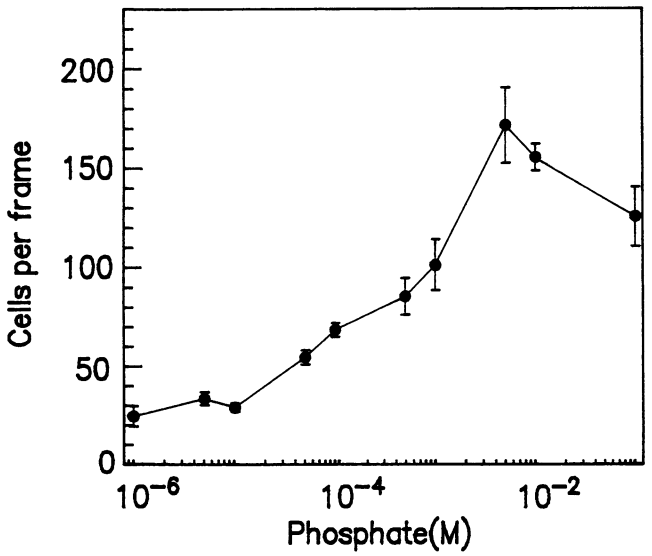

FIG. 2. Concentration-response curve for phosphate. Videotaped images were sampled at 30 s after the start of observation. Vertical bars represent the standard deviations of measurements done at each phosphate concentration in four separate experiments.

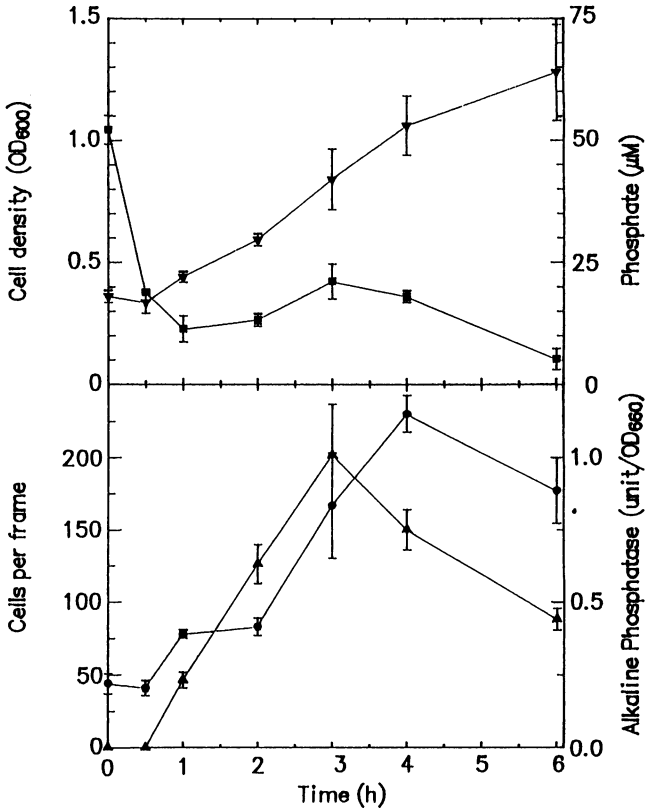

FIG. 3. Time course of cell growth (▼), phosphate concentration (■), alkaline phosphatase activity (▲), and strength of chemotaxis to phosphate (●) during growth in phosphate-limiting medium. Vertical bars represent the standard deviations of measurements done in four separate experiments. OD_{600}, optical density at 600 nm.

Phosphate taxis appears to be under the control of the same set of regulatory genes as *phoA*. Figure 3 shows the time course data on cell growth, the phosphate concentration, the alkaline phosphatase activity, and the strength of chemotaxis to phosphate during growth in the phosphate-limiting medium. Phosphate initially detected in the medium was that brought in from the preculture. The activity of alkaline phosphatase started to increase about 0.5 h after the start of incubation. In parallel with the alkaline phosphatase activity, the strength of the response to phosphate increased, through the peak was observed 1 h after that of the alkaline phosphatase activity.

In order to check further this point, we selected mutants constitutive for alkaline phosphatase by the procedure of Poole and Hancock (11). Of 11 alkaline phosphatase-constitutive mutants obtained, all showed a chemotactic response to phosphate, regardless of whether they were starved for phosphate (Table 1). It becomes of interest to further assess mechanisms of bacterial phosphate taxis, particularly in connection with the *pho* regulon system in *P. aeruginosa* (3).

Bacterial chemotaxis and sensory transduction have been the subject of intensitive investigation (1, 12). Rapid progress toward detailing the molecular mechanisms of chemotaxis in enteric bacteria has been made in recent years (4). Though relatively little is known about the chemotactic responses in *P. aeruginosa*, this organism is likely to exhibit responses and regulatory strategies different from those of the enteric bacteria (5, 8, 9). Whether the taxis to phosphate

TABLE 1. Comparison of the alkaline phosphatase activities and the strength of phosphate taxis of the *P. aeruginosa* wild type (PAO1) and mutant strains (AP01 and AP02)[a]

Strain	Alkaline phosphatase activity (U/OD_{600})[b] in:		Chemotactic response to phosphate (cells/frame)[c] in:	
	T_0 medium[d]	T_{10} medium[e]	T_0 medium	T_{10} medium
PAO1	0.76 ± 0.08	0	135 ± 13	35 ± 5
APO1	0.75 ± 0.02	0.21 ± 0.01	139 ± 9	115 ± 2
APO2	0.93 ± 0.22	0.55 ± 0.05	156 ± 8	126 ± 10

[a] Data from two randomly chosen mutants are shown.
[b] The activity was determined by the method of Torriani (13), and 1 U of enzyme activity was defined as the amount of enzyme which liberated 1 μmol of *p*-nitrophenol per min. OD_{600}, optical density at 600 nm.
[c] Videotaped images were sampled at 60 s after the start of observation.
[d] T medium (7) without phosphate.
[e] T medium with 10 mM phosphate.

generally occurs in the other bacterial species is still unknown. However, it is possible that the chemotaxis toward phosphate has an important role in scavenging phosphate under conditions of phosphate limitation.

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