JOURNAL OF BACTERIOLOGY, Apr. 1994, p. 2133–2135
0021-9193/94/$04.00+0

Vol. 176, No. 7

Reconstitution of Glucose Uptake and Phosphorylation in a Glucose-Negative Mutant of *Escherichia coli* by Using *Zymomonas mobilis* Genes Encoding the Glucose Facilitator Protein and Glucokinase†

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Received 15 November 1993/Accepted 1 February 1994

Expression of the *Zymomonas mobilis glf* (glucose facilitator protein) and *glk* (glucokinase) genes in *Escherichia coli* ZSC113 (glucose negative) provided a new functional pathway for glucose uptake and phosphorylation. Both genes were essential for the restoration of growth in glucose minimal medium and for acid production on glucose-MacConkey agar plates.

In *Escherichia coli*, the primary route for glucose uptake is the phosphoenolpyruvate-dependent phosphotransferase system (PTS) (16). Glucose is concomitantly phosphorylated during transport to produce intracellular glucose 6-phosphate. *E. coli* also contains redundant systems for glucose transport, which include an overlap in specificity, allowing glucose uptake also by the mannose-PTS system (10), as well as a pyrroloquinoline quinone-dependent glucose dehydrogenase system which oxidizes glucose to gluconate prior to uptake (14, 21). For this latter system to function in *E. coli*, pyrroloquinoline quinone must be added as a supplement (12, 15).

In contrast to *E. coli*, *Zymomonas mobilis* (5, 17) and *Saccharomyces cerevisiae* (6) utilize a facilitated diffusion system with a glucose facilitator protein (GLF). After entry, glucose is intracellularly phosphorylated by glucokinase (GLK) (*Z. mobilis*) or hexokinase (*S. cerevisiae*) to produce glucose 6-phosphate. Genes encoding GLF (*glf*) and GLK (*glk*) have recently been cloned from *Z. mobilis* on separate DNA fragments (pTC111 and pTC120) (3, 4). In the present study, we have investigated the abilities of these two genes to provide a functional heterologous system for glucose transport and phosphorylation in a glucose-negative mutant of *E. coli*, strain ZSC113 (10).

Plasmid constructions. Plasmids were constructed by standard methods (19) with *E. coli* DH5α (*thi lacZΔM15 recA* Nal^r) as the host. The full *Z. mobilis glf-zwf-edd-glk* operon was assembled from the original clones, pTC111 and pTC120 (3, 4), to produce pLOI740 as shown in Fig. 1. Two derivatives in which frameshift mutations were inserted into either *glf* or *glk* (by digestion with *Eag*I or *Mlu*I, treatment with DNA polymerase I, or self-ligation) were constructed to produce pLOI744 and pLOI746 (data not shown). A third derivative in which large parts of *zwf* and *edd* were deleted by digestion with *Bgl*II and *Nsi*I (after conversion to blunt ends) was constructed to produce pLOI790. *Z. mobilis* DNA fragments (*Sac*I to *Xba*I) from these plasmids were used to replace pUC18 in pLOI707EH (*Sac*I-*Xba*I), an RSF1010-based (broad-host-range) expression vector containing the *tac* promoter and a *lacI*^{q} repressor gene (2). The resulting plasmids were designated pLOI742 (*glf zwf edd glk*), pLOI763 (*zwf edd glk*), pLOI767 (*glf zwf edd*), and pLOI792 (*glf glk*), respectively. PCR was used to construct a derivative of pLOI142 (pUC18 containing a *Not*I linker in the *Sma*I site) which contained only the coding region and ribosome-binding site for *glf*. The resulting plasmid has been designated pLOI670. The primers used to construct pLOI670, 5′ GCG AGC TCA AGG CGG GAG AGG AAT 3′ (5′ end of the *glf* gene) and 5′ GTG GCG GCC GCC TAC TTC TGG GAG CG 3′ (3′ end of the *glf* gene), included *Sac*I and *Not*I sites, respectively.

Restoration of growth and acid production in ZSC113 by expression of *glf* and *glk*. *E. coli* ZSC113 (*lacZ82 ptsM12 ptsG22glk-7 rha-4 rpsL223*) is a glucose-negative strain which contains mutations in glucose-specific and mannose-specific

TABLE 1. Growth rates and expression of *Z. mobilis glk* in recombinants of glucose-negative *E. coli* ZSC113 mutant[a]

Plasmid	Active genes	Amt of IPTG (mM)[b]	GLK activity (IU mg^{-1})[c]	Specific growth rate (h^{-1})	Acid production[d]
None		0	<0.01	0.01	−
pLOI742	*glf zwf edd glk*	0	1.2	0.30	+
		1	1.7	0.53	+
pLOI763	*zwf edd glk*	0	1.4	0.05	−
		1	1.4	0.03	−
pLOI767	*glf zwf edd*	0	<0.01	0.02	−
		1	<0.01	0.02	−
pLOI792	*glf glk*	0	0.2	0.16	+
		1	1.1	0.53	+

[a] Cells were grown overnight in M9 minimal medium containing trace metals (13) and 10 g liter^{-1} of gluconate to prepare inocula and for biochemical analyses. Gluconate-grown cells were diluted (optical density at 550 nm of 0.1) into glucose minimal medium to measure growth rates. Values are averages from two experiments.

[b] IPTG, isopropyl β-D-thiogalactoside.

[c] GLK activity was determined as described by Doelle (11); the amount of protein was estimated by the Bradford method (7).

[d] Acid production on glucose-MacConkey agar plates is indicated by + for growth as dark red colonies; −, white colonies scored as negative.

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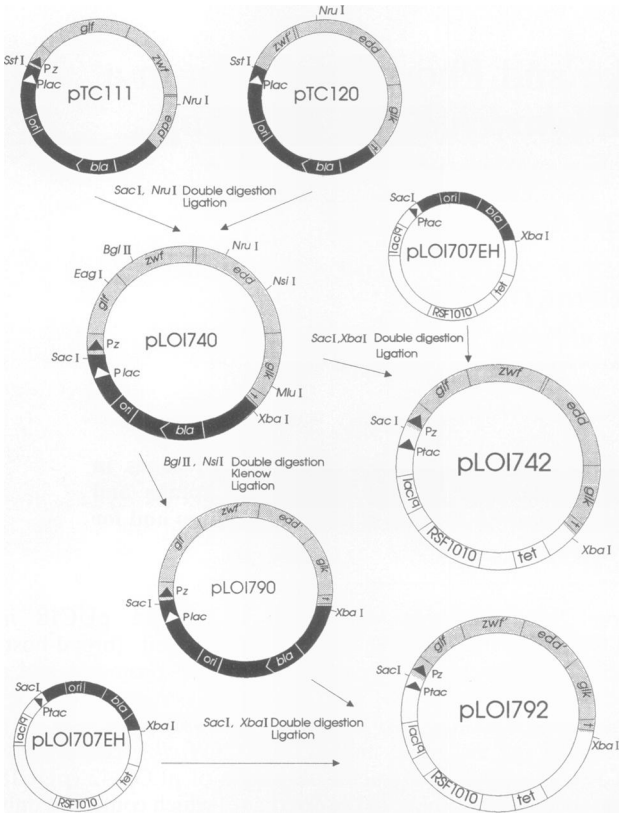

FIG. 1. Construction of plasmids. DNA from *Z. mobilis* is indicated by stippling. Solid regions represent DNA derived from pUC18. Open regions represent DNA derived from pLOI706EH. Triangles within plasmids indicate the directions of transcription. *t*, the terminator for the *glf* operon; Pz, P*tac*, and P*lac*, the *Z. mobilis glf* promoter, the *tac* promoter, and the *lac* promoter, respectively. Primes are used to indicate incomplete genes.

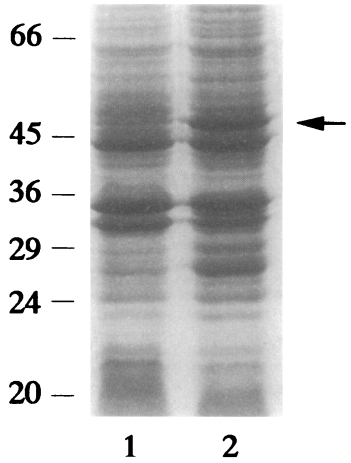

FIG. 2. Sodium dodecyl sulfate-polyacrylamide (8%) gel of proteins from the membrane fraction of *E. coli*. Protein gels were prepared essentially as described elsewhere (1). Positions of molecular weight markers (in thousands) are indicated on the left. Arrow indicates the region containing GLF. Lanes: 1, *E. coli* DH5α(pUC18); 2, *E. coli* DH5α(pLOI670).

phosphotransferase genes and in GLK (10). As summarized in Table 1, growth on glucose minimal medium and acid production on glucose-MacConkey agar were restored only by pLOI742 and pLOI792. Both recombinants contained GLK activity and the native genes for *glf* and *glk*. Although expression was not fully controlled by the *tac* promoter and *lacI*^{q}, the addition of inducer increased the growth rates of these recombinants.

GLF was not observed in denaturing gels containing membranes or soluble proteins from RSF1010-based constructs. However, this protein was clearly evident as an overexpressed band (apparent M_r of 51,300) when membrane fractions from DH5α(pLOI670) were compared (Fig. 2). Other low-molecular-weight bands were also present and may represent degradation products. The addition of pLOI670 containing only *glf* was sufficient to restore glucose utilization in ZSC113 (pLOI763) but not in ZSC113(pLOI767), confirming that *glf* had been selectively inactivated (not shown).

Conclusions. The glucose uptake (*glf*) and phosphorylation genes (*glk*) from *Z. mobilis* functioned well in *E. coli* and provide an alternative to the native glucose-PTS system. This is somewhat surprising when one considers the differences in plasma membrane lipids between these two organisms. *Z. mobilis* contains large amounts of phosphatidylcholine and an extremely high proportion of vaccenic acid (8, 20). Large amounts of hopanoids which may be needed for ethanol tolerance are also present (18). In contrast, *E. coli* contains a balanced mixture of 16- and 18-carbon fatty acids, lacks phosphatidylcholine (9), and lacks hopanoids. Thus, it appears that the insertion and functioning of *Z. mobilis* GLF are tolerant of variations in membrane lipid composition. Portable operons encoding this permease together with *glk* may prove useful for the genetic engineering of other organisms by providing an alternative or supplemental route for glucose entry into glycolysis.

We thank Barbara Bachmann and the *E. coli* Genetic Stock Center for providing *E. coli* ZSC113.

This research was supported by grants from the Department of Energy, Division of Basic Energy Sciences (DE-FG05-86ER13575 and DE-FG02-90ER20002).

REFERENCES

1. **An, H., R. K. Scopes, M. Rodriguez, K. F. Keshav, and L. O. Ingram.** 1991. Gel electrophoretic analysis of *Zymomonas mobilis* glycolytic and fermentative enzymes: identification of alcohol dehydrogenase II as a stress protein. J. Bacteriol. **173:**5975–5982.
2. **Arfman, N., V. Worell, and L. O. Ingram.** 1992. Use of the *tac* promoter and *lacI*^{q} for the controlled expression of *Zymomonas mobilis* fermentative genes in *Escherichia coli* and *Z. mobilis*. J. Bacteriol. **174:**7370–7378.
3. **Barnell, W. O., J. Liu, T. L. Hesman, M. C. O'Neill, and T. Conway.** 1992. The *Zymomonas mobilis glf*, *zwf*, *edd*, and *glk* genes form an operon: localization of the promoter and identification of a conserved sequence in the regulatory region. J. Bacteriol. **174:**2816–2823.
4. **Barnell, W. O., K. C. Yi, and T. Conway.** 1990. Sequence and genetic organization of a *Zymomonas mobilis* gene cluster that encodes several enzymes of glucose metabolism. J. Bacteriol. **172:**7227–7240.
5. **Belaich, J. P., J. Senez, and M. Murgier.** 1968. Microcalorimetric study of glucose permeation in microbial cells. J. Bacteriol. **95:**1750–1757.
6. **Bisson, L. F., and D. G. Fraenkel.** 1983. Involvement of kinases in glucose and fructose uptake by *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **80:**1730–1734.
7. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72:**248–254.

8. **Carey, V. C., and L. O. Ingram.** 1983. Lipid composition of *Zymomonas mobilis*: effects of ethanol and glucose. J. Bacteriol. **154:**1291–1300.
9. **Cronan, J. E., Jr., and C. O. Rock.** 1987. Biosynthesis of membrane lipids, p. 474–497. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
10. **Curtis, S. J., and W. Epstein.** 1975. Phosphorylation of D-glucose in *Escherichia coli* mutants defective in glucosephosphotransferase, mannosephosphotransferase, and glucokinase. J. Bacteriol. **122:**1189–1199.
11. **Doelle, H. W.** 1982. Kinetic characteristics and regulatory mechanisms of glucokinase and fructokinase from *Zymomonas mobilis*. Eur. J. Appl. Microbiol. Biotechnol. **14:**241–246.
12. **Fliege, R. S., S. Tong, A. Shibata, K. W. Nickerson, and T. Conway.** 1992. The Entner-Douderoff pathway in *Escherichia coli* is induced for oxidative glucose metabolism via pyrroloquinoline quinone-dependent glucose dehydrogenase. Appl. Environ. Microbiol. **58:**3826–3829.
13. **Guimaraes, W. V., G. L. Dudey, and L. O. Ingram.** 1992. Fermentation of sweet whey by ethanologenic *Escherichia coli*. Biotechnol. Bioeng. **40:**41–45.
14. **Hommes, R. W. J., P. W. Postma, O. M. Neijssel, D. W. Tempest, P. Dokter, and J. A. Duine.** 1984. Evidence of a quinoprotein glucose dehydrogenase apoenzyme in several strains of *Escherichia coli*. FEMS Microbiol. Lett. **24:**329–333.
15. **Hommes, R. W. J., J. A. Simons, J. L. Snoep, P. W. Postma, D. W. Tempest, and O. M. Neijssel.** 1991. Quantitative aspects of glucose metabolism by *Escherichia coli* B/r, grown in the presence of pyrroloquinoline quinone. Antonie Leeuwenhoek **60:**373–382.
16. **Postma, P. W., J. W. Lengeler, and G. R. Jacobson.** 1993. Phosphoenolpyruvate:carbohydrate phosphotransferase systems of bacteria. Microbiol. Rev. **57:**543–594.
17. **Romano, A. H.** 1986. Microbial sugar transport systems and their importance in biotechnology. Trends Biotechnol. **4:**207–213.
18. **Sahm, H., M. Rohmer, S. Bringer-Meyer, G. A. Sprenger, and R. Welle.** 1993. Biochemistry and physiology of hopanoids in bacteria. Adv. Microb. Physiol. **35:**247–273.
19. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
20. **Tornabene, T. G., G. Holzer, A. S. Bittner, and K. Grohmann.** 1982. Characterization of the total extractable lipids of *Zymomonas mobilis* var. *mobilis*. Can. J. Microbiol. **28:**1107–1118.
21. **van Schie, B. J., K. J. Hellingwerf, J. P. van Dijken, M. G. L. Elferink, J. M. van Dijl, J. G. Kuenen, and Wil N. Konings.** 1985. Energy transduction by electron transfer via a pyrrolo-quinoline quinone-dependent glucose dehydrogenase in *Escherichia coli*, *Pseudomonas aeruginosa*, and *Acinetobacter calcoaceticus* (var. *lwoffi*). J. Bacteriol. **163:**493–499.