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Properties of the Galactose Binding Protein of *Salmonella typhimurium* and *Escherichia coli*†

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ABSTRACT: The galactose binding protein implicated in transport and in chemotaxis has been purified to homogeneity from the shock fluids of *Salmonella typhimurium* and *Escherichia coli.* Both proteins are monomers of molecular weight 33 000 and exhibit cross-reactivity with antibody. The *Salmonella* galactose receptor showed binding of 1 mol of [^{14}C]galactose or 1 mol of [^{14}C]glucose at saturation. The dissociation constants were 0.38 and 0.17 μM, respectively. In light of the previously published report that the *E. coli* protein contains two binding sites with two different affinities, the binding characteristics of this protein were reexamined. Using highly purified radiolabeled substrate and homogeneous protein, a single binding site and single binding affinity were seen for galactose (K_D = 0.48 μM) or for glucose (K_D = 0.21 μM). The competition between glucose and galactose for the same site is intriguing in view of the competition between ribose and galactose at the receptor level.

Binding proteins for many different substances such as inorganic ions (Pardee, 1966, 1968; Medveczky and Rosenberg, 1970), sugars (Anraku, 1968a,b; Schleif, 1969; Boos, 1974), and amino acids (Penrose et al., 1968; Ames and Lever, 1972) have been isolated from bacteria by osmotic shock and have been implicated in the mechanisms for transport and chemotaxis. The galactose binding protein (GBP)[1] was first isolated from *E. coli* by Anraku (1968a,b) and was identified as one of the components of the β-galactoside permease system (Anraku, 1968a,b; Boos, 1969). GBP was also identified by genetic studies as the galactose chemoreceptor by Hazelbauer and Adler (1971). The purified protein has been studied and was found to bind 2 mol of galactose per mol of protein at two independent but nonequal sites (Boos and Gordon, 1971). The possible functions of such a dual affinity system were discussed in subsequent papers (Boos et al., 1972; Silhavy et al., 1974).

In the case of *Salmonella typhimurium,* a ribose receptor was isolated by Aksamit and Koshland (1974) which had a molecular weight similar to that of other binding proteins, but which binds only 1 mol of ribose per mol of protein. In the course of studying the properties of this protein in its interactions with galactose, Strange and Koshland (1975) demonstrated that there was competition between the galactose receptor and the ribose receptor in the signalling systems of *S. typhimurium* and *E. coli.* The galactose receptor of *Salmonella* was then isolated and preliminary results indicated that only one molecule of galactose was bound per molecule of protein. In view of the similarities of *E. coli* and *Salmonella,* the importance of this receptor interaction phenomena, and the apparent discrepancy between the two systems, the galactose binding proteins from both *Salmonella* and *E. coli* were purified and their properties investigated. The results are reported in this paper.

Materials and Methods

General. All chemicals were of the highest purity available. Radiolabeled sugars were obtained from Amersham/Searle and were routinely purified by preparative thin-layer chromatography on silica gel in 1-butanol–ethanol–water, 7:4:2. The concentration of radiolabeled sugar was assayed using the method of Park and Johnson (1949). *Salmonella typhimurium* ST1 is a derivative of LT2 and was obtained as described previously (Aksamit and Koshland, 1974). *E. coli* W3092, a strain constitutive for the galactose binding protein, was obtained from Clement Furlong.

Liquid scintillation counting was carried out in aquasol–toluene, 2:1 (10 ml; New England Nuclear) on a Packard TriCarb 3320 liquid scintillation spectrometer. Protein was determined by the method of Lowry et al. (1951) using bovine serum albumin as a standard. All chromatography, diaflow concentrations, and dialyses were carried out at 4 °C and pH's and conductivities were determined at 25 °C.

Purification of Galactose Binding Protein. For purification of the galactose binding protein from *Salmonella typhimurium,* 135 l. of ST1 were grown at 30 °C in Vogel–Bonner citrate based medium (Vogel and Bonner, 1956) to a cell density of about 8 × 10^8 cells/ml. Bacteria were harvested using a steam-driven Sharples continuous centrifuge after the growth medium had been preconditioned by the additions of molar Tris-HCl buffer, pH 7.3, and molar NaCl to final concentrations of 33 mM. The cells were then osmotically shocked ac-

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[1] Abbreviations used: GBP, galactose binding protein; DEAE, diethylaminoethyl; Tris, tris(hydroxymethyl)aminomethane; Temed, *N,N,N',N'*-tetramethylethylenediamine.

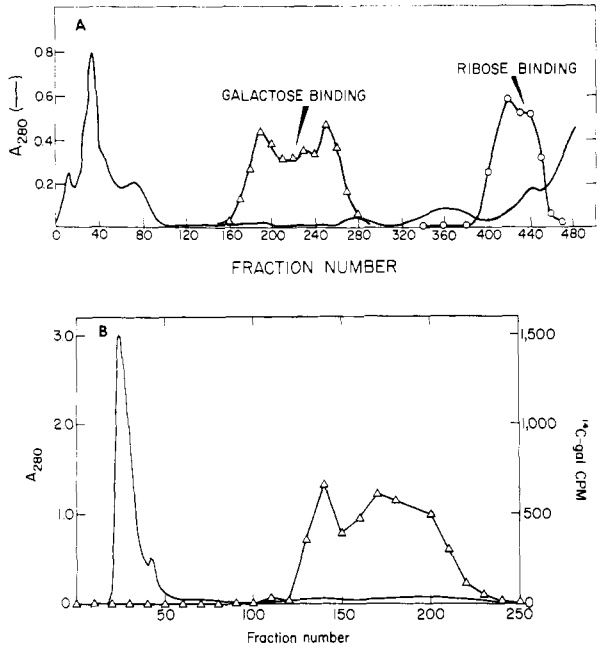

FIGURE 1: Initial purification of galactose binding protein from shock fluid. (A) Shock fluid from *S. typhimurium* was applied to a 6 × 40 cm column of sulfoethyl-Sephadex (a cation exchanger) at 4 °C. Protein was eluted with 10 mM NaCl–30 mM phosphate buffer, pH 5.05, and then a linear gradient of 5 l. of 30 mM phosphate, pH 5.05, from 10 to 120 mM NaCl. Fractions were assayed for protein concentration (—) by determination of absorbance at 280 nm and for galactose and ribose binding activity (△) by the nitrocellulose filter assay. Counts of [^{14}C]galactose bound for each 80 μl of eluate are recorded. (B) Same procedure applied to shock fluid of *E. coli*.

cording to the procedure of Willis et al. (1974). The shock fluid (2 l.) was treated with antibacterial agent (1,1,1-trichloro-2-methylpropanol) and concentrated to 60 ml by ultrafiltration using an Amicon Diaflo apparatus (UM 10 membrane, 50 psi). The concentrated shock fluid was centrifuged to remove any cells and dialyzed against 600 volumes of 10 mM NaCl, 30 mM phosphate, pH 5.05. The total protein (about 600 mg) was fractionated by cation-exchange chromatography using a sulfoethyl-Sephadex column equilibrated with the same buffer at 4 °C. The column was eluted with 1 l. of starting buffer and then a linear gradient of 5 l. of 30 mM phosphate, pH 5.05, from 10 to 120 mM NaCl. Fractions were assayed for ribose and galactose binding activities using the nitrocellulose filter assay (Lever, 1972).

The pooled galactose binding fractions were concentrated by ultrafiltration to 30 ml and dialyzed against 600 volumes of 10 mM Tris-HCl buffer, pH 8.35 at 4 °C. The protein (approximately 23 mg) was loaded on a DEAE-cellulose (Whatman DE 52) column equilibrated in the same buffer. After elution at 4 °C with 10 column volumes of starting buffer, a linear gradient of 50 column volumes of 0 to 150 mM NaCl in 10 mM Tris-HCl buffer, pH 8.35, was applied at a rate of 0.3 ml/min. The galactose binding protein was assayed by polyacrylamide gel electrophoresis and by the filter assay. The fractions containing GBP activity were pooled once more, concentrated to 15 ml, and dialyzed against 600 volumes of 10 mM sodium phosphate buffer–150 mM NaCl, pH 6.0 (PSS6). The dialyzed protein was stored with a few crystals of antibacterial agent at 4 °C.

Galactose binding protein was isolated from *E. coli* W3092 by the above procedure except that 30 l. of Tryptone medium (Boos, 1972) supplemented with 1.0% glucose was inoculated with 10 l. of log phase bacteria and the bacteria were harvested at late log phase (after about 8 h growth).

Polyacrylamide Gel Electrophoresis. The homogeneity of the galactose binding protein was assessed and its molecular weight was estimated by polyacrylamide gel electrophoresis. Gels were either 10% polyacrylamide discontinuous slab gels containing 0.4% sodium dodecyl sulfate, prepared according to the procedures of Laemmli (1970) and Ames (1974), or 7.5% native polyacrylamide slab gel. The latter was prepared with Tris-HCl buffer, pH 8.7, acrylamide, methylenebisacrylamide and polymerized with ammonium persulfate and *N,N,N′,N′*-tetramethylethylenediamine (Temed). Fairly large samples (5–10 μg) of protein were run to assess homogeneity.

Molecular Weight Determination by Analytical Ultracentrifugation. The molecular weight of the galactose binding protein purified from *S. typhimurium* was determined by sedimentation equilibrium analysis using a Beckmann Model E analytical ultracentrifuge. Samples of the protein were dialyzed extensively against physiological saline buffer, pH 6.0, at 4 °C for 72 h. They were then centrifuged at 32 000 rpm, 4.7 °C for 25 h, to ensure that equilibrium had been reached and were then visualized by means of a photoelectric scanner recording at 280 nm.

Amino Acid Analyzer. The amino acid composition of the *S. typhimurium* and *E. coli* galactose binding proteins was determined using a Beckman Model 120C amino acid analyzer. Molecular weights of 33 000, determined by sedimentation equilibrium for both proteins, were used.

Filter Assay. A rapid estimate of galactose binding activity was made by filter assay as described by Lever (1972) using Schleicher and Schuell B-6, 0.45-μm nitrocellulose filters. Samples were buffered in 10 mM phosphate–150 mM NaCl, pH 6.0, and 100-μl samples were filtered. The samples were washed with 600 μl of deionized water and the filters dried at 110 °C for 10 min. For competition binding studies, the appropriate concentration of competing sugar was included in the incubation mixtures. The nonspecific binding of radiolabeled sugar to the filters was always tested whenever different label concentrations were used.

Equilibrium Dialysis. Galactose binding activity for all binding studies was determined by equilibrium dialysis with the apparatus described previously (Englund et al., 1970).

Results

I. Purification and Composition of the Galactose Binding Proteins. The galactose binding proteins from *S. typhimurium* and *E. coli* were isolated from shock fluid by conventional chromatographic techniques as described in the Materials and Methods section. Bacteria were osmotically shocked in both cases using the modified procedure of Willis et al. (1974), and the shock fluid was fractionated on an SE-Sephadex column which separated galactose and ribose binding activities well (Figures 1A and 1B). Further fractionation of the galactose binding activity from the SE-Sephadex column was achieved in each case using DEAE-cellulose (Figures 2A and 2B). For both protein preparations, elution from the DEAE-cellulose column yielded a homogeneous preparation of the protein as judged both by polyacrylamide gel electrophoresis and ultracentrifugation. Both techniques gave single sharp bands without trace of impurities. Polyacrylamide gels for the purification of the *S. typhimurium* galactose receptor are shown in Figure 3.

In the case of *S. typhimurium* ST1, 5 mg of the purified protein was isolated from 250 g of wet weight cells. In the case

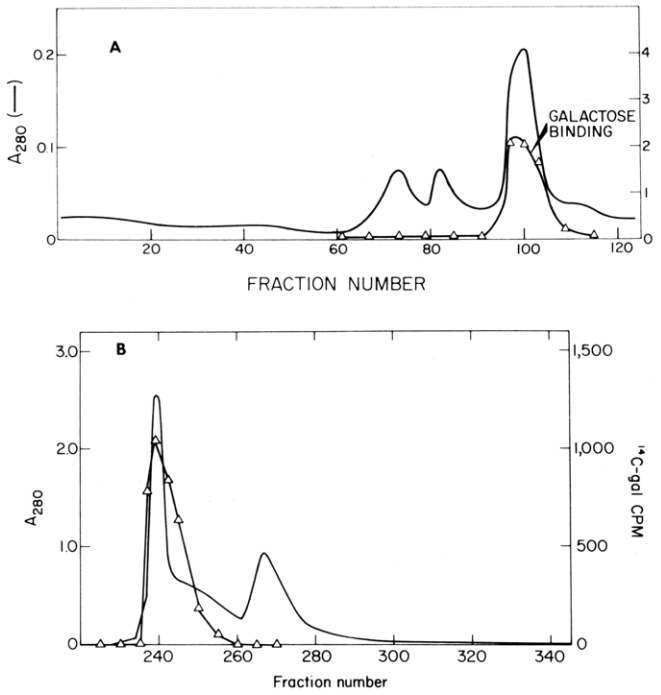

FIGURE 2: Further purification of galactose binding protein. (A) Galactose binding fractions from sulfoethyl-Sephadex fractionation of *Salmonella typhimurium* were placed on DEAE-cellulose (1.5 × 90 cm) columns at 4 °C and were eluted with 10 mM Tris-HCl buffer, pH 8.35, and then with a linear gradient of 0–150 mM NaCl in the same buffer. Fractions were assayed for protein concentration (—) by absorbance at 280 nm and for galactose binding activity (Δ) by polyacrylamide gels and the filter assay. (B) Same procedure for *E. coli* protein.

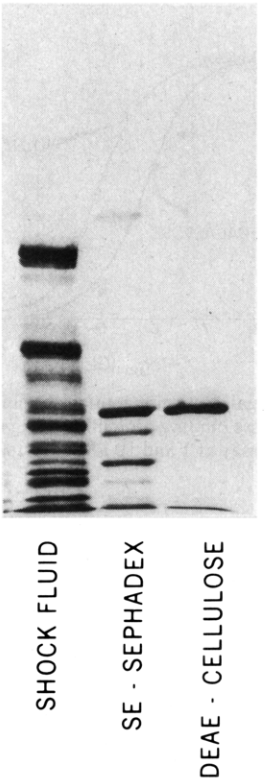

FIGURE 3: Electrophoretic analysis during purification of galactose binding protein. Polyacrylamide gel electrophoreses of samples representing initial, middle, and final stages in the purification of the galactose binding protein from *S. typhimurium* are presented. Electrophoresis was performed using 10% polyacrylamide discontinuous slab gels containing 0.4% sodium dodecyl sulfate, prepared according to the procedures of Laemmli (1970) and Ames (1974), at 30 mA for 1.5 h. Gels were stained with Coomasie brilliant blue. (Left) Concentrated shock fluid, obtained by osmotic shock of ST1 as described in Materials and Methods, dialyzed against 10 mM NaCl–30 mM phosphate, pH 5.05; (center) pooled galactose binding fractions from the sulfoethyl-Sephadex column dialyzed against 10 mM Tris-HCl buffer, pH 8.35; (right) pooled galactose binding fractions from the DEAE-cellulose column, dialyzed against 10 mM sodium phosphate buffer–150 mM NaCl, pH 6.0. Stained line at bottom of figure is solvent front.

TABLE I: Amino Acid Compositions of Some Transport Binding Proteins and Chemotactic Receptors.

Amino Acid	*S. typhimurium* Ribose Binding Protein[a] (mol/29 000 g)	*S. typhimurium* Galactose Binding Protein[a] (mol/33 000 g)	*E. coli* Galactose Binding Protein[c] (mol/35 000 g[b])	*E. coli* Arabinose Binding Protein[d] mol/38 000 g)
Lys	26.0	31.7	27.3	29.6
His	3.0	3.1	3.0	2.7
Arg	5.8	6.0	5.7	8.4
Asp	35.6	50.0	43.4	34.2
Thr	12.2	12.9	12.2	17.9
Ser	8.0	13.3	11.0	14.4
Glu	26.1	29.0	25.2	38.4
Pro	8.2	10.3	8.0	17.9
Gly	23.7	22.0	19.4	33.1
Ala	36.7	39.0	37.7	36.1
Val	25.5	25.9	26.1	33.8
Met	4.9	5.7	5.2	11.4
Ile	12.0	13.3	13.2	16.0
Leu	24.0	22.7	21.2	23.6
Tyr	2.7	7.6	5.0	7.6
Phe	6.7	6.0	5.8	16.0
Cys	0	0	0	1.7
Trp	0	1.0	3.5	7.0

[a] Threonine was corrected for 5% loss; serine was corrected for 10% loss; and methionine was corrected for a 30% loss. Tryptophan was detected by UV absorbance and by fluorescence. [b] Data originally reported as residues/100 residues were converted to residues/35 000 daltons, the molecular weight reported for *E. coli* GBP by these workers. [c] Anraku, 1968b. [d] Parsons and Hogg, 1974a.

of *E. coli* 3092, about 25 mg of the purified protein was obtained from 125 g of wet weight cells. These values correspond to approximately 10^3 and 10^4 molecules of galactose receptor per *Salmonella* and *E. coli* bacterium, respectively. The presence of fewer receptors in *Salmonella* is consistent with a diminished response in this bacterium, as compared with that of *E. coli*.

The molecular weights of the galactose binding proteins from both *S. typhimurium* and *E. coli* were estimated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (Weber and Osborn, 1969). The molecular weight of the *Salmonella* protein is estimated by this method to be 33 000. This value was corroborated by equilibrium ultracentrifugation analysis, which also shows the protein to be monomeric over the range of 0.5–1.0 μM. The molecular weight of the *E. coli* protein is estimated here to be 33 000 also, which may be compared with the previously reported value of 36 000 (Anraku, 1968b).

The amino acid compositions of the *Salmonella* galactose binding protein, the *E. coli* galactose binding protein, and other binding proteins are shown in Table I. It is interesting to note that the compositions of the galactose binding proteins from *E. coli* and *Salmonella* are very similar. The *Salmonella*

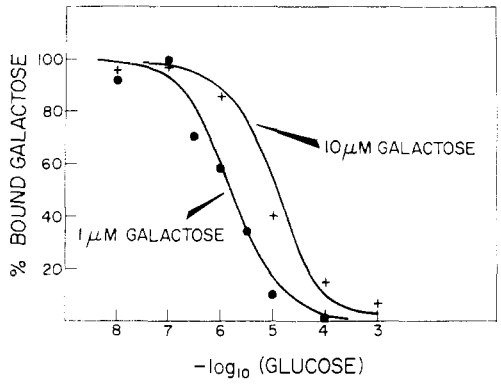

FIGURE 4: Glucose–galactose competition for binding site of purified *S. typhimurium* galactose binding protein. Binding was determined by the nitrocellulose filter assay at 1 and 10 μM galactose concentrations.

TABLE II: Binding of Saccharides to the Purified Galactose Receptor of *S. typhimurium*.

Substrate	Dissociation Constant (M)
Galactose	2×10^{-7} [a,b]
Glucose	10^{-7} [a,b]
Arabinose	4×10^{-5} [b]
Lactose	6×10^{-4} [b]
Fucose	6×10^{-3} [b]
Methyl galactoside	Not detectable
Ribose	No binding[b]
Allose	No binding[b]

[a] Direct. [b] By competition.

TABLE III: Galactose and Glucose Binding to the *E. coli* and *S. typhimurium* Galactose Receptors (Determined by Equilibrium Dialysis at 4 °C).[a]

Protein	Substrate	Dissociation Constant (M) × 10^7	Maximum Moles Bound per Mole of Protein
S. typhimurium GBP	Galactose	3.8×10^{-7}	0.9
	Glucose	1.7×10^{-7}	0.7
E. coli GBP	Galactose	4.8×10^{-7}	0.9
(pH 8.0)	Galactose	4.3×10^{-7}	0.9
	Glucose	2.1×10^{-7}	1.0

[a] Protein concentration, 2 μM, pH 6.0 unless otherwise indicated.

protein contains no SH groups, consistent with the composition of the *E. coli* protein.

II. Specificity and Binding Study. Binding of sugars to the purified galactose binding protein of *Salmonella typhimurium* was observed by the nitrocellulose filter assay, either directly or indirectly by competition studies, or by equilibrium dialysis. The results of the filter assay studies are shown in Table II. Galactose and glucose are shown to bind strongly, whereas D-arabinose, D-lactose, and D-fucose bind more weakly, and D-ribose not at all.

The results of one key competition study are shown in Figure 4. Galactose at two different concentrations was subjected to competition by glucose. The results are fit by a simple binding competition between galactose and glucose for a single site.

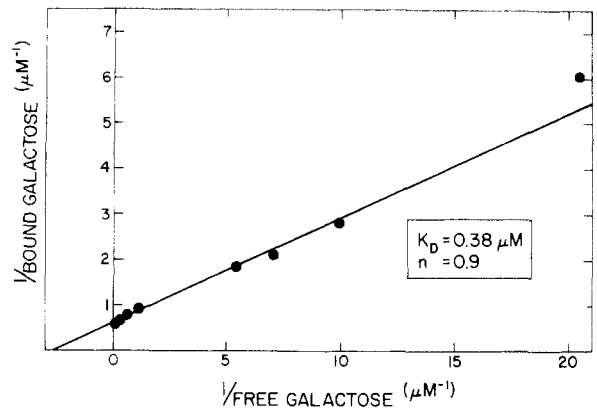

FIGURE 5: Equilibrium dialysis measurement of galactose binding to *S. typhimurium* galactose binding protein. Double chambers separated by dialysis tubing were filled with 250 μl of galactose binding protein (0.06 mg/ml) in physiological saline buffer, pH 6.0, and 250 μl of repurified [1-^{14}C]galactose in the same buffer. The dialysis was performed at 4 °C for at least 16 h. Fifty microliters from each chamber was counted for radioactivity.

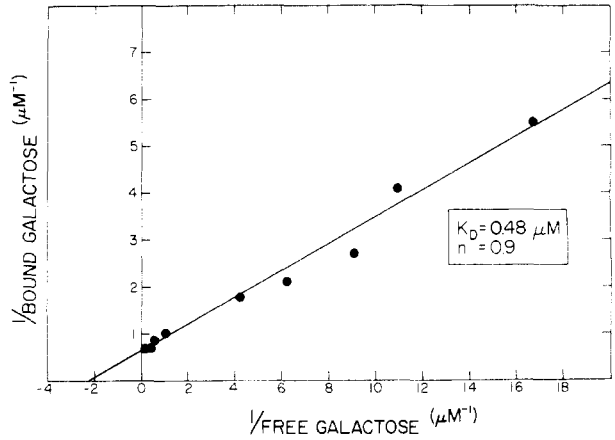

FIGURE 6: Equilibrium dialysis measurement of galactose binding to the *E. coli* galactose binding protein. Chambers were filled with protein and repurified [1-^{14}C]galactose as described in Figure 5 with galactose binding protein concentration of 0.35 mg/ml. Inset is expanded view of lower left-hand corner of double-reciprocal plot.

Results of equilibrium dialysis for galactose (Figure 5) and glucose in protein concentrations of 2 μM or higher gave linear double-reciprocal plots for the *Salmonella* protein. In both cases the data are fit by single-binding affinities which are summarized in Table III. Glucose binds more strongly than galactose to this protein, as indicated by the dissociation constants of 0.17 and 0.38 μM, respectively. Previous studies had shown glucose binds more strongly (Hazelbauer and Adler, 1971; Boos et al., 1972; Silhavy et al., 1974). There is no evidence in either our direct binding studies or the indirect competition studies of more than 1 mol of galactose bound per mol of protein.

These results were somewhat surprising in view of the reports of two binding sites per mole of protein for the *E. coli* protein. The binding of galactose to the purified *E. coli* protein was, therefore, reexamined with both repurified glucose and repurified galactose at several protein concentrations. Typical data are shown in Figures 6 and 7. The possibility of a discrepancy caused by the concentration of protein was examined carefully and the protein concentration varied from 1 to 12 μM. Variation in the pH was also studied. The K_d for galactose was 0.48 μM at pH 8.0 (Table III). K_d for binding of glucose at pH

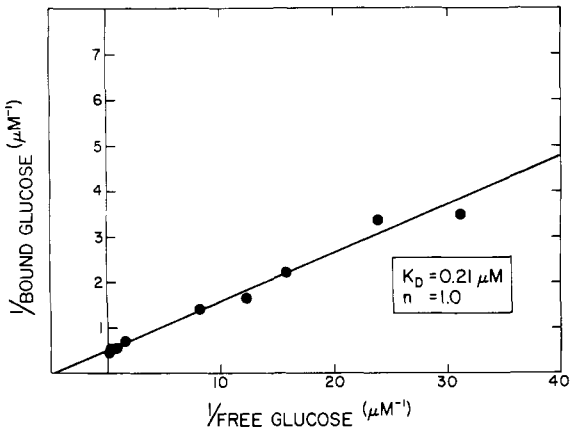

FIGURE 7: Binding of glucose of *E. coli* galactose binding protein. Equilibrium dialysis carried out as described in Figure 5 at protein level of 0.06 mg/ml.

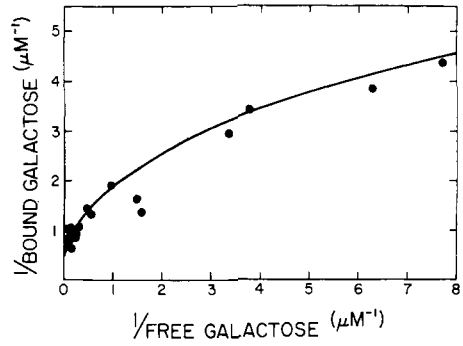

FIGURE 8: Binding of commercial galactose to galactose binding protein of *S. typhimurium*. Galactose binding to the *S. typhimurium* galactose binding protein carried out precisely as in Figure 5, except that commercial [1-^{14}C]galactose was used without further purification.

6.0 (Figure 7) was 0.21 μM. In all cases and conditions, the pattern of one galactose or glucose bound per mole of protein was obtained. The possibility that the biphasicity previously observed for galactose binding to the *E. coli* protein (Boos and Gordon, 1971) was a result of a monomer–oligomer equilibrium which had been examined and excluded by these workers.

III. Explanation of the Reported Two Binding Sites. The conflict beween the above results and the previously reported findings of two binding affinities and two binding sites for the *E. coli* galactose binding protein (Boos et al., 1972) was examined carefully. If the final purification of the galactose binding protein by DEAE-cellulose chromatography is omitted, we find a small protein impurity in either *Salmonella* or *E. coli*. Such an impurity could account for some deviation from linearity in the double-reciprocal binding plots. More significantly, we find that the radiolabeled galactose substrate obtained commercially contains a substrate-like impurity which gives highly artifactual binding curves. The results in Figure 8 show the binding of galactose to the *Salmonella* protein in an experiment identical with that described in Figure 5 with the exception that [^{14}C]galactose obtained from Amersham/Searle without further repurification was used. A clear curvature in the double-reciprocal binding plot is seen. The results in Figure 8 are strikingly similar to those reported earlier by Boos et al. (1972) for galactose binding to the *E. coli* protein, a result which led to the suggestion of two sugar binding affinities and two binding sites per mole of protein. Such curvature is to be expected if a radioactive impurity in the [^{14}C]galactose substrate binds more tightly than galactose to the binding protein. Thin-layer chromatography of the [^{14}C]galactose obtained from Amersham/Searle showed an impurity which migrates very close to galactose at about a 10% level. The present results, therefore, indicate that there is only one chemoeffector site per protein molecule and that the previous reports were caused by artifacts in the measuring process.

The *E. coli* maltose binding protein has been reported (Hazelbauer, 1975) to exhibit two binding affinities. In light of our present findings of artifactual curvature in double-reciprocal binding plots, reexamination of the maltose protein results would seem indicated.

Discussion

The galactose binding protein appeared to be unique among the binding proteins of *E. coli* in containing two binding sites per molecule of 36 000 molecular weight (Boos et al., 1972). Arguments were developed that this dual binding capacity had certain advantages for the organism; such arguments raised interesting questions in regard to the lack of two binding sites for the other proteins (Boos et al., 1972). The present results indicate that the galactose binding protein is not unique and that, in fact, it only contains a single substrate binding site per mole of protein, as do the other analogous proteins. The reason for the discrepancy as indicated by these studies is probably an impurity in the substrate used in the original binding studies. It appears that all the periplasmic binding proteins that have been purified have a single binding site for the chemoeffector.

A comparison of the binding proteins indicates a general similarity in molecular weight and amino acid composition. All the proteins appear to be quite high in content of alanine, glycine, valine, leucine, aspartate, and glutamic acids and quite low in histidine, methionine, and cystine. In fact, the galactose binding proteins of *Salmonella typhimurium* and *E. coli* are completely lacking in cystine residues and the same is true of the ribose binding protein of *Salmonella typhimurium*. The amino acid compositions of the *Salmonella* galactose binding protein and the *E. coli* galactose binding protein are strikingly similar, the most notable discrepancy being the presence of 3.5 tryptophan residues per mole of protein in the case of the *E. coli* protein and only one tryptophan per mole of protein in the case of the *Salmonella* protein. A later study showed 5 tryptophans per protein molecule for the *E. coli* galactose protein (McGowan et al., 1974). A general similarity of these amino acid compositions to that of the *E. coli* arabinose binding protein is also observed. Cross-reactivity has been observed between antisera directed against the *E. coli* galactose and arabinose-binding proteins (Parsons and Hogg, 1974b). Nevertheless, there appears to be more similarity in antigenicity in proteins from the different bacterial strains than between analogous proteins in the same strain. Thus we find that the galactose binding proteins of *Salmonella* and *E. coli* cross-react with antibody, whereas the ribose binding protein and galactose binding protein of *Salmonella* fail to cross-react with the same antibody.

The tighter binding of glucose to both the *Salmonella* galactose binding protein and the *E. coli* galactose binding protein is noteworthy since this protein is programmed in the β-methyl galactoside operon and is believed to be a galactose transporting protein (Boos, 1969). In addition, glucose transport in *E. coli* and a chemotactic receptor have been identified with part of the phosphotransferase system (Kundig and Roseman, 1971; Adler and Epstein, 1974). Chemotaxis

can also occur through the "galactose binding protein." This undoubtedly explains the residual activity of glucose transport when the PTS system is inactivated (Adler and Epstein, 1974).

This finding of glucose and galactose competition adds a further interest to the regulation patterns of the biological systems. In a previous paper from this laboratory, Strange and Koshland (1975) showed that galactose and ribose can compete with one another in the *Salmonella typhimurium* and *E. coli* chemotaxis systems by competition between the corresponding receptors. In that case, it was clear that ribose did not bind to the galactose receptor and galactose did not bind to the ribose receptor. Rather, each induced a conformation change in their respective receptors which allowed the receptors to bind to a common third component identified as component I in the signalling system. In contrast, glucose and galactose compete with each other for the same site on the same receptor protein.

Obviously, direct competition of two substrates for the same site is the most economical way of controlling the total level of two substrates which are structurally similar. If glucose and galactose provide the same function for the cell, then a cell which is saturated in its needs for glucose would not need galactose. Hence it is quite logical that the two compete for the same site and thus eliminate excessive intake of the superfluous compound. Such a mechanism can only be achieved readily if the two sugars are very similar in structure, as in the case of glucose and galactose, where inversion of a single carbon atom converts one sugar to the other. Ribose and galactose are not nearly so similar and yet they may each provide a source of carbon to the cell of approximately equal value. In that case, saturation with one sugar can turn off intake of another sugar by the device alluded to above, i.e., competition of the receptor molecules for a third component in limiting supply. This second mechanism is obviously more expensive in terms of protein synthesis, but it is also appreciably more general. In this case the structures of the chemoeffectors are irrelevant in the final competition since the competition occurs between proteins. A site for substrate and a mechanism to induce the proper conformational change are all that are needed. A substrate–substrate competition mechanism is, therefore, more economical but a receptor–receptor competition mechanism is appreciably more general.

Note Added in Proof

Dr. Gerald Hazelbauer has kindly informed us that recent work has indicated that the maltose binding protein has only one binding site for maltose.

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