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Receptors for Chemotaxis in *Bacillus subtilis*

M. H. DE JONG,* C. VAN DER DRIFT, AND G. D. VOGELS

Laboratory of Microbiology, Faculty of Science, University of Nijmegen, Nijmegen, The Netherlands

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At least three receptors for chemotaxis toward L-amino acids in *Bacillus subtilis* could be found with the aid of taxis competition experiments. They are called the asparagine receptor, which detects asparagine and glutamine, the isoleucine receptor, which detects isoleucine, leucine, valine, phenylalanine, serine, threonine, cysteine, and methionine, and the alanine receptor, which detects alanine and proline. Histidine and glycine could not be assigned to one of these receptors. Cysteine and methionine were found to be general inhibitors of chemotaxis and serine was found to be a general stimulator of chemotaxis. Some structural analogues of amino acids were tested for chemotactic activity. The chemotactic activity of *B. subtilis* is compared with that of *Escherichia coli*.

In a previous study (5) we reported on chemotaxis of *Bacillus subtilis* toward amino acids. To investigate this in more detail we tried to determine the number and the specificity of the receptors involved. This can be done in several ways (1, 2, 8): (i) by taxis competition experiments between different amino acids (8); (ii) through isolation of mutants lacking chemotaxis toward one or several amino acids (6); (iii) by studying the chemotaxis toward structural analogues of amino acids (8).

In general only a combination of these different approaches delivers a clear picture of the number and specificity of the receptors involved (9).

In this publication we describe a classification of receptors obtained by taxis competition experiments (8). Also a few structural analogues of amino acids were tested for chemotactic activity.

MATERIALS AND METHODS

Chemicals. L-Amino acids and glycerol were obtained from Merck A. G., Darmstadt; Tween 80 was from Sigma Chemical Co., St. Louis, Mo.; all other chemicals were analytical grade. Amino acids were checked for purity on an amino acid analyzer and contained no detectable contaminant except for an 0.15% alanine impurity in L-threonine.

Media. The inorganic portion of the growth medium contained 14 g of K_2HPO_4, 6 g of KH_2PO_4, 2 g of $(NH_4)_2SO_4$, 0.25 g of $MgSO_4 \cdot 7H_2O$, and 17 mg of $MnSO_4 \cdot 7H_2O$ per liter of distilled water. The growth medium consisted of the inorganic portion supplemented with 5 g of glycerol, 1 g of casein hydrolysate (acidic, Nutritional Biochemicals Corp.) and 25 mg of L-tryptophan per liter. The final pH was 7.0. Taxis medium consisted of the inorganic portion of the growth medium to which 5 g of glycerol and 1 g of Tween 80 were added per liter. The final pH was 7.0. Solutions of amino acids were, if necessary, adjusted to this pH.

Bacteria. In all taxis competition experiments *B. subtilis* Marburg strain 60015, which requires indole or L-tryptophan, and L-methionine, for growth, was used. Chemotaxis toward structural analogues of L-amino acids was tested with *B. subtilis* W23.

Chemotaxis assay. Cells were grown until the suspension reached an optical density of about 0.1 at 600 nm and were harvested by centrifugation (2,800 × *g* for 8 min at room temperature). The pellets were washed twice with taxis medium, and finally resuspended in taxis medium (5). For a normal chemotaxis assay a capillary containing an attractant dissolved in taxis medium was inserted in a cell suspension and after 30 min of incubation at 30 C the number of bacteria that had entered the capillary was determined by plating (5).

In taxis competition experiments (8) attractant at 1 mM was present only in the capillary and both the capillary and the cell suspension contained a competitor at 10 mM. The attractant concentration was lower than one-fifth of the peak concentration to prevent saturation of the receptor (5). The response was at least 10 times the blank. For taxis competition experiments a freshly prepared cell suspension was divided in two parts. One part was further diluted with taxis medium and was used for a normal chemotaxis assay. The other part was diluted with taxis medium containing the appropriate amount of competitor and was used in the competition experiment. The final optical density of both cell suspensions at 600 nm was 0.02 (approximately 3×10^6 cells/ml) (5).

The results reported in this paper are based on measurements with four capillaries per single assay. The percentage of standard deviation for the response in a single assay is about 15% (5). Taxis inhibition is said to occur when the remaining response is less than 100% minus two times the standard deviation, i.e., 70%. Remaining responses were calculated after sub-

straction of the blank, which was the number of bacteria accumulated in the capillary in the absence of attractant. In every experiment the attraction toward 10 mM glutamine (30,000 bacteria/capillary) served as a control.

RESULTS AND DISCUSSION

The results of the taxis competition experiments are given in Table 1. All L-amino acids toward which chemotaxis was found (5), except for tryptophan and tyrosine, were tested as an attractant and competitor.

The procedure has the following rationale. As bacteria can only respond toward a concentration gradient of an attractant, no response can occur toward the competitor, which is present in a uniform concentration both in the capillary and in the cell suspension (8). This means that in the competition experiments a chemotactic response can only occur to the attractant present in the capillary. However, when the competitor and attractant share a common receptor, taxis inhibition due to partial saturation of the receptor by the competitor occurs. An attractant could be detected by two chemoreceptors; competition for one of the receptors might have a small or large effect on the response of the cells to the attractant, depending on the sensitivities of the receptors for the attractant. Examples of such behavior in *Escherichia coli* (toward glucose, benzoate, and several amino acids) have been reported (2, 8, 9). If the competitor and the attractant are recognized by two different receptors, the uniform presence of the competitor should have no effect on the response to a gradient of the attractant. It is also possible that a competitor influences the response to the attractant in other ways than by competition for a common receptor. Therefore, only a mutual inhibition of response should be considered as evidence for a shared receptor (2).

The results in Table 1 indicate the presence of at least three different receptors for chemotaxis toward L-amino acids. The receptors which are named for the amino acid with the lowest threshold detected by them (8) are: (i) the asparagine receptor which detects glutamine and asparagine; (ii) the alanine receptor which detects alanine and proline; (iii) the isoleucine receptor which detects phenylalanine, valine, leucine, isoleucine, methionine, cysteine, threonine, and serine. Glycine and histidine could not be assigned to one of these receptors. The fact that the chemotaxis to these amino acids is not inhibited by any amino acid, except by methionine and cysteine, suggests that they have their own receptor, or that they are slightly detected by more than one receptor. At this point it should be noted that for these two amino acids ambiguous transport data were obtained (7). The poor self-competition of histidine is as yet not understood. The data in Table 1 show that for a distinct receptor, in general, the better attractants are the stronger inhibitors and the weaker attractants the poorer inhibitors. This does not apply to methionine, cysteine, and serine, which have a general effect on chemotaxis.

Most L-amino acids, except cysteine and methionine, stimulate the response toward amino acids with which they do not share their receptor. The observed stimulation of chemotaxis caused by competitors outside their own receptors results from an enhancement of velocity and not from a repression of twiddling, as is evident from motility tracks and motility assays (M.H. de Jong, C. van der Drift, C. Stumm, and J.J.A. Arends, manuscript in preparation). An effect on velocity and twiddling was also observed for *E. coli* in homogeneous solutions of amino acids (4). Serine even stimulates the response toward amino acids recognized by its own receptor. Still serine could only be assigned to the isoleucine receptor because the chemotaxis toward it was inhibited by all amino acids sharing this receptor, whereas serine was only an inhibitor for chemotaxis toward cysteine, threonine, and itself. It is of interest that in *E. coli* serine is a general inhibitor of chemotaxis (8). Cysteine and methionine inhibited chemotaxis toward all other amino acids. They could be confined to the isoleucine receptor because as an attractant they were only inhibited by the amino acids of this receptor. The methionine auxotroph used in this study does not require methionine to be chemotactically responsive as is the case for methionine-requiring mutants of *E. coli* (3).

The anomalous effects caused by serine, cysteine, and methionine deserve further investigation, especially since transport of amino acids in *B. subtilis* is inhibited by cysteine, probably due to the interaction of the sulfhydryl group with some membrane component needed for active transport (7). Preliminary results indicate that motility in cell suspensions containing serine, methionine, and cysteine is not impaired. Moreover, these amino acids seem to exert a similar effect on all chemoreceptors. Therefore, their site of action is probably located somewhere between the receptors and the flagella.

The three receptors differ widely in the number of amino acids detected by them and the

TABLE 1. *Competition experiments for chemotaxis toward amino acids*[a]

Competitor (10 mM)	Fold above threshold	Remaining responses with competitor (percentage of response without competitor)													
		Attractant (1 mM) fold above threshold in parentheses													
		GLU-NH$_2$ (3,333)	ASP-NH$_2$ (2,000)	PHE (14)	VAL (500)	LEU (500)	ILEU (5,000)	MET (2,500)	CySH (125)	THR (10)	SER (200)	ALA (10,000)	PRO (1,000)	GLY (16)	HIS (12)
GLU-NH$_2$	33,333	2	8	370	115[b]	104	164	150	200	84	240	180	87	140	90
ASP-NH$_2$	20,000	9	10	—[c]	—	—	248	—	—	—	—	280	250	69	175
PHE	140	450	—	14	10	7	28	8	—	8	20	46	—	140	250
VAL	5,000	—	—	21[b]	—	0.7	0.6	4	15	3.5	16	—	—	—	—
LEU	5,000	—	—	9[b]	0.5[b]	—	0.7	5	10	0	12	—	—	—	—
ILEU	50,000[d]	210[d]	159[d]	3[b]	0.7[b]	0.8	—	1	13	0.3	7	130[d]	110[d]	100[d]	170[d]
MET	25,000	16	45	15[b]	0[b]	0.9	0.8	—	6	2	5	9	8	45	78
CySH	1,250	10	37	3	1.4	0.4	4	1	—	2	1	5	7	3	16
THR	100	—	—	15[b]	5[b]	21	47	7	7	—	26	65	135	264	123
SER	2,000	220	230	166[b]	98	160	125	168	22	10	21	81	99	365	620
ALA	100,000	—	102	116	—	—	130	200	53	18	56	6	6	110	200
PRO	10,000	—	—	—	—	—	125	260	52	—	39	15	12	—	280
GLY	165	330	120	—	—	—	334	123	51	37	28	150	240	11	230
HIS	125	—	—	—	—	—	260	153	165	258	150	250	147	—	67

[a] Inhibition of chemotaxis toward amino acids was measured by comparing the response toward an attractant at 1 mM in the capillary alone to the response toward the same attractant at 1 mM in the capillary and a competitor at 10 mM both in the capillary and the cell suspension (see Results and Discussion). Responses are given in percentage of the value of the normal chemotaxis assay, i.e., without competitor.

[b] Attractant at 2 mM instead of 1 mM.

[c] —, Not tested.

[d] Competitor at 1 mM instead of 10 mM.

closeness of the structural relationships between the amino acids detected by them. The asparagine receptor detects two chemically closely related amino acids, which also appear to have the same transport system (7). The alanine receptor detects two chemically different amino acids, which appear to have separate transport systems (7). The inhibition of chemotaxis toward cysteine, threonine, and serine by alanine, proline, and glycine seems to be partly mutual, due to the general inhibition by cysteine. However, alanine, proline, and glycine cannot be assigned to the isoleucine receptor since other amino acids detected by this receptor expose no inhibition, except for the general inhibition by methionine and the one-way inhibition of phenylalanine versus alanine. Possibly cysteine, threonine, and serine are detected by both the isoleucine and the alanine receptor.

The isoleucine receptor detects eight chemically widely different amino acids. Four different transport systems were reported for these amino acids (7). It seems quite possible that the isoleucine receptor is a cluster of several receptors. There are data for *E. coli* that are consistent with the view that clustering of receptors occurs (9).

To clarify this in our case, it will be necessary to isolate two kinds of specific nonchemotactic mutants, as Tso and Adler have recently found for *E. coli* (9): (i) specific mutants which lack one receptor only; (ii) multiple defective mutants which lack receptor activity for a whole cluster. Isolation of such mutants is in progress.

In Table 2 the chemotactic behavior toward a number of structural analogues of L-amino acids is given. Ornithine and citrulline, which can be considered as analogues of L-lysine and L-arginine, themselves not attractants, fail to elicit a chemotactic response. Among the analogues of the amino acids presumably detected by the isoleucine receptor, only L-homoserine, L-norleucine, and DL-penicillamine are attractants, whereas isobutylamine, isovaleric acid, isoamylamine, and isocaproic acid are not. From the results it is evident that only those amino acid analogues with an intact amino group are attractants. So it seems that at least for the isoleucine receptor, an intact amino group is necessary for a compound to serve as an attractant. In the experiments with structural analogues *B. subtilis* W23 was used. This strain exhibits the same chemotactic behavior as *B. subtilis* 60015.

At least two receptors, the aspartate and the serine receptor, for chemotaxis toward amino acids in *E. coli* were found (8). Several amino acids are detected exclusively by one of the receptors, whereas other amino acids are detected by both receptors, albeit with different effectiveness. Whereas hydrophilic amino acids are attractants for *E. coli* (8), hydrophobic amino acids are repellents (9). In contrast to *E. coli* the best attractants for *B. subtilis* are the hydrophobic amino acids. It is unknown if some amino acids act as repellents for *B. subtilis*. Although the chemical nature of the attractants and the specifity of the receptors in both bacteria differ widely, in our opinion there are no reasons to believe that the general mechanism of chemotaxis is different for *E. coli* and *B. subtilis*.

TABLE 2. *Chemotaxis toward structural analogues of amino acids*[a]

Compound	Threshold (M)	Peak (M)	Peak response
Isobutylamine	$>10^{-2}$		
Isovaleric acid	$>10^{-2}$		
Isoamylamine	$>10^{-2}$		
Isocaproic acid	$>10^{-2}$		
L-Homoserine	4×10^{-5}	$>10^{-1}$	31,000
L-Norleucine	10^{-5}	10^{-1}	3,500
DL-Penicillamine	2×10^{-5}	10^{-1}	7,000
L-Ornithine	$>10^{-2}$		
L-Citrulline	2×10^{-3}	$>10^{-1}$	1,200

[a] For each compound a concentration response curve was determined. Compounds with thresholds lower than 10^{-4} M are named attractants (5). The blank value (assay without attractant) amounted up to 100 bacteria/capillary. Peak concentration is the concentration of an attractant in the capillary which gives the largest response (peak response) on a concentration response curve.

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