JOURNAL OF BACTERIOLOGY, Nov. 1983, p. 922–925
0021-9193/83/110922-04$02.00/0

Vol. 156, No. 2

Cloning of the Glucokinase Gene in *Escherichia coli* B

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Received 13 June 1983/Accepted 19 August 1983

A gene for glucokinase (Glk) in *Escherichia coli* B was cloned onto vector plasmid pBR322, and the hybrid plasmid obtained was designated pGK100. The gene for Glk was located in the central *Mlu*I fragment (0.82 megadalton) of the 6.0-megadalton chromosomal DNA inserted in the *Hin*dIII site of the vector. The introduction of pGK100 into *E. coli* 112L having a decreased level of Glk activity resulted in the about 15-fold increase in this enzyme activity. The poor growth rate of 112L cells on glucose or mannose was also improved by the introduction of pGK100. However, removal of some portion near the *glk* gene prevented the growth of 112L cells, although Glk activity was high enough to support growth. Therefore, some function of Glk may be regulated by a gene(s) near the *glk* gene.

Escherichia coli has three distinct enzymes able to phosphorylate glucose to allow growth on glucose. These enzymes are ATP-dependent glucokinase (Glk), glucose phosphotransferase (GPT), and mannose phosphotransferase (MPT). GPT and MPT are species of enzyme II of the phosphotransferase. GPT is highly specific for glucose, and MPT is active on glucose, mannose, and several other sugars (5). These enzymes II have been thought to be of major importance in permitting rapid growth on glucose and mannose. Almost all studies of *E. coli* glucose metabolism have been carried out on enzyme II, and little information on Glk has been obtained.

However, one *E. coli* mutant deficient in GPT, MPT, and Glk activities does not grow on glucose, although a mutant lacking only GPT and MPT does (2). This indicates that Glk can function for glucose catabolism. Our purpose in this study was to clarify the role of Glk in glucose catabolism in *E. coli* cells. This article describes preliminary results on the cloning of the *glk* gene in *E. coli* B.

For the cloning of the gene responsible for Glk activity, the *E. coli* B chromosomal DNA extracted by the method of Saito and Miura (8) was digested with restriction endonuclease *Hin*dIII, and the resultant DNA fragments were ligated with vector plasmid pBR322, digested with *Hin*dIII, and treated with bacterial alkaline phosphatase by the method of Ullrich et al. (9).

E. coli ZSC 112L (*gpt mpt glk*), which was obtained from Curtis and Epstein (2) and had a decreased level of Glk activity, was transformed with the whole ligation mixture prepared above, and the cells were spread on glucose-MacConkey (2) agar plates supplemented with 20 μg of ampicillin per ml. The plates were incubated at 37°C overnight, and the transformants were selected as ampicillin-resistant, very light pink colonies on the plates. The colonies of transformants were white on mannose-MacConkey agar and blue on Davis-Mingioli minimal medium (3) supplemented with 1.0% glucose, 0.2% lactose, and 0.002% 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside, indicating that the cloned gene was for Glk, not MPT or GPT, both of which show different colors in these media (2).

The hybrid plasmid in one of the transformants was isolated by the method of Murata and Kimura (7) and was designated pGK100. To construct a physical map of pGK100, restriction analysis was carried out. The circular restriction map of pGK100 was determined as shown in Fig. 1. This hybrid plasmid was 8.8 megadaltons and had an insert of 6.0 megadaltons of a chromosomal DNA fragment of *E. coli* B in the *Hin*dIII site of the vector. The cloned fragment had four susceptible sites for *Eco*RI, *Pst*I, and for *Mlu*I.

To locate the *glk* gene on this insert, pGK100 was completely digested with *Mlu*I. After electrophoresis of digestion mixtures on agarose gels, the DNA fragments were extracted by the method of Young et al. (10). Four kinds of linear DNA fragments (F1, F2, F3, and F4) of 1.2, 0.82, 0.5, and 6.2 megadaltons, respectively, were obtained (Fig. 1). Each fragment (F1, F2, and F3) was ligated with F4 by T_4 DNA ligase. F4 was self-ligated. The ligation products thus prepared were used for the transformation of 112L cells to select the hybrid plasmid carrying the *glk* gene. Transformants were selected as colonies resistant to ampicillin and sensitive to

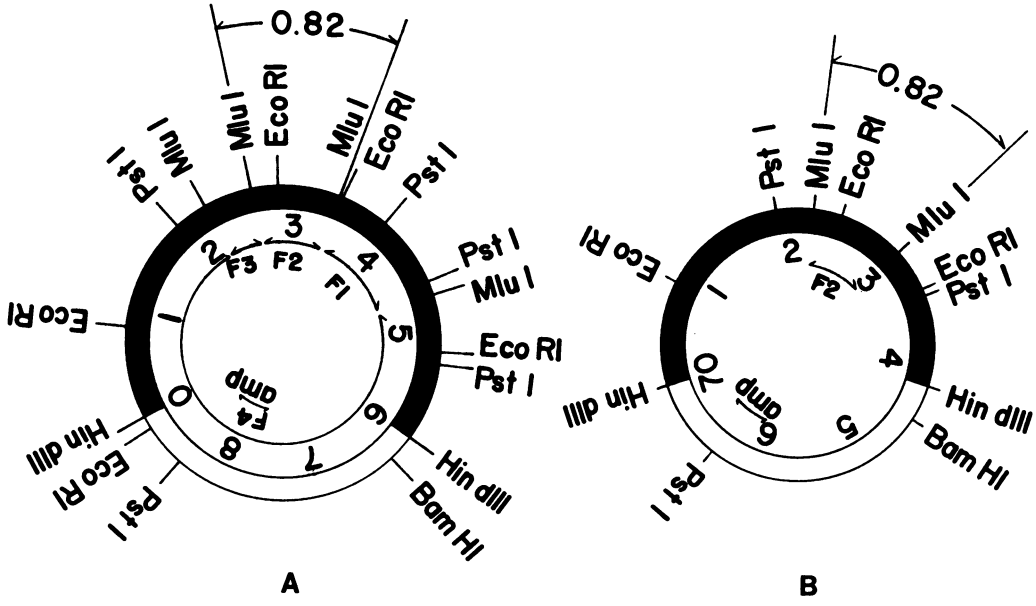

FIG. 1. Circular restriction maps of hybrid plasmids pGK100 (A) and pGK100-5 (B) (8.8 and 7.02 megadaltons, respectively). Hybrid plasmids were digested with various restriction endonucleases, and the molecular sizes of the linear DNA fragments generated were determined by agarose gel electrophoresis by the method of Helling et al. (4). The restriction sites were drawn to scale on a circular map. The number in the circle represents megadaltons. The 0.82-megadalton *Mlu*I fragment (F3) is depicted outside of the circle. (□) Vector plasmid pBR322; (■) chromosomal DNA fragment of *E. coli* B.

tetracycline on glucose-MacConkey agar plates, and Glk activities in transformants were determined by the method of Curtis and Epstein (2). An increase in Glk activity was found only in the transformants harboring the ligation product of F2 with F4. The introduction of the self-ligation product of F4 itself caused no increase in Glk activity of transformants. Therefore, the *glk* gene was located in 0.82 megadalton (F2) of the *Mlu*I fragment on the chromosomal DNA segment in pGK100. A hybrid plasmid consisting of F2 and F4 was designated pGK100-5 (Fig. 1).

Table 1 shows the Glk activities and growth rates of *E. coli* 112L and *E. coli* B with or without the hybrid plasmid. In strains of 112L(pGK100) and 112L(pGK100-5), appreciably high Glk activities (about 10- to 15-fold higher than that of 112L) were observed, al-

TABLE 1. Effects of hybrid plasmids on Glk activity[a] and growth rate[b]

E. coli strain	Glk activity (μmol/min per mg of protein)	Generation time (h) on:			
		Glucose	Glycerol	Fructose	Mannose
112L	0.012	33	2.4	2.7	15.8
112L(pGK100)	0.190	4.1	2.5	2.8	8.4
112L(pGK100-5)	0.127	37	2.5	2.8	14.8
B	0.093	1.2	1.9	1.6	1.9
B(pGK100)	0.182	1.2	2.0	1.6	2.3
B(pGK100-5)	0.143	1.2	2.0	1.6	1.9

[a] Glk activities were assayed with extracts prepared from cells growing exponentially on Davis-Mingioli minimal medium (3) [0.7% KH_2PO_4, 0.3% K_2HPO_4, 0.1% $(HN_4)_2SO_4$, 0.01% $MgSO_4 \cdot 7H_2O$, 0.5% glucose] at 37°C with shaking. Glk reaction was carried out in 3.0 ml of the mixture containing 50 mM glucose, 2.5 mM ATP, 2.0 mM $MgCl_2$, 0.25 mM NADP, 50 mM Tris-hydrochloride buffer (pH 7.5), 0.6 U of glucose-6-phosphate dehydrogenase, and cell extracts at 25°C. Reaction was followed by recording the change in absorbance at 340 nm. The preparation of cell extracts was as described by Curtis and Epstein (2). Protein was determined by the method of Lowry et al. (6).

[b] The cells were grown on Davis-Mingioli minimal medium (3) containing various carbon sources at 0.5%. Incubation was carried out at 30°C with shaking. Growth was monitored by measuring the turbidity at 610 nm.

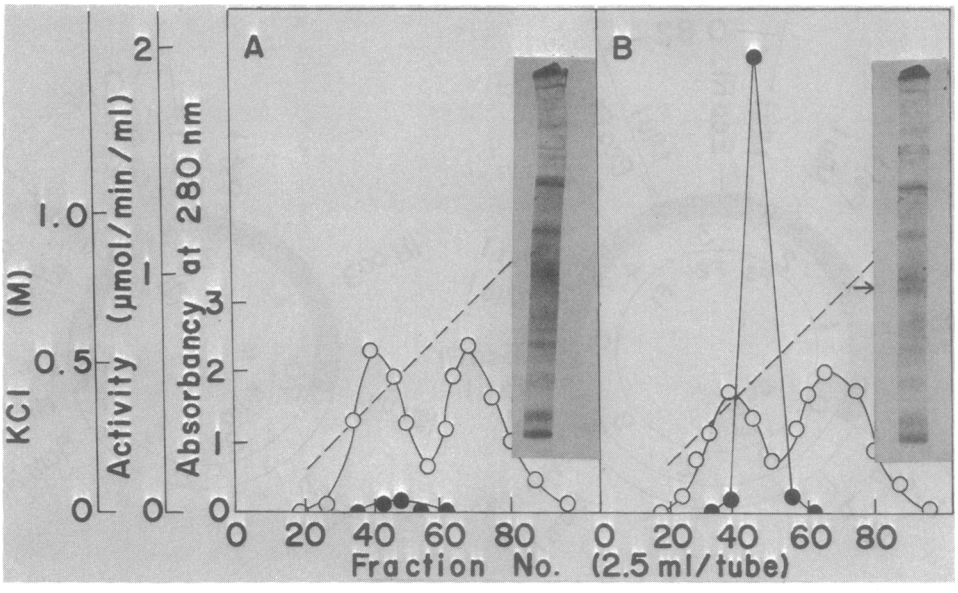

FIG. 2. Analysis of cell extracts of *E. coli* 112L and 112L(pGK100) on DEAE-cellulose column chromatography (A and B) and polyacrylamide gel electrophoresis (insets). The crude cell extracts were prepared from strains 112L and 112L(pGK100) and applied to the DEAE-cellulose column equilibrated with 5.0 mM Tris-hydrochloride buffer (pH 7.5). The absorbed proteins were eluted with a linear gradient of the same buffer containing KCl at increasing concentrations from 0 to 1.0 M. Glk activity of each fraction was determined as described in footnote *a* of Table 1. Protein was estimated by measuring the absorbance at 280 nm. Symbols: (●) Glk activity; (○) extinction coefficient at 280 nm; (– – –) KCl concentration. Polyacrylamide gel electrophoresis was carried out by the method of Andrews (1); the arrow shown in inset B indicates a plasmid-dependent protein band. (A) Crude cell extracts of 112L; (B) crude cell extracts of 112L(pGK100).

though the effects of these hybrid plasmids on Glk activities were slight in *E. coli* B cells. The poor growth rate of 112L on glucose was also improved by the introduction of pGK100. The generation time of 112L was 55 h on glucose at 30°C, whereas the generation time of strain 112L(pGK100) was only 4 h on glucose and about one-third of that of wild *E. coli* B. An improvement of generation time of 112L by pGK100 was observed only when the cells were grown on glucose or mannose. No effect of pGK100 on the growth rate of 112L was found with fructose or glycerol. However, hybrid plasmid pGK100-5 did not enhance the growth rate of 112L on glucose or mannose, although 112L(pGK100-5) cells showed high Glk activity. These results indicated that the increase in Glk activity alone was not always sufficient to allow growth on glucose. Another factor, which might be coded by genes in the F1 or F3 region (Fig. 1), might be necessary for glucose catabolism through the Glk reaction in *E. coli*. Studies on the effects of such regions are now under way, using various derivatives of pGK100 with or without the F2 region.

To analyze products of the cloned gene, the cell extracts of 112L and 112L(pGK100) were subjected to DEAE-cellulose column chromatography and polyacrylamide gel electrophoresis (Fig. 2). The crude extracts of 112L(pGK100) showed a single large peak with Glk activity on the DEAE-cellulose column chromatograph (Fig. 2B), whereas the activity in 112L was very low (Fig. 2A). Polyacrylamide gel electrophoresis of cell extracts of 112L(pGK100) showed a plasmid-dependent protein band (indicated by an arrow in Fig. 2B) having Glk activity assayed by using the gel slice with this protein band. Direct staining of the gel showed the existence of one Glk band at the same position as that indicated by the arrow in Fig. 2B (data not shown). Crude cell extracts of 112L(pGK100-5) also showed one plasmid-dependent protein band with Glk activity at the same position as that shown in Fig. 2B (data not shown).

Thus, we succeeded in cloning a gene responsible for Glk activity. Furthermore, factor(s) regulating the function of Glk in glucose catabolism seem to be present in *E. coli* B. Further studies are now in progress.

We thank Morihiko Sakaguchi, Associate Professor of Research Institute for Food Science, Kyoto University, for valuable discussions on this work. We also thank Noriko Okamoto for helpful assistance.

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