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Selection of *Escherichia coli* Mutants Lacking Glucose-6-Phosphate Dehydrogenase or Gluconate-6-Phosphate Dehydrogenase

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Glucose is metabolized in *Escherichia coli* chiefly via the phosphoglucose isomerase reaction; mutants lacking that enzyme grow slowly on glucose by using the hexose monophosphate shunt. When such a strain is further mutated so as to yield strains unable to grow at all on glucose or on glucose-6-phosphate, the secondary strains are found to lack also activity of glucose-6-phosphate dehydrogenase. The double mutants can be transduced back to glucose positivity; one class of transductants has normal phosphoglucose isomerase activity but no glucose-6-phosphate dehydrogenase. An analogous scheme has been used to select mutants lacking gluconate-6-phosphate dehydrogenase. Here the primary mutant lacks gluconate-6-phosphate dehydrase (an enzyme of the Enter-Doudoroff pathway) and grows slowly on gluconate; gluconate-negative mutants are selected from it. These mutants, lacking the nicotinamide dinucleotide phosphate-linked glucose-6-phosphate dehydrogenase or gluconate-6-phosphate dehydrogenase, grow on glucose at rates similar to the wild type. Thus, these enzymes are not essential for glucose metabolism in *E. coli*.

Pathways of glucose and gluconate metabolism in *Escherichia coli* are outlined in Fig. 1. According to present information, glucose is first phosphorylated, and the glucose-6-phosphate may either be isomerized to fructose-6-phosphate or oxidized to gluconate-6-phosphate (7). Gluconate, after phosphorylation, may either be oxidized to pentose phosphate or dehydrated to 2 - keto - 3 - deoxygluconate - 6 - phosphate (4, 7). A mutant lacking phosphoglucose isomerase grows slowly on glucose (7), and a mutant lacking gluconate-6-phosphate dehydrase grows slowly on gluconate (14). In both, metabolism is exclusively by the hexose monophosphate shunt. In this study, the above two mutants were used as starting strains to select mutants unable to grow on glucose or on gluconate, respectively. The new mutations affect glucose-6-phosphate dehydrogenase or gluconate-6-phosphate dehydrogenase. Glucose or gluconate positivity may be returned by transduction of the double mutants; among the transductants are ones with single enzyme deficiencies, in glucose-6-phosphate dehydrogenase or gluconate-6-phosphate dehydrogenase. Some characteristics of these strains are described. Perhaps the most striking one is that a deficiency in either of the oxidative enzymes of the hexose monophosphate shunt has little effect on the growth rate of *E. coli* on glucose.

A preliminary report of this work has been presented (Federation Proc. **26**:679, 1967).

MATERIALS AND METHODS

The wild-type parental strain for these mutant selections was K-10, a prototrophic Hfr. From it have been previously derived a mutant lacking phosphoglucose isomerase activity, strain DF40 (7; previously called L40), and a mutant lacking gluconate-6-phosphate dehydrase activity, strain DF10 (14; previously called RZ10); these were the starting strains in the present selections. Media, culture methods, transduction technique, and enzyme assays have been described previously (5, 7).

Genetic nomenclature. Abbreviations for two of the genes used in this work have been suggested previously: *pgi*, for phosphoglucose isomerase (7), and *edd*, for gluconate-6-phosphate dehydrase (14). For the new mutations, we suggest *zwf*, for glucose-6-phosphate dehydrogenase (after *zwischenferment*), and *gnd*, for gluconate-6-phosphate dehydrogenase. Separately isolated mutations are given numbers, in accord with the suggestions of Demerec et al. (3); e.g., strain DF1800 carries the *zwf-1* mutation.

RESULTS

Selection of mutants lacking glucose-6-phosphate dehydrogenase. The rationale for this selection was that, since a mutant lacking phosphoglucose isomerase (*pgi*) grows slowly on glucose, utilizing only the hexose monophosphate shunt

(7), a derivative of that strain that lacks also an enzyme of the shunt ought not to grow on glucose at all. Strain DF40 (*pgi-2*), grown in minimal medium containing gluconate as sole carbon source, was treated in this medium (lacking gluconate) with 0.035 volume of ethyl methane sulfonate (9) at 37 C for 20 min. Survival was 70%. The treated cells were subcultured in gluconate minimal medium and then were resuspended in minimal medium containing glucose, 4 mg/ml, and glucose-6-phosphate, 4 mg/ml. (In this medium the double mutants lacking glucose-6-phosphate dehydrogenase and phosphoglucose isomerase should not grow; certain other glucose-negative mutants—e.g., those lacking a phosphorylation function (13)—would be able to grow on the glucose-6-phosphate (6) and thus, in the penicillin treatment, should be killed.) After two mass doublings, penicillin (2,000 units/ml) was added; 2.5 hr later, dilutions of the lysed culture were spread on gluconate minimal plates. The colonies obtained were tested by replica-plating for ability to grow on glucose and on glucose-6-phosphate. Isolates unable to grow on either of these two carbon sources, but still forming colonies on gluconate, were assayed for phosphoglucose isomerase, glucose-6-phosphate dehydrogenase, and gluconate-6-phosphate dehydrogenase. Of four such isolates, all had no activity or very low activity (<10% that of wild type) of glucose-6-phosphate dehydrogenase; like their immediate parent, DF40, they lacked phosphoglucose isomerase activity and contained gluconate-6-phosphate dehydrogenase activity. Data for two of these double mutants, DF1800 and DF2000, are included in Table 1.

Cells of both double mutant strains were transduced from an inability to an ability to grow on glucose by infection with phage P1 grown on the parent strain, K-10 (*pgi*$^+$ *zwf*$^+$), and selection of transductants on glucose minimal plates. The transductants were assayed for their enzyme content (Table 1). Two classes of glucose-positive transductants were recognized. The major class (e.g., DF1801 and DF2001) appeared on the primary transduction plate within 48 hr; on purification, they appeared as normal-size colonies on glucose minimal plates. The minor class (e.g., DF2002) were found as small colonies on the primary plates after 3 days of incubation. The first class contained phosphoglucose isomerase but no glucose-6-phosphate dehydrogenase; the second class lacked phosphoglucose isomerase but contained glucose-6-phosphate dehydrogenase, and thus resembled the grandparent strain, DF40. Thus, the double mutants contained independent mutations which probably are not closely linked. [This inference is confirmed in the accompanying paper, in which the *zwf* gene is mapped (11).]

Selection of mutants lacking gluconate-6-phosphate dehydrogenase. The rationale of this selection was similar to that used for the glucose-6-phosphate dehydrogenase mutants. A mutant lacking gluconate-6-phosphate dehydrase (an inducible enzyme of the Entner-Doudoroff pathway) was earlier shown to grow slowly on gluconate by using the hexose monophosphate shunt exclusively (14); among derivatives of this strain unable to grow at all on gluconate should be ones lacking also gluconate-6-phosphate dehydrogenase. Strain DF10 (*edd*$^-$ *gnd*$^+$) was grown on glycerol minimal medium and treated with ethyl methane sulfonate, as above. Survival was 10%. The treated cells were subcultured in glycerol minimal medium, resuspended in gluconate minimal medium, and treated with penicillin. The survivors of this treatment were grown on glycerol

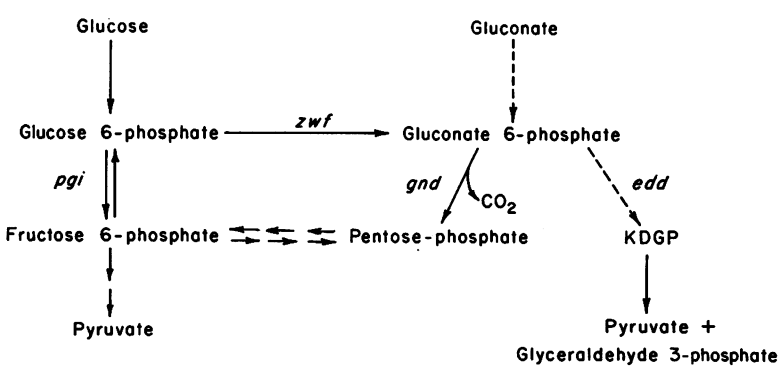

FIG. 1. *Pathways of glucose and gluconate metabolism. Not all reactions are included. Dashed arrows represent inducible enzymes. Abbreviations: KDGP, 2-keto-3-deoxygluconate-6-phosphate; pgi, a gene for phosphoglucose isomerase; zwf, a gene for glucose-6-phosphate dehydrogenase; gnd, a gene for gluconate-6-phosphate dehydrogenase; edd, a gene for gluconate-6-phosphate dehydrase (Entner-Doudoroff dehydrase).*

TABLE 1. *Isolation of zwf⁻ mutants*[a]

Strain	Origin	Phosphoglucose isomerase	Glucose-6-phosphate dehydrogenase	Gluconate-6-phosphate dehydrogenase	Genotype
K-10	Wild type	1,010	103	138	*pgi⁺ zwf⁺ gnd⁺*
DF40	Mutant of K-10	<1	145	158	*pgi-2 zwf⁺ gnd⁺*
DF 1800	Mutant of DF40	<1	0	149	*pgi-2 zwf-1 gnd⁺*
DF 2000	Mutant of DF40	<1	0	65	*pgi-2 zwf-2 gnd⁺*
DF 1801	Transductant of DF1800	652	0	105	*pgi⁺ zwf-1 gnd⁺*
DF2001	Transductant of DF2000	488	0	76	*pgi⁺ zwf-2 gnd⁺*
DF2002	Transductant of of DF2000	<1	130	78	*pgi-2 zwf⁺ gnd⁺*

[a] Extracts were prepared from broth-grown cultures. Enzyme activities are given as millimicromoles per minute per milligram of protein.

minimal plates, and then were tested by replication to gluconate, glucose, and fructose minimal plates.

One class of gluconate-negative derivatives of DF10 (e.g., strain DF1070) found in this selection lacked gluconate-6-phosphate dehydrogenase as well as gluconate-6-phosphate dehydrase (Table 2). [All the gluconate-negative derivatives of this type grow on glucose, but not on fructose, minimal plates. The reason for fructose negativity is under study. There is another type of gluconate-negative derivative of DF10. In contrast to the type exemplified by DF1070, these mutants grow normally on both fructose and glucose plates and have gluconate-6-phosphate dehydrogenase activity. The additional lesion in this class has not yet been identified.] When phage P1 grown on the parent strain K-10 (*edd⁺ gnd⁺*) was used to transduce DF1070 to the ability to grow on gluconate, two classes of transductants were recognized (Table 2); examples are DF1071 (*edd⁺ gnd⁻*) and DF1072 (*edd⁻ gnd⁺*). Thus, the double mutants contained independent mutations which are not closely linked. This inference is confirmed in the following paper (11).

The remainder of this paper concerns some of the properties of the single-gene mutants: DF1801 (*gnd⁺ pgi⁺ zwf-1*) and DF1071 (*gnd-1 pgi⁺ zwf⁺*).

Growth of the dehydrogenase mutants. The mutants lacking either dehydrogenase of the hexose monophosphate shunt were tested for growth on plates containing the following single carbon sources: glucose, fructose, gluconate, glycerol, succinate, xylose, and L-arabinose. They grew on all these compounds, forming colonies of size similar to those of the wild-type strain K-10. Table 3 shows the growth rates in liquid minimal medium with glucose, fructose, or gluconate as sole carbon source. Neither of the mutants grew appreciably slower than the wild type on any of these three media.

TABLE 2. *Isolation of gnd⁻ mutant*[a]

Strain	Origin	Gluconate-6-phosphate dehydrase	Gluconate-6-phosphate dehydrogenase	Genotype
K-10	Wild type	121	121	*edd⁺ gnd⁺*
DF10[b]	Mutant of K-10	0	180	*edd-1 gnd⁺*
DF1070	Mutant of DF10	0	0	*edd-1 gnd-1*
DF1071	Transductant of DF 1070	161	0	*edd⁺ gnd-1*
DF1072	Transductant of DF1070	0	156	*edd-1 gnd⁺*

[a] Extracts were prepared from cultures grown in gluconate-containing broth. Enzyme activities are given as millimicromoles per minute per milligram of protein.

[b] These data are taken from Zablotny and Fraenkel (14).

Enzyme content in various media. To ascertain whether the enzyme deficiency in the mutant extracts could be an artefact resulting from unusual lability in stationary-phase cells, or from an altered regulation such that they do not appear in broth-grown cells, the enzymes were assayed in extracts prepared from logarithmic-phase cells grown in minimal media. Such extracts had the same enzyme deficiency as found in the extracts from stationary-phase broth cultures (Table 4). The absence of one of these enzymes did not affect the constitutivity of the other two.

DISCUSSION

Glucose-6-phosphate dehydrogenase and gluconate-6-phosphate dehydrogenase are very widespread enzymes. Several possible roles in metabolism have been suggested for them (10). These include glucose oxidation by the pentose oxidation cycle, the formation of pentose, and the

TABLE 3. *Growth rates*[a]

Strain	Carbon source		
	Glucose	Fructose	Gluconate
K-10 (wild type)[b]	62	78	56
DF1801 (*zwf*⁻)	65	74	62
DF1071 (*gnd*⁻)	69	78	76

[a] Mass doubling times are given in minutes. Strains K-10 and DF1801 were pregrown in fructose; strain DF1071 was pregrown in gluconate (7).
[b] These data are from Fraenkel and Levisohn (7).

TABLE 4. *Enzyme activities from logarithmic-phase cultures*[a]

Strain	Medium	Phosphoglucose isomerase	Glucose-6-phosphate dehydrogenase	Gluconate-6-phosphate dehydrogenase
K-10	Glucose	729	226	110
DF1801 (*zwf*⁻)	Glucose	689	0	118
DF1801 (*zwf*⁻)	Fructose	580	0	61
DF1801 (*zwf*⁻)	Gluconate	614	0	101
DF1071 (*gnd*⁻)	Glucose	868	195	0
DF1071 (*gnd*⁻)	Fructose	776	141	0
DF1071 (*gnd*⁻)	Gluconate	857	191	0

[a] Extracts were prepared from cultures harvested in minimal medium with the indicated carbon sources. Activities are expressed as millimicromoles per minute per milligram of protein.

reduction of nicotinamide adenine dinucleotide phosphate. [Various terminologies have been used (8, 10, 12) for the pentose oxidation cycle, by which is meant the complete metabolism of glucose-6-phosphate to CO_2 and water by the sequence of reactions including the two dehydrogenases, pentose phosphate isomerase, pentose phosphate epimerase, transketolase, transaldolase, and phosphoglucose isomerase. This sequence, or part of it, is also sometimes called the pentose phosphate pathway, the gluconate-6-phosphate pathway, the hexose monophosphate pathway, or the hexose monophosphate shunt. With respect to pentose formation or utilization, it is usual to refer to the oxidative pentose phosphate pathway (the irreversible two dehydrogenation reactions yielding pentose-5-phosphate from glucose-6-phosphate) and the nonoxidative pentose phosphate pathway (the reversible reactions interconverting pentose-5-phosphate, fructose-6-phosphate, and glyceraldehyde-3-phosphate).] To sort out these roles it would be useful to compare the characteristics of normal cells and mutants lacking these enzymes. Mutations are known affecting the levels of glucose-6-phosphate dehydrogenase in man, and there has been extensive study of the metabolism of tissue from such individuals (1). To my knowledge the only microbial mutant affected in these dehydrogenases is in *Neurospora crassa*, in which an altered glucose-6-phosphate dehydrogenase has been shown to cause pleiotropic effects, including morphological variation (2). [*Species* of microbe are known which lack activity of one or both of these enzymes (1*a*).]

In the present work, mutants of *E. coli* were selected which completely lacked activity of glucose-6-phosphate dehydrogenase or gluconate-6-phosphate dehydrogenase. It is hoped that the relative ease of physiological and genetic experimentation afforded by *E. coli* will be useful in studying the roles of these two enzymes. The present work shows that, in *E. coli*, they are not indispensable. Some glucose metabolism by the hexose monophosphate shunt does occur in the wild type. This is shown by the finding that the specific activity of pyruvate during growth on glucose-*1*-^{14}C is less than one-half the specific activity of the glucose (7). However, it is not known whether this pathway provides energy, and there is no reason to suppose that it is obligatory. Ribose formation in *E. coli* growing on glucose can be mainly ascribed to the oxidative pentose phosphate pathway (12). Results of radioisotope labeling experiments, however, are in accord with the formation of ribose solely by the nonoxidative pentose phosphate pathway in the dehydrogenase-negative mutants (to be reported). The results with the mutants also show that *E. coli* has adequate sources of the reduced form of nicotinamide adenine dinucleotide phosphate besides the hexose monophosphate shunt.

It was shown earlier that, in a mutant lacking phosphoglucose isomerase, the levels of the two dehydrogenases of the hexose monophosphate shunt remained constitutive (7). Similarly, in the present experiments mutation in one dehydrogenase did not affect constitutivity of the other, or of phosphoglucose isomerase. These results militate against one model of constitutivity—that it is merely an expression of induction by internally formed substrates. Thus, suppose the inducer of gluconate-6-phosphate dehydrogenase were gluconate-6-phosphate. In a mutant lacking glucose-6-phosphate dehydrogenase growing on glucose, the level of gluconate-6-phosphate should be zero, whereas, during growth on gluconate, this strain should contain gluconate-6-phosphate; nonetheless, the levels of gluconate-6-phosphate dehydrogenase were similar in the two growth conditions. Such experiments do not, however, exclude the possibility of an internal inducer metabolically unrelated to the enzyme.

Genetic mapping of the genes of the two dehydrogenases is described in an accompanying paper (11).

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