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uhp-Directed, Glucose 6-Phosphate Membrane Receptor in *Escherichia coli*

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Membrane vesicles were characterized for their ability to specifically bind [^{14}C]glucose 6-phosphate. Membranes prepared from a strain carrying a ColE1 *uhp* hybrid plasmid showed significantly enhanced glucose 6-phosphate binding. It is hypothesized that glucose 6-phosphate binding to these membranes is due to a *uhpR*-directed, membrane-bound receptor which functions in regulation of the inducible *uhpT* gene product: the hexose phosphate permease.

Escherichia coli can actively transport exogenous hexose phosphates into the cell via an inducible permease (2). This permease is the product of the *uhpT* gene, and its synthesis is induced only by the presence of external glucose 6-phosphate (G6P) (12). Intracellular G6P or other hexose phosphates, such as fructose 6-phosphate (F6P), found externally do not induce permease production (3). This regulatory phenomenon is termed exogenous induction, and others (13) have hypothesized the involvement of a membrane receptor which would specifically bind to exogenous G6P and then somehow allow *uhpT* gene activation. Kadner (7) has identified a regulatory element (*uhpR*) of the *uhp* genes. We present evidence that the *uhpR* gene product is a membrane-bound receptor, specific for external G6P.

E. coli strain CSR603 (F^- Str^r Gal^- Uhp^+ Rec^-) was provided by A. Sancar and has been described elsewhere (11). *E. coli* JA200 (F^+ Str^s Gal^+ Uhp^+) was provided by J. Carbon and described elsewhere (1). Four JA200 strains were provided, each carrying a hybrid ColE1 plasmid known to contain the *uhp* genes (R. Essenberg, personal communication). These four JA200 strains are designated 17-47, 35-4, 40-33, and 14-9. Conjugation between each of the four JA200 donors and the CSR603 recipient was carried out (10), and conjugates receiving the ColE1 *uhp* plasmid were selected by screening on nutrient agar-yeast extract plates containing streptomycin (200 μg/ml) and colicin E1 (10%, vol/vol). ColE1 colicin was prepared from chloroform-treated cultures of *E. coli* strain DB 1187. This strain has been shown to be a highly efficient producer of colicin E1 and was provided by D. Berg, Washington University, St. Louis, Mo., whose procedure for colicin preparation was used. Selected conjugates were also tested to demonstrate their Gal^- phenotype. The largest number of conjugates was found resulting from the cross with JA200-17-47, and one of these colonies was chosen for further study. This conjugate, containing the ColE1 *uhp* plasmid, was designated CSR603-17-47.

The conjugate strain was tested in our laboratory for the presence of multiple copies of the *uhp* genes. It was found (data not shown) that the induced rate of G6P uptake (initial velocity) was 5.5 times greater in the conjugate than in the same strain which did not contain the ColE1 hybrid plasmid. Both strains displayed similar degrees of *uhp* inducibility, indicating the presence of normal regulation of the hexose phosphate transport process. These findings indicate that the ColE1 hybrid plasmid contains both the *uhpR* and *uhpT* genes.

Cultures of CSR603 and CSR603-17-47 were grown on glucose-minimal medium (5), and membrane vesicles were prepared from each by the method of Kaback (6). [*U*-^{14}C]G6P (ICN Pharmaceuticals, Irvine, Calif., 198 mCi/mmol) was purified (4) before use by ion-exchange chromatography.

The binding of [^{14}C]G6P to vesicles was assayed in 12- by 75-mm tubes at 0°C for 10 min. Assay tubes contained (in a final volume of 0.1 ml) vesicle suspension, 50 mM KPO_4 buffer (pH 6.6), 10 mM $MgSO_4$, 10 μM F6P, and [^{14}C]G6P. F6P was added before the [^{14}C]G6P to fill any nonspecific hexose phosphate binding sites. The reaction was terminated by filtering the contents on 25-mm, 0.2-μm pore size Amicon microporous filters. A washing step was not used. The filters were dried and counted in toluene scintillation fluid (4). Background controls were prepared by using reaction mixtures without vesicles added. Vesicle protein was assayed (8) from vesicles digested in warm 0.1 N NaOH.

Results showed that for both vesicle prepara-

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tions, the amount of [^{14}C]G6P bound was a linear function of the amount of vesicle protein present over the test range of 0 to 27 μg of protein. The vesicles made from CSR603-17-46 bound 3.6 times more G6P per mg of protein than those from CSR603. Data in Table 1 show that an approximate 40-fold excess of unlabeled G6P resulted in almost eliminating [^{14}C]G6P binding. An approximate 125-fold excess of F6P only inhibited [^{14}C]G6P binding by about 3%. Excess glucose had no effect on G6P binding. Everted (inside-out) vesicles bound only 15% as much G6P as did normally oriented vesicles. This percentage closely matches the efficiency of the eversion procedure (9). After a pretreatment of mild trypsin digestion, vesicles were markedly diminished in their ability to bind G6P. Also shown, vesicles made from CSR603 (lacking the plasmid) bound only 29% as much G6P as those from the strain harboring the plasmid.

Figure 1 shows a double reciprocal plot of the binding of [^{14}C]G6P as a function of G6P concentration for both sets of vesicles. Analysis of this plot shows a K_d value of about 16 μM G6P for both sets of vesicles. This value is very close to the K_m of induction of the *uhp* system, which has been reported as 20 μM G6P (13). Maximal values of G6P binding (from the *y* axis intercepts) are 1.41 pmol of G6P per mg of vesicle protein for CSR603 and 5.05 pmol of G6P per mg of vesicle protein for CSR630-17-46. These values also differ by a factor of 3.6.

TABLE 1. *Binding of [^{14}C]G6P to vesicles prepared from E. coli C5R603 and C5R603-17-47*[a]

Strain	Addition/treatment	[^{14}C]-G6P bound[b]	% of maximal amount
C5R603-17-47	None	57	100
	G6P (unlabeled)	3	6
	F6P	55	97
	Glucose	57	100
	Eversion[c]	8	15
	Trypsin[d]	5	9
C5R603	None	17	29

[a] Each reaction tube contained 2.34 μM [^{14}C]G6P (198 mCi/mmol). Values shown are averages of multiple assays. The amount of vesicle protein per tube was 6.4 μg for CSR603-17-47 and 10.2 μg for C5R603. When present, the following were used at the indicated final concentration: unlabeled G6P, 0.1 mM; F6P, 0.3 mM; glucose, 0.1 mM.

[b] Expressed in terms of picomoles × 10^{-2} of [^{14}C]G6P bound per milligram of vesicle protein.

[c] Vesicles were everted as described elsewhere (9).

[d] Vesicles were pretreated at 37°C for 10 min with 0.05% trypsin, then sedimented at 27,000 × *g*, and washed with phosphate buffer. These vesicles were then resuspended in phosphate buffer to the original volume and chilled to 0°C before being assayed.

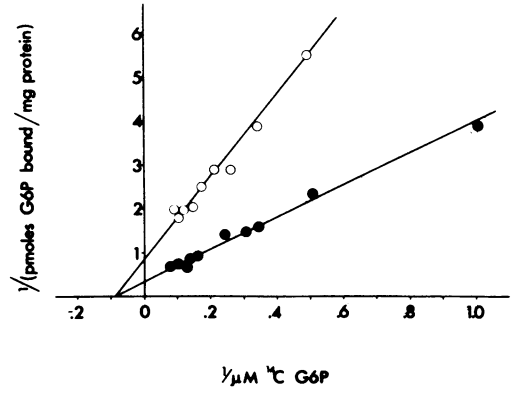

FIG. 1. *Double reciprocal plot of the binding of [^{14}C]G6P to membrane vesicles as a function of G6P concentration. Symbols: ○, C5R603 vesicles; ●, C5R603-17-47 vesicles. C5R603 reaction tubes each contained 10.2 μg of membrane protein, and C5R603-17-47 tubes contained 6.4 μg of membrane protein. [^{14}C]G6P concentrations varied in 10 even increments from 1.17 to 11.7 μM.*

The vesicles used in these studies were prepared from cells grown in the absence of exogenous G6P and therefore were uninduced for hexose phosphate permease production and lack this protein. Any *uhp*-directed product in these membranes would thus be synthesized without the requirement of induction. Our results show that membranes made from cells containing multiple copies of the *uhp* genes (ColE1 *uhp* plasmid) have the ability to bind almost four times more G6P per milligram of vesicle protein than do vesicles made from a strain containing a single set of *uhp* genes. The cells used were not induced for *uhp* transcription, so this binding ability is not likely due to the *uhpT* gene product (permease). The fact that G6P binding was not inhibited by F6P also helps eliminate the permease as the G6P binding site, for it has a broad specificity for hexose phosphates (2).

We hypothesize that the *uhpR* gene is expressed in the absence of inducer and codes for a membrane-bound G6P receptor. This receptor in some way regulates expression of the *uhpT* gene, perhaps by serving as a repressor analogous to the *lacI* gene product, but membrane bound. Its G6P binding site would be on the external side of the cytoplasmic membrane. As seen in Table 1, everted vesicles have a considerably diminished G6P binding ability, indicating that the G6P binding site is on external membrane surface. Trypsin digestion essentially eliminated G6P binding. Because the *uhpT* gene is induced by G6P and not F6P, a receptor fulfilling a *uhp* regulatory rule must reflect this specificity. This is shown in Table 1.

The K_d for G6P binding is the same for both sets of vesicles, indicating binding to the same receptor in both. Vesicles from CRS603-17-46, whose cells contained multiple copies of the *uhp* genes, however, can bind almost fourfold more G6P. We interpret this as indicating that the G6P binding ability of these vesicles is due to a product of their multiple *uhp* genes and not another, or nonspecific binding ability of the membranes.

In summary we interpret our data as showing that a *uhp* gene, probably *uhpR*, directs the synthesis of a membrane-bound receptor which binds G6P and not F6P. The G6P binding site is on the external membrane surface, and the receptor is most likely a protein. Further work is under way to determine how this receptor regulates *uhpT* gene activity.

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