Genetic identification of chemotactic transducers for amino acids in *Pseudomonas aeruginosa*

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Two chemotactic transducer genes (termed *pctB* and *pctC*) and an open reading frame (*orf1*) were found in the *pctA*-flanking region which was previously identified as a chemotactic transducer gene in *Pseudomonas aeruginosa*. The *pctB* and *pctC* genes encode predicted polypeptides of 629 and 632 amino acids, respectively. Overall, PctB and PctC had 81 and 75% amino acid identities with PctA, respectively. A null mutant strain PCT2, which contained a deletion in the entire *pctC*, *orf1*, *pctA* and *pctB* genes, did not show chemotaxis towards all 20 commonly occurring L-amino acids. This mutant strain also failed to respond to amino acid catabolites (cadaverine, 4-aminobutyrate and putrescine) that are strong attractants for the wild-type strain PAO1. To study the role of each gene product in L-amino acid taxis, plasmids harbouring the *pctC*, *orf1*, *pctA*, or *pctB* genes were constructed and introduced into strain PCT2 by transformation. The *orf1* gene did not complement the defect in chemotaxis of strain PCT2. The *pctA* gene restored the ability of strain PCT2 to respond to 18 L-amino acids, suggesting that PctA plays a major role in detecting L-amino acids in *P. aeruginosa*. The *pctB* and *pctC* genes complemented the defect in chemotaxis to only seven (Ala, Arg, Glu, Lys, Met, Tyr, Gln) and two (His, Pro) L-amino acids, respectively.

Keywords: chemotaxis, amino acids, transducer, *Pseudomonas aeruginosa*

INTRODUCTION

Many bacteria show chemotactic responses towards L-amino acids commonly found in proteins (Boyd *et al.*, 1983; Mesibov & Adler, 1972; Ordal & Gibson, 1977). *Escherichia coli* is strongly attracted to L-alanine, L-asparagine, L-aspartate, L-cysteine, L-glutamate, glycine and L-serine, but is repelled by hydrophobic amino acids including L-isoleucine, L-leucine, L-phenylalanine, L-tryptophan and L-valine (Hedblom & Adler, 1983; Mesibov & Adler, 1972; Tso & Adler, 1974). The *E. coli* chemotactic response for L-amino acid attractants is mediated by two methyl-accepting chemotaxis proteins (MCPs) which are the products of the *tar* and *tsr* genes

(Boyd *et al.*, 1983; Clark & Koshland, 1979). *Salmonella typhimurium*, which has a sensory system very similar to that of *E. coli*, is strongly attracted to five of the L-amino acids that are also strong attractants for *E. coli* (Melton *et al.*, 1978). All 20 commonly occurring L-amino acids serve as attractants for *Bacillus subtilis* (Ordal & Gibson, 1977). In *B. subtilis*, the McpB protein is responsible for detecting a subset of L-amino acids that includes L-asparagine, L-aspartate, L-glutamine and L-histidine, although only chemotaxis towards L-asparagine is completely deficient in the absence of this transducer (Hanlon & Ordal, 1994).

Pseudomonas aeruginosa is strongly attracted to 20 commonly occurring L-amino acids (Kuroda *et al.*, 1995). A nonmetabolizable amino acid, α -aminoisobutyric acid, also elicits a strong chemotactic response from *P. aeruginosa* (Craven & Montie, 1985). The taxis towards L-amino acids is subjected to control by nitrogen availability in a manner similar to the control of various enzymes of nitrogen metabolism (Craven &

Abbreviations: HCD, highly conserved domain; MCP, methyl-accepting chemotaxis protein.

The DDBJ accession number for the sequences of *pctC*, *orf1* and *pctB* reported in this paper is D86947.

Fig. 1. (a) Restriction map of the 24 kb *EcoRI*-*HindIII* fragment from *P. aeruginosa* chromosomal DNA and the subcloned derivatives. Horizontal bars show the DNA fragments subcloned into vector plasmids. The location and orientation of the *pctB*, *pctA*, *orf1* and *pctC* genes is indicated by horizontal arrows. Vertical bars below the restriction map show proposed transcriptional terminators. Restriction sites: *B*, *Bam*HI; *Bg*, *Bgl*II; *Cl*, *Cl*aI; *E*, *Eco*RI; *H*, *Hind*III; *P*, *Pst*I; *S*, *Sal*I; *Xb*, *Xba*I. (b) Disruption construct map of chromosomal DNA of strains PCT2, PCTC1, ORF1Km, PCTA1 and PCTB1. The kanamycin-resistance gene is denoted by *Km'*. (c) Plasmids used for complementation analysis. Horizontal bars show the inserts derived from the 24 kb *EcoRI*-*HindIII* fragment.

Montie, 1985). Recently, we cloned a chemotactic transducer gene, designated *pctA* (*Pseudomonas* chemotactic transducer A), by phenotypic complementation of a *P. aeruginosa* mutant defective in taxis towards L-serine but responsive to peptone (Kuroda *et al.*, 1995). PctA is an integral membrane protein of 629 amino acids which shows high similarity to the enteric MCPs. *In vivo* methyl labelling experiments with L-[methyl-³H]methionine showed that the chromosomal *pctA* mutant lacked an MCP with a molecular mass of approximately 68 kDa.

MCPs from a wide variety of organisms have been shown to possess a region of high conservation within the cytoplasmic portion of the MCP, referred to as the highly conserved domain (HCD). We previously showed that PctA possesses the HCD within its C-terminal domain (Kuroda *et al.*, 1995). Here, we report the cloning, sequencing and characterization of two other chemotactic transducer genes, designated *pctB* and *pctC*, from *P. aeruginosa*. The newly identified chemotactic transducers also possessed the HCD within their C-terminal domain and were involved in chemotaxis towards L-amino acids and some amino acid catabolites.

METHODS

Bacterial strains and plasmids. The bacterial strains and plasmids used in this study are listed in Table 1. *E. coli* MV1184 was used for plasmid construction and DNA manipulation. *P. aeruginosa* and *E. coli* strains were grown at 37 °C with shaking in 2 × YT medium (Sambrook *et al.*, 1989). This medium was also used for preparation of *P. aeruginosa* cells for chemotaxis assays and electroporation. For *E. coli*, antibiotics used for selection were 50 µg kanamycin ml⁻¹, 50 µg ampicillin ml⁻¹ and 20 µg tetracycline ml⁻¹. *P. aeruginosa* transformants were selected with 1 mg kanamycin ml⁻¹, 300 µg carbenicillin ml⁻¹ or 200 µg tetracycline ml⁻¹.

Chemotaxis assay. The computer-assisted capillary assay method was carried out as described previously (Nikata *et al.*, 1992). Cells in a 20 µl suspension were placed on a coverslip, and the assay was started by placing the coverslip upside down on the U-shaped spacer to fill the chemotaxis chamber with the cell suspension. Cells were videotaped through a phase-contrast microscope (IMT-2, magnification × 300, bright field; Olympus) fitted with a 20 × objective (LWDCDPlan; Olympus) and a video camera (BR9000; Hitachi). Digital image processing was used to count the number of bacteria accumulating towards the mouth of a capillary containing a known concentration of an attractant plus 1% agarose. The strength of chemotactic response was determined by the number of bacteria accumulating around the mouth of capillary within 1 min after the start of microscopic observation. The lower limit of detection was 50. The chemotaxis buffer used was 10 mM HEPES buffer (pH 7). All chemicals used for chemotaxis assays were reagent grade.

DNA manipulation and sequencing. Standard procedures were used for plasmid DNA manipulation and agarose gel electrophoresis (Sambrook *et al.*, 1989). *P. aeruginosa* chromosomal DNA was prepared as described previously (Kato *et al.*, 1994). *P. aeruginosa* was transformed by electroporation (Kato *et al.*, 1994). DNA sequence was determined by dideoxy chain-termination method using an Auto Cycle Kit (Pharmacia) and an ALFred DNA sequencer (Pharmacia).

Plasmid construction. To obtain a DNA fragment containing downstream sequences of the *pctA* gene, *Eco*RI fragments of 6–10 kb were recovered from PAO1 chromosomal DNA and inserted into an *Eco*RI site of pCP19 (Goldberg & Ohman, 1987). Plasmid pPT09 was selected by Southern hybridization with the 3' end of the *pctA* gene as a DNA probe.

To construct a null mutant, strain PCT2, which contains a deletion in the entire *pctC*, *orf1*, *pctA* and *pctB* genes, a 1.4 kb *Xba*I-*Eco*RI (blunt-ended) fragment of plasmid pPT08 (Kuroda *et al.*, 1995) and a 4.4 kb *Bgl*II (blunt-ended)-*Eco*RI fragment of plasmid pPT09 were ligated with a blunt-ended 1.3 kb *Pst*I fragment containing a kanamycin-resistance (*Km'*)

gene cassette from pUC4K (Pharmacia). The resulting 7.1 kb *Xba*I–*Eco*RI fragment was cloned into pBluescriptIIKS(+). This recombinant plasmid was then transformed into strain PAO1 to construct the null mutation by the direct gene replacement technique (Kuroda *et al.*, 1995). Km^r transformants were selected on 2 × YT plates supplemented with kanamycin (1 mg ml⁻¹). The deletion–insertion mutation of strain PCT2 was confirmed by Southern blot analysis.

Plasmid pPT08.8 was constructed by inserting a 6.5 kb *Xba*I–*Hind*III fragment of pPT08 into pUC119. Plasmid pPT09.3 was constructed by inserting a 7.0 kb *Eco*RI fragment of pPT09 into an *Eco*RI site of plasmid pUC119. Plasmids pPT09.4 and pPT09.5 were constructed from pPT08.8 by interrupting the *pctC* gene at the *Eco*RI site and the *orf1* gene at *Cl*aI site with a Km^r gene from pUC4K, respectively. Plasmid pPT09.6 was made from pPT09.3 by interrupting the *pctB* gene at the *B*amHI site with a Km^r gene from pUC4K. Plasmids pPT09.4, pPT09.5 and pPT09.6 were transformed into strain PAO1 to make mutations of the *pctC*, *orf1* and *pctB* genes, respectively. Strain PCTA1, which contained an insertional mutation of the *pctA* gene, has been described previously (Kuroda *et al.*, 1995).

To generate plasmid pPT10, which contained the entire *pctC*, *orf1*, *pctA* and *pctB* genes, a 2.8 kb *Hind*III–*S*alI fragment of pPT09 was ligated with a 6.5 kb *Hind*III–*Xba*I fragment of pPT08. The resulting 9.3 kb *Xba*I–*S*alI fragment was then cloned into pCP19. Plasmids pPT08.12, pPT08.13, pPT08.14 and pPT09.1 were constructed by cloning the *pctC*, *orf1*, *pctA* and *pctB* genes into vector pMMB66EH, respectively. These plasmids were introduced into strain PCT2 by transformation, and the transformants were grown without IPTG in 2 × YT medium containing 0.3 mg carbenicillin ml⁻¹ for 18 h.

RESULTS

Isolation of chemotactic transducer genes

To determine whether the *pctA*-flanking region contains additional genes encoding MCPs, plasmids pPT08 and pPT09 (Fig. 1a) were first digested with several restriction enzymes and subcloned into pUC119. The resulting plasmids were then analysed by Southern blot analysis using the 3' end of the *pctA* gene as a DNA probe. The DNA probe hybridized with a 2.8 kb *Hind*III–*S*alI fragment of pPT09 and a 6.5 kb *Hind*III–*Xba*I fragment of pPT08 (data not shown). However, no positive hybridization signal was detected with a 3.9 kb *Eco*RI–*S*alI fragment of pPT09 and a 10 kb *Hind*III–*Xba*I fragment of pPT08. These results suggested that there were *P. aeruginosa* genes encoding proteins homologous to PctA in the 2.8 kb *Hind*III–*S*alI and 4.3 kb *Xba*I–*S*alI regions (Fig. 1a). These DNA fragments were subcloned for sequence analysis.

DNA sequence analysis

DNA sequence analysis revealed three open reading frames, designated *pctC* (633 codons), *orf1* (496 codons) and *pctB* (630 codons), in the region flanking the *pctA* gene (Fig. 1a). These ORFs contained the codon-choice pattern characteristic of *P. aeruginosa* (Coyne & Gold-

		TM1	
PctA	MIKSLKFSHLLLAASLVVFAAPALFTLYN	DYLORNAIREDESYLEMGLVTSNINQW	60
PctB	MIKSLKFSHLLLAASLVVFAAPALFTLYN	DYLORNAIREDESYLEMGLVTSNINQW	60
PctC	MLSLKFSKHLILAASLVVFAAPALFTLYN	DYLORNAIREDESYLEMGLVTSNINQW	60
	*****	*****	
PctA	LGGRLLVBEQTAQLARDHSPETVSAL--	LEQPALSTFTSYLQGGD-GVFTMRPDSFM	117
PctB	LSGRILLTENLAQTLARDHSPETVSAL--	LEQPLLGSTFTLYLQGGD-GTYTARPTSDI	117
PctC	LEGRHLVBLASQJALLDQPDENIARQLBQ	PVFSRNFASVYLGEAASGTFTMRPYDAM	120
	*****	*****	
PctA	PAGYDPRSRPWYKDAVAGGLTLTEFYVDAATQELI	ITAATPVKAAGNYLGVVGGDLSLK	177
PctB	PADYDPRRRPWYNAATSAGQTLTEFYMEPAIH	ELVLTIASTPARQGGQPGVVGGLSLQ	177
PctC	PEGYDPRTRAMYKDAAADRLIVTEPFVDAGTG	QELAMSLPVRHAGQLLVGAAGMKLE	180
	*****	*****	
PctA	TLVQIINSLDFSGMGYAFVLSGDKILVHPDKQV	MKTLSEVYPQNTPKIATGFSSEALH	237
PctB	TVVKIINSLDFSGMGYAFVLSGDKILVHPDKQV	MKTLSDVYPRNTPKIGSGFSSEALH	237
PctC	TLTALINSLDFSGMGYAFVLSGDKILVHPDKQV	MLTAEAYPKGAPNTPGVHEVELD	240
	*****	*****	
		TM2	
PctA	GHRILAFPTINGLPSVITWYLAISIDKDAYAML	SKPFSATAAALISTVAIILVLLGLI	297
PctB	GHRILAFPTINGLPSVITWYLAISIDKDAYAML	SKPFSATAAALISTVAIILVLLGLI	297
PctC	GSSQVPSFTPVKGLPGVITWYLAISIDKDAYAML	SKPFSATAAALISTVAIILVLLGLI	300
	*****	*****	
PctA	RLIMQPLHLMGRAMQDIAQGGDLTKRLAVTS	RDEFVLGDAFNQFVERIHRSIREVAGT	357
PctB	RLVMQPLHLMGRAMQDIAQGGDLTKRLAVTS	RDEFVLGDAFNQFVERIHRSIREVAGT	357
PctC	RLVMQPLHLMGRAMQDIAQGGDLTKRLAVTS	RDEFVLGDAFNQFVERIHRSIREVAGT	360
	*****	*****	
		K1	
PctA	AHKLHVSQLVVNAISNSMANSDEQSNIN	SVAAAINELGAAQEIARNADASHHASDA	417
PctB	ARQLHVSQLVVNAISNSMANSDEQSNIN	SVAAAINELGAAQEIARNADASHHASDA	417
PctC	ARQLHVSQLVVNAISNSMANSDEQSNIN	SVAAAINELGAAQEIARNADASHHASDA	420
	*****	*****	
PctA	NHQAEKGQVVBQTIKRNELSEKISASCANIEAL	NSRTVNIGQILEVTKGISBQTNLLA	477
PctB	NHQAEKGQVVBQTIKRNELSEKISASCANIEAL	NSRTVNIGQILEVTKGISBQTNLLA	477
PctC	NHQAEKGQVVBQTIKRNELSEKISASCANIEAL	NSRTVNIGQILEVTKGISBQTNLLA	480
	*****	*****	
		HCD	
PctA	LNAATEAARAGEAGRGFAVVADEVRLN	LAHQESAQQIQKMIELQVGAREAVATMTESQ	537
PctB	LNAATEAARAGEAGRGFAVVADEVRLN	LAHQESAQQIQKMIELQVGAREAVATMTESQ	537
PctC	LNAATEAARAGEAGRGFAVVADEVRLN	LAHQESAQQIQKMIELQVGAREAVATMTESQ	540
	*****	*****	
		R1	
PctA	RYSLSEVEIANRAGESLSSVTRIGEIDQNNQSV	ATATEEQTAVVDSLANMDITEINTLNQ	597
PctB	RYSLSEVEIANRAGERLSSVTRIGRIEIDQNNQSV	ATATEEQTAVVDSLANMDITEINTLNQ	597
PctC	RYSLSEVEIANRAGERLSSVTRIGRIEIDQNNQSV	ATATEEQTAVVDSLANMDITEINTLNQ	600
	*****	*****	
PctA	EGVENLQATLRACGELETQAGRLRLQVDSFKI		629
PctB	EGVENLQATLRACGELETQAGRLRLQVDSFKI		629
PctC	EGVENLQATLRACGELETQAGRLRLQVDSFKI		631
	*****	*****	

Fig. 2. Amino acid sequence comparison of PctA, PctB and PctC. Dash marks denote gaps introduced into the sequence to improve the alignment. The alignment was generated by the M-AUGN program (DDBJ). Boxed amino acid sequences show possible transmembrane domains (TM1, TM2), methylation domains (K1, R1) and HCD. Asterisks indicate identical amino acids among three predicted proteins.

berg, 1995); a strong preference for either G or C was found in codon positions 3 and 1 in this order, and the second codon position was far less biased. Each gene was preceded by a Shine–Dalgarno sequence and followed by an inverted repeat, suggestive of a transcriptional terminator (Fig. 1a).

The *pctB* and *pctC* genes encode polypeptides with calculated masses of 68.1 kDa and 68.4 kDa, respectively. Computer analysis revealed that PctB and PctC were highly similar to PctA (Fig. 2). Overall, PctB and PctC had 81 and 75% identity (97 and 91% similarity),

Table 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant characteristics	Source or reference
Strains		
<i>E. coli</i> MV1184	<i>ara</i> Δ (<i>lac-proAB</i>) <i>rpsL thi</i> (Φ 80 <i>lacZ</i> Δ M15) Δ (<i>srl-recA</i>)306::Tn10(Tet ^r) F' [<i>traD36 proAB⁺ lacI^a lacZ</i> Δ M15]	Vieira & Messing (1987)
<i>P. aeruginosa</i>		
PAO1	Prototroph, FP ⁻ (sex factor minus)	Holloway <i>et al.</i> (1979)
PCT2	PAO1 derivative containing a deletion of the entire <i>pctC</i> , <i>orf1</i> , <i>pctA</i> and <i>pctB</i> genes	This study
PCTA1	PAO1 derivative, <i>pctA</i> ::Km ^r	Kuroda <i>et al.</i> (1995)
PCTB1	PAO1 derivative, <i>pctB</i> ::Km ^r	This study
PCTC1	PAO1 derivative, <i>pctC</i> ::Km ^r	This study
ORF1Km	PAO1 derivative, <i>orf1</i> ::Km ^r	This study
Plasmids		
pUC119	Cloning vector; Ap ^r ; <i>lacPOZ'</i>	Vieira & Messing (1987)
pUC4K	pUC4 containing 1.3 kb Km ^r cassette; Ap ^r ; Km ^r	Pharmacia
pBluescriptII KS(+)	Cloning vector; Ap ^r ; <i>lacPOZ'</i>	Stratagene
pCP19	Broad-host-range vector; Tet ^r ; IncP	Goldberg & Ohman (1987)
pMMB66EH	Broad-host-range vector; <i>Ptac</i> , <i>lacI^q</i> , Ap ^r , IncQ	Furste <i>et al.</i> (1986)
pPT08	pCP19 harbouring a 17.0 kb <i>HindIII</i> fragment from <i>P. aeruginosa</i> PAO1; Tet ^r ; <i>pctC</i> , <i>orf1</i> and <i>pctA</i>	Kuroda <i>et al.</i> (1995)
pPT09	pCP19 harbouring a 7.0 kb <i>EcoRI</i> fragment from <i>P. aeruginosa</i> PAO1; Tet ^r ; <i>pctB</i>	This study
pPT08.8	pUC119 harbouring a 6.5 kb <i>XbaI-HindIII</i> fragment from pPT08; Ap ^r ; <i>pctC</i> , <i>orf1</i> and <i>pctA</i>	This study
pPT08.12	pMMB66EH harbouring a 2.9 kb <i>XbaI-ClaI</i> fragment from pPT08; Ap ^r ; <i>pctC</i>	This study
pPT08.13	pMMB66EH harbouring a 2.0 kb <i>BglII-SalI</i> fragment from pPT08; Ap ^r ; <i>orf1</i>	This study
pPT08.14	pMMB66EH harbouring a 2.2 kb <i>SalI-HindIII</i> fragment from pPT08; Ap ^r ; <i>pctA</i>	This study
pPT09.1	pMMB66EH harbouring a 2.8 kb <i>HindIII-SalI</i> fragment from pPT09; Ap ^r ; <i>pctB</i>	This study
pPT09.3	pUC119 harbouring a 7.0 kb <i>EcoRI</i> fragment from pPT09; Ap ^r ; <i>pctB</i>	This study
pPT09.4	pUC119 containing a Km ^r cassette in <i>EcoRI</i> site of <i>pctC</i>	This study
pPT09.5	pUC119 containing a Km ^r cassette in <i>ClaI</i> site of <i>orf1</i>	This study
pPT09.6	pUC119 containing a Km ^r cassette in <i>BamHI</i> site of <i>pctB</i>	This study
pPT10	pCP19 harbouring a 9.3 kb <i>XbaI-SalI</i> fragment from <i>P. aeruginosa</i> PAO1; Tet ^r ; <i>pctC</i> , <i>orf1</i> , <i>pctA</i> and <i>pctB</i>	This study

respectively, to PctA. Hydropathy plots (Kyte & Doolittle, 1982) of PctB and PctC revealed two hydrophobic segments (TM1 and TM2) at N-terminal and central portions (data not shown). These segments are characteristic of transmembrane regions separating the cytoplasmic and periplasmic domains. The putative cytoplasmic domain of PctA, PctB and PctC was strikingly conserved (94% amino acid identity and 98% similarity). Two potential methylation sites (K1 and R1) were also conserved among these proteins (Fig. 2).

Chemotaxis assays with deletion-insertion mutants

A series of deletion-insertion mutants was constructed by direct gene replacement. Computer-assisted capillary

assays were performed with these mutant strains to quantitate chemotaxis towards 20 commonly occurring L-amino acids (Table 2). Strain PCT2, which contains a deletion of the entire *pctC*, *orf1*, *pctA* and *pctB* genes (Fig. 1b), was defective in taxis towards all L-amino acids. Plasmid pPT10, which harbours the entire *pctC*, *orf1*, *pctA* and *pctB* genes (Fig. 1c), restored the ability of strain PCT2 to respond to all L-amino acids, indicating that the mutation phenotype of strain PCT2 was not due to a polar effect (Table 2). The *pctA* mutant PCTA1 was impaired in taxis to 17 amino acids. The *pctB* mutant PCTB1 failed to respond to L-glutamine, while no chemotactic defect was detected with the *pctC* mutant PCTC1. The *orf1* mutant ORF1Km also showed no defect in chemotaxis towards 20 L-amino acids.

Table 2. Chemotactic response of *P. aeruginosa* strains towards 20 commonly occurring L-amino acids at 10 mM

The strength of chemotactic response was determined by the number of bacteria accumulating around the mouth of a capillary within 1 min after the start of microscopic observation (Nikata *et al.*, 1992). ++, More than 150 cells per videotape frame; +, less than 150 cells per videotape frame; –, no detectable response.

Amino acid	PAO1	PCT2	PCT2 (pPT10) <i>pctABC, orf1</i>	PCT2 (pPT08.14) <i>pctA</i>	PCT2 (pPT09.1) <i>pctB</i>	PCT2 (pPT08.12) <i>pctC</i>	PCT2 (pPT08.13) <i>orf1</i>	PCTA1	PCTB1	PCTC1	ORF1Km
L-Alanine	++	–	++	++	+	–	–	+	++	++	++
L-Arginine	++	–	++	++	++	–	–	+	++	++	++
L-Asparagine	++	–	++	++	–	–	–	–	++	++	++
L-Aspartate	+	–	+	–	–	–	–	+	+	+	+
L-Cysteine	++	–	++	++	–	–	–	–	++	++	++
L-Glutamine	++	–	++	–	++	–	–	++	–	++	++
L-Glutamate	+	–	+	+	+	–	–	+	+	+	+
Glycine	+	–	+	+	–	–	–	–	+	+	+
L-Histidine	++	–	++	++	–	++	–	+	++	++	++
L-Isoleucine	++	–	++	++	–	–	–	–	++	++	++
L-Leucine	++	–	++	++	–	–	–	–	++	++	++
L-Lysine	++	–	++	++	+	–	–	+	++	++	++
L-Methionine	++	–	++	++	+	–	–	+	++	++	++
L-Phenylalanine	++	–	++	++	–	–	–	–	++	++	++
L-Proline	++	–	++	++	–	++	–	+	++	++	++
L-Serine	++	–	++	++	–	–	–	–	++	++	++
L-Threonine	++	–	++	++	–	–	–	–	++	++	++
L-Tryptophan	++	–	++	++	–	–	–	–	++	++	++
L-Tyrosine	++	–	++	++	+	–	–	+	++	++	++
L-Valine	++	–	++	++	–	–	–	–	++	++	++

To further examine the role of each gene product in L-amino acid chemotaxis, recombinant plasmids were constructed by cloning each of the four genes into plasmid pMMB66EH (Table 1) and used to transform strain PCT2 by electroporation. Plasmid pPT08.14, which carried the *pctA* gene, restored the ability of strain PCT2 to respond towards all L-amino acids except L-glutamine and L-aspartate (Table 2). Strain PCT2 bearing plasmid pPT09.1, which contains the *pctB* gene, responded well towards L-arginine and L-glutamine and responded weakly towards L-alanine, L-glutamate, L-lysine, L-methionine and L-tyrosine. Introducing plasmid pPT08.12, which contains the entire *pctC* gene, resulted in restoration of PCT2 chemotaxis towards L-histidine and L-proline. Plasmid pPT08.13, bearing the *orf1* gene, did not alter the ability of strain PCT2 to respond to L-amino acids.

To determine whether products of L-amino acid catabolism are also sensed by the PctABC system, amino acids catabolites, including urocanate, pyruvate, 2-oxoglutarate, agmatine, cadaverine, putrescine and 4-aminobutyrate, were tested for their ability to attract *P. aeruginosa* cells. Urocanate and pyruvate, which are products of L-histidine and L-alanine catabolism, respectively, were not attractive to strain PAO1 grown in 2 × YT medium (data not shown). Cadaverine, which is a product of L-lysine catabolism, was attractive to strain PAO1. Strain PAO1 was attracted to 4-aminobutyrate but not to 2-oxoglutarate, though both compounds are products of L-glutamate catabolism. *P. aeruginosa* is able to utilize agmatine as a carbon and nitrogen source (Tricot *et al.*, 1990) but is not attracted to this compound. Interestingly, putrescine, which is a product of agmatine catabolism, was attractive to strain PAO1.

PCT2 failed to respond towards cadaverine, 4-aminobutyrate or putrescine, indicating that the PctABC system is involved in chemotaxis towards these amino acid catabolites.

DISCUSSION

In the present study, together with our previous work (Kuroda *et al.*, 1995), we cloned and characterized a *P. aeruginosa* gene cluster encoding chemotactic transducers for L-amino acids, designated PctA, PctB and PctC. The predicted products of the *pctA*, *pctB* and *pctC* genes have the typical structural features of chemotactic transducers (Boyd *et al.*, 1983), which are as follows: 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 (Fig. 2). Previously, we showed that PctA is a major MCP of *P. aeruginosa*, based upon the loss of methylated bands visualized on SDS-polyacrylamide gels in the *pctA* null mutant (Kuroda *et al.*, 1995). Two potential methylation sites (K1 and R1), which were identified in the cytoplasmic domain of PctA, are well conserved in both PctB and PctC. On comparing the periplasmic domains, the similarity between PctA and PctB was higher (70% amino acid identity) than that between PctA and PctC (53% amino acid identity). In addition, PctC is larger by three amino acids than PctA and PctB. We also performed a Southern blot analysis of PCT2 genomic DNA with the 3' end of the *pctA* gene (data not shown). However, no significant band was detected in PCT2 genomic DNA, suggesting that *P. aeruginosa* contains no additional *pctA*-like genes.

Fig. 3. Representation of 20 commonly occurring L-amino acids detected by *P. aeruginosa* chemotactic transducers. All chemotaxis assays were performed at a concentration of 10 mM.

PctA, PctB and PctC have individual specificities for L-amino acid attractants despite their relatively high similarity in the periplasmic domain. Chemotaxis assays revealed that PctA is likely a major receptor for L-amino acids in *P. aeruginosa*. This transducer detects 18 of 20 commonly occurring L-amino acids. PctB and PctC respond to seven and two L-amino acids, respectively (Table 2). Redundancies in the chemotaxis transducers were observed in *P. aeruginosa* (Fig. 3). In the chemotaxis assays, all L-amino acids were tested at a concentration of 10 mM, since a peak response towards L-serine was previously observed at 1–10 mM (Nikata *et al.*, 1992). Therefore, it cannot be ruled out that the observed specificities of PctA, PctB and PctC may reflect their different peak concentrations for L-amino acids. It is also possible that PctA may be a relatively abundant protein among *P. aeruginosa* transducers for L-amino acids. In *E. coli*, Tsr and Tar are relatively abundant, at approximately 1600 and 900 copies per cell, respectively. Trg and Tap are significantly less abundant, at approximately 150 copies per cell. Plasmid pPT08.12, which contained the *pctC* gene, complemented the chemotaxis of strain PCT2 towards L-histidine and L-proline. However, the chromosomal *pctC* mutation did not affect the chemotaxis of strain PAO1 in the presence of the *pctA* gene. These results suggest that PctC may be less abundant compared to PctA. Based upon DNA sequence analysis, it appears that all of the *pct* genes are monocistronic.

Taxis towards L-aspartate was not restored in strain PCT2 harbouring each *pct* gene. However, strain PCT2 harbouring the entire *pctC*, *orf1*, *pctA* and *pctB* genes was attracted to L-aspartate as strongly as PAO1 was. Since the L-aspartate chemotactic transducer is known to form a dimer (Milburn *et al.*, 1991), a combination of Pct proteins might play an important role in detecting L-aspartate. However, we do not have any data indicating that Pct proteins form a heterodimer.

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