JOURNAL OF BACTERIOLOGY, Sept. 1975, p. 1000-1005
Vol. 123, No. 3

Printed in U.S.A.

# Soluble and Membrane-Bound Aspartate-Binding Activities in *Salmonella typhimurium*

ROBERT R. AKSAMIT,[1]* BARBARA J. HOWLETT, AND D. E. KOSHLAND, JR.
*Department of Biochemistry, University of California, Berkeley, California 94720*

Received for publication 25 March 1975

The specificities of the soluble and membrane aspartate-binding activities were compared with each other and with the specificity of aspartate chemotaxis and were found to be distinct. The soluble aspartate-binding protein was purified to homogeneity and had a molecular weight of 30,000. The dissociation constant was $10^{-6}$ M for aspartate, and the protein bound glutamate, cysteic acid, and 2-amino-3-phosphonopropionate. Aspartate transport was inhibited by cysteic acid.

Competition studies have shown that receptors exist in bacterial chemotaxis that are able to recognize only a limited number of chemicals (1, 3, 21, 22). The galactose-binding protein in *Escherichia coli* (7-9, 11) and the ribose-binding protein in *Salmonella* (5) have been identified as the receptors for these organisms. Preliminary evidence has been presented that a maltose-binding protein is the receptor for maltose chemotaxis in *E. coli* (3). These receptor molecules belong to a class of small-molecular-weight proteins that are characterized by the fact that they are readily released upon osmotic shock. Also, by specificity correlation and genetic studies they can be implicated as the recognition proteins in bacterial transport (12, 14, 20). Recently, Adler and Epstein identified the glucose-specific enzyme II of the phosphotransferase system in *E. coli* as the recognition protein for glucose chemotaxis (2).

Although Adler has evidence that there are at least two receptors for chemotaxis to amino acids, the serine and aspartate receptors, none have been identified (18). In *E. coli* the strongest response to the sugars and amino acids is nearly the same; however, Aksamit and Koshland have found that in *Salmonella* the strongest ribose response is 10 times less than the strongest aspartate response (5). Therefore, it appears that the properties of the ribose receptor and the aspartate receptor are very different. The difference in the intensity of response could be due to a difference in the number of receptor molecules, to a difference in the conformational state induced by the attractant, or to an entirely different mechanism. Therefore, the isolation of the aspartate receptor seemed an important step.

Whether an aspartate receptor is easily released by osmotic shock or is an integral component of the bacterial envelope, it must specifically bind aspartate in order to confer specificity upon the chemotactic system. In this paper we have investigated the specificity of two binding activities, one released by osmotic shock and one membrane bound. The purification of an aspartate-binding protein released by osmotic shock is reported. Since this work was started we have learned that a glutamate-aspartate-binding protein from *E. coli* has been isolated (R. L. Willis and C. F. Furlong, personal communication).

## MATERIALS AND METHODS

**Chemicals.** Radioactive [U-$^{14}$C]aspartate (170 mCi/mmol) and [1-$^{14}$C]ribose (49.9 mCi/mmol) were purchased from New England Nuclear Corp.

The medium used was either Vogel-Bonner salts (23), containing citrate (VBC) or C-minus medium. C-minus medium contained 3.94 g of $KH_2PO_4$, 4.98 g of $K_2HPO_4$, 3.5 g of $NaHNH_4PO_4 \cdot 4H_2O$, and 0.2 g of $MgSO_4 \cdot 7H_2O$ per liter.

**Preparation of soluble and membrane fractions.** *Salmonella typhimurium* strain ST1 was grown at 30 C in VBC to stationary phase. The total wet weight of the cells after harvesting was 100 g. The bacteria were osmotically shocked (4, 12), aspartate-binding protein was purified from the shock fluid, and membranes were prepared from the shocked bacteria.

**Purification of the soluble aspartate-binding protein.** The shock fluid was chromatographed on SE-Sephadex as described by Aksamit and Koshland for the ribose-binding protein (4) (Fig. 1). Aspartate-binding activity eluting from the column was well separated from the major proteins and the ribose-binding protein. The fractions with activity were

[1] Present address: Flow Laboratories, Rockville, Md. 20852.

pooled from the SE-Sephadex column, concentrated by ultrafiltration through a UM2 membrane, dialyzed against 20 mM triethanolamine-20 mM NaCl, pH 8.5, and applied to a diethylaminoethyl (DEAE)-Sephadex column (3 by 40 cm) equilibrated in the dialysis buffer. The highly purified aspartate-binding protein passed through the column while the impurities remained on the column.

**Preparation of membranes.** Spheroplasts were formed from the shocked cells by incubation in 10 mM tris(hydroxymethyl)aminomethane (tris)-hydrochloride, pH 8.0, 200 $\mu$g of lysozyme per ml, and 10 mM ethylenediaminetetraacetic acid (EDTA) for 30 min at 25 C. The spheroplasts were centrifuged at 16,000 $\times$ *g* until the supernatant was clear, suspended in 0.1 M potassium phosphate, pH 6.6, 20 mM $MgSO_4$ at 4 C, and sonicated for 4 min at 30-s intervals. Deoxyribonuclease was added to a final concentration of 1 mg/ml, and the sonic fluid was incubated for 15 min. Whole cells were removed by centrifugation at 2,000 $\times$ *g* for 10 min and the membranes were centrifuged at 54,000 $\times$ *g* for 1 h. The membranes were washed and purified on a sucrose gradient as described by Kaback (15). The purified membranes were washed in 0.1 M potassium phosphate, pH 6.6, 10 mM EDTA and stored in 1-ml aliquots in liquid nitrogen. The protein concentration was 6 mg/ml as determined by the method of Lowry et al. (16). This preparation was not intended to give membrane vesicles with good transport activity, but to give a membrane preparation with good aspartate-binding activity.

**Assay of aspartate-binding activity.** Soluble aspartate-binding activity was measured by a filter paper assay or by equilibrium dialysis (4). The final concentration of aspartate in the filter assay was 3.51 $\times 10^{-7}$ M.

The membrane-bound aspartate-binding activity was measured by incubating the membrane suspension with 3.51 $\times 10^{-7}$ M aspartate in a total volume of 50 $\mu$l. The suspension was centrifuged at 17,600 $\times$ *g* for 20 min at 4 C, and 25 $\mu$l of the supernatant was counted in a toluene scintillation fluid. The amount of aspartate bound to the membranes was approximately the same at 25 or 4 C. This suggested that binding and not transport was being measured since transport is highly temperature dependent. The bound radioactive aspartate was readily released by the addition of excess nonradioactive aspartate. Controls showed that there was no radioactive histidine or ribose bound to the membranes under these conditions. For inhibition studies, 1.75 $\times 10^{-3}$ M inhibitor was added to the suspension in addition to radioactive aspartate.

**Assay of chemotaxis.** For chemotaxis assays, *Salmonella* ST1 was grown to exponential phase in VBC at 30 C. Chemotaxis was measured quantitatively by a capillary assay (1, 5) or qualitatively by subjecting the bacteria to a rapid concentration change and observing the resulting motility (17).

For the qualitative assay, the bacteria were diluted into VBC containing either aspartate or the aspartate analogue at a 1 mM concentration. If the bacteria swam smoothly, the compound was scored as an attractant. To measure inhibition, the bacteria were incubated in the compound (100 mM) for 10 min to allow the bacteria to return to normal swimming, and then L-aspartate was rapidly added to a final concentration of 1 mM. Thus the bacteria experienced a sudden concentration change of aspartate from 0 to 1 mM and swam smoothly for 4 min in the absence of an inhibitor. The converse experiment was also done, whereby the bacteria were incubated in 100 mM aspartate and then subjected to a concentration change of an analogue. This experiment allowed more toxic analogues to be tested. The capacity of a compound to act as an attractant and to inhibit aspartate chemotaxis was measured and a qualitative estimate made by assigning "++++" to a strong attractant or inhibitor.

**Measurement of uptake.** *S. typhimurium* ST1 was grown at 30 C in VBC to 2 $\times 10^8$ bacteria/ml. The cells were harvested and washed twice in C-minus medium by centrifugation. The cell pellet was suspended to 0.1 mg (dry weight) of bacteria per ml and stored until assayed at 4 C for not longer than 4 h. The bacteria were preincubated at 30 C for 10 min.

**Electrophoresis.** Sodium dodecyl sulfate-polyacrylamide slab gel electrophoresis was performed and the slab gel was stained as described by Ames (6). Nondenaturating polyacrylamide slab gel electrophoresis had a 7.5% separating gel and a 2.5% stacking gel. Either the Tris-glycine, pH 8.5, buffer system or the $\beta$-alanine/acetic acid, pH 4.3, buffer system was used, and uptake was initiated by the addition of radioactive aspartate to a final concentration of 2 $\times 10^{-5}$ M or radioactive ribose to a final concentration of 2 $\times 10^{-6}$ M. Aliquots (0.5 ml) were removed at 10, 15, 20, 30, 40, and 50 s, delivered to membrane filters (Millipore Corp.), and washed three times with 0.6 ml of C-minus medium at 25 C. Blank values were obtained by treating the bacteria with 1% formaldehyde for 30 min at 25 C before preincubation at 30 C. The inhibition of transport by cysteic acid was studied by the simultaneous addition of cysteic acid (final concentration, 1.88 $\times 10^{-3}$ M) and radioactive substrate.

**Purity of aspartate analogues.** Analogues of aspartate that inhibited the soluble aspartate-binding protein were tested for contamination by aspartate or glutamate by amino acid analysis. The maximum amount of possible aspartate or glutamate contaminant was calculated by using the experimentally determined binding constant. Enough inhibitor was then applied to detect any aspartate or glutamate that may have been present. Unless indicated otherwise, all compounds reported as inhibitors were free from significant contamination by aspartate or glutamate. The fact that a compound did not inhibit aspartate-binding indicated that it contained less than a 0.1% impurity.

## RESULTS

**Properties of the soluble aspartate-binding protein.** The purified aspartate-binding protein

can be stored at 4 or −20 C at pH 6.0 for several months without a significant loss in activity. The amount of aspartate bound to the protein was relatively unchanged from pH 5 to pH 9 as measured by the filter assay. Treatment with 1 mM disodium EDTA or 5 mM dithiothreitol did not affect the binding activity of the protein.

The aspartate-binding protein released by osmotic shock showed a single band on polyacrylamide gel electrophoresis in acid gels. Disc gel electrophoresis in the Tris buffer system at pH 8.5 did not yield any bands. This fact and the finding that the protein did not bind to a DEAE-Sephadex column at pH 8.5 suggests that the protein has an isoelectric point above pH 8.5. The ribose-binding protein with an isoelectric point of 7.8 (4) showed a high mobility into polyacrylamide gels under these conditions.

Sodium dodecyl sulfate electrophoresis showed only one protein band with a molecular weight of 30,000. Thus, it appears that the aspartate-binding protein is homogeneous with a monomeric molecular weight similar to those of other binding proteins that have been isolated from osmotic shock fluid.

**Comparison of the aspartate-binding activities with the aspartate chemotactic response.** The binding constant of the soluble aspartate-binding protein after purification through the SE-Sephadex chromatography and storage at 4 C for 2 months was estimated by the filter assay to be $10^{-6}$ M. Measurement of the binding constant by equilibrium dialysis on a preparation purified through the DEAE-Sephadex step and stored at 4 C for 10 months gave a binding constant of $4.5 \times 10^{-6}$ M. The difference between these determinations was not investigated and could be due to the difference in purity of the preparations, in the age of the preparation or in the assay techniques.

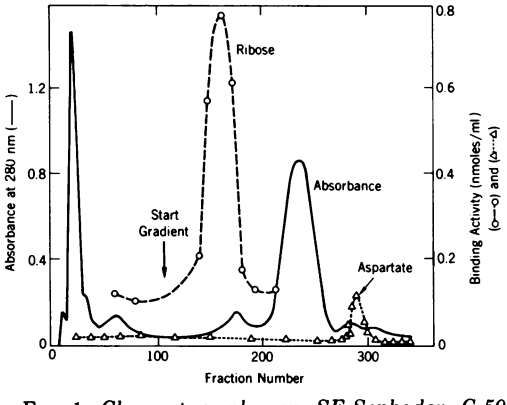


FIG. 1. *Chromatography on SE-Sephadex C-50. Concentrated, dialyzed shock fluid from 200 g (wet weight) of cells was applied to a column (6 by 37 cm) equilibrated in 30 mM sodium phosphate, pH 5.05, 40 mM NaCl and eluted with a linear NaCl gradient from 40 to 130 mM NaCl in 30 mM sodium phosphate, pH 5.05; 21-ml fractions were collected. Symbols: ——, Absorbancy at 280 nm; - - - -, ribose-binding activity; · · · ·, aspartate-binding activity.*

Competition of unlabeled inhibitor with $3.5 \times 10^{-7}$ M radioactive aspartate for binding to the aspartate-binding protein indicated that glutamate, systeic acid, and 2-amino-3-phosphonopropionate will bind to the aspartate-binding protein (Table 1). From the inhibition data and assuming that the dissociation constant for aspartate is $10^{-6}$ M, dissociation constants of $1.8 \times 10^{-5}$ and $4.3 \times 10^{-4}$ M were calculated for cysteic acid and 2-amino-3-phosphonopropionate, respectively. The binding of glutamate was as strong as or stronger than the binding by aspartate ($K_D = 10^{-6}$ M). The inhibition data suggest that the $\beta$-carboxyl group of aspartate can be modified, and the lack of inhibition by homoserine indicates that the negative charge of the carboxyl is important to binding.

In contrast to the soluble aspartate-binding protein, the binding specificity of the membrane preparation (Table 1) indicates that the membrane-bound activity will tolerate modification at the $\alpha$-carboxyl group. Both L-aspartyl-L-phenylalanine and the $\alpha$-benzylester of aspartate were very good inhibitors of membrane-bound aspartate-binding activity. There was some inhibition of the membrane-bound system by cysteic acid. Glutamate was not an inhibitor, indicating that the membranes did not contain small amounts of the soluble aspartate-binding protein.

The inhibition of aspartate chemotaxis by aspartate analogues showed a specificity that was different from that of both the soluble and membrane aspartate-binding activities (Table 2). Cysteic acid, a good inhibitor of the soluble aspartate-binding protein, did not inhibit chemotaxis. Cysteic acid should be accessible to the aspartate-binding protein in vivo since cysteic acid inhibits the uptake of aspartate (Table 3). Although the concentration of cysteic acid ($1.88 \times 10^{-3}$ M) was sufficient to saturate the aspartate-binding protein, uptake was only inhibited by 51%. This suggests more than one transport system for the uptake of $2 \times 10^{-5}$ M aspartate. Aspartate chemotaxis was strongly inhibited by glutamate, malate, $\alpha$-methylaspartate, and D-tartrate, and some inhibition was observed for methylsuccinate. However, L-glutamate and methylsuccinate did not sig-

TABLE 1. *Inhibition of soluble and membrane aspartate-binding activity*

| Analogue | % Remaining | |
|---|---|---|
| | Soluble[a] | Membrane[b] |
| None | 100 | 100 |
| L-Aspartate | 4.0[c] | 0 |
| L-Glutamate | 0.4 | 97 |
| D-Aspartate[d] | 56.5 | 95 |
| 2-Amino-3-phosphonopropionate | 53.7 | 135, 75 |
| D,L-Homoserine | 93.1 | 70, 171 |
| L-Alanine | 82.8 | 105 |
| L-Cysteic acid | 4.7 | 39 |
| D,L-*threo*-β-Hydroxyaspartate[d] | 77.3 | 122 |
| β-Methyl-D,L-aspartate[d] | 57.6 | 132 |
| β-Hydroxy-D,L-glutamate[d] | 55.3 | 104 |
| Citrate | 104.7 | 109 |
| L-Malate | 98.1 | 97 |
| *N*-methyl-D,L-aspartate | 60.7 | 79, 104 |
| α-Ketoglutarate | 93.0 | 117, 93 |
| Succinate | 88.1 | 60, 31 |
| Oxaloacetate | 109.2 | 128 |
| L-Aspartyl-L-phenylalanine | 92.5 | 0, 0 |
| L-Aspartate, α-benzyl ester | 28.2 | 0, 0 |
| β-Alanine | 103.4 | 123 |
| D,L-α-Aminobutyric acid | 97.6 | 150 |
| α-Methyl-D,L-aspartate | 98.0[c] | 22, 156 |
| L-Tartrate | NT[e] | 83, 87 |
| D-Tartrate | NT | 52, 88 |
| Methylsuccinate | 103.4 | 164 |
| Asparagine[f] | (20)[c] | NT |

[a] Concentration of L-[U-$^{14}$C]aspartate was $3.5 \times 10^{-7}$ M and the concentration of aspartate analogue was $5 \times 10^{-4}$ M unless otherwise indicated. When a D,L mixture was used the concentration was $10^{-3}$ M for the mixture.

[b] Second values are from a second experiment. The concentration of L-[U-$^{14}$C]aspartate was $3.5 \times 10^{-7}$ M and the concentration of aspartate analogue was $1.75 \times 10^{-3}$ M. When a D,L mixture was used the concentration was $3.5 \times 10^{-3}$ M for the mixture.

[c] Concentration of analogue was $3 \times 10^{-5}$ M.

[d] Purity could not be determined by amino acid analysis.

[e] NT, Not tested.

[f] Inhibition of asparagine could be accounted for by a contaminant of aspartate found by amino acid analysis.

nificantly inhibit the membrane aspartate-binding activity. Thus, the specificity of aspartate chemotaxis correlates with neither the membrane-bound activity nor the soluble aspartate-binding activity. In addition, L-malate (and possibly D-tartrate) does not inhibit either of the aspartate-binding activities, although malate is a strong inhibitor of aspartate chemotaxis. This could indicate that the receptor for aspartate chemotaxis is distinct from either of the binding activities examined, or it could indicate that L-malate inhibits at a site distinct from the aspartate receptor.

Chemotaxis to α-methylaspartate and cysteic acid was tested by the capillary assay method as well as by temporal gradients. Cysteic acid was not an attractant between $10^{-6}$ and $10^{-1}$ M cysteic acid initially present in the capillary. A capillary response curve to α-methylaspartate (Fig. 2) indicated that it is an attractant (optimum at $10^{-2}$ M), although not nearly as good an attractant as aspartate. The possibility of a contaminant of aspartate was not eliminated. In contrast, Mesibov and Adler found that the response curve of *E. coli* to α-methylaspartate was indistinguishable from the response to aspartate (18).

Although aspartate and glutamate bound to the soluble aspartate-binding protein to a similar extent, the concentration of glutamate giving the strongest response was about $10^{-1}$ M (the highest tested, Fig. 2), whereas for aspartate it was between $10^{-3}$ and $10^{-2}$ M. If the aspartate-binding protein were the receptor and behaved like the ribose chemotactic receptor (5), then the optimal concentration in a capillary response curve for glutamate would be expected to be the same as or less than that for aspartate. Since this was not the case, it supports the contention that the aspartate-binding protein is not the receptor or that the mechanism of aspartate reception is different from that of ribose.

Glutamate-binding activity has been found in shock fluid from *E. coli* (10, 19), and Willis and Furlong (personal communication) have recently purified a glutamate-aspartate-binding protein from *E. coli* osmotic shock fluid. The properties of the *E. coli* protein—high isoelectric point (pH 9.69), 30,000 molecular weight, and a dissociation constant for aspartate of $1.2 \times 10^{-6}$ M—are very similar to those of the *Salmonella* aspartate-binding protein. In addition, Willis and Furlong (personal communication) found that antisera to *E. coli* glutamate-aspartate-binding protein cross-reacted with a component in shock fluid from *S. typhimurium*, presumably the aspartate-binding protein. Although the *E. coli* glutamate-aspartate-binding protein appeared to be inhibited by asparagine, we found that inhibition of aspartate binding to the *Salmonella* aspartate-binding protein by asparagine could be accounted for by an impurity of aspartate.

## DISCUSSION

Aspartate-binding activity has been found in both the soluble fraction (released by osmotic

TABLE 2. *Comparison of inhibition by aspartate analogues of soluble asparte-binding, membrane aspartate-binding, and aspartate chemotaxis*

| Analogue | Inhibitor of soluble aspartate binding[a] | Inhibitor of membrane aspartate binding[a] | Aspartate chemotaxis | |
|---|---|---|---|---|
| | | | Inhibitor | Attractant |
| L-Glutamate | ++++[b] | − | ++++ | + |
| L-Cysteic acid | ++++ | + | − | ± |
| 2-Amino-3-phosphonopropionate | ++ | − | − | ± |
| β-Methyl-D,L-aspartate | ++ | − | ++++ | ± |
| L-Aspartyl-L-phenylalanine | − | ++++ | —[c] | + |
| L-Aspartate, α-benzylester | ++ | ++++ | − | ± |
| L-Malate | − | − | ++++ | ++++ |
| D-Tartrate | NT | ± | ++++ | ++++ |
| Succinate | − | + | − | ± |
| Methylsuccinate | − | − | ++ | ++ |
| α-Methyl-D,L-aspartate | —[d] | ± | ++++ | ++ |
| D-Aspartate | ++ | − | − | ± |

[a] Data taken from Table 1.
[b] Strong inhibition; −, no inhibition; ±, data is not sufficiently accurate to conclude; NT, not tested.
[c] Inhibition concentrations were toxic to bacteria.
[d] The concentration of inhibitor was 100 times higher than the concentration of aspartate; for the other analogues inhibition was more than 1,000 times higher.

TABLE 3. *Inhibition of aspartate uptake by cysteic acid*

| Substrate | Initial velocity of uptake | |
|---|---|---|
| | μmol/min per 0.05 mg (dry wt) of bacteria | % |
| Aspartate ($2 \times 10^{-5}$ M) | $3.72 \times 10^{-5}$ | 100 |
| Aspartate ($2 \times 10^{-5}$ M) + cysteic acid ($1.88 \times 10^{-3}$ M) | $1.90 \times 10^{-5}$ | 51 |
| Ribose ($2 \times 10^{-6}$ M) | $1.91 \times 10^{-4}$ | 100 |
| Ribose ($2 \times 10^{-6}$ M) + cysteic acid ($1.88 \times 10^{-3}$ M) | $2.30 \times 10^{-4}$ | 120 |

shock) and in an isolated membrane preparation. The soluble aspartate-binding activity was associated with a protein with a monomeric molecular weight of 30,000 that was released upon osmotic shock. Thus, it would appear that this protein is similar to other binding proteins isolated from osmotic shock fluid. From its chromatographic and electrophoretic behavior, the protein was shown to have an isoelectric point greater than 8.5. The protein has a very strong affinity for aspartate and glutamate and binds cysteic acid and 2-amino-3-phosphonopropionate.

The specificity of the soluble aspartate-binding protein indicates that modification at the β-carboxyl group can be tolerated, whereas the membrane aspartate-binding activity can tolerate modification at the α-carboxyl group. Although the membrane-binding activity could be

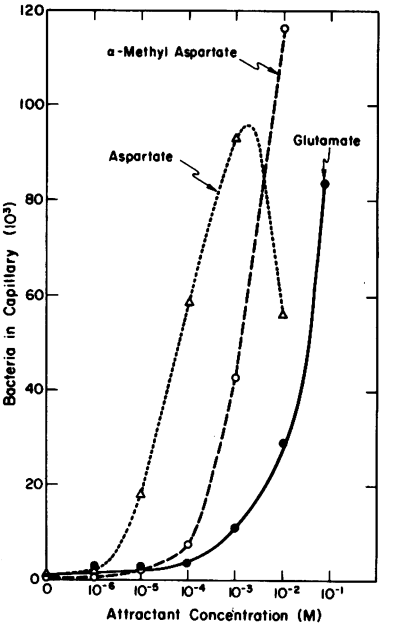


FIG. 2. *Capillary response of ST1 to aspartate, α-methylaspartate, and glutamate. The capillary was incubated in a bacterial suspension containing $5 \times 10^6$ bacteria/ml at 30 C for 30 min. Symbols: Δ, aspartate; O, α-methylaspartate; ●, glutamate. The aspartate and α-methylaspartate responses were measured simultaneously. The response to glutamate was a separate experiment. The background (600 bacteria/capillary measured in the absence of attractant) was subtracted.*

partly due to transport, we would not expect this to greatly affect the specificity data. In fact, since the membrane binding was insignificantly affected by a temperature change from 25 to 4 C, and since the only energy source present was aspartate or the inhibitor, the activity was undoubtedly due primarily to binding. In contrast to the soluble and membrane-binding activities, the chemotactic response is strongly affected by modification of either carboxyl group.

A comparison of the specificities of the two aspartate-binding activities with the specificity of the aspartate chemotactic response suggests that each binding activity is due to a different protein and that neither activity correlates with the chemotactic response. Therefore, the simplest explanation for these results is that there are at least three different recognition proteins for aspartate on the bacterial surface—one for chemotaxis and possibly transport, and two others that are probably involved in transport. Other possibilities such as the modification of the specificity of either of the aspartate-binding proteins in vivo cannot be eliminated. However, the recent demonstration that the specificity and dissociation constants of the purified ribose receptor is the same as the biological response in the living system makes this possibility seem remote (5).

Two binding activities associated with the bacterial envelope are found, and these may be similar to the soluble and membrane-bound aspartate transport systems reported by Kay in *E. coli* (13). However, on the basis of specificity data, neither is the receptor for aspartate chemotaxis in *Salmonella*, assuming that a single protein is responsible for the specificity as demonstrated for the ribose-binding and galactose-binding proteins. Inhibition of aspartate transport by cysteic acid suggests that the soluble system at least is involved in transport. This inhibition with cysteic acid and the inhibition of aspartyl-phenylalanine make it possible to differentiate between the soluble and membrane activities.

## ACKNOWLEDGMENTS

This work was supported by Public Health Service grant AM 09765 from the National Institute of Arthritis, Metabolism and Digestive Diseases and by National Science Foundation grant GB7057.

We wish to thank Bernard Cantau for his many helpful suggestions in the preparation and assay of the membrane particles.

## LITERATURE CITED

1. Adler, J. 1969. Chemoreceptors in bacteria. Science **166**:1588–1597.
2. Adler, J., and W. Epstein. 1974. Phosphotransferase system enzymes as chemoreceptors for certain sugars in *Escherichia coli* chemotaxis. Proc. Natl. Acad. Sci. U.S.A. **71**:2895–2899.
3. Adler, J., G. L. Hazelbauer, and M. M. Dahl. 1973. Chemotaxis towards sugars in *Escherichia coli*. J. Bacteriol. **115**:824–847.
4. Aksamit, R. R., and D. E. Koshland, Jr. 1972. A ribose binding protein of *Salmonella typhimurium*. Biochem. Biophys. Res. Commun. **48**:1348–1353.
5. Aksamit, R. R., and D. E. Koshland, Jr. 1974. Identification of the ribose binding protein as the receptor for ribose chemotaxis in *Salmonella typhimurium*. Biochemistry **13**:4473–4478.
6. Ames, G. F. 1974. Resolution of bacterial proteins by polyacrylamide gel electrophoresis on slabs. J. Biol. Chem. **249**:634–644.
7. Anraku, Y. 1968. Transport of sugars and amino acids in bacteria. I. Purification and specificity of the galactose and leucine binding proteins. J. Biol. Chem. **243**:3116–3122.
8. Anraku, Y. 1968. Transport of sugars and amino acids in bacteria. II. Properties of galactose and leucine binding proteins. J. Biol. Chem. **243**:3123–3127.
9. Anraku, Y. 1968. Transport of sugars and amino acids in bacteria. III. Studies on the restoration of active transport. J. Biol. Chem. **243**:3128–3135.
10. Barash, H., and Y. S. Halpern. 1971. Glutamate-binding protein and its relation to glutamate transport in *Escherichia coli* R12. Biochem. Biophys. Res. Commun. **45**:1681–1688.
11. Hazelbauer, G. L., and J. Adler. 1971. Role of the galactose binding protein in chemotaxis of *Escherichia coli* towards galactose. Nature (London) New Biol. **230**:101–104.
12. Heppel, L. A. 1967. Selective release of enzymes from bacteria. Science **156**:1451–1455.
13. Kay, W. W. 1971. Two aspartate transport systems in *Escherichia coli*. J. Biol. Chem. **246**:7373–7382.
14. Kaback, H. R. 1970. Transport. Annu. Rev. Biochem. **39**:561–598.
15. Kaback, H. R. 1972. Bacterial membranes. Methods Enzymol. **22**:99–120.
16. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
17. Macnab, R. M., and D. E. Koshland, Jr. 1972. The gradient-sensing mechanism in bacterial chemotaxis. Proc. Natl. Acad. Sci. U.S.A. **69**:2509–2512.
18. Mesibov, R., and J. Adler. 1972. Chemotaxis towards amino acids in *Escherichia coli*. J. Bacteriol. **112**:315–326.
19. Miner, K. M., and L. Frank. 1974. Sodium-stimulated glutamate transport in osmotically shocked cells and membrane vesicles of *Escherichia coli*. J. Bacteriol. **117**:1093–1098.
20. Pardee, A. B. 1968. Membrane transport proteins. Science **162**:632–637.
21. Tsang, N., R. Macnab, and D. E. Koshland, Jr. 1973. Common mechanism for repellents and attractants in bacterial chemotaxis. Science **181**:60–63.
22. Tso, W., and J. Adler. 1974. Negative chemotaxis in *Escherichia coli*. J. Bacteriol. **118**:560–576.
23. Vogel, H. J., and D. M. Bonner. 1956. Acetylornithinase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218**:97–106.