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Purification and Properties of the Periplasmic Glucose-Binding Protein of *Pseudomonas aeruginosa*

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A glucose-binding glycoprotein (GBP) from the periplasm of *Pseudomonas aeruginosa* was purified to homogeneity as judged by polyacrylamide gel electrophoresis, molecular sieve chromatography, and double-diffusion gel precipitation. It had an average molecular weight of 44,500 and an isoelectric point of 4.7. One mole of glucose was bound per mole of GBP with a dissociation constant of 0.35 μM. The binding of radioactive glucose by GBP was not significantly inhibited by 10-fold-higher concentrations of other carbohydrates; however, a number of related compounds were found to compete at 100-fold-higher concentrations. Amino acid analyses revealed predominant amounts of alanine, glutamate, and glycine and a low content of sulfur-containing amino acids. The carbohydrate moiety of GBP, comprising nearly 16% of the total weight, contained galactosamine, glucosamine, fucose, galactose, glucose, and mannose. A GBP-deficient mutant, strain MB723, was found to be defective in both membrane transport and glucose chemotaxis. Strain MB724, a revertant to GBP-positive phenotype, simultaneously recovered normal levels of both membrane functions.

Escherichia coli contains a number of binding proteins that are released from the periplasm by the osmotic shock procedure of Neu and Heppel (20). These proteins have been implicated in transport processes (4, 5) and in chemotaxis (2). In a previous study (29), we demonstrated that inducible binding proteins for C_4-dicarboxylic acids and for glucose could also be isolated from *Pseudomonas aeruginosa* by extraction with 0.2 M $MgCl_2$ and by an osmotic shock procedure that does not affect cell viability. The synthesis of the glucose-binding protein (GBP) and the glucose transport system were found to be co-regulated. In this report we describe the purification and properties of GBP. The properties of a *P. aeruginosa* mutant that lacks a fundamental GBP are also described. This mutant was defective in both glucose transport and chemotaxis.

MATERIALS AND METHODS

Chemicals. D-[*U*-^{14}C]glucose (200 mCi/mmol), methyl-α-D-[*U*-^{14}C]glucopyranoside (αMeGlc; 3 mCi/mmol), and ^{14}C-labeled amino acid mixture (52 mCi/matom of carbon) were purchased from Amersham/Searle Corp. Unlabeled αMeGlc was obtained from Pfanstiehl Laboratories, Inc. *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NG) was purchased from K & K Laboratories, Inc, Plainview, N.J. All other compounds were from local distributors and were analytical grade.

Bacteria. The bacterial strains used are listed in Table 1.

Growth conditions. Bacteria were grown in basal salts medium (29) containing 20 or 40 mM concentrations of the indicated carbon sources. The medium was supplemented with 0.5 mM tryptophan for the cultivation of tryptophan-requiring strains. Cultures (400 ml in 2-liter flasks) were incubated on a New Brunswick gyratory shaker at 30°C until late logarithmic growth, usually 16 to 18 h. Large-scale cultures (16 liters per 20-liter carboy) were grown at 30°C with continuous forced aeration. Growth was followed by measuring turbidity with a Klett-Summerson photoelectric colorimeter (no. 66 filter). Cultures with optical densities between 250 and 300 Klett units (2×10^9 cells/ml; 0.7 mg of cell dry weight/ml) were harvested by centrifugation at 22°C. Chemotaxis experiments were conducted with the mineral salts medium of Ordal and Adler (21).

Mutagenesis. Mutagenesis with NG followed the procedure of Adelberg et al. (1). Log-phase cells were suspended to 4×10^9/ml in 50 mM citrate buffer (pH 6.0) containing 100 μg of NG per ml and incubated at 30°C for 45 min. Cell survival under these conditions was approximately 10%. After mutagenesis, the bacteria were washed with saline and incubated in nutrient broth (Difco) for 4 h. The cells were then centrifuged and washed with and suspended in basal medium containing 40 mM concentrations of the appropriate carbon sources. Mutants defective in membrane-bound glucose dehydrogenase were selected by using a modification of the indicator medium described by Korman and Berman (16). Bacte-

TABLE 1. *P. aeruginosa strains*

Strain	Phenotype[a]	Derivation
MB720	Wild	ATCC 15692
MB721	Trp	From MB720 by NG[b]
MB722	TrpGdh	From MB721 by NG
MB723	TrpGdhTglc	From MB722 by NG
MB724	TrpGdh (Tglc revertant)	From MB723 by NG

[a] Symbols: Trp, tryptophan auxotroph; Gdh, glucose dehydrogenase deficient; Tglc, glucose transport deficient.

[b] NG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine mutagenesis.

ria were plated onto nutrient agar (Difco) (pH 7.0) containing 0.1% glucose and 0.005% bromothymol blue indicator and incubated at 37°C for 36 h.

Binding-protein extraction. GBP cell-free extracts were prepared from packed log-phase cells as previously described (29).

Cell extracts. Soluble and particulate cell fractions were prepared from log-phase cells as described in a previous report (29).

Binding assays. Equilibrium dialysis and nitrocellulose filtration assays were used to measure binding activity of GBP preparations. For quantitative determinations, the equilibrium dialysis assay was performed as previously described (29). The more rapid filtration assay was used primarily to monitor activity of various column fractions during GBP purification. Solutions of 1 to 50 μg of protein were incubated for 5 min at 22°C in 1.0 ml of 1 of mM $MgCl_2$–10 mM tris(hydroxymethyl)aminomethane (Tris)-hydrochloride (pH 7.5) (TM buffer) containing 1 μM [^{14}C]glucose (100 mCi/mmol). The mixture was rapidly filtered through a premoistened 24-mm filter (Millipore Corp., HAMK-02414) and then washed with 5 ml of TM buffer. The filter was removed while still under vacuum and transferred to a scintillation vial containing 1.5 ml of absolute ethanol. After the addition of 6 ml of scintillation fluid, radioactivity was measured in a Nuclear-Chicago Mark II spectrophotometer. The liquid scintillation solvent contained 4.0 g of 2,5-diphenyloxazole and 0.1 g of 1,4-bis-2(4-methyl-5-phenyloxazolyl)benzene per liter of toluene. One unit of binding activity is equivalent to 1 nmol of substrate bound. Specific activities are expressed as units per milligram of protein. A comparison of the two assay systems indicated that under standard conditions, the filtration assay reproducibly bound 35% of the GBP-glucose complexes determined by equilibrium dialysis.

Enzyme assays. Glucose dehydrogenase (EC 1.1.99A) was assayed spectrophotometrically by the procedure of Hauge (10). Glucokinase activity was determined by the method of Hylemon and Phibbs (13). One enzyme unit catalyzes the conversion of 1 μmol of substrate per min at 22°C. The specific activity is expressed as units per milligram of protein.

Transport assays. The uptake of αMeGlc by *P. aeruginosa* cells was measured as previously described (29).

Polyacrylamide gel electrophoresis. Polyacrylamide gel electrophoresis was conducted in a Canalco model 1200 apparatus at pH 8.3 according to the method of Davis (7). GBP samples (1 to 150 μg of protein) containing bromophenol blue dye and 5% sucrose or glycerol were layered onto 7.5% polyacrylamide gel columns (0.4 by 8.0 cm) and developed at 4 mA per gel. The current to each column was interrupted when the marker dye had migrated 7 cm into the gel. The gels were then fixed and stained with Coomassie brilliant blue. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis was performed according to the method of Weber and Osborn (31). Cytochrome *c* (12,400 daltons), chymotrypsinogen A (25,000 daltons), pepsin (35,000 daltons), ovalbumin (45,000 daltons), and bovine serum albumin (67,000 daltons) were used as molecular-weight standards. Gels were stained with Coomassie brilliant blue or periodic acid-Schiff reagent as described by Segrest and Jackson (25).

The isoelectric focusing of GBP was performed by the procedure of Wrigley (32). GBP (5 to 10 μg of protein) was mixed with 7.5% acrylamide solution containing 1% Ampholine carrier ampholytes, pH range 3.5 to 10 (LKB Instruments, Inc.). The resulting mixture was polymerized in glass columns (7 by 125 mm) and developed at 0.75 mA per gel for 6 to 8 h. The gels were removed, fixed, and stained with Coomassie brilliant blue as described above.

Chemical analysis of binding protein. Amino acid analyses were performed on a Beckman model 120C amino acid analyzer according to the method of Moore and Stein (19). The protein was hydrolyzed in sealed vials for 24 to 28 h at 105°C in 6 N HCl under an atmosphere of nitrogen. The mixture was evaporated to dryness at reduced pressure and dissolved in distilled water. Tryptophan was estimated spectrophotometrically in the unhydrolyzed protein (27).

Samples for the determination of sugar residues were hydrolyzed in 1 N HCl for 6 h at 100°C in sealed tubes. For neutral-sugar determinations, the hydrolysate was passed through coupled columns of Dowex 50-X4, H^+ (200/400 mesh) and Dowex 1-X8 formate (200/400 mesh). Analysis was performed by means of automated borate complex anion-exchange chromatography (28). To determine amino sugars, Dowex 50-X4-eluted material was examined, using a Beckman model 120C amino acid analyzer with a pH 5.0 sodium citrate gradient (28).

Motility and chemotaxis assays. Cell motility in nutrient broth cultures was examined by phase-contrast microscopy. More than 90% of all cell strains were actively motile and demonstrated frequent changes in direction.

Bacterial migration in soft-agar plates (21) was used as a measure of both motility and chemotaxis. Bacteria from a fresh nutrient agar slant culture were spotted with a wire needle onto 0.4% agar plate media (mineral salts medium plus 1 μM glucose or gluconate), which were then incubated at 37°C for 18 to 24 h.

Bacterial chemotaxis in vertical liquid gradients was performed in test tubes according to the general procedure of Aswad and Koshland (3). Bacteria were grown to mid-logarithmic phase in basal medium

containing 20 mM pyruvate, 10 mM αMeGlc, and 0.01% Casamino Acids. The culture was diluted with fresh medium to 2×10^5 cells/ml, and 1 ml of this suspension was transferred to a conical centrifuge tube. A mixture of ^{14}C-labeled amino acids (1 μCi; 52 mCi/matom of carbon) was added, and the suspension was incubated for 1 h at 37°C. The bacteria were centrifuged at 4,000 × *g* for 10 min, washed twice with 5 ml of swarm medium, and suspended in 1 ml of swarm medium. Phase-contrast microscopic examination indicated that approximately 50 to 70% of the bacteria were motile at this point. The radioactively labeled bacteria were then placed into a preformed glucose-glycerol gradient in swarm medium. First, 5 ml of gradient solution was pumped through a 6-inch (about 15-cm) stainless-steel hypodermic needle (20 gauge) that extended to the bottom of a cellulose nitrate test tube (100 by 16 mm). The glucose concentration was initially 1 mM and decreased linearly to 0, whereas glycerol increased linearly from 0 to 0.1 M. Next, 0.2 ml of the ^{14}C-labeled cell suspension containing 0.15 M glycerol was pumped in, followed by 5 ml of glycerol, which increased linearly from 0.2 to 0.3 M. The resulting gradient was incubated at 37°C for 90 min. The location of the cells in the gradient was determined by direct visual observation and by measuring radioactivity. For the latter procedure, the bottoms of the gradient tubes were punctured with a needle and 0.5-ml fractions were collected. Radioactivity was counted as described above.

Antiserum. Antisera were prepared in two adult New Zealand white rabbits. One milligram of partially purified GBP, obtained from Sephadex G-200 chromatography, was emulsified in Freund complete adjuvant (Difco) and injected intradermally at 1-week intervals for 5 weeks. The rabbits were bled before each injection, and precipitating antibody was checked qualitatively by double-diffusion gel precipitation.

Other assays. Protein was measured by the method of Lowry et al. (17), using crystalline bovine serum albumin as a standard.

RESULTS

Purification of glucose-binding protein. (i) Ammonium sulfate fractionation. GBP was extracted from 80 g (wet weight) of logarithmic-phase *P. aeruginosa* and concentrated as previously described (29). All purification steps were performed at 4°C. Ammonium sulfate (20 g) was added to the concentrated extract (50 ml, 5 mg of protein/ml) over a period of 1 h to bring the concentration to 65% saturation. After 60 min, the resulting precipitate was collected by centrifugation for 30 min at 27,000 × *g*. The supernatant fluid was adjusted to 95% saturation with 12 g of ammonium sulfate, and after 60 min the resulting precipitate was collected by centrifugation. The precipitates were dissolved separately in a small volume (5 to 20 ml) of TM buffer and dialyzed for 24 h against the same buffer. After dialysis, the dialysis bags contained precipitates, which were collected by centrifugation and discarded. The supernatant liquids from the 0 to 65% and the 65 to 95% ammonium sulfate fractions contained 8 and 92% of the glucose-binding activity, respectively. Specific activity was increased threefold in the latter fraction (Table 2).

(ii) Sephadex G-200 chromatography. The dialyzed 65 to 95% ammonium sulfate fraction (5 ml, 7 mg of protein/ml) was subjected to gel filtration on a Sephadex G-200 column (3 by 96 cm) that had previously been equilibrated with TM buffer. The fractions containing glucose-binding activity were pooled, concentrated by Amicon PM-10 ultrafiltration, and dialyzed overnight against TM buffer. A threefold purification was attained, with a 94% recovery (Table 2).

(iii) Polyacrylamide disc gel electrophoresis. The concentrated material from the Sephadex G-200 column was separated on preparative polyacrylamide gel columns (1.5 by 8.0 cm). The GBP component was identified in preliminary studies as follows. A gel was run in the standard manner, and a thin vertical slice of the gel was fixed and stained with Coomassie brilliant blue. The remaining unstained gel was sectioned into 3-mm lateral slices, which were then placed into separate test tubes containing 2 ml of TM buffer and held at 4°C overnight. The buffer baths containing eluted proteins were then assayed for GBP activity. The active region of the gel was matched with a band in the stained vertical slice. In subsequent preparative runs, a stained vertical slice was

TABLE 2. *Purification of glucose-binding protein*

Purification step	Vol (ml)	GBP (U)	Protein (mg)	Sp act (U/mg)	Yield (%)
Crude concentrated extract[a]	50	36	281	0.13	100
60-95% ammonium sulfate	11	32	65	0.41	88
Sephadex G-200	3	34	25	1.40	94
Preparative disc gel electrophoresis	2	22	1.4	16	61

[a] Extracted from 80 g of cell wet weight.

prepared from each gel column and used to identify the GBP-containing region of the remaining unstained gel. After sectioning, GBP was routinely eluted from the slices by suspending them in approximately 5 volumes of TM buffer at 4°C for 36 h, with three buffer changes. Traces of polyacrylamide gel were removed from the pooled GBP solutions by filtration (0.2 μm; Nucleopore Corp.). Finally, the preparation was dialyzed against TM buffer for 24 h. This preparation had a specific activity of 16 and was purified 123-fold over the concentrated crude extract (Table 2). The purified GBP fraction showed only one band when analyzed by polyacrylamide disc gel electrophoresis in the presence and absence of SDS (Fig. 1) and by agar gel double diffusion against rabbit anti-GBP extract serum (Fig. 2). Analyses by isoelectric focusing in polyacrylamide gels also showed a single protein band (data not shown).

Molecular weight. The molecular weight of the glucose-binding protein determined by SDS-disc gel electrophoresis (31) and by Bio-Gel P-100 gel filtration (5) was 44,000 and 45,000, respectively (Fig. 3).

pI. An isoelectric point of 4.7 was determined for the GBP, using electrofocusing separation in polyacrylamide gels with a pH gradient of 3.5 to 10.

Binding constant. The binding of [^{14}C]glucose as a function of substrate concen-

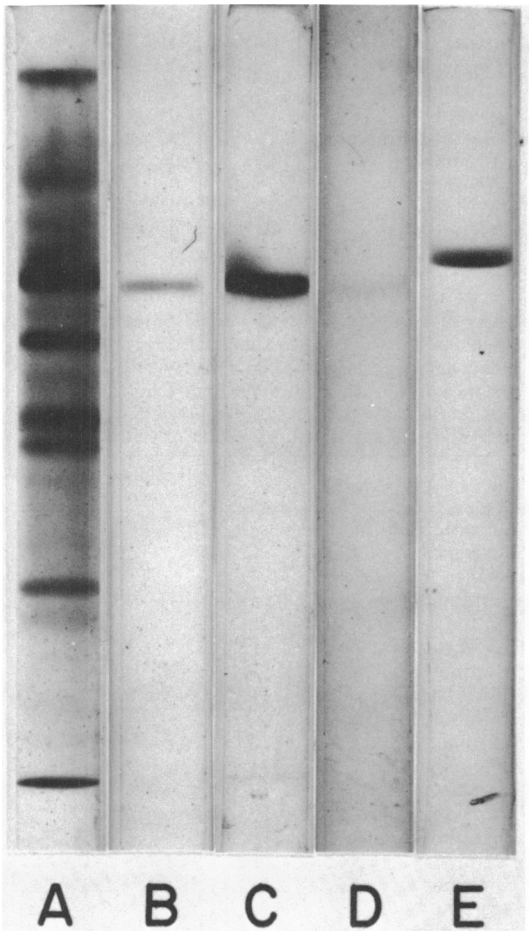

FIG. 1. *Polyacrylamide disc gel electrophoresis of GBP. Gels: (A) 100 μg of concentrated crude extract protein; (B) 2 μg of purified GBP; (C and D) 50 μg of purified GBP; (E) 10 μg of purified GBP in the presence of SDS. Gels A, B, C, and E were stained with Coomassie brilliant blue. Gel D was stained with periodic acid-Schiff reagent.*

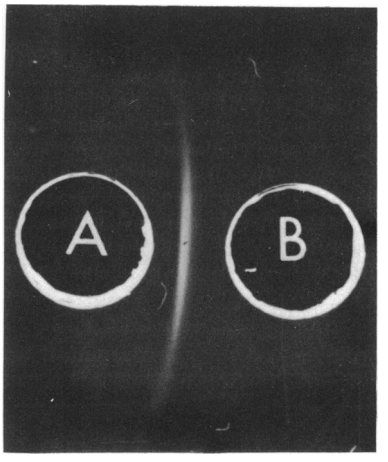

FIG. 2. *Double-diffusion gel precipitation. Wells: (A) Antiserum to partially purified GBP (from Sephadex G-200); (B) 4 μg of purified GBP.*

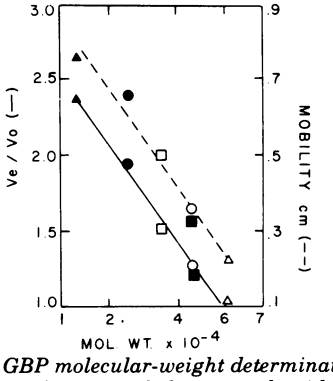

FIG. 3. *GBP molecular-weight determination. Ve/Vo indicates the ratio of elution and void volumes determined by P-100 gel filtration. Mobility signifies protein migration during SDS-polyacrylamide disc gel electrophoresis. Proteins: ▲, cytochrome c; ●, chymotrypsinogen A; □, pepsin; ■, ovalbumin; △, albumin; ○, GBP.*

tration was examined using equilibrium dialysis. The data plotted according to the method of Scatchard (24) indicated a K_D of 0.35 μM and that 1 mol of glucose was bound per mol of GBP (Fig. 4).

Binding specificity. The specificity of the GBP was investigated by measuring the ability of unlabeled carbohydrates to compete with the binding of [^{14}C]glucose in the filtration assay (Table 3). At 10-fold-higher concentrations of unlabeled competitor relative to [^{14}C]glucose, only unlabeled glucose was an effective inhibitor. At 100-fold excess, a number of sugars and glucose derivatives had significant affinity for the binding protein.

Effect of selected compounds on binding. The binding of glucose by GBP was unaffected by 10 to 100 mM concentrations of mono- and divalent cations, 10 mM ethylenediaminetetraacetic acid, and 1 mM sulfhydryl compounds or reagents. It was also unaffected by pH in the range of 6 to 10 or by alteration of ionic strength over the range of 0.001 to 0.6.

Stability. GBP activity was measured after heating 3 μg of protein in 0.5 ml of TM buffer at 100°C for various periods of time. The solution was quickly cooled to room temperature and assayed by membrane filtration. GBP was 75% inactive after 5 min and completely inactive after 30 min. The GBP was stored at −20°C for several months without loss of activity.

Chemical composition. The chemical composition of the GBP is shown in Table 4. The

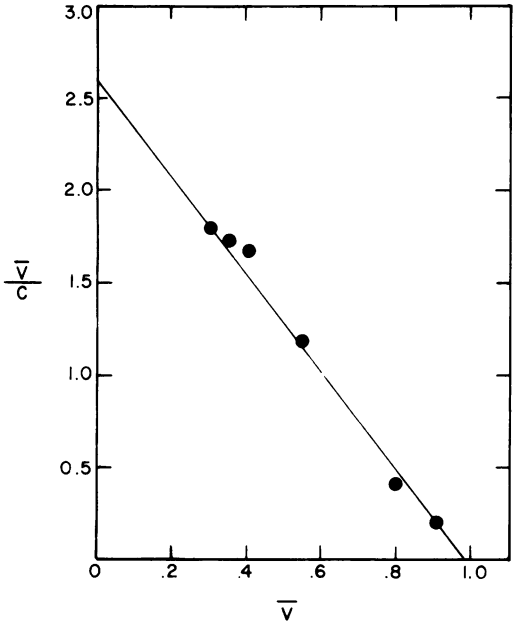

FIG. 4. *Scatchard plot of GBP activity. Binding assays were performed with 9 μg of GBP and varying amounts of glucose, $\bar{V}$ represents the moles of glucose bound per mole of protein. C signifies the concentration of glucose (micromolar concentration).*

TABLE 3. *Specificity of glucose-binding protein*[a]

Addition	Inhibition (%)	
	10 μM	100 μM
D-Glucose	88	97
α-Methyl-D-glucose	6	31
2-Deoxy-D-glucose	0	17
D-Glucose 1-phosphate	0	32
D-Glucose 6-phosphate	0	35
Gluconate	0	45
D-Fucose	0	47
Lactose	4	9
D-Galactose	0	23
D-Glucosamine	0	15
Glycerol	0	0
N-Acetyl-D-glucosamine	0	0
D-Fructose	0	5
L-Rhamnose	0	0
D-Mannose	0	8
D-Mannitol	0	1
L-Arabinose	0	6

[a] Activity was measured by membrane filtration assay of 4 μg of GBP with 1 μM [^{14}C]glucose in the presence of 10 or 100 μM concentrations of the unlabeled compounds. Binding activity of GBP in the absence of inhibitor was 6,590 cpm/4 μg of protein.

TABLE 4. *Composition of GBP*

Residue	Residues/100 amino acid residues
Aspartic acid	11.2
Threonine	4.1
Serine	5.4
Glutamic acid	13.0
Proline	5.2
Glycine	10.1
Alanine	14.7
Half-cystine	0.8
Valine	6.6
Methionine	0.3
Isoleucine	2.9
Leucine	7.3
Tyrosine	1.4
Phenylalanine	4.5
Lysine	7.1
Histidine	0.9
Arginine	2.1
Tryptophan	3.2
Glucosamine	3.1
Galactosamine	0.6
Fucose	2.0
Galactose	2.8
Glucose	2.3
Mannose	1.3

larger number of asparate and glutamate residues accounted for the acidic nature of the protein. Based on one residue of methionine per mole of GBP, a minimal molecular weight of 44,000 was calculated. Three half-cystine residues were detected per residue of methionine.

Of particular interest is the presence of neutral and amino sugars, constituting nearly 16% of the GBP. The carbohydrate moiety of GBP contained glucosamine, mannose, galactose, fucose, and glucose. A trace amount of galactosamine was also detected.

Since it was possible that a contaminating polysaccharide was being coincidentally purified with the GBP, we subjected the purified protein to Pronase treatment (100 μg of GBP plus 25 μg of Pronase for 24 h at 37°C). The digested protein was then examined by polyacrylamide disc gel electrophoresis. Under these conditions, no component was detected by either Coomassie brilliant blue or periodic acid-Schiff reagent.

Glucose transport mutant isolation. The strongest evidence linking periplasmic binding proteins to membrane transport in bacteria has been obtained with mutant strains that have lost the capacity to synthesize functional binding proteins. These mutants exhibit greatly reduced uptake of the homologous substrates. Moreover, revertants of these mutants simultaneously recover a functional protein and near-normal transport activity (4, 5). Two distinct mechanisms have been described for the transport of glucose by *P. aeruginosa*: the glucose-αMeGlc transport system (8, 9) and the gluconate transport system (18), which involves oxidation of glucose to gluconate by membrane-bound glucose dehydrogenase and the subsequent translocation of gluconate. To separate the two systems, a mutant defective in the membrane-bound glucose dehydrogenase (strain MB722) was isolated as a derivative of the tryptophan auxotroph MB721 after NG mutagenesis. Suitable dilutions of the mutagenized cell suspension were made in basal medium, and 0.1-ml portions were plated onto bromothymol blue indicator medium. The colonies that did not produce acid after 36 h of incubation at 37°C were selected and analyzed. One such colony (MB722) was isolated for further study. This mutant strain lacked glucose dehydrogenase (Table 5) but grew normally (2-h generation) on the glucose basal salts medium.

A glucose transport-deficient strain MB723 was isolated from strain MB722 by selection for loss of glucose chemotaxis and the ability to grow on gluconate but not on glucose. NG-mutagenized bacteria were grown to mid-log phase (100 Klett units, 5×10^8 cells/ml) in gluconate-basal medium at 30°C. Cells were harvested and suspended in an equal volume of basal salts medium containing 10 mM glucose and incubated at 30°C for 2 h. The cells were centrifuged, washed gently with chemotaxis medium at 22°C, and suspended to 10^8 cells per ml in the same medium. A 0.2-ml portion of the cell suspension was then placed in a vertical preformed liquid gradient for selection of motile nonchemotactic mutants as described by Aswad and Koshland (3). The bacteria unresponsive to the glucose attractant were harvested and grown overnight in gluconate-basal medium. The gradient enrichment procedure was repeated twice, allowing for turbid growth of the culture between each enrichment. Bacteria from the third gradient were plated onto a gluconate-basal salts medium and, after the appearance of visible colonies, replicated onto a glucose-basal salts medium. Strain MB723 was unable to grow on glucose but grew normally on gluconate (3-h generation) and was actively motile. Strain MB724 was selected from NG-mutagenized strain MB723 for its ability to utilize glucose as a sole source of carbon. This revertant grew normally on glucose-basal medium as compared with wild strain MB720.

Enzyme activity. Whole-cell extracts and membrane preparations from the mutant strains were examined for the presence of the metabolic enzymes glucose dehydrogenase and glucokinase (Table 5). Glucose dehydrogenase activity could not be detected in membrane preparations from MB722, MB723, or MB724.

Transport activity. Earlier studies (9, 29) have shown that the glucose transport system of *P. aeruginosa* is induced by the non-metabol-

TABLE 5. *Glucose dehydrogenase and glucokinase activities of P. aeruginosa mutant strains*[a]

Cell	Sp act	
	Glucose dehydrogenase	Glucokinase
MB721	0.05	0.12
MB722	0	0.09
MB723	0	0.09
MB724	0	ND[b]

[a] Soluble and particulate fractions from each mutant bacterium were assayed for glucokinase and glucose dehydrogenase, respectively. One enzyme unit catalyzes the conversion of 1 μmol of substrate per min at 22°C. Specific activity is the units of enzyme per milligram of protein.

[b] ND, Not determined.

izable glucose analog αMeGlc during growth on pyruvate. The ability of the various mutant strains to accumulate αMeGlc is shown in Fig. 5. Glucose-negative strain MB723 was unable to accumulate significant quantities of substrate. Strain MB722 and glucose revertant MB724 showed normal levels of active transport, as demonstrated by MB721.

GBP production. The ability of the various *P. aeruginosa* strains to synthesize GBP was determined with cells grown on pyruvate plus αMeGlc or on gluconate. (Table 6). The glucose transport mutant (MB723) did not produce detectable quantities of GBP in either medium. The failure to synthesize GBP when grown on αMeGlc was not expected since *P. aeruginosa* apparently does not have alternative uptake systems for glucose such as those present in *E. coli* (4, 22). Consequently, the cell may not be able to accumulate adequate quantities of the inducer. To circumvent this problem, we examined the synthesis of GBP by gluconate-grown cells. Wild-type cells grown on gluconate synthesized low but measurable levels of GBP. The inability of MB723 to synthesize GBP under these conditions, therefore, suggests that the binding protein is in some way linked to the glucose transport system.

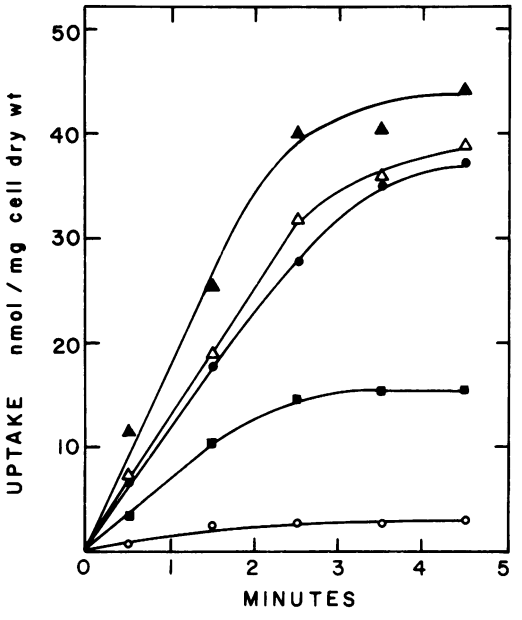

FIG. 5. *Uptake of αMeGlc by P. aeruginosa mutants. Bacteria were grown to logarithmic phase on mineral salts medium containing 20 mM pyruvate plus 10 mM αMeGlc. The cells were harvested, washed, and then suspended (1 mg of cell dry weight/ml) in mineral salts medium plus 5 mM ^{14}C-labeled αMeGlc. At various intervals, 0.05-ml samples were withdrawn, and the cells were collected and washed by membrane filtration. Radioactivity was determined by liquid scintillation spectrometry. Symbols: ▲, MB721; △, MB722; ○, MB723; ●, MB724; ■, MB721 grown on pyruvate only.*

Strain MB723 was examined for the production of an altered nonfunctional GBP, using specific rabbit anti-GBP serum. No component cross-reactive with GBP was detected in concentrated Mg extracts or in spent culture medium.

Chemotaxis. A number of binding proteins of *E. coli* have been shown to be involved in the phenomenon of chemotaxis (2). Loss of a binding protein results in impaired responses of the bacterium to gradients of the homologous attractant. The ability of the various mutants of *P. aeruginosa* to detect and migrate toward glucose was tested in two ways. Figure 6 shows chemotaxis in a semisolid medium containing glucose as the attractant. These conditions require that the bacteria create a gradient by metabolizing glucose as they swim outwardly from the site of inoculation. Since strain MB723 is unable to transport glucose, no chemotaxis was observed. This strain did demonstrate normal taxis when gluconate was substituted as the attractant (Fig. 6). The other strains, including revertant MB724, responded equally well to both attractants. Chemotaxis was also examined in preformed liquid gradients. It has been shown that motile gram-negative enteric bacteria utilize a temporal gradient-sensing mechanism (2, 3) that regulates directional swimming in preformed gradients. Under these environmental conditions, a bacterium with the proper receptor protein should exhibit positive chemotaxis independently of transport. Figure 7 shows the response of radioactively labeled bacteria when placed at the edge of a glucose gradient. Strain MB721 and glucose revertant MB724 moved up-gradient as visible bands migrating 1.5 cm in 90 min. Binding protein-deficient MB723 was not attracted, and

TABLE 6. *GBP production by P. aeruginosa mutant strains*[a]

Cell	Pyruvate + αMeGlc		Gluconate	
	U	Sp act (U/mg)	U	Sp act (U/mg)
MB721	1.2	0.52	0.11	0.02
MB722	1.0	0.48	0.10	0.06
MB723	0	0	0	0
MB724	1.3	0.35	0.12	0.03

[a] Bacteria were grown in 1.6 liters of basal salts medium containing 20 mM gluconate or 20 mM pyruvate plus 10 mM αMeGlc. GBP was isolated and assayed as previously described.

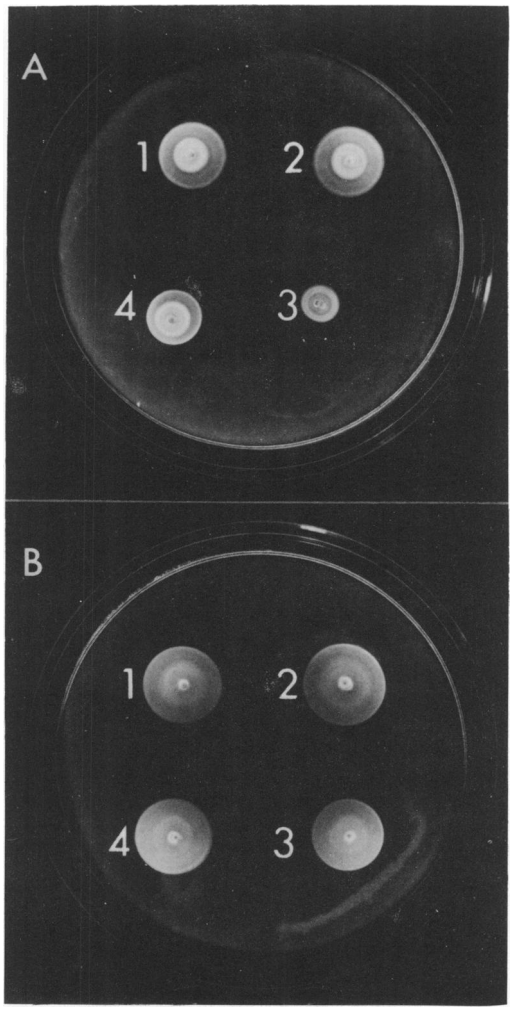

FIG. 6. *Chemotactic behavior of P. aeruginosa mutants in semisolid media. Bacteria were inoculated into mineral salts medium containing 0.4% agar, 0.1% tryptone, and 1 mM glucose (plate A) or 1 mM gluconate (plate B). The plates were incubated at 37°C for 24 h. Strains: (1) MB721; (2) MB722; (3) MB723; (4) MB724.*

its response resembled that of MB721 when no attractant was present. These observations indicate that GBP may function as the receptor protein for chemotaxis as well as for transport.

DISCUSSION

GBP of *P. aeruginosa* has been purified and characterized as a glycoprotein of 44,500 daltons. The presence of a carbohydrate moiety distinguishes the GBP from the previously described binding proteins of *E. coli* and *Salmonella typhimurium*. These latter periplasmic components have been characterized as homogeneous proteins with molecular weights between 22,000 and 42,000 (4). Cell envelope glycoproteins, however, have recently been detected in gram-negative bacteria. Kidroni and Weinbaum (15) have isolated several glycoproteins of undetermined function from both the inner and outer cell membranes of *E. coli*. The receptor protein for colicin E3 in the envelope of *E. coli* has been shown by Sabet and Schnaitman (23) to contain covalently linked carbohydrate. Binding activity of the glycoprotein was destroyed by periodate oxidation, suggesting that carbohydrate was required for receptor activity. Glycoproteins with enzymatic activity

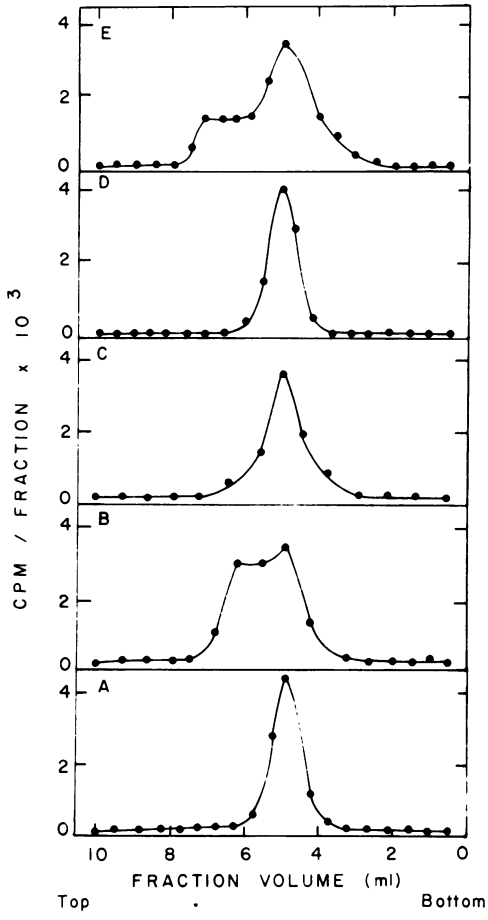

FIG. 7. *Chemotactic behavior of P. aeruginosa mutants in performed liquid gradients. Radioactively labeled bacteria were placed at the edge of a glucose gradient and incubated at 37°C for 5 or 90 min. Fractions were collected, and radioactivity was measured. Gradients: (A) MB721 at 5 min; (B) MB721 at 90 min; (C) MB721 at 90 min in the absence of glucose; (D) MB723 at 90 min; (E) MB724 at 90 min.*

have also been reported in gram-negative bacteria. *Pseudomonas fluorescens* produces cell-bound and extracellular cellulases that contain between 7 and 28% carbohydrate (33).

The presence of glycoproteins as binding components of cell surfaces has also been reported in eucaryotic cells. Two glycoproteins have been isolated from conidia of *Neurospora crassa* with high affinities for arginine (30). Synthesis of these binding proteins appeared to be requisite for arginine transport. Initial chemical characterization indicated a weight composition of approximately 50% carbohydrate in both glycoprotein components. More recently, Ashwell and associates (12, 14) have reported that glycoprotein components of the hepatic membranes of rabbit liver function in the clearance of serum glycoproteins. The carbohydrate moiety of the chemoreceptors is suspected of participating in the binding reaction at the cell surface, since enzymatic removal of terminal sialic acid residues from the isolated binding protein reduced its activity. The carbohydrate-binding activities of plant lectins have also attracted much recent attention. Many of these chemoreceptors have been purified and characterized as glycoproteins (26).

The physiological role of the binding proteins of gram-negative bacterial envelopes has been extensively studies in recent years. Biochemical and genetic evidence has strongly implicated a number of *E. coli* binding proteins as chemoreceptors for both active transport and chemotaxis (2, 4, 11). The present study suggests that these membrane phenomena are also closely linked in *P. aeruginosa*. The inability of mutant strain MB723 to synthesize GBP was accompanied by severe deficiencies in both glucose transport and chemotaxis. The failure to detect an altered GBP in cell extracts and spent culture medium suggests that these physiological defects may be the result of a regulatory mutation rather than an altered structural gene.

Several binding proteins of *E. coli* have been shown to undergo conformational changes upon binding of substrate, a phenomenon posulated for transport carrier proteins (4, 5). These substrate-induced conformational changes may result in alterations in the electrophoretic mobility of the protein in polyacrylamide disc gels and in the intensity of the protein's fluorescence emission spectrum (5). Similar studies in our laboratory to detect glucose-induced conformational changes in the GBP of *P. aeruginosa* were unsuccessful.

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