

# The *Bacillus subtilis* Chemoreceptor McpC Senses Multiple Ligands Using Two Discrete Mechanisms<sup>\*S</sup>

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**Background:** The loss of McpC has been shown to reduce chemotaxis to 19 of the 20 amino acids.

**Results:** McpC can directly bind 11 amino acids and indirectly sense four others.

**Conclusion:** McpC can sense a variety of amino acids by using two discrete mechanisms.

**Significance:** We elucidate the mechanisms by which a single receptor can sense a wide variety of ligands.

*Bacillus subtilis* can perform chemotaxis toward all 20 L-amino acids normally found in proteins. Loss of a single chemoreceptor, McpC, was previously found to reduce chemotaxis to 19 of these amino acids. In this study, we investigated the amino acid-sensing mechanism of McpC. We show that McpC alone can support chemotaxis to 17 of these amino acids to varying degrees. Eleven amino acids were found to directly bind the amino-terminal sensing domain of McpC *in vitro*. Sequence analysis indicates that the McpC sensing domain exhibits a dual Per-Arnt-Sim (PAS) domain structure. Using this structure as a guide, we were able to isolate mutants that suggest that four amino acids (arginine, glutamine, lysine, and methionine) are sensed by an indirect mechanism. We identified four candidate binding lipoproteins associated with amino acid transporters that may function in indirect sensing: ArtP, GlnH, MetQ, and YckB. ArtP was found to bind arginine and lysine; GlnH, glutamine; MetQ, methionine; and YckB, tryptophan. In addition, we found that ArtP, MetQ, and YckB bind the sensing domain of McpC, suggesting that the three participate in the indirect sensing of arginine, lysine, methionine, and possibly tryptophan as well. Taken together, these results further our understanding of amino acid chemotaxis in *B. subtilis* and gain insight into how a single chemoreceptor is able to sense many amino acids.

The Gram-positive bacterium *Bacillus subtilis* can perform chemotaxis toward all 20 L-amino acids normally found in proteins (1). These amino acids are sensed by at least two transmembrane chemoreceptors, McpB and McpC, which contain an extracellular sensing domain and a cytoplasmic signaling domain (2, 3). These chemoreceptors form ternary complexes with the CheA histidine kinase and the CheW and CheV scaffolding proteins (4). The binding of amino acids to these

chemoreceptors increases the rate of CheA autophosphorylation (2, 5). The phosphoryl group is then passed to the CheY response regulator protein, which in its phosphorylated form can bind to the cytoplasmic face of the flagellar motor and induce a chemotactic response (6).

McpB is the sole chemoreceptor for asparagine, and it additionally supports chemotaxis toward aspartate, glutamine, and histidine (2). Previous work has demonstrated that the sensing domain of McpB contains a dual Per-Arnt-Sim (PAS)<sup>3</sup> domain architecture and that asparagine binds in the upper PAS domain (7). This architecture is found in other chemoreceptors, including *B. subtilis* McpC and *Vibrio cholerae* McpN, as well as numerous other sensor histidine kinases (7, 8). McpC is the sole receptor for proline and was previously found to support chemotaxis to all other amino acids except asparagine (3). In particular, an *mcpC*-null mutant showed reduced chemotaxis to 19 amino acids. These results suggest that McpC senses 19 amino acids. The goal of this study is to investigate amino acid sensing by McpC. In particular, we sought to determine whether a single receptor can indeed sense 19 different amino acids and, if so, to begin to elucidate the mechanisms involved.

Previous work from *Escherichia coli* demonstrated that chemotaxis sensing may be direct, where the chemoreceptor directly binds a ligand, or indirect, where a second protein is involved. A well studied example of indirect sensing is the maltose-binding protein from *E. coli* involved in maltose chemotaxis (9–11). This periplasmic protein binds maltose and interacts with the sensing domain of the Tar chemoreceptor (10). Given the diversity of amino acid ligands for McpC, an indirect sensing mechanism is likely involved as well. However, the sensing domains of the *E. coli* chemoreceptors exhibit a different structure than those of the *B. subtilis* McpB and McpC chemoreceptors (7). An example of indirect sensing by a dual PAS domain sensor comes from LuxQ, a sensor histidine kinase involved in quorum sensing in *Vibrio harveyi* (12). LuxQ indirectly senses AI-2 through its interactions with LuxP, a periplasmic binding protein. LuxP directly binds the small molecule AI-2 and can then activate the LuxQ sensor kinase. Both

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<sup>3</sup> The abbreviations used are: PAS, Per-Arnt-Sim; ABC, ATP-binding cassette; ITC, isothermal titration calorimetry.

PAS domains of LuxQ are involved in the interaction with LuxP, and there are numerous residues along the LuxQ/LuxP interface that are crucial for this interaction (12).

In this work we demonstrate that *McpC* alone can support chemotaxis to 17 amino acids. Of these, *McpC* directly binds 11 amino acids. Using the structure of LuxQ bound to LuxP, we were able to identify mutants that suggest four amino acids are sensed by an indirect mechanism. Moreover, we were able to show three binding lipoproteins that interact with the sensing domain of *McpC* and a fourth that may as well. We also found that these four lipoproteins respectively bind these four amino acids, suggesting that they enable indirect sensing by *McpC*.

## EXPERIMENTAL PROCEDURES

**Strains and Plasmids**—All *B. subtilis* strains are derived from the chemotactic strain (*che+*) OI1085 (13). All cloning and plasmid propagations were performed in *E. coli* strain TG1 (Amersham Biosciences). Recombinant protein overexpression was done in *E. coli* strain BL-21 (Amersham Biosciences). Plasmids used for protein purification were expressed in pGEX-6P-2 (GE Healthcare).

All *B. subtilis* strains were created by using QuikChange (Stratagene) mutagenesis on pAIN750 variants that contained the full-length *mcpC* gene (14). These plasmids were then transformed into the *amyE* locus of the  $\Delta 10mcp$  strain (OI3545) and selected for spectinomycin resistance. Expression of *McpC* was confirmed by Western blotting, and its levels were found to be equal to the wild type (data not shown).

The GST fusion pGEX-6P-2 plasmids were made by amplifying the amino terminus from the *mcpC* gene (residues 33–276) or the binding protein genes without the transmembrane anchor (*artP* residues 34–322, *glnH* residues 48–297, *metQ* residues 33–317, *yckB* residues 47–314) from OI1085 genomic DNA by PCR. The primers used were engineered to create a 5' EcoRI and 3' NotI site. This fragment was then ligated into pGEX-6P-2.

**Capillary Assay**—The capillary assay was performed as described previously to quantitatively measure the chemotactic ability of various strains (15, 16). Briefly, cells were grown overnight at 30 °C on TBAB plates (1% tryptone, 0.3% beef extract, 0.5% NaCl, 1.5% agar). The cells were then scraped from the plate and resuspended to  $A_{600\text{ nm}} = 0.03$  in 5-ml capillary assay minimal medium (50 mM  $K_3PO_4$ , pH 7.0, 1.2 mM  $MgCl_2$ , 0.14 mM  $CaCl_2$ , 1 mM  $(NH_4)_2SO_4$ , 0.01 mM  $MnCl_2$ , 20 mM sorbitol, and 0.02% tryptone, supplemented with 50  $\mu g/ml$  histidine, methionine, and tryptophan). The cultures were grown to  $A_{600\text{ nm}} = 0.4$  at 37 °C and 250 rpm shaking, after which 50  $\mu l$  of the 5% glycerol, 0.5 M sodium lactate solution was added, and the cells were incubated a further 15 min. The cells were then washed three times with chemotaxis buffer (10 mM  $K_3PO_4$ , pH 7.0, 0.14 mM  $CaCl_2$ , 0.3 mM  $(NH_4)_2SO_4$ , 0.1 mM EDTA, 5 mM sodium lactate, 0.05% (v/v) glycerol) and diluted to  $A_{600\text{ nm}} = 0.001$ . The resuspended cells were aliquoted into 0.3-ml ponds on a temperature-controlled plate at 37 °C, and closed-end capillary tubes filled with appropriate chemoattractant were inserted and incubated for 30 min. Cells in the capillaries were harvested and transferred to 0.5 ml of top agar (1% tryptone, 0.8% NaCl, 0.8% agar, 0.5 mM EDTA) and plated onto TBr (1%

tryptone, 0.5% NaCl, 1.5% agar) plates. These plates were incubated at 37 °C for 16 h at which point colonies were counted to derive the data. Experiments were performed in duplicate and on 2 different days to assure reproducibility.

**Protein Purification**—To purify the GST fusion proteins, cells containing pGEX-6P-2 with the assorted chemotaxis proteins cloned in the multiple cloning site were grown in 6 liters of LB medium (1% tryptone, 0.5% yeast extract, 0.5% NaCl) supplemented with 100  $\mu g/ml$  ampicillin at 37 °C and with 250 rpm shaking until  $A_{600\text{ nm}} = 0.8$ . Expression was then induced by addition of 1 mM isopropyl  $\beta$ -D-1-thiogalactopyranoside, and the culture was grown at 25 °C with 250 rpm shaking for 12 h. Cells were collected by centrifugation at  $7000 \times g$  for 10 min. The cell pellet was resuspended in 3 ml of TBS (50 mM Tris, pH 7.5, 150 mM NaCl) + 1% Triton X-100 + 1 mM dithiothreitol (DTT) for every 1-g cell pellet. The cells were then disrupted by sonication ( $5 \times 10$ -s pulse), and the cellular debris was removed by centrifugation at  $10,000 \times g$  for 11 min. Lysate was further centrifuged for 1 h at  $40,000 \times g$ . Finally for further clarification, the lysate was passed through a 0.2- $\mu m$  filter to remove any other aggregates or insoluble particles.

The cell lysate was then passed through a 5-ml GSTrap column (GE Healthcare) and washed with at least 5 bed volumes of TBS. The fusion proteins were eluted from the column with 20 ml of GEB (50 mM Tris, pH 8.0, 10 mM glutathione). If necessary, the GST tag was removed by digestion with 100 units of PreScission protease for 12 h at 4 °C. This solution was again passed over the GSTrap column to remove the GST and protease. The pure Che protein flow-through was collected and stored in TKMDmod (50 mM Tris, pH 8.0, 50 mM KCl, 5 mM  $MgCl_2$ ) buffer at  $-80$  °C.

**Isothermal Titration Calorimetry (ITC)**—All ITC measurements were carried out using a VP-ITC titration calorimeter (MicroCal) at 25 °C, and data were extracted and processed using the Origin software package. During the titration reaction, the 1.4-ml reaction cell was constantly stirred at 300 rpm. A total of 28 injections, all 10  $\mu l$  in volume, were performed. Prior to analysis, the pH of all solutions used was checked and adjusted so that every solution had an identical pH the day of the reaction (the pH of the solutions was between 7.2 and 7.3). All solutions were also degassed for 15 min prior to the experiment.

**GST Pulldowns**—The pulldowns were performed as described previously (17). Briefly, 100  $\mu l$  of 30  $\mu M$  GST-*McpCn* was added to 50  $\mu l$  of prewashed (with 400  $\mu l$  of TBS-X (TBS + 1% Triton X-100)) glutathione beads (GE Healthcare) in a Handee spin cup column (Pierce) and incubated for 10 min. The beads were washed with 800  $\mu l$  of TBS-X, and 100  $\mu l$  of 150  $\mu M$  of purified binding protein was added and incubated for 10 min. The beads were again washed with 800  $\mu l$  of TBS-X, and the protein was eluted with 75  $\mu l$  of GEB. 25  $\mu l$  of 4 $\times$  SDS solubilizer was added, and the samples were run on SDS-polyacrylamide gels and visualized with Coomassie stain. A GST control was also run, as was a nonspecific GST-tagged protein control, and no binding of the binding protein was observed (data not shown).

# Chemotaxis Receptor McpC Senses Multiple Ligands

**TABLE 1**  
ITC results and capillary assays of all 20 amino acids

Amino acid	<i>mcpC</i> mutant <sup>a</sup>	Capillary assay accumulation <sup>b</sup>	$K_D^c$	ITC $K_D$	McpC E115A mutant	McpC K195A mutant
	% <i>reduced</i>		$\mu\text{M}$	$\mu\text{M}$	% <i>reduced</i>	% <i>reduced</i>
Cys	100	28,175 ± 5,408	10	NA <sup>d</sup>	>99	20
Pro	99	29,119 ± 4,845	1	14	>99	8
Thr	99	30,120 ± 6,375	5.6	21	97	11
Gly	99	13,081 ± 6,440	320	699	94	51
Ser	99	9,395 ± 816	32	90	95	9
Lys <sup>h</sup>	99	15,500 ± 5,348	1,000	NB <sup>e</sup>	50	88
Val	98	15,653 ± 6,372	32	154	96	60
Arg <sup>h</sup>	98	12,887 ± 1,574	100	NB <sup>e</sup>	<1	95
Ala	92	12,225 ± 3,766	0.32	18	97	4
Tyr	92	2,378 ± 701	320	360	95	<1
Glu	89	3,892 ± 2,515	32,000	NB <sup>e</sup>	98	54
Ile	87	3,355 ± 219	100	1,000	97	67
Met <sup>h</sup>	79	8,650 ± 2,036	100	NB <sup>e</sup>	62	91
Trp	78	254 ± 42	3,200	3,700	<1	86
Phe	72	8,713 ± 1,061	320	492	95	77
Leu	70	8,713 ± 1,061	32	72	97	80
Asp	35	3,355 ± 1,061	10,000	NB <sup>e</sup>	97	51
Gln <sup>h</sup>	34	1,475 ± 369	32	NB <sup>e</sup>	55	95
His	27	26 ± 12	3,200	320 <sup>f</sup>	NA <sup>g</sup>	NA <sup>g</sup>
Asn	0	0 ± 35	56	NB <sup>e</sup>	NA <sup>g</sup>	NA <sup>g</sup>

<sup>a</sup> As previously determined experimentally (3).

<sup>b</sup> Strain OI4007 ( $\Delta 10$  *mcp amyE::mcpC*). All amino acids were tested at 0.01 M, except for Glu and Asp, which were tested at 0.1 M.

<sup>c</sup> As previously determined experimentally (26).

<sup>d</sup> Could not be tested.

<sup>e</sup> No binding under the conditions tested.

<sup>f</sup> Exothermic binding.

<sup>g</sup> Accumulation too low.

<sup>h</sup> Chemotaxis mediated by an amino acid binding protein.

## RESULTS

*McpC Alone Can Support Chemotaxis to 17 Amino Acids*—*B. subtilis* can perform chemotaxis toward all 20 L-amino acids normally found in proteins. Previous work suggested that a single receptor, McpC, supports taxis to 19 of these amino acids; an *mcpC* mutant exhibited reduced chemotaxis to all amino acids except asparagine (Table 1, column 2). One possible interpretation of these results is that McpC alone is capable of sensing 19 amino acids. The other would be that McpC may somehow enable one of the other nine chemoreceptors present in *B. subtilis* to sense these amino acids. We first sought to examine whether McpC alone is capable of sensing these amino acids by expressing it in a strain lacking the other nine chemoreceptors ( $\Delta 10$  *mcp amyE::mcpC*, strain OI4007) (14). We then employed the capillary assay to test for chemotaxis toward all 20 amino acids using this strain.

The results from the capillary assay show that McpC alone is able to support chemotaxis to 17 amino acids (Table 1, column 3). Accumulation to tryptophan and histidine was very low, suggesting chemotaxis to these two attractants might require an additional chemoreceptor. In the case of histidine, McpB is the likely chemoreceptor involved (3). However, in the case of tryptophan, the additional chemoreceptor is not known.

*The Sensing Domain of McpC Binds 11 Amino Acids Directly*—We next sought to test whether these 17 amino acids are sensed directly by McpC. The amino-terminal sensing domain of McpC (McpCn, residues 33–276) was purified, and then ITC was used to determine which amino acids it bound *in vitro*. Previous work using ITC showed that the sensing domain of McpB binds asparagine *in vitro* with affinities comparable with those inferred from *in vivo* experiments (7).

The ITC results show that the sensing domain of McpC directly binds 12 amino acids *in vitro*, and the binding is endothermic for 11 of them (Fig. 1 and Table 1, column 5). The *in vitro* affinities derived from the binding curves generally agree with the previously reported *in vivo* affinities determined using a specialized capillary assay (Table 1, column 4). The exceptions are proline, alanine, and isoleucine, where the *in vitro* affinities are at least 10-fold lower than the *in vivo* one. McpC clearly binds proline and alanine because *in vitro* binding was strong ( $K_D = 14$  and  $18 \mu\text{M}$ , respectively). In the case of isoleucine, *in vitro* binding was much weaker ( $K_D = 1 \text{ mM}$ ), although still sufficiently strong to suggest that McpC directly binds it as well. The other exception was histidine, which exhibited exothermic binding, unlike the endothermic change observed with the other 11 amino acids. McpC alone does not support chemotaxis to histidine. In addition, it appears to be a shared ligand with McpB so we did not explore it further.

Of the 20 amino acids tested, 7 did not bind the sensing domain of McpC, and 1, cysteine, could not be tested because DTT, necessary to prevent disulfide bond formation, interferes with ITC analysis. Aside from asparagine, the amino acids that did not bind *in vitro* are lysine, arginine, glutamate, methionine, aspartate, and glutamine. Both glutamate and aspartate have weak *in vivo* affinity for the chemoreceptors (Table 1, column 4); likely, *in vitro* binding is too weak to be detected by ITC. The simplest explanation for the remaining four (lysine, arginine, methionine, and glutamine) is that McpC senses them by an indirect mechanism, most likely one involving ancillary proteins.

*Identification of Mutants That Separate Direct from Indirect Sensing*—The sensing domains of *B. subtilis* McpB and *V. cholerae* McpN consist of two dual PAS domains; the ligand binds in



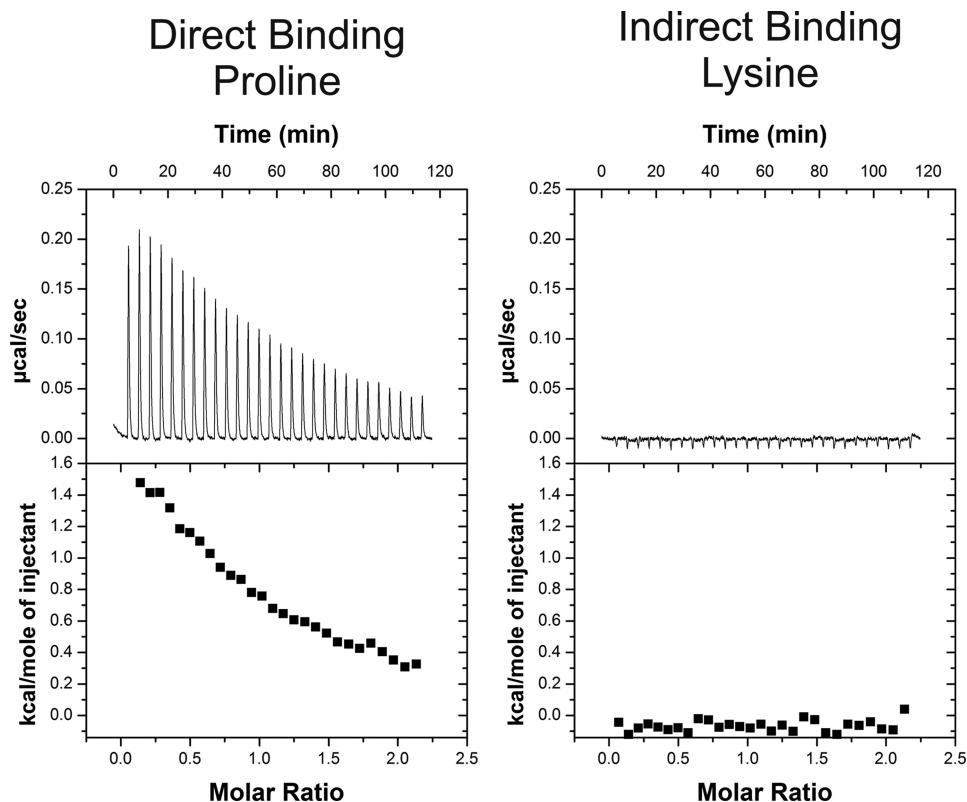


FIGURE 1. ITC binding isotherms for the purified amino-terminal sensing domain of *McpC* reveals direct binding to many amino acids. Proline binds directly to *McpC*, whereas lysine does not bind. The ITC results for all 20 amino acids are summarized in Table 1.

the upper PAS domain. Homology modeling predicts that the sensing domain of *McpC* also adopts the same dual PAS domain structure (Fig. 2). Given the structural similarity among the sensing domains of these three chemoreceptors, we expect that the upper PAS domain of *McpC* is also the site of direct binding. Using the homology model, we identified residues on *McpC* that might play a role in direct ligand binding in a putative upper PAS domain binding pocket (Fig. 2). Indirect binding, on the other hand, likely involves a second binding protein. Based on the structure of *LuxQ* and *LuxP*, we would expect that this binding protein interacts with both PAS domains of *McpC*. Previous work has shown that certain residues on *LuxQ* are vital for its interaction with *LuxP* (12). We were able to identify similar residues on *McpC* that might play a role in its interaction with a putative binding protein (Fig. 2). Initially, we made alanine substitutions in these putative binding pocket and binding protein interface residues in a strain expressing the mutated *McpC* as the sole chemoreceptor.

By screening these numerous mutants using capillary assays (data not shown), we were able to identify one binding pocket mutant and one binding protein interface mutant. The first point mutant, E115A ( $\Delta 10$  *mcp amyE::mcpC E115A*), is located in the binding pocket of the upper PAS domain. It exhibited reduced chemotaxis to all amino acids tested as determined using the capillary assay. However, the reduction was less for lysine, arginine, methionine, and glutamine (Table 1, column 6; Fig. 3). The second point mutant, K195A ( $\Delta 10$  *mcp amyE::mcpC K195A*), is located in the lower PAS domain along the putative binding protein interface and is distal to the upper PAS domain binding pocket. This mutant also exhibited vary-

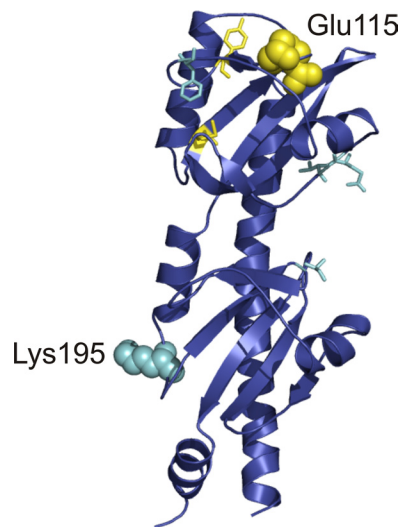


FIGURE 2. Identification of residues involved in ligand sensing on the extracellular amino-terminal domain of the *McpC* chemoreceptor. Putative residues that were tested for direct binding (yellow) and indirect binding (cyan) are highlighted on a structural model of the *McpC* sensing domain. The two residues chosen for further study, Glu-115 and Lys-195, are shown in a space-filling representation for clarity.

ing levels of reduced chemotaxis to all amino acids tested (Table 1, column 7; Fig. 3). However, the reduction was greater for lysine, arginine, methionine, and glutamine. When the reductions for both mutants are plotted on the same figure, these four amino acids clearly segregate from the others for the two mutants (supplemental Fig. S1).

These results are entirely consistent with the ITC results if we discount glutamate and aspartate due to their high apparent

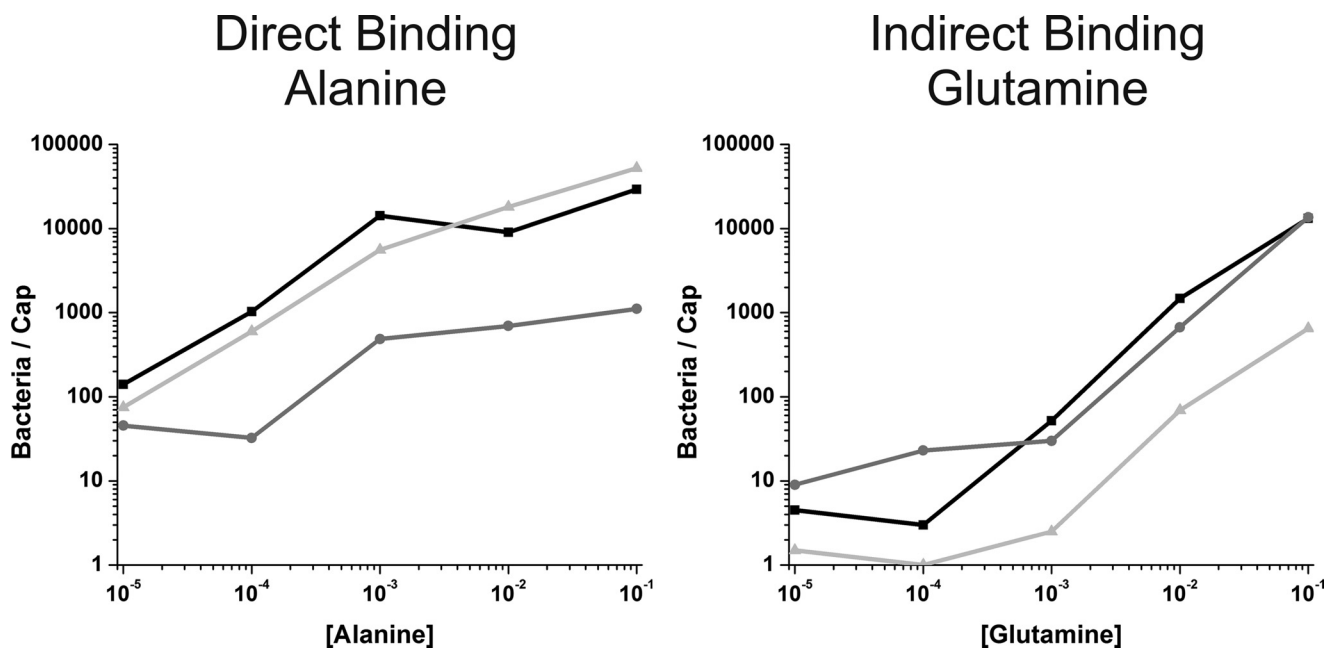


FIGURE 3. **Capillary assays toward alanine and glutamine show bacterial accumulation over a range of attractant concentrations.** The wild-type McpC chemoreceptor is shown in black ( $\Delta 10 mcp amyE::mcpC$ ), the E115A mutant ( $\Delta 10 mcp amyE::mcpC E115A$ ) is shown in dark gray, and the K195A mutant ( $\Delta 10 mcp amyE::mcpC K195A$ ) is shown in light gray. Accumulation to alanine is indicative of direct ligand binding, whereas the results for glutamine are an example of indirect ligand binding. The results of capillary assays to all 20 amino acids are summarized in Table 1.

$K_D$  values. Moreover, when we locate these two residues on the structural model for McpC sensing domain, they are entirely consistent with our hypothesis derived from structural analysis. Residue 115 is located in the upper PAS domain in the putative ligand binding pocket, and residue 195 is located in the lower PAS domain on the external face of the protein (Fig. 2). Taken together, these results suggest that McpC directly senses the binding of at least 11 amino acids and indirectly senses the binding of 4 amino acids.

**Identification of the Ancillary Proteins Involved in Indirect Sensing**—What is the indirect sensing mechanism for McpC? Based on results from *E. coli* chemotaxis and the LuxQ/LuxP interaction, ancillary proteins are expected to directly bind these four amino acids and interact with McpC. The *B. subtilis* genome contains >40 binding lipoproteins associated with ATP-binding cassette (ABC) transporters that are potential candidates. Eight of these proteins are known or have been hypothesized to be involved in amino acid transport (18, 19). Of these eight, four appear to be involved in cysteine uptake: TcyA, TcyJ, TcyK, and YxeM (20, 21). The other four are: ArtP (arginine), GlnH (glutamine), MetQ (methionine), and YckB (unknown) (22–24). These four were chosen for further study. Three of them are responsible for the same amino acids that do not directly bind McpC; and the fourth, YckB, is annotated as a binding lipoprotein associated with an amino acid transporter (18). These proteins all contain a hydrophobic amino-terminal region, presumably to anchor these proteins to the membrane.

To test whether these four binding proteins interact with the sensing domain of McpC, we purified them without the anchor region and then used the glutathione *S*-transferase (GST) pull-down assay to test for binding. Briefly, the McpC sensing domain tagged to GST was bound to glutathione beads and incubated with the binding protein, both in the presence or

absence of their cognate amino acid. For instance, ArtP was tested in the presence or absence of 1 mM arginine. The beads were then washed twice and eluted using glutathione.

These results showed that McpC binds to ArtP, MetQ, and YckB (Fig. 4). The results for GlnH are ambiguous as GST, the likely product of degradation due to a labile linker, runs at the same molecular mass. Additionally, we found that the presence of amino acid did not appear to have any significant effect on this binding. The ability of the McpC sensing domain to bind three of the four binding proteins tested suggests that an indirect binding mechanism is indeed plausible for chemotaxis toward lysine, arginine, glutamine, and perhaps methionine as well. As a control, we also tested whether the McpC sensing domain binds CheC and BSA and found that it did not (supplemental Fig. S2), indicating that the interaction with the binding lipoproteins is specific.

We also tested whether the K195A mutant sensing domain was able to bind ArtP, MetQ, and YckB and found that it still did. These results are not surprising as multiple residues on McpC are predicted to interact with the binding lipoproteins. However, the affinity appears to be somewhat less for the K195A mutant than for the wild-type sensing domain (supplemental Fig. S3). Likely, the K195A mutation affects the allosteric coupling between receptor and binding lipoproteins, including by reducing the affinity between the two.

Finally, we tested whether these proteins bind amino acids using ITC. We found that ArtP bound arginine tightly ( $K_D = 43$  nM) and lysine with a much lower affinity ( $K_D = 657$   $\mu$ M). GlnH bound glutamine ( $K_D = 120$   $\mu$ M) and MetQ bound methionine ( $K_D = 3.8$   $\mu$ M). We additionally found that YckB bound tryptophan very weakly ( $K_D = 2020$   $\mu$ M); however, McpC alone does not support chemotaxis to this amino acid. Taken together, these results show that ABC transport-binding lipoproteins

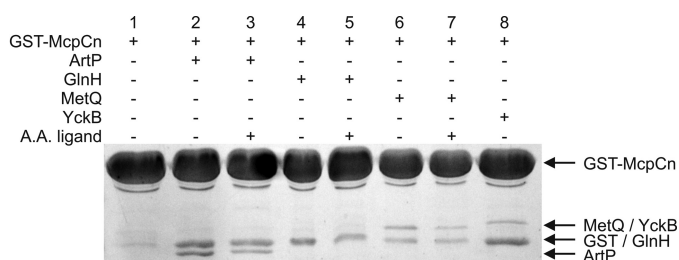


FIGURE 4. **GST pull-down assays show an interaction between the amino-terminal sensing domain of McpC and four different ABC transport-binding proteins.** GST-McpCn was immobilized on glutathione beads, incubated with a binding protein (in the absence of presence of its cognate amino acid ligand), washed, and eluted with glutathione.

bind both the McpC chemoreceptor and an amino acid ligand, demonstrating that indirect sensing by McpC likely involves ABC transport-binding lipoproteins.

## DISCUSSION

McpC is a nearly universal amino acid chemoreceptor, alone capable of supporting chemotaxis to 17 amino acids in *B. subtilis*. Eleven of these amino acids were found to directly bind the sensing domain of McpC as determined using *in vitro* ITC analysis. As the *in vitro* affinities were similar to those inferred from *in vivo* analysis, we conclude that all are directly sensed by McpC. Mutational analysis further revealed that four amino acids (arginine, glutamine, lysine, and methionine) are likely sensed by an indirect mechanism involving ancillary proteins. We were able to identify and characterize three lipoproteins (ArtP, GlnH, and MetQ) associated with amino acid transporters that may function as ancillary proteins involved in the indirect sensing of arginine, glutamine, lysine, and methionine. Although our results show that these proteins provide a sufficient mechanism for indirect sensing, we were unable to demonstrate that they are necessary as null mutations to these binding lipoproteins appear to affect chemotaxis toward all amino acids (data not shown).

How is the sensing domain of McpC capable of directly binding 11 different amino acids? Clearly, high resolution structures of the sensing domain of McpC in the apo and bound form for all 11 amino acids are needed to definitively elucidate the binding mechanism. However, based on previous work, we can speculate on possible mechanisms. In particular, previous work has demonstrated that ligand binds in the upper PAS domain in a dual PAS domain sensor (8). Moreover, the mutational analysis from this study supports this conclusion. If true, then the binding pocket of the McpC sensing domain is fairly flexible and likely requires multiple contacting residues. In the crystal structure of the sensing domain of the McpN chemoreceptor from *V. cholerae* bound to alanine, at least eight residues contact this ligand (Protein Data Bank code 3C8C). Assuming that McpC binds ligand in a similar fashion, multiple residues would be available to contact these 11 amino acids, which suggests that multiple ligands can be accommodated in the same binding pocket. Furthermore, the 11 amino acids identified in this study are all relatively small in size and uncharged, so all could feasibly physically fit in the same binding pocket.

Our results suggest that four amino acids (lysine, arginine, methionine, and glutamine) are sensed by McpC using an indi-

rect binding mechanism involving ABC transport-binding lipoproteins. Lysine and arginine are both charged ligands, which might make them poor ligands for the McpC binding pocket. Glutamate and aspartate are also charged, and perhaps the weak affinity (Table 1) also reflects poor binding in the McpC binding pocket. The ArtP-binding lipoprotein was able to bind both lysine and arginine and is likely responsible for the ability of McpC to sense both of these ligands. As for methionine and glutamine, an indirect sensing mechanism may enable the cells to couple chemotaxis to transport as recently proposed by Neumann and co-workers (25). Methionine is a sulfur-containing amino acid, and perhaps sulfur is limiting for *B. subtilis*. Cysteine, the other sulfur-containing amino acid, is sensed directly by McpC. However, *B. subtilis* possesses four binding lipoproteins specific for cysteine, suggesting that it may be more important for sulfur scavenging. Alternatively, methionine may be expensive for the cell to synthesize, as it requires both cobalamin and a folic acid derivative. Glutamine may be an important source of nitrogen for the *B. subtilis*. This amino acid is also a ligand for the McpB receptor and actually binds McpB *in vitro* (data not shown), so it appears that glutamine can be sensed indirectly by McpC and directly by McpB.

The protein binding interface of the dual PAS domain may be conserved among diverse sensor histidine kinases and chemoreceptors. Our results show that residues along the protein binding interface of LuxQ are conserved in McpC, in particular the lysine at residue 195. This suggests that these two sensors employ similar mechanisms for binding accessory proteins. Moreover, the signaling mechanisms are similar. We found that the amino acid ligand does not alter the interaction between the McpC sensing domain and binding lipoprotein, implying that the two proteins interact without ligand present. The same is seen with LuxQ and LuxP, where two are bound in the absence of ligand; activation is induced when AI-2 binds LuxP (12). Presumably the same occurs with McpC and the binding lipoproteins. Finally, we were not able to examine whether the presence of bound binding protein might affect the affinity of McpC for ligands binding in the upper PAS domain, although this is certainly an intriguing area for future work.

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## Chemotaxis Receptor McpC Senses Multiple Ligands

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