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# Identification of the Ribose Binding Protein as the Receptor for Ribose Chemotaxis in *Salmonella typhimurium*†

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ABSTRACT: The ribose binding protein of *Salmonella typhimurium* has been shown to be the receptor for ribose chemotaxis by thermodynamic, kinetic, specificity, and genetic correlations. D-Allose, which binds about 1000 times less tightly than ribose, can compete with ribose for the receptor and can also serve as an attractant. Transport or metabolism of ribose is not required or correlated with the chemotactic response. The biological response correlates qualitatively and quantitatively with the properties of the pure isolated receptor protein.

Receptors are the initial component of sensory systems and they have the specificity characteristics of protein molecules. Whether in the taste and odor responses of man or the chemotactic responses of bacteria, the discrimination between highly similar structures is characteristic of enzyme and antibody specificities. In bacterial chemotaxis, Adler has established by specificity studies that bacteria have chemoreceptors and that *Escherichia coli* specifically responds to ribose (Adler, 1969). Hazelbauer and Adler (1971) showed that the binding protein for galactose transport (Anraku, 1968; Kalckar, 1971; Boos, 1972) was the receptor for galactose. Binding proteins for ribose (Aksamit and Koshland, 1972) and maltose (Adler *et al.*, 1973) have also been identified with chemotaxis.

Receptors for transport are not necessarily receptors for chemotaxis, however, since neither histidine nor lysine is a significant attractant or repellent but both have active transport systems and specific binding proteins which are released upon osmotic shock (Ames and Lever, 1970, 1972; Mesibov and Adler, 1972; Rosen, 1971).[1]

The receptors of bacteria seemed to be of particular value since they could be isolated in a pure state, and are subject to genetic manipulation. Moreover, their properties as pure proteins can be compared to the biological response of the whole organism. In this paper some of the properties of the ribose binding protein of *Salmonella* and its relation to chemotactic behavior are examined.

## Materials and Methods

3-*O*-Methylribose and 3-deoxyribose were prepared by acid hydrolysis of the corresponding adenine nucleosides. 3-*O*-Methyladenosine and 1,5-anhydroribitol were gifts of Dr. C. A. Dekker and Dr. Robert Barker, respectively. [1-$^{14}$C]Ribose was obtained from New England Nuclear and had a specific activity of 49.9 Ci/mol. All other reagents were obtained from commercial sources.

*Soft Agar Chemotaxis Assay.* A gradient was formed on a soft agar plate containing 0.3% agar in VBC (Vogel–Bonner medium containing citrate (Vogel and Bonner, 1956)) by applying a disc containing 20 μmol of ribose to the center of the plate. The bacteria were applied 2.5 cm from the center of the disc by placing one drop of a suspension in exponential growth on the plate or by spotting directly from another plate using a wooden stick. Chemotactic bands could be observed after 1 hr. When spotted directly from a plate, the bacteria were allowed to begin growth on the soft agar plate for 12 hr at 30° and then the disc of attractant was applied. A major advantage of this assay is that 20 different mutants per plate can be screened for chemotactic bands and for motility as indicated by the diameter of the swarm. Figure 4 shows an example of four motile mutants tested for chemotaxis. Other types of soft agar assays have been described (Adler, 1966, 1973).

*Mutant Selection. S. typhimurium* ST1, a wild type with good motility and chemotaxis, was mutagenized by the frameshift mutagen ICR-191 (Ames and Whitfield, 1966) and screened for ribose fermentation on MacConkey plates containing 0.5% ribose (Difco Manual, 1953). White colonies were picked, purified, and tested for growth on glucose, glycerol, arabinose, and uridine by the radial streak method (Ames and Roth, 1968). Those mutants which grew nor-

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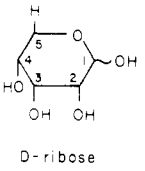
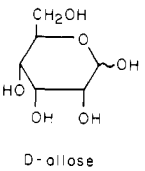
[1] Tso and Adler (1974) have recently shown that histidine is a mild repellent in *E. coli*.




D-ribose




D-allose

| Position | Ribose Modification | Saccharide | Binding to Ribose Binding Protein |
|---|---|---|---|
| C1 | -OH replaced by | | |
| | -H | 1,5-anhydroribitol | No |
| | O | | |
| | O-P-O⁻ | ribose-1-P | No |
| | O⁻ | | |
| C2 | epimer | D-arabinose | No |
| | -OH replaced by | | |
| | -H | 2-deoxy-D-ribose | No |
| C3 | epimer | D-xylose | No |
| | -OH replaced by | | |
| | -H | 3-deoxy-D-ribose | No |
| | $-OCH_3$ | 3-O-methyl-D-ribose | No |
| C4 | epimer | L-lyxose | No |
| C5 | -H replaced by | | |
| | $-CH_2OH$ | D-allose | Yes |

FIGURE 1: Specificity of ribose binding protein. D-Ribose and D-allose are presented in the pyranose form. Binding to the ribose binding protein was measured by competition with D-[1-$^{14}$C]ribose as described in the text.

mally on the above carbon sources, but poorly on ribose, were then tested for chemotaxis by the soft agar chemotaxis assay or by the capillary assay (Adler, 1973). *P22 int4* phage grown on each of the isolated mutants was used to transduce an *ilv A* mutant to prototrophy on plates without isoleucine.

The presence of ribose binding protein was determined by antigenic activity using a modification of the method of Lengeler *et al.* (1971). Mutants were grown in 5 ml of VBC to about $5 \times 10^8$ cells/ml, harvested by centrifugation, and incubated with 50 $\mu$l of 0.5 mg/ml of lysozyme, 10 mM $Na_2EDTA$, and 30 mM Tris (pH 8.0) at 25°. After 30 min 10 $\mu$l of toluene–chloroform (1:1, v/v) was added and the suspension shaken at 37° for 60–90 min. Lysozyme treatment ensured complete lysis and was necessary to give reproducible results. The suspensions were pipetted directly into the wells of an Ouchterlony plate (Ouchterlony, 1949) with rabbit antiserum to the ribose binding protein in the center well.

*Other Methods.* Chemotaxis was measured quantitatively by the capillary assay described by Adler (1973), using a chamber formed from a glass tube (0.5 × 3.7 cm) and suspending the capillary in the center of the tube by an appropriate adapter. The bacteria were grown in VBC at 30° to about $10^8$ cells/ml and immediately diluted to $5 \times 10^6$ bacteria/ml with VBC at 30° for the assay. Chemotaxis to ribose is the same whether the bacteria are grown on citrate or ribose, since it was found that the same amount of ribose binding protein is synthesized during growth on either carbon source. Osmotic shock was performed as described previously (Nossal and Heppel, 1966; Aksamit and Koshland, 1972). Ribose binding activity (Aksamit and Koshland, 1972) and histidine binding activity (Lever, 1972) were quantitatively measured by a filter paper assay.

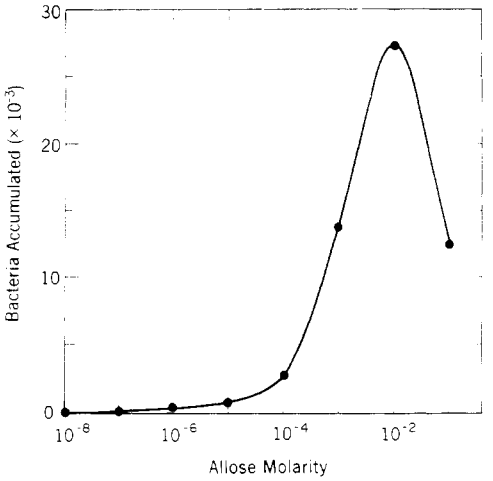



FIGURE 2: Capillary response curve of ST1 to allose. The capillary containing allose was incubated in a bacterial suspension containing $5 \times 10^6$ bacteria/ml at 30° for 30 min. Background was 484 bacteria/capillary.

The purity of the allose used was tested by thin-layer chromatography using butanol–ethanol–$H_2O$ (50:32:18) as a solvent and a $AgNO_3$ detection system. In addition a sample of allose was chromatographed and the areas corresponding to allose and ribose were removed and eluted with water. These fractions were then tested for inhibition of the ribose binding assay under conditions in which a 0.1% impurity of ribose could have been easily detected. Only the area which corresponded to allose inhibited the binding assay indicating that ribose contamination was insignificant.

## Results

*Correlation between Specificity of Binding Protein and of Chemotactic Response.* In a previous study, Aksamit and Koshland (1972) indicated that none of a large number of ribose analogs were bound by the ribose binding protein. Additional studies have shown that allose will inhibit the binding of ribose to the ribose binding protein. Using the per cent inhibition of ribose binding measured by the filter assay, a dissociation constant for allose of $3 \times 10^{-4}$ M was calculated. Further studies showed that 1 mM 3-*O*-methyl-

TABLE I: Capillary Assay Parameters for Ribose and Allose Chemotaxis.[a]

| Attractant | Initial Significant Response (M) | Maximum (M) | Response at Maximum $\left(\frac{\text{bacteria}}{\text{capillary}}\right)$ | % Total Bacteria at Maximum Response |
|---|---|---|---|---|
| Ribose (ST29) | $1 \times 10^{-7}$ | $3 \times 10^{-6}$ | 42,000 | 1.7 |
| Ribose (ST1) | $1 \times 10^{-7}$ | $1 \times 10^{-4}$ | 41,000; 55,000 | 1.6, 2.2 |
| Allose (ST1) | $3 \times 10^{-5}$ | $1 \times 10^{-2}$ | 28,000 | 1.1 |

[a] Data for calculations are taken from Figures 2, 3, and 5.

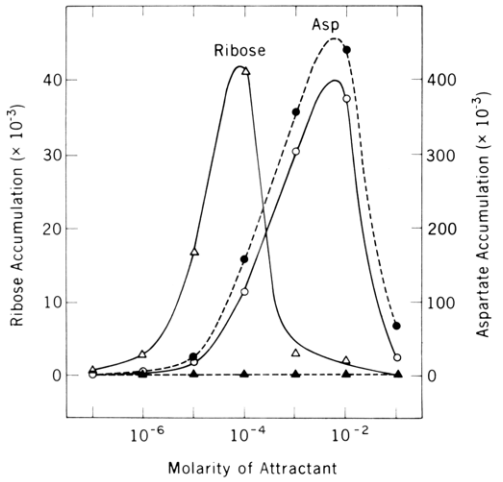



FIGURE 3: Capillary response of ST48 and ST1 to ribose and aspartate. The capillary was incubated in a bacterial suspension containing 5 × $10^6$ bacteria/ml at 30° for 30 min. (- - -) Response of ST48 (which is missing a functional ribose binding protein) to ribose (▲) or aspartate (●); (—) response of ST1 (wild type) to ribose (△) or aspartate (○). Backgrounds were *ca.* 850 bacteria/capillary.

ribose, 3-deoxyribose, L-lyxose, ribulose, talose, *myo*-inositol, or 1,5-anhydroribitol did not inhibit the binding of 1 μM ribose to the pure ribose binding protein (Figure 1).

The chemotactic response curves of ST1 (wild-type) to allose is shown in Figure 2. The response curve for allose occurs at concentrations which are approximately 1000 times higher than the concentrations for ribose. This is in excellent agreement with the dissociation constants which are 3 × $10^{-7}$ M for ribose and 3 × $10^{-4}$ M for allose. The number of bacteria which enter the capillary at the optimal concentration of attractant is the same within experimental error for ribose and allose (Table I). This suggests that the fraction of the receptors occupied is the primary factor in determining the magnitude of the response and that conformational changes induced by the ligand must be similar for ribose and allose. Chemotaxis to 1 mM allose in the capillary was completely eliminated by including 1 mM ribose in both the capillary and medium, providing additional evidence that allose is binding at the ribose receptor.

*Isolation of Mutants.* Thirty mutants of ST1 were isolated using two separately mutagenized cultures. The mutants grew slowly on 0.5% ribose plates, but normally on glucose, glycerol, arabinose, and uridine. Since uridine is metabolized to form ribose-5-P (Kammen, 1967) only permease and kinase mutants should be obtained. All of the 30 mutants were cotransducible with *ilv A* by *P22 int4* transduction. Cotransduction frequencies of 31% (12/39), 32% (10/31), 40% (16/40), and 42% (18/43) were found for four of the mutants which lacked the ribose binding protein. A close linkage of *ilv* and *rbsP* (ribose permease) and *rbsK* (ribokinase) has been found in *E. coli* using *P1* transduction (Anderson and Cooper, 1970; Taylor and Trotter, 1967).

All of the mutants were tested for ribose binding protein by the antibody test and for ribose chemotaxis using the capillary assay. Four mutants failed to give a positive antibody test and only these four mutants failed to display chemotaxis toward ribose. These four mutants which apparently did not contain ribose binding protein were subjected to osmotic shock and no ribose binding activity was released into the shock fluid.

The chemotactic response of one such mutant, ST48, to ribose and aspartate is shown in Figure 3. ST48 does not respond to a ribose or allose gradient, but responds normally to an aspartate gradient. Thus the absence of an identifiable ribose binding protein specifically eliminates the bacterial response to ribose but not to other attractants.

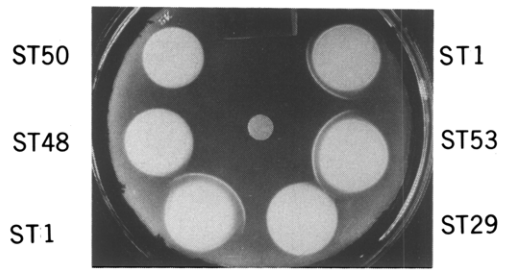



FIGURE 4: Soft agar chemotaxis assay. A drop of bacterial suspension was applied to a 0.3% soft agar plate as described in the Materials and Methods section. ST1 and ST53 (a revertant of ST48) are wild type for ribose growth and ribose chemotaxis. ST29 and ST48 are deficient in ribose growth and do not show chemotactic bands. ST50 grows normally on 0.05% ribose plates, but does not contain the ribose binding protein.

Spontaneous revertants of ST48 which have regained the ability to grow normally on 0.05% ribose were isolated by plating ST48 on 0.05% ribose agar. Two different classes were found. The first class appeared to be true revertants which had regained the ribose binding protein, normal ribose chemotaxis, and normal ribose growth (Figure 4). Capillary assays of these revertants show response curves identical with wild type. The second class did not contain ribose binding protein by the antibody assay or by assaying the shock fluid for ribose binding activity. Although these bacteria grew normally on ribose, they did not regain ribose chemotaxis when grown on ribose or citrate as measured by the capillary assay or by the soft agar chemotaxis assay (Figure 4). The number of independent revertants of the first class was 3 and of the second class, 173.

One explanation for the latter mutants which are missing the ribose binding protein, but grow normally on ribose, is that a second ribose permease which is inducible has been mutated so that it is now produced constitutively. There is precedent for this since two ribose permease systems, one constitutive and one inducible, and an inducible ribokinase have been found in *E. coli* by David and Wiesmeyer (1970). Anderson and Cooper (1970) have isolated mutants of *E. coli* with a constitutive ribose permease.

This raises the question as to why the ST48 mutant fails to grow normally on ribose. Lacking the high affinity transport system, for which ribose binding protein is the recognition component, ribose can only enter the cell by diffusion or by utilizing a small percentage of a low affinity transport system. The entry of ribose at low concentrations (less than 0.1%) is not sufficient for the accumulation of an internal concentration of ribose which will induce a second high affinity ribose transport system and ribokinase. This predicts that these ribose binding protein mutants should grow at high ribose concentrations where the entry rate would be sufficient to accumulate an internal concentration of ribose that would induce the remaining ribose permease. In agreement with this prediction, ST48 grows after an initial lag period at the same rate as ST1 in liquid minimal media containing 0.4% ribose as a sole carbon source. Ribose uptake studies using standard Millipore filtrating techniques confirmed that ST48 does not accumulate significant concentrations of ribose at an external concentration of 3 × $10^{-5}$ M.

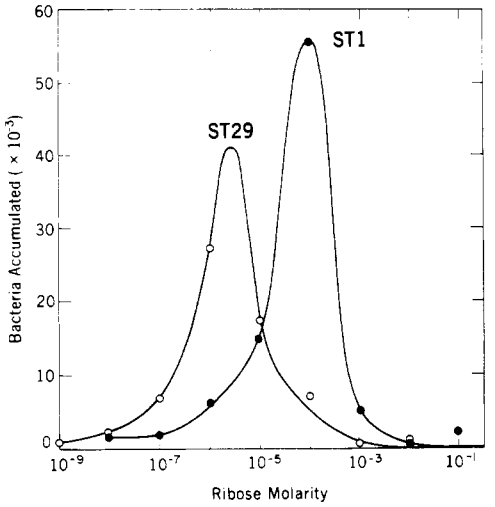



FIGURE 5: Capillary response curve of ST1 and ST29. The capillary was incubated in a bacterial suspension containing 5 × $10^6$ cells/ml at 30° for 30 min (○——○) ST29 is a mutant defective in ribose growth; (●——●) ST1 (wild type). Backgrounds were (bacteria/capillary): 250 for ST1 and 550 for ST29.

*Effect of Ribose Utilization.* Most of the mutants obtained after screening the ICR-191 mutagenized culture on ribose MacConkey agar were defective in ribose growth, but contained normal amounts of ribose binding protein. The chemotactic properties of one of them, ST29, was examined in detail. ST29 was osmotically shocked and the ribose binding activity was the same as ST1 and had the same dissociation constant.

ST29 does not show a chemotactic band to ribose using the soft agar chemotaxis assay as shown in Figure 4. However, when analyzed by the capillary assay, the mutant is chemotactic toward ribose (Figure 5). The failure of ST29 to form chemotactic bands by the soft agar chemotaxis assay suggests that metabolism of the gradient is required for bands to form in confirmation of Adler's postulation of travelling bands in chemotaxis of *E. coli* (Adler, 1966, 1973). Apparently the gradient formed by diffusion from the disc is not sufficient for chemotaxis and the bacteria which are able to grow on ribose respond to the much stronger gradient formed by utilization of the attractant.

A shift in the response curve of ST29 occurs relative to the ST1 wild type. The former which cannot metabolize ribose responds between $10^{-8}$ and $10^{-3}$ M ribose in the capillary assay whereas the latter responds between $10^{-7}$ and 3 × $10^{-3}$ M. A similar shift has been observed by Adler (1969, 1973) using a mutant in which galactose metabolism was reduced.

The reason for a shift in the response curve can be explained by considering what happens in the capillary assay at the lowest and highest attractant concentrations that give a detectable response. At the lowest concentration wild-type bacteria at the mouth of the capillary will utilize the ribose as it diffuses from the capillary, and therefore those bacteria at a distance from the capillary will not sense the gradient. Hence, the apparent threshold should be lower when ribose utilization is reduced. At the highest concentration the ribose receptor is nearly saturated at the mouth of the capillary; and the bacteria do not migrate into the capillary. A reduction in ribose utilization by the bacteria should result in the bacteria becoming saturated at lower concentrations of attractant. Thus the overall capillary response curve in a ribose utilization mutant, such as ST29, should be shifted to lower concentrations of attractant.

TABLE II: Effect of Sucrose and Ribose Shock Upon the Release of Ribose Binding Activity.[a]

| Suspension Medium | Ribose Binding Protein Released (nmol of ribose bound/g of dry weight cells) |
|---|---|
| 20% sucrose | 12.5 |
| 8.75% ribose | 0 |

[a] ST1 was grown on VBC at 30° and osmotically shocked using sucrose or ribose as the suspension medium. Stage II of the osmotic shock was concentrated by vacuum dialysis, dialyzed against 150 mM NaCl and 10 mM sodium phosphate (pH 6.0), and assayed for ribose binding activity.

*Release of Ribose Binding Protein. Salmonella* ST1 was grown at 30° in VBC and subjected to osmotic shock using 20% sucrose and an equal osmolarity, 8.75%, of ribose. The results are shown in Table II. There was no ribose binding activity released in cells shocked with ribose. To assess whether or not ribose was actually stabilizing a ribose binding protein-membrane complex, the shock was repeated using 20% sucrose and 1 mM ribose as the shock medium. Although 1 mM ribose has little effect on the osmolarity of the solution, it is sufficient to completely saturate the ribose binding protein (Aksamit and Koshland, 1972). L-Arabinose (20%) was used as a control. The results are presented in Table III. The presence of ribose binding protein was determined by reaction with antiserum to the ribose binding protein in Ouchterlony plates. In this experiment, the release of ribose binding protein is dependent upon the presence of sucrose. A much higher osmolarity of D-ribose or L-arabinose did not release the ribose binding protein although similar amounts of protein were released as measured by uv absorption. Most importantly, the inclusion of ribose in the 20% sucrose shock did not significantly reduce the amount of binding protein released.

These results are interesting in relation to those of Nossal

TABLE III: Effect of Osmotic Shock by Sugars upon the Release of Ribose Binding Protein.[a]

| Suspension Medium | Presence of RBP as Indicated by Antigenic Activity |
|---|---|
| 20% sucrose | ++++ |
| 20% sucrose + 1 mM D-ribose | ++++ |
| 20% D-ribose | 0 |
| 20% L-arabinose | 0 |

[a] ST1 was grown on VBC at 30° and osmotically shocked using the indicated suspension medium. When 1 mM ribose was included in the sucrose suspension medium, 1 mM ribose at 0° was used as the shock medium. In all other cases water at 0° was the shock medium. The shock fluid was analyzed for ribose binding protein (RBP) by the double diffusion technique of Ouchterlony using antiserum prepared against the purified ribose binding protein.

TABLE IV: Release of Ribose Binding and Histidine Binding Activity.[a]

| | Concentrate | | | | |
|---|---|---|---|---|---|
| | SA722 | R60 | R67 | R71 | R104 |
| | Ribose Binding Activity | | | | |
| Culture medium | 0.8 | 4.0 | 0.3 | 22.8 | 0.3 |
| Stage I of osmotic shock | 0.0 | 0.0 | 0.0 | 1.7 | 0.0 |
| Stage II of osmotic shock | 20.0 | 0.1 | 4.1 | 0.8 | 7.6 |
| | Histidine Binding Activity | | | | |
| Culture medium | 0.11 | 0.37 | 0.14 | 0.55 | 0.0 |
| Stage I of osmotic shock | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Stage II of osmotic shock | 0.94 | 0.37 | 0.56 | 0.06 | 0.86 |

[a] Values are expressed as nmoles bound/g of dry weight bacteria. Cells were grown on 0.4% ribose at 30° and subjected to osmotic shock. The culture medium, stage I, and stage II were concentrated by vacuum dialysis, dialyzed against 150 mM NaCl and 10 mM sodium phosphate (pH 6.0), and assayed for binding activity.

and Heppel (1966) in which 0.4 M NaCl could replace 20% sucrose in the shock procedure, suggesting that osmotic pressure was the most important element. Apparently sucrose has some specific effects such as changing the cell wall, or dissociating the ribose binding protein in the periplasmic space.

Several mutants were obtained from R. A. Weigand and L. Rothfield which were characterized by the ability to excrete the periplasmic enzymes ribonuclease and cyclic phosphodiesterase into the media during growth without releasing the cytoplasmic enzyme, glucose-6-phosphate dehydrogenase (R. A. Weigand and L. Rothfield, unpublished results). Four strains, which contain different mutations, and the parental strain were grown on 0.4% ribose containing auxotrophic requirements for 24 hr at 30° and shocked with 20% sucrose. The media and shock fluids (both stage I and stage II) were assayed for ribose binding protein and histidine binding protein. The results are summarized in Table IV. Ribose chemotaxis was not measured since the parental strain was not strongly chemotactic to ribose.

Two of the strains, R60 and R71, released ribose binding protein into the medium with less than 10% additional binding activity released into the shock fluid. The other two strains, R67 and R104, behaved like the parent.

The pattern of release of binding protein into the medium and by osmotic shock was the same as for histidine binding activity except for strain R60. In this strain 50% of the total histidine binding activity was recovered in the media and 50% was released in the shock fluid. The results for histidine binding activity are in contrast to previous strains, R18 and R19, in which the amount of histidine binding activity in the shock fluid of the mutants was higher than the amount of histidine binding protein in shock fluid of the parental strain (Lopes *et al.*, 1972).

The results of the experiments with the mutants which leak periplasmic proteins into the media support a model in which the ribose binding protein is in the periplasmic region or is only loosely bound. Ribose shows no effect in stabilizing the binding protein to the membrane or cell wall during osmotic shock.

## Discussion

*Identification of the Ribose Binding Protein as the Receptor in Ribose Chemotaxis.* The identification of the ribose binding protein as the receptor in the chemotactic response to ribose appears to be complete. The specificity of the purified protein to ribose analogs is the same as the chemotactic response. The quantitative relationship of binding between allose and ribose are the same as the quantitative alterations in the chemotactic response curves. Mutants which lack the ribose binding protein fail to chemotax to ribose even though they respond to other attractants and have unimpaired motility. Finally revertants were obtained in which the chemotactic response to ribose was regenerated as was the reappearance of the normal ribose binding protein. Thus, it appears that the monomeric protein of 29,000 molecular weight isolatable by sucrose shock procedures is the receptor on the bacterial membrane which provides the first component of the chemotactic response.

*Relation of Transport and Chemotaxis.* The chemotactic response is apparently not dependent on a particular transport scheme even though the binding protein apparently is the primary component in both chemotaxis and transport. Thus ST48, a mutant lacking the binding protein, had altered transport as well as altered chemotaxis, as found by Adler in the galactose system of *E. coli*. However, the parallel disappearance of chemotaxis and transport could allow the possibility of a relationship, *e.g.*, that chemotaxis depends on differential concentrations inside and outside the membrane, or changes in the internal pool of ribose. The fact that the mutants such as ST50 had a second ribose transport system eliminated these possibilities. Since these mutants could grow normally on ribose they would have differential ribose concentrations inside and outside the cell and their internal ribose pool would be sensitive to the external ribose concentration. Yet they failed to respond chemotactically to ribose because they lacked the ribose chemotaxis receptor. Moreover the mutant which could not utilize ribose, but did chemotax to ribose, had different inside-outside ratios from the wild type. Hence it appears certain that chemotaxis and transport are quite separate response systems even though they may share a common binding protein. This supports the similar conclusion of Adler for the galactose binding protein of *E. coli* on the basis of other types of evidence.

Mutants lacking the ribose binding protein are cotransducible with *ilv A* as are the ribose permease and ribokinase mutants of *E. coli* (Anderson and Cooper, 1970; Taylor and Trotter, 1967). This suggests further that the ribose binding protein is the recognition component for one ribose permease.

*Specificity of the Ribose Binding Protein and the Chemotactic Response.* The specificity of the ribose receptor is summarized in Figure 1. It appears that all of the hydroxyls of the ribose are important in the binding process. Moreover, the finding that allose is an inhibitor indicates that the effective stimulant is the pyranose form of ribose, not the furanose form.

It is of considerable interest that the specificity of the chemotactic response is so similar to the binding pattern of the pure protein. Since binding studies were made on the pure protein in aqueous solution, a receptor buried in a hy-

drophobic membrane might be expected to have quite different binding properties. Yet the chemotactic response occurs in the range indicated by the dissociation constant of the binding protein, and the ribose analogs which fail to bind to the pure protein fail to elicit the biological response (Aksamit and Koshland, 1972). Moreover, allose which is bound less poorly shows a quantitatively parallel shift in binding to the pure protein and the biological response. Hence it appears probable that the binding site of the ribose receptor is in an essentially aqueous environment even if the other end of the molecule is embedded in the membrane. A very loose association of the protein with the membrane is suggested since it is readily released by osmotic shock. From the yield of pure protein it can be calculated that there are approximately $10^4$ ribose binding protein molecules per bacterium.

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