JOURNAL OF BACTERIOLOGY, June 2000, p. 3400–3404 Vol. 182, No. 12
0021-9193/00/$04.00+0

Identification and Characterization of Two Chemotactic Transducers for Inorganic Phosphate in *Pseudomonas aeruginosa*

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Received 18 January 2000/Accepted 23 March 2000

Two chemotactic transducers for inorganic phosphate (P_i), designated CtpH and CtpL, have been identified in *Pseudomonas aeruginosa*. The corresponding genes (*ctpH* and *ctpL*) were inactivated by inserting kanamycin and tetracycline resistance gene cassettes into the wild-type genes in the *P. aeruginosa* PAO1 genome. Computer-assisted capillary assays showed that the *ctpH* single mutant failed to exhibit P_i taxis when the concentration of P_i in the capillary was higher than 5 mM. Conversely, the *ctpL* single mutant could not respond to P_i at the concentration of 0.01 mM. The *ctpH ctpL* double mutant was defective in P_i taxis at any concentration ranging from 0.01 to 10 mM. To investigate regulation of P_i taxis, the *ctpH* and *ctpL* genes were also disrupted individually in the *P. aeruginosa phoU* and *phoB* single mutants. The *ctpH phoU* and *ctpH phoB* double mutants were defective in P_i taxis, regardless of whether the cells were starved for P_i. The *ctpL phoU* double mutant was constitutive for P_i taxis, whereas the *ctpL phoB* double mutant was induced by P_i limitation for P_i taxis. The region upstream of *ctpL*, but not *ctpH*, contained a putative *pho* box sequence. Expression of *ctpL*::*lacZ* was induced by P_i limitation in PAO1, while it was constitutive in the *phoU* mutant. In contrast, the *phoB* mutant showed only background levels of *ctpL*::*lacZ* expression. These results showed that *ctpL* is involved in the *pho* regulon genes in *P. aeruginosa*. The *ctpH phoU* mutant, which failed to exhibit P_i taxis, was constitutive for *ctpL*::*lacZ* expression, suggesting that the P_i detection by CtpL requires PhoU. Like PAO1, the *phoB* and *phoU* single mutants were constitutive for expression of *ctpH*::*lacZ*. Thus, the evidence that the *ctpL phoU* mutant, but not the *ctpL phoB* mutant and PAO1, was constitutive for P_i taxis raised the possibility that PhoU exerts a negative control on P_i detection by CtpH at the posttranscriptional level.

Pseudomonas aeruginosa PAO1 is attracted to inorganic phosphate (P_i) (10). P_i taxis is induced when the cells are starved for P_i. Although the chemoreceptor for P_i has never been identified, its specificity for P_i appears to be relatively high. No other phosphorus compounds have been shown to elicit responses similar to those for P_i (10). P_i-starved cells are also attracted to arsenate (AsO_4^{3-}) (11). Since P_i competitively inhibits the response to AsO_4^{3-}, both P_i and AsO_4^{3-} are likely detected by the same chemoreceptors. The enteric bacterium *Enterobacter cloacae*, but not *Escherichia coli* and *Salmonella enterica* serovar Typhimurium, also exhibits P_i taxis under conditions of P_i limitation (11). Experimental evidence shows that the *E. cloacae* genes encoding the P_i-specific transport (Pst) system and the PhoU protein are required for P_i taxis.

Previously, we showed that *P. aeruginosa* mutants PHOB1 (*phoB*::*kan*) and PHOR1 (*phoR*::*kan*) were not induced by P_i limitation for alkaline phosphatase synthesis but exhibited P_i taxis under conditions of P_i limitation (12). Interestingly, the *P. aeruginosa phoU* mutant, which was constructed by *N*-methyl-*N*′-nitro-*N*′-nitrosoguanidine mutagenesis, showed P_i taxis regardless of whether the cells were starved for P_i (constitutive P_i taxis) (12). We also found that *P. aeruginosa* mutants lacking the Pst complex were constitutive for P_i taxis (17). Based on these results, it has been suggested that the Pst complex, interacting with the PhoU protein, exerts a negative control on P_i taxis, even though it is not positively regulated by the PhoB and PhoR proteins (12). In the present study, we found that *P. aeruginosa* possesses two chemoreceptors for P_i, designated CtpH and CtpL. CtpH was required for exhibiting P_i taxis at high concentrations of P_i, while CtpL could serve as the major chemoreceptor for P_i at low concentrations. Expression of the gene coding for CtpL (*ctpL*) was induced by P_i limitation, depending on the PhoB and PhoU proteins. In contrast, the gene coding for CtpH (*ctpH*) was expressed constitutively.

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MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains and plasmids used in this study are listed in Table 1. *E. coli* MV1184 and HB101 were used for plasmid construction and DNA manipulation. *P. aeruginosa* and *E. coli* were grown at 37°C with shaking in 2× YT medium (19) supplemented with appropriate antibiotics. *P. aeruginosa* was also grown at 37°C with shaking in T_5 minimal medium (7) containing 5 mM P_i. For P_i limitation, *P. aeruginosa* cells grown overnight in 2× YT medium were inoculated (a 2.5% inoculum) into T_0 medium, which was prepared by omitting P_i from T_5 medium, and incubated at 37°C with shaking.

Chemotaxis assays. The computer-assisted capillary assays were carried out as described previously (16). Cells were videotaped over the first 3 min. Digital image processing was used to count the number of bacteria accumulating toward the mouth of a capillary containing a known concentration of an attractant plus 1% agarose. The chemotaxis buffer used was T_0 medium supplemented with 2.78 mM glucose. All chemicals used for chemotaxis assays were reagent grade.

DNA manipulation. Standard procedures were used for plasmid DNA manipulations and agarose gel electrophoresis (19). *P. aeruginosa* chromosomal DNA was prepared as described previously (12). *P. aeruginosa* was transformed by electroporation (12). TaKaRa Ex Taq DNA polymerase (Takara Shuzo, Shiga, Japan) was used for PCR. The search for DNA sequences corresponding to the highly conserved domain (HCD) of chemotactic transducers was done with the *P. aeruginosa* genome sequence at the *Pseudomonas* genome project website (http://www.pseudomonas.com) by using the program TBLASTIN (1). Alignment of amino acid sequences was performed with the program FASTA (18).

Cloning of the *ctpH* and *ctpL* genes. The *ctpH* and *ctpL* genes were cloned from the PAO1 genome by using PCR. A 3.6-kb DNA fragment, which contained the entire *ctpH* gene, was amplified with the PCR primers TR1 (5′-TCTGTTTCA GCGTCTGTAGCATCG) and TR2 (5′-ACATCGGTACCAATAGCGAAGTC G). The PCR product was cloned into pGEM-T Easy (Promega) to make pPT10.1. Similarly, a 3.3-kb DNA fragment, which contained the entire *ctpL* gene, was amplified with the PCR primers TR3 (5′-TCGACGATGTTGTAGT AGACCTCG) and TR4 (5′-GATCATCCTCGACATGTACATGCC). This

TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant characteristics	Reference or source
E. coli		
MV1184	*ara*Δ(*lac-proAB*) *rpsL thi* (ϕ80 *lacZ*ΔM15) Δ(*srl-recA*)*306*::Tn*10*(Tc^r) F′[*traD36 proAB*$^+$ *lacI*^{q} *lacZ*ΔM15]	23
HB101	*hsdS20 recA13 proA2 leu6 thi-1 rpsL20 ara-14 galK2 lacY1 xyl-5 mtl-1 supE44*	3
P. aeruginosa		
PAO1	Prototroph, FP$^-$ (sex factor minus)	8
PP1	PAO1 derivative, *ctpH*::*kan*	This study
PP2	PAO1 derivative, *ctpL*::*kan*	This study
PP3	PAO1 derivative, *ctpH*::*tet ctpL*::*kan*	This study
APC1	PAO1 derivative, *phoU*	12
PHOB1	PAO1 derivative, *phoB*::*kan*	12
PP4	PAO1 derivative, *phoU ctpH*::*kan*	This study
PP5	PAO1 derivative, *phoU ctpL*::*kan*	This study
PP6	PAO1 derivative, *phoB*::*kan ctpH*::*tet*	This study
PP7	PAO1 derivative, *phoB*::*kan ctpL*::*tet*	This study
Plasmids		
pGEM-T Easy	PCR cloning vector; Ap^r *lacPOZ′*	Promega
pUC118Tc	pUC118 containing a 1.3-kb *tet* cartridge from pBR322; Ap^r Tc^r	This study
pUC4K	pUC4 containing a 1.3-kb *kan* cartridge; Ap^r Km^r	Pharmacia
pCP19	Broad-host-range cosmid; Tc^r IncP	6
pPT10.1	pGEM-T Easy with a 3.6-kb PCR fragment containing *ctpH*; Ap^r	This study
pPT11.1	pGEM-T Easy with a 3.3-kb PCR fragment containing *ctpL*; Ap^r	This study
pPT10.2	pPT10.1 derivative containing a 1.3-kb *kan* cartridge	This study
pPT11.2	pPT11.1 derivative containing a 1.3-kb *kan* cartridge	This study
pPT10.3	pPT10.1 derivative containing a 1.3-kb *tet* cartridge	This study
pPT10.4	pCP19 derivative containing a 3.6-kb *Eco*RI fragment of pPT10.1; Tc^r	This study
pPT11.4	pCP19 derivative containing a 2.3-kb *Eco*RV fragment of pPT11.1; Tc^r	This study
pKZ27	Broad-host-range transcriptional fusion vector; Km^r *lacZ* IncQ	This study
pKZ27.1	pKZ27 containing a 1.2-kb fragment with *amp* gene from pBR322; Km^r Cb^r	This study
pPT10.5	pKZ27 derivative containing a 1.8-kb *Kpn*I fragment of pPT10.1; Km^r	This study
pPT10.6	pKZ27.1 derivative containing a 1.8-kb *Kpn*I fragment of pPT10.1; Km^r Cb^r	This study
pPT11.5	pKZ27 derivative containing a 1.6-kb *Eco*RV-*Sal*I fragment of pPT10.1; Km^r	This study
pPT11.6	pKZ27.1 derivative containing a 1.6-kb *Eco*RV-*Sal*I fragment of pPT10.1; Km^r Cb^r	This study

PCR product was also cloned into pGEM-T Easy to make pPT11.1. For complementation experiments, the 3.6- and 3.3-kb DNA fragments were also cloned into pCP19 (6) to make pPT10.4 and pPT11.4, respectively.

Construction of deletion-insertion mutants. Deletion-insertion mutants were constructed by the direct gene replacement technique (12). Plasmid pPT10.1 was digested by *Xho*I and ligated to a 1.3-kb *Sal*I fragment containing a *kan* (conferring kanamycin resistance [Km^r]) cassette from pUC4K (Pharmacia) to make pPT10.2. Similarly, pPT11.1 was digested by *Eco*RI and ligated to a 1.3-kb *Eco*RI fragment containing a *kan* cassette from pUC4K to make pPT11.2. Plasmids pPT10.2 and pPT11.2 were individually introduced into PAO1 by electroporation, and Km^r transformants were selected on 2× YT plates containing 1 mg of kanamycin per ml. The resulting *ctpH* and *ctpL* single mutants were designated PP1 and PP2, respectively. To construct the *ctpH ctpL* double mutant, pPT10.1 was digested by *Xho*I and ligated to a 1.3-kb *Xho*I fragment containing a *tet* (conferring tetracycline resistance [Tet^r]) cassette from pUC118Tc. The resulting plasmid, designated pPT10.3, was then introduced into PP2 by electroporation, and Km^r Tc^r transformants were selected on 2× YT plates containing kanamycin (1 mg/ml) and tetracycline (100 μg/ml). The *ctpH ctpL* double mutant was designated PP3. The deletion-insertions were confirmed by Southern hybridization with a digoxigenin nonradioactive DNA labeling and detection kit (Boehringer Mannheim).

Transcriptional fusion experiments. Transcriptional fusion vectors pKZ27 and pKZ27.1 were derivatives of pKTK40 (9). They contained a multicloning site upstream of *lacZ*. They differed from each other only in that pKZ27 contained a *kan* marker, while pKZ27.1 had a carbenicillin resistance (Cb^r) marker. Plasmid pPT10.1 was digested with *Kpn*I, and a 1.8-kb *Kpn*I fragment was cloned in front of the promoterless *lacZ* gene of pKZ27 and pKZ27.1 to make pPT10.5 and pPT10.6, respectively. Similarly, pPT11.1 was digested with *Eco*RV and *Sal*I, and a 1.6-kb *Eco*RV-*Sal*I fragment was inserted upstream of the promoterless *lacZ* gene of pKZ27 and pKZ27.1 to make pPT11.5 and pPT11.6, respectively. β-Galactosidase activities of *P. aeruginosa* cells were determined as described by Miller (15), with the modification that the enzymatic reaction was carried out at 28°C.

Nucleotide accession numbers. The sequences of the *P. aeruginosa* PAO1 *ctpH* and *ctpL* genes are deposited in the GSDB, DDBJ, EMBL, and NCBI nucleotide sequence databases under accession numbers AB039333 and AB039332, respectively.

RESULTS AND DISCUSSION

Cloning of the *ctpH* and *ctpL* genes. We initially identified the *ctpH* and *ctpL* genes as open reading frames (ORFs) discovered in the *P. aeruginosa* genome sequencing project. The methyl-accepting chemotaxis proteins (MCPs) from phylogenetically diverse bacteria have been shown to possess the HCD which is likely to be important for intracellular chemotactic signaling (21). Based on the conserved amino acid sequence (IADQTNILALNAAIEAARAGDQGRGFAVVADEVRKLA),computer analysis of the PAO1 genome sequence predicted that PAO1 possesses 26 ORFs which likely encode proteins containing the HCD (Table 2). Among them were the known genes such as *pctA* (13), *pctB*, *pctC* (22), and *pilJ* (5). Thirteen randomly chosen ORFs were individually amplified by PCR using the sequence-specific primers and cloned into the vector plasmid pGEM-T Easy (Promega). Individual genes were then disrupted by inserting a *kan* cassette into the wild-type genes in the PAO1 genome, and Km^r mutants and PAO1 were examined for the ability to exhibit P_i taxis by using the computer-assisted capillary assay technique (16).

P. aeruginosa PAO1 was attracted to P_i in the concentration range of 0.01 to 10 mM when the cells were starved for P_i (Fig. 1). PAO1 cells grown under P_i excess did not show P_i taxis at any concentration ranging from 0.01 to 10 mM. The accumu-

TABLE 2. *P. aeruginosa* PAO1 potential genes which likely encode proteins containing the HCD

ORF	Position[a]	Predicted size of gene product	
		kDa	Amino acid residues
tlpA	ATG282182/280224TAG	69.8	652
tlpB	ATG321757/323367TGA	57.7	536
tlpC	ATG373201/374880TGA	61.8	559
tlpD	ATG523573/524928TAG	51.1	451
tlpE	ATG713912/715537TGA	58.5	541
piJ[b]	ATG1622181/1620139TGA	72.5	682
tlpF	ATG1867469/1868653TGA	43.2	394
tlpG[b]	ATG1871671/1873710TGA	72.6	679
tlpH[b]	ATG2628762/2626819TAA	69.7	647
tlpI	ATG2822989/2821370TAG	57.8	541
ctpL[b]	ATG2899931/2898031TAG	68.4	632
tlpJ	ATG3137228/3155090TAG	76.6	712
tlpK[b]	GTG3273964/3275985TAG	72.3	673
pctC[b]	ATG3500156/3498435TGA	68.6	633
pctA[b]	ATG3502450/3500543TGA	68.0	629
pctB[b]	ATG3506342/3504444TGA	68.3	632
tlpL[b]	ATG3523500/3525116TAG	58.4	538
tlpM	GTG4185369/4186997TGA	59.0	542
tlpN[b]	ATG5061480/5063117TGA	58.3	545
tlpO	ATG5118106/5116634TGA	51.5	490
tlpP[b]	ATG5191707/5193302TGA	56.4	531
tlpQ	ATG5334256/5332112TGA	77.1	714
tlpR	ATG5335954/5337639TGA	60.3	561
tlpS	ATG5427096/5428703TGA	57.7	535
ctpH[b]	ATG5442805/5441099TGA	61.7	568
tlpT[b]	ATG6227481/6226186TGA	47.9	431

[a] Numbers indicate the first and last nucleotides of start and stop codons, respectively, and correspond to the PAO1 genome sequence (http://www.pseudomonas.com).

[b] Genes which were insertionally inactivated by using a *kan* cassette in this study.

lation patterns of bacteria differed depending on the concentration of P_i. At P_i concentrations of 0.01 and 0.1 mM, the bacterial numbers reached a maximum about 70 s after the start of observation and then gradually decreased because of the competitive attraction due to oxygen. A *kan* insertional mutant, designated PP1, showed P_i taxis when the concentration of P_i in the capillary was lower than 0.1 mM but was not attracted to P_i at concentrations higher than 5 mM. In contrast, mutant PP2 showed P_i taxis at concentrations higher than 5 mM but failed to respond to P_i at 0.01 mM. The disrupted genes in PP1 and PP2 were thus designated *ctpH* and *ctpL* (chemotactic transducer for P_i H and L), respectively. Plasmids pPT10.4 (carrying the entire *ctpH* gene) and pPT11.4 (carrying the entire *ctpL* gene) complemented the mutation of PP1 and PP2, respectively (data not shown), showing that these mutation phenotypes were not due to polar effects. We further constructed the double mutant PP3 by inserting a *tet* cassette into the wild-type *ctpL* gene in the PP1 genome. The *ctpH ctpL* double mutant failed to exhibit P_i taxis at any concentration ranging from 0.01 to 10 mM (Fig. 1). These results suggest that *P. aeruginosa* possesses two P_i chemoreceptors, CtpH and CtpL. CtpH is likely required for exhibiting P_i taxis at high concentrations of P_i, while CtpL could serve as the major chemoreceptor for P_i at low concentrations.

The potential products of *ctpH* and *ctpL* were 568-amino-acid CtpH (predicted 61.6 kDa) and 632-amino-acid CtpL (predicted 68.4 kDa), respectively (Table 2). They exhibited typical structural features of MCPs (4): a positively charged N terminus followed by a hydrophobic membrane-spanning region, a hydrophilic periplasmic domain, a second hydrophobic membrane-spanning region, and a hydrophilic cytoplasmic domain. CtpH residues 400 to 443 and CtpL residues 489 to 533 are 75 and 49%, respectively, identical to the 44-amino-acid HCD sequence of the *E. coli* chemotaxis transducer Tsr (2). These features strongly supported the conclusion that CtpH and CtpL are chemotactic transducers in *P. aeruginosa*. The potential periplasmic domain of CtpL was larger by 127 amino acids than that of CtpH. No significant homology was detected in the potential periplasmic domains between CtpH and CtpL. Furthermore, these regions had no significant similarity to any known proteins.

Effects of the *phoU* and *phoB* mutations on P_i taxis. We previously showed that chromosomal *phoU* mutant APC1, which had been selected after *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine mutagenesis, was constitutive for P_i taxis (12). To further investigate the effect of *phoU* on P_i taxis, we inactivated the *ctpH* and *ctpL* genes individually by inserting a *kan* cassette

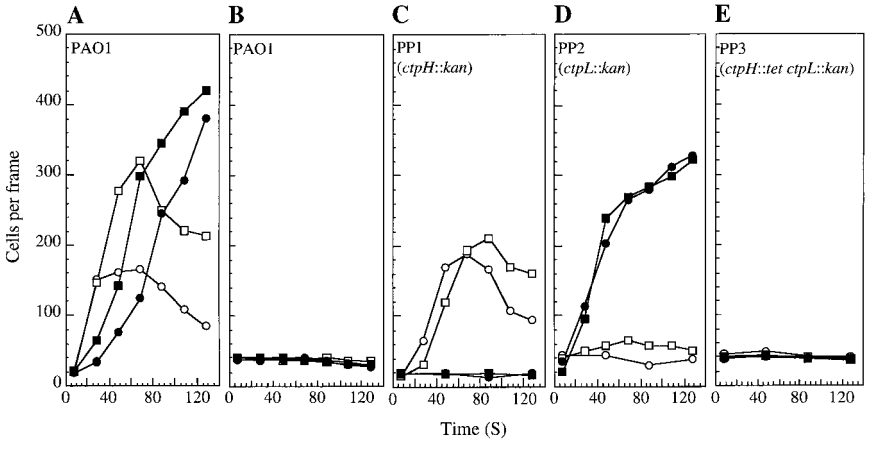

FIG. 1. Chemotactic responses to P_i by P_i-starved (A) and P_i-sufficient (B) cells of *P. aeruginosa* wild-type strain PAO1 and P_i-starved cells of *ctpH* single mutant PP1 (C), *ctpL* single mutant PP2 (D), and *ctpH ctpL* double mutant PP3 (E). Digital image processing was used to count the number of bacteria accumulating around the mouth of the capillary containing a known concentration of P_i plus 1% agarose. One videotape frame was analyzed at each time point. The chemotactic response is presented at the number of bacteria per videotape frame as described previously (16). P_i concentrations (millimolar) in the capillary: ○, 0.01; □, 0.1; ●, 5; ■, 10.

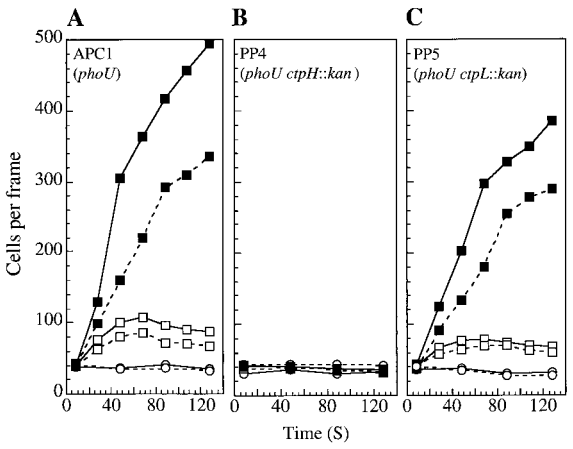

FIG. 2. Chemotactic responses to P_i by *P. aeruginosa phoU* single mutant APC1 (A), *ctpH phoU* double mutant PP4 (B), and *ctpL phoU* double mutant PP5 (C). *P. aeruginosa* cells were grown with either P_i excess (dotted lines) or P_i limitation (solid lines). P_i concentrations (millimolar) in the capillary: ○, 0.01; □, 0.1; ■, 10.

into the wild-type genes in the APC1 genome. The *ctpH phoU* and *ctpL phoU* double mutants were designated PP4 and PP5, respectively. The computer-assisted capillary assays revealed that PP4 failed to exhibit P_i taxis at any concentration ranging from 0.01 to 10 mM even under conditions of P_i limitation, whereas PP5, like APC1, was constitutive for P_i taxis at P_i concentrations higher than 5 mM (Fig. 2). As expected, the *ctpL phoU* double mutant PP5 was unable to respond to P_i at 0.01 mM. We also previously showed that a chromosomal *phoB* mutant PHOB1 (*phoB*::*kan*) exhibited chemotactic responses toward 10 mM P_i under conditions of P_i limitation (12). Interestingly, it was now found that the *phoB* single mutant could not respond to 0.01 mM P_i (Fig. 3). To further investigate the effect of *phoB* on P_i taxis, we also disrupted the *ctpH* and *ctpL* genes individually in the genome of PHOB1 by insertional mutagenesis. The *ctpH phoB* double mutant, designated PP6, did not show P_i taxis at any concentration ranging from 0.01 to 10 mM, even when the cells were starved for P_i limitation (Fig. 3). Like PHOB1, the *ctpL phoB* double mutant, designated PP7, exhibited strong chemotactic responses toward P_i at concentrations higher than 5 mM when the cells were starved for P_i. However, both PHOB1 and PP7 failed to exhibit P_i taxis under conditions of P_i excess.

TABLE 3. β-Galactosidase activities in *P. aeruginosa* strains

Strain	Relevant characteristics	β-Galactosidase activity[a] (Miller units)	
		P_i limited	P_i excess
PAO1(pKZ27)	Control	3 ± 1	1 ± 1
PAO1(pPT10.5)	*ctpH*::*lacZ*	473 ± 21	278 ± 8
APC1(pPT10.5)	*phoU ctpH*::*lacZ*	554 ± 7	378 ± 3
PHOB1(pPT10.6)	*phoB ctpH*::*lacZ*	499 ± 19	256 ± 15
PAO1(pPT11.5)	*ctpL*::*lacZ*	130 ± 1	3 ± 1
APC1(pPT11.5)	*phoU ctpL*::*lacZ*	139 ± 4	126 ± 5
PHOB1(pPT11.6)	*phoB ctpL*::*lacZ*	6 ± 0	2 ± 1

[a] Measured by the method of Miller (15). Values are the means ± standard deviations of at least three separate assays.

Expression of *ctpH*::*lacZ* and *ctpL*::*lacZ*. The nucleotide sequences upstream of *ctpH* and *ctpL* were scanned for the presence of a *pho* box, the consensus sequence shared by the *pho* promoters, using the consensus sequence published previously (20). A putative *pho* box was found in the sequence upstream of *ctpL* (data not shown). There was a 13/18-bp match with the consensus *pho* box sequence (21). On the other hand, no *pho* box sequence was found with the promoter region of *ctpH*. To investigate the *ctpH* and *ctpL* promoter activities, the promoter regions of *ctpH* and *ctpL* were inserted individually upstream from the promoterless *lacZ* gene in transcriptional fusion vectors pKZ27 and pKZ27.1. The control strain PAO1 and *phoU* single mutant APC1 were transformed with either pPT10.5 (Km^r; carrying *ctpH*::*lacZ*) or pPT11.5 (Km^r; carrying *ctpL*::*lacZ*). Since the *phoB* single mutant PHOB1 had a Km^r marker, this strain was transformed by either pPT10.6 (Cb^r; carrying *ctpH*::*lacZ*) or pPT11.6 (Cb^r; carrying *ctpL*::*lacZ*). β-Galactosidase activities were then measured in the wild-type and transformant strains of *P. aeruginosa* under conditions of P_i excess and P_i limitation (Table 3).

High levels of β-galactosidase activities were detected with PAO1(pPT10.5), APC1(pPT10.5), and PHOB1(pPT10.6) even under conditions of P_i excess. The enzyme levels were further increased by P_i limitation in these strains. It was unexpected

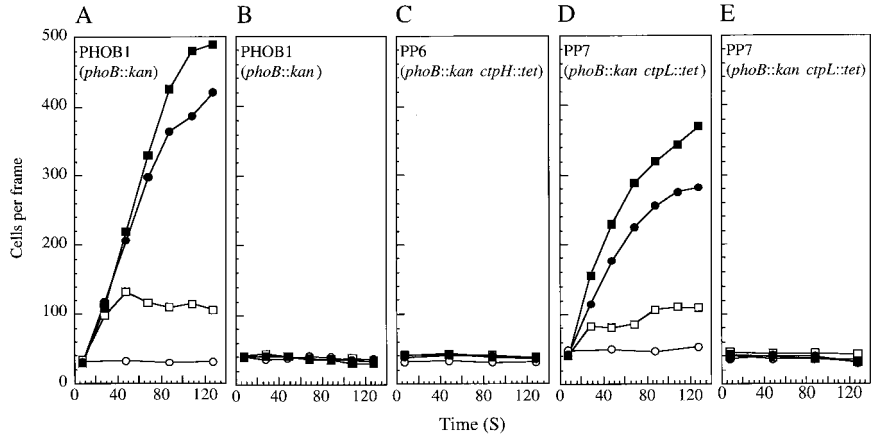

FIG. 3. Chemotactic responses to P_i by P_i-starved (A) and P_i-sufficient (B) cells of *P. aeruginosa phoB* single mutant PHOB1, P_i-starved cells of *ctpH phoB* double mutant PP6 (C), and P_i-starved (D) and P_i-sufficient (E) cells of *ctpL phoB* double mutant PP7. P_i concentrations (millimolar) in the capillary: ○, 0.01; □, 0.1; ●, 5; ■, 10.

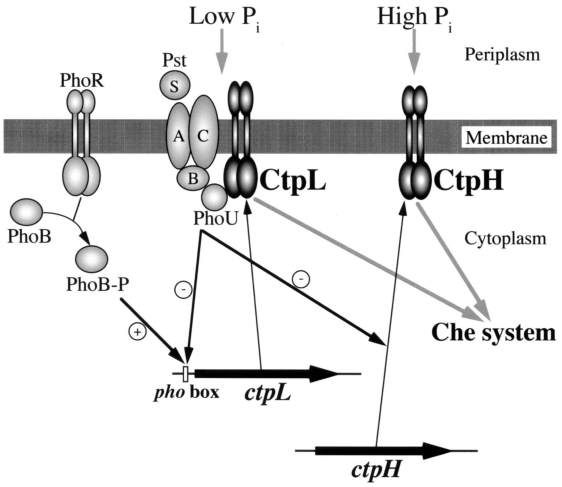

FIG. 4. Model for P_i taxis in *P. aeruginosa*. *P. aeruginosa* possesses two P_i chemoreceptors, CtpH and CtpL. CtpH is required for exhibiting P_i taxis at high concentrations of P_i, while CtpL serves as the major chemoreceptor for P_i at low concentrations. The Pst complex, together with PhoU, is likely to exert a negative control on P_i detection by CtpH at high concentrations of P_i at the posttranscriptional level. In contrast, these proteins are required for P_i detection by CtpL at low concentrations of P_i. A putative *pho* box sequence exists in the promoter region of *ctpL*. The two-component regulatory proteins, PhoR and PhoB, activate its transcription under conditions of P_i limitation. The Pst complex, together with PhoU, also causes the repression of CtpL synthesis under conditions of P_i excess. A plus sign signifies gene activation, while a minus sign means inhibition or repression.

that PHOB1 and PAO1, both of which were inducible for P_i taxis, constitutively expressed *ctpH*::*lacZ*. However, since the *ctpL phoU* mutant PP5 was constitutive for P_i taxis at 10 mM (Fig. 2), this result may suggest that PhoU exerts a negative control on the P_i detection by CtpH. If this is the case, the Pst complex is also likely involved in this negative control, because *P. aeruginosa* mutants lacking the Pst system, but not PhoU, were constitutive for P_i taxis at 10 mM P_i (12). The fact that the *ctpH*::*lacZ* was expressed constitutively in both PHOB1 and PAO1 also suggests that the P_i detection by CtpH is controlled at posttranscriptional level. Alternatively, CtpH-mediated P_i taxis may require additional components whose expression is negatively regulated by PhoU.

The β-galactosidase levels were also approximately 40-fold higher in PAO1(pPT11.5) than in the control strain PAO1 (pKZ27) when the cells were starved for P_i. When pPT11.5 was introduced into the *phoU* single mutant APC1, the enzyme levels were high regardless of whether the cells were starved for P_i. In contrast, when pPT11.6 was introduced into the *phoB* single mutant PHOB1, only background levels of β-galactosidase activities were detected. These results, together with the fact that a putative *pho* box sequence existed in the region upstream of *ctpL*, suggest that the *ctpL* gene is involved in the *pho* regulon genes in *P. aeruginosa* (20). Despite inducible expression of *ctpL*::*lacZ* in the *phoU* mutant APC1, the *ctpH phoU* mutant PP4 failed to exhibit P_i taxis even under conditions of P_i limitation (Fig. 2). This is probably because P_i detection by CtpL requires PhoU. In fact, the *phoU* single mutant APC1 was unable to respond to P_i at 0.01 mM (Fig. 3). In this respect, it is noteworthy that *E. cloacae* absolutely requires the Pst complex, together with PhoU, for P_i taxis which is induced by P_i limitation.

In summary, *P. aeruginosa* possesses two P_i chemoreceptors, CtpH and CtpL, which are functional at different concentrations of P_i (Fig. 4). The Pst complex, together with PhoU, is likely to exert a negative control on the P_i detection by CtpH at high concentrations of P_i at posttranscriptional level. In contrast, these proteins are likely required for the P_i detection by CtpL at low concentrations of P_i. Thus, the Pst system, together with PhoU, seems to play a complex role in P_i taxis in *P. aeruginosa*. A putative *pho* box sequence exists in the promoter region of *ctpL*, and the two-component regulatory proteins PhoR and PhoB likely activate its transcription under conditions of P_i limitation.

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