Regulation of Glucose-6-Phosphate Dehydrogenase in *Brevibacterium flavum*

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NADP-Specific G6P dehydrogenase was partially purified from *Brevibacterium flavum*. Its activity, with an optimum pH of 7.5, was stabilized by KCl or Mg^{2+} and inhibited by diamide, a sulfhydryl reagent. It was also inhibited by oxaloacetate, FBP, PRPP, acetyl-CoA, Ru5P, xylulose 5-phosphate and NADPH. Among them, oxaloacetate showed the strongest inhibition. The concentration of oxaloacetate giving 50% inhibition was 0.09 mM. The inhibitions by oxaloacetate, FBP, PRPP, and NADPH were non-competitive, mixed, and competitive for both the substrates, respectively. Oxaloacetate in combination with FBP, PRPP, or Ru5P inhibited the activity cumulatively. The sensitivities to the oxaloacetate, FBP, and PRPP inhibitions were lost on ammonium sulfate treatment, whereas that to NADPH inhibition was not affected at all. The inhibition by oxaloacetate was specific to glutamate-producing bacteria belonging to the genera, *Brevibacterium* and *Corynebacterium*, in contrast to those by FBP and PRPP, which were found in almost all bacteria tested. G6P dehydrogenase in *B. flavum* was induced by glucose when it was cultured on acetate, succinate, or glutamate.

The contribution of the pentose phosphate pathway to glucose metabolism in *Brevibacterium flavum* has been indicated by the metabolism of various ^{14}C -labeled glucoses¹⁾ and supported by the presence of G6P dehydrogenase activity in the cell-free extract.²⁾ However, its contribution to pyruvate formation from glucose was estimated to be only about 10%.¹⁾ Furthermore, glucose phosphate isomerase-deficient mutants of *B. flavum* did not grow on glucose but did on gluconate.³⁾ These results suggest that in *B. flavum* the pentose phosphate pathway is not a main route for glucose metabolism, but that it operates to supply erythrose 4-phosphate and PRPP, precursors for aromatic amino acid biosynthesis. Since some mutants of *B. flavum* have been bred as aromatic amino acid-producing strains,⁴⁾ it is interesting to elucidate the regulatory mechanisms for the biosynthesis of these precursors via the pentose phosphate

pathway.

This paper deals with the control of the activity and synthesis of G6P dehydrogenase, the first step enzyme of the pathway in *B. flavum*.

MATERIALS AND METHODS

Organisms. *Brevibacterium flavum* No. 2247 (ATCC 14067) was used. In addition, the organisms listed in Table V were used for comparison with *B. flavum* as to the regulation of G6P dehydrogenase.

Media. A complex medium, Medium 7,⁵⁾ and a minimal medium, Medium 13,⁵⁾ were used. Medium 40, which was a modification of Medium 29,⁶⁾ was composed of 50 g of monosodium glutamate or 20 g of an organic acid plus 15 g of $(NH_4)_2SO_4$, 5 g of KH_2PO_4 , 1.2 g of $MgSO_4 \cdot 7H_2O$, 100 mg of $FeSO_4 \cdot 7H_2O$, 8 mg of $MnSO_4 \cdot 4H_2O$, 100 μ g of thiamine HCl, 500 μ g of *d*-biotin, and 3 g of yeast extract (Daigo Eiyo) in a total volume of 1 liter. The pH was adjusted to 7.0 with NaOH and then the medium was sterilized at 115°C for 10 min.

Abbreviations: G6P, glucose 6-phosphate; TES, *N*-tris-(hydroxymethyl)methyl-2-aminoethane sulfonic acid; PRPP, phosphoribosyl pyrophosphate; FBP, fructose 1,6-bisphosphate; Ru5P, ribulose 5-phosphate; acetyl-CoA, acetyl coenzyme A.

Chemicals. G6P, NADP and NADPH were purchased from Boehringer Mannheim. Oxaloacetate and DEAE-cellulose (DE-52) were from ICN Pharmaceuticals and Whatman, respectively. Other chemicals were purchased from Sigma Chemical Co. and Boehringer Mannheim.

Enzyme preparation. Cells of *B. flavum* No. 2247 cultured in 500-ml flasks containing 50 ml of Medium 13 at 30°C for 24 hr were harvested, washed twice with 0.2% KCl and then suspended in 0.1 M Tris-buffer, pH 7.5, containing 10 mM MgCl₂. Then, the cells were sonically disrupted at 10 kc for 20 min. The cell-free extract obtained by centrifugation at 32,000 × *g* for 30 min (Fraction I) was placed on a DEAE-cellulose column (2.5 × 22 cm) equilibrated with 0.05 M Tris-HCl buffer, pH 7.5, containing 30 mM MgCl₂. The column was washed with 100 ml of the same buffer. G6P dehydrogenase was eluted with a KCl concentration gradient consisting 500 ml of the same buffer and the latter containing 0.4 M KCl. In order to stabilize the enzyme, KCl solution was added to the test tubes before the elution, to give a final concentration of 0.5 M. Fractions of 8 ml were collected. The fractions containing G6P dehydrogenase activity were pooled, concentrated with an ultrafiltration apparatus (Amicon PM-10) and then used as the enzyme preparation (Fraction II). Since the enzyme preparation contained small amounts of the activities of aldolase, glucose phosphate isomerase, phosphoglucumutase, and 6-phosphogluconate dehydrogenase, the effects of fructose 6-phosphate, FBP, glyceraldehyde 3-phosphate, dihydroxyacetone phosphate and glucose 1-phosphate on the activity, as shown in Table II, were determined after the contaminating enzymes had been removed from the preparation by rechromatography under the same conditions as for the first chromatography (Fraction III).

Assay method. G6P dehydrogenase was assayed by measuring the increase in absorbancy at 340 nm of the reaction product, NADPH. The standard reaction mixture contained 0.05 M Tris-HCl buffer, pH 7.5, 10 mM MgCl₂, 2 mM G6P, 0.5 mM NADP and the enzyme in a total volume of 0.5 ml. One unit of the activity was defined as that catalyzing the formation of 1 nmol of NADPH per min.

Protein was determined by the method of Lowry *et al.*⁷⁾

RESULTS

General properties

As shown in Table I, when G6P dehydrogenase in cell-free extracts was transferred into Tris-HCl buffer, pH 7.5, by gel filtration, it lost its activity completely, but was stabilized by the addition of 0.5 M KCl or 10 mM MgCl₂. G6P and NADP, the substrates, and dithio-

TABLE I. STABILITY OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE

The cell-free extract (Fraction I) was passed through a Sephadex G-25 column (1.5 × 5 cm) equilibrated with 0.05 M Tris-HCl buffer, pH 7.5, containing the additions indicated in the table. Then, the activity was measured under the standard assay conditions. The specific activities relative to that of the cell-free extract are listed in the table. The specific activity of the cell-free extract was 85 units/mg protein.

Addition to buffer	Activity (%)
None	0
0.5 M KCl	130
10 mM MgCl ₂	118
1 mM Dithiothreitol	25
1 mM G6P	15
0.1 mM NADP	21

threitol showed slight stabilizing effects. Then, the enzyme was partially purified in the presence of KCl and Mg²⁺ from the cell-free extract by DEAE-cellulose column chromatography. It was eluted from the column with 0.12 M KCl and purified about 3-fold. When further purified by column chromatography on Sephadex G-200 or hydroxyapatite in the presence of KCl and Mg²⁺ but in the absence of an SH group stabilizing agent, it was inactivated or desensitized to the regulation, respectively. Therefore, the fraction obtained on DEAE-cellulose column chromatography was used as the enzyme preparation.

The enzyme reaction completely depended on the substrates, G6P and NADP, and did not proceed with NAD at all, similarly to the previous results obtained using cell-free extracts.²⁾ The enzyme required Mg²⁺ for its activity and showed the maximum activity at more than 6 mM. The activity without Mg²⁺ was only 1% of that with 10 mM Mg²⁺. Mn²⁺ also developed the enzyme activity, which was, however, only 77% of that with 10 mM Mg²⁺ at the optimum concentration of 3 mM.

The optimum pH of the reaction was 7.5 with both TES-NaOH and Tris-HCl buffers (Fig. 1).

When the enzyme reaction was started after preincubation of the enzyme with diamide, a

FIG. 1. Effects of pH Glucose-6-phosphate Dehydrogenase Activity and on the Inhibition by Oxaloacetate.

The reactions were carried out using Fraction II in the presence and absence of 1 mM oxaloacetate under the standard assay conditions except that buffers of various pHs, 0.15 mM G6P and 0.15 mM NADP were used. —●— and —○—, Tris-HCl buffer; —▲—, and —△—, TES-NaOH buffer. —○— and —△—, enzyme activity; —●— and —▲—, relative activity in the presence of 1 mM oxaloacetate.

sulfhydryl reagent, for 90 sec, the activity decreased to 0.8% of that without diamide but was completely recovered on further incubation with 30 mM dithiothreitol. This indicates that a sulfhydryl group is involved in the active site of the enzyme.

Figure 2 shows double reciprocal plots of the reaction rate against the substrate concentrations. Plots against both the substrates gave straight lines that intersected at a point, indicating that the reaction mechanism was sequential. The *K_m*s for G6P and NADP were calculated from the plots of the intercepts at the vertical lines against the reciprocals of the second substrate concentrations to be 0.14 and 0.024 mM, respectively.

Regulation of glucose-6-phosphate dehydrogenase activity

The effects of various metabolites on the

TABLE II. EFFECTS OF METABOLITES ON GLUCOSE-6-PHOSPHATE DEHYDROGENASE

The activity was measured using Fraction II (327 units/mg protein) in the presence and absence of the additions under the standard assay conditions except that the concentrations of G6P and NADP were 0.15 mM. The relative activities in the presence of fructose 6-phosphate, FBP, glyceraldehyde 3-phosphate, dihydroxyacetone phosphate and glucose 1-phosphate were determined using the rechromatographed enzyme preparation (Fraction III, 626 units/mg protein), as described in MATERIALS AND METHODS.

Addition (1 mM)	Relative activity (%)	Addition (1 mM)	Relative activity (%)
None	100	Fructose-1P	106
6-Phosphogluconate	102	Citrate	94
Ru5P	73	2-Oxoglutarate	101
Xylulose-5P	74	Succinate	97
Ribose-5P	80	Fumarate	97
PRPP	64	Malate	97
Sedoheptulose-7P	103	Oxaloacetate	33
Erythrose-4P	90	L-Glutamate	97
Fructose-6P	94	L-Aspartate	100
FBP	60	ATP	96
Glyceraldehyde-3P	82	ADP	90
Dihydroxyacetone-P	93	AMP	94
3-Phosphoglycerate	95	GMP	90
2-Phosphoglycerate	93	L-Tryptophan	99
Phosphoenolpyruvate	90	L-Phenylalanine	96
Pyruvate	98	L-Tyrosine	92
Acetyl-CoA	74	L-Histidine	99
Glucose-1P	100		
Glucose 1,6-bisP	74	NADPH ^a	48
Fructose 2,6-bisP	73	NADH ^b	111

^a 0.1 mM.

^b 0.15 mM. Abbreviation: P, phosphate.

FIG. 2. Double Reciprocal Plots of the Glucose-6-phosphate Dehydrogenase Reaction Rate against the Substrate Concentration.

The activities were assayed using Fraction II under the standard conditions except for the substrate concentration. In (A), the NADP concentrations were 0.5 (—○—), 0.05 (—△—), 0.025 (—□—), 0.0167 (—●—), and 0.0125 mM (—▲—). ----, secondary plots of the intercept at the vertical line in (B). In (B), the G6P concentrations were 2.0 (—○—), 0.4 (—△—), 0.2 (—□—), 0.13 (—●—), and 0.10 mM (—▲—). ----, secondary plots of the intercept at the vertical line in (A).

G6P dehydrogenase activity were examined using a modified assay system, in which the concentrations of both substrates were decreased to 0.15 mM, almost the same as the K_m value for G6P. As shown in Table II, the enzyme activity was inhibited specifically by Ru5P and xylulose 5-phosphate among the intermediates of the pentose phosphate pathway, by PRPP, by FBP among the intermediates of glycolysis, by acetyl-CoA, and by oxaloacetate among the intermediates of the tricarboxylic acid cycle. The activity was also inhibited by NADPH, one of the reaction products. NADPH showed the strongest inhibition among the metabolites tested (52% at 0.1 mM), while oxaloacetate caused 67% inhibition at 1.0 mM. Whereas analogs of FBP, glucose 1,6-bisphosphate and fructose 2,6-bisphosphate, showed slight inhibitory effects, purine nucleotides and aromatic amino acids had no effect on the activity at all.

The inhibition by oxaloacetate, the strongest in Table II except for that by the reaction product, NADPH, was further examined. As shown in Fig. 1, the inhibition by oxaloacetate increased at a lower pH and was stronger in

FIG. 3. Effect of Oxaloacetate on the Glucose-6-phosphate Dehydrogenase.

The activity was assayed using Fraction II under the standard assay conditions except that TES-NaOH buffer, pH 7.0, 0.15 mM G6P, and 0.15 mM NADP were used. Oxaloacetate was added at the concentrations indicated.

FIG. 4. Double Reciprocal Plots of the Glucose-6-phosphate Dehydrogenase Reaction Rate against the Substrate Concentration in the Presence and Absence of Inhibitors.

The activity was assayed using Fraction II under the standard conditions except for the substrate concentration. To the assay mixture, 2 mM oxaloacetate (—■—), 5 mM FBP (—▲—), 5 mM PRPP (—□—), 0.1 mM NADPH (—△—), or nothing (—○—) was added. The concentration of NADP in (A) and (C) was 0.5 mM and that of G6P in (B) and (D) was 2.0 mM.

TES-NaOH buffer than in Tris-HCl buffer, presumably due to the formation of Tris oxaloacetate. Thus, the inhibition by 1 mM oxaloacetate increased 30% in TES-NaOH buffer, pH 7.0, compared with in the case of the assay conditions in Table II. As shown in Fig. 3, oxaloacetate inhibited the activity completely

at 1.0 mM in TES-NaOH buffer, pH 7.0. The concentration giving 50% inhibition was estimated to be 0.09 mM. This suggests the possible operation of the oxaloacetate inhibition under physiological conditions.

Figure 4 shows double reciprocal plots of the reaction rate against the substrate concentrations in the presence and absence of oxaloacetate, FBP, PRPP, or NADPH. They showed the same inhibition types for both the substrates. The inhibition by oxaloacetate, FBP, PRPP, or NADPH was non-competitive, mixed, mixed, or competitive for the substrates, respectively. These results suggest that the former three inhibitors are allosteric inhibitors that bind to different site(s) from the active site, whereas NADPH bound at the active site as the reaction product. The competitive inhibition by the reaction product, NADPH, indicates the reaction mechanism of rapid equilibrium random BiBi⁽⁸⁾

The effect of the simultaneous presence of

inhibitors on the enzyme activity was examined, as shown in Table III. Among five inhibitors tested, the combination of oxaloacetate with FBP, PRPP, or Ru5P caused cumulative inhibition. This suggests that the inhibitions by oxaloacetate and FBP, PRPP or Ru5P operate independently.

Desensitization to the allosteric inhibition

When the enzyme was precipitated with ammonium sulfate and then removed by dialysis repeatedly three times, the inhibitions by oxaloacetate, FBP, and PRPP were markedly reduced, whereas that by the reaction product, NADPH, as well as the enzyme activity, was not affected by the treatment at all (Table IV). This suggests that the former three inhibitors bind to site(s) different from the active site and labile to the ammonium sulfate treatment. As described previously, the enzyme was also desensitized to the oxaloacetate inhibition on hydroxyapatite chromatography.

Effects of allosteric inhibitors on enzymes from other bacteria

Since the effects of oxaloacetate, FBP,

TABLE III. CUMULATIVE INHIBITION OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE

The activity was assayed using Fraction II in the simultaneous presence of two inhibitors under the same conditions as in Fig. 3. The theoretical values for cumulative inhibition are given in parentheses.

Additions	Concentration (mM)	Relative activity (%)
None	—	100
Oxaloacetate	0.06	65
+ FBP	0.06 + 1.0	34 (36)
+ PRPP	0.06 + 1.0	40 (42)
+ Ru5P	0.06 + 1.0	41 (41)
+ Acetyl-CoA	0.06 + 1.0	66 (45)
FBP	1.0	55
+ PRPP	1.0 + 1.0	64 (36)
+ Ru5P	1.0 + 1.0	60 (35)
+ Acetyl-CoA	1.0 + 1.0	56 (38)
PRPP	1.0	65
+ Ru5P	1.0 + 1.0	63 (41)
+ Acetyl-CoA	1.0 + 1.0	55 (45)
Ru5P	1.0	63
+ Acetyl-CoA	1.0 + 1.0	58 (44)
Acetyl-CoA	1.0	70

TABLE IV. EFFECT OF AMMONIUM SULFATE TREATMENT ON THE SENSITIVITY TO INHIBITION AND THE ACTIVITY OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE

Ammonium sulfate and EDTA were added to Fraction II to 0.7 saturation and 80 mM, respectively. After standing overnight at 5°C, the enzyme was dialyzed against 0.05 M Tris-HCl buffer, pH 7.5, containing 0.5 M KCl. This procedure was further repeated twice. Then, the activity was assayed in the presence and absence of each inhibitor under the same conditions as in Fig. 3. The specific activity was determined under the standard assay conditions.

Inhibitor	Concentration (mM)	Inhibition(%)	
		Treated	Untreated
		(212) ^a	(280) ^a
Oxaloacetate	0.6	34	96
FBP	5.0	29	65
PRPP	5.0	16	55
NADPH	0.06	60	59

^a Specific activity (units/mg protein).

TABLE V. INHIBITION BY OXALOACETATE, FRUCTOSE 1,6-BISPHOSPHATE, AND PHOSPHORIBOSYL PYROPHOSPHATE OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE IN VARIOUS BACTERIA

The bacterial strains were cultured in Medium 7 at 30°C for 24 hr. The cells were sonically disrupted by the method described in MATERIALS AND METHODS. Supernatant solutions obtained on centrifugation of the sonicates were passed through a Sephadex G-25 column (1.5 × 5 cm) equilibrated with 0.05 M Tris-HCl buffer, pH 7.5, containing 0.5 M KCl, and then used as enzyme preparations. The specific activity was determined under the standard assay conditions and percent inhibition was determined under the same conditions as in Fig. 3 in the presence of 1 mM oxaloacetate, 5 mM FBP, or 5 mM PRPP. The inhibitions in *P. putida*, *B. ammoniagenes* and *C. xerosis* were determined with the same substrate concentrations as for the standard assay conditions due to their low enzyme activities.

Enzyme source	Inhibition (%)		
	Oxaloacetate	FBP	PRPP
<i>Brevibacterium flavum</i> ATCC 14067	94	80	51
<i>Escherichia coli</i> ATCC 10798	3	57	50
<i>Bacillus subtilis</i> ATCC 6051	19	47	34
<i>Salmonella typhimurium</i> AJ 3224	3	29	34
<i>Pseudomonas putida</i> ATCC 17390	2	42	24
<i>Streptococcus faecalis</i> ATCC 12984	-1	52	43
<i>Lactobacillus casei</i> ATCC 7469	2	51	52
<i>Lactobacillus plantarum</i> ATCC 8014	4	29	42
<i>Leuconostoc mesenteroides</i> ATCC 8042	1	6	25
<i>Zymonas mobilis</i> ATCC 29191	0	18	6
<i>Corynebacterium equi</i> ATCC 6939	-8	68	48
<i>Corynebacterium xerosis</i> ATCC 373	16	56	2
<i>Corynebacterium glutamicum</i> ATCC 13032	89	69	61
<i>Corynebacterium acetoacidophilum</i> ATCC 13870	91	69	65
<i>Corynebacterium lilium</i> ATCC 15990	88	70	59
<i>Brevibacterium ammoniagenes</i> ATCC 6871	8	40	3
<i>Brevibacterium lactofermentum</i> ATCC 13869	89	77	52
<i>Brevibacterium divaricatum</i> ATCC 14020	91	63	53
<i>Brevibacterium roseum</i> ATCC 13825	81	68	61

and PRPP, found in this study, have not been previously reported for any bacteria, they were examined in 10 genera of bacteria (Table V). The inhibitions by FBP and PRPP were found in almost all bacteria tested as in *B. flavum*. However, the inhibition by oxaloacetate was found only in glutamate-producing species of the genera, *Brevibacterium* and *Corynebacterium*.

Regulation of glucose-6-phosphate dehydrogenase synthesis

The specific G6P dehydrogenase activities of *B. flavum* grown on various carbon sources were determined in order to examine regulation of the enzyme synthesis, as shown in Table VI. The activities of cells grown on acetate, glutamate, and succinate, the assimi-

lation of which might not require the G6P dehydrogenase reaction, decreased to 4 to 9% of those of cells grown on glucose. The addition of the same amounts of glucose to these carbon sources increased the specific activity.

DISCUSSION

The G6P dehydrogenase of *B. flavum* was found to have similar properties to those of other microorganisms reported.^{9,10} That is, the enzyme was stabilized by KCl, specific to NADP, required Mg^{2+} , and inhibited by a sulfhydryl reagent. The activity was inhibited by various metabolites such as oxaloacetate, FBP, PRPP, acetyl-CoA, Ru5P, xylulose 5-phosphate, and NADPH. Furthermore, the inhibitions by oxaloacetate, FBP, and PRPP

TABLE VI. EFFECTS OF CARBON SOURCES IN THE MEDIUM ON THE SPECIFIC ACTIVITY OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE

B. flavum No. 2247 was cultured at 30°C for 24 hr with various carbon sources. In Expt. I, Medium 13, of which the concentration of urea was 1.5 g/liter, was used, and Medium 40 was used in Expt. II and III. When glutamate, succinate, and citrate were used as carbon sources in Expt. II, the strain was cultured for 48 hr. The enzyme was prepared by the same method as described in Table V. The specific activity was determined under the standard assay conditions.

Expt.	Carbon source	Growth (A_{562})	Specific activity (units/mg protein)
I	Glucose	0.495	97
	Fructose	0.360	81
	Ribose	0.260	58
	Gluconate	0.440	87
II	Glucose	0.345	93
	Lactate	0.395	14
	Acetate	0.430	8.7
	Glutamate	0.315	3.5
	Succinate	0.190	3.9
	Citrate	0.330	40
III	Acetate + glucose	0.540	74
	Glutamate + glucose	0.670	49
	Succinate + glucose	0.670	27

were not competitive for the substrates. The sensitivities to oxaloacetate, FBP, and PRPP inhibitions were lost on ammonium sulfate treatment, which did not affect the activity, in contrast to that to NADPH inhibition. Oxaloacetate showed cumulative inhibition in combination with FBP, PRPP, or Ru5P. These results indicate that the G6P dehydrogenase of *B. flavum* is an allosteric enzyme.

Oxaloacetate showed the strongest inhibition among the inhibitors tested and its concentration giving 50% inhibition was 0.09 mM. Moreover, the inhibition by oxaloacetate was further enhanced by the simultaneous presence of other inhibitors. It is strongly suggested that the inhibition actually operates under physiological conditions in *B. flavum*. These inhibitions of G6P dehydrogenase seem to have the following physiological significance; the inhibition by PRPP is a

feedback inhibition of its biosynthesis, that by NADPH is a product inhibition, and those by FBP and oxaloacetate are feedback control of the central carbon metabolism.

Whereas the inhibitions of G6P dehydrogenase by NADPH, ATP, ADP, AMP, inorganic phosphate, acetyl-CoA, and pyridoxal 5-phosphate have been found previously in various microorganisms,⁹⁻¹³⁾ those by oxaloacetate, FBP, and PRPP found in *B. flavum* have not been known in any microorganisms. The inhibitions by FBP and PRPP were found in almost all strains tested, suggesting their general operation in bacteria. On the contrary, the inhibition by oxaloacetate was highly specific to glutamate-producing bacteria belonging to the genera, *Brevibacterium* and *Corynebacterium*. In *Streptococcus faecalis*,¹⁴⁾ FBP was reported not to inhibit G6P dehydrogenase activity, whereas in this study, it was found to inhibit the activity of a strain of *S. faecalis*, which was not the same strain as in the report.¹⁴⁾ This seems to be due to that the reaction system contained lower concentrations of the substrates than in that previous study.¹⁴⁾

Compared with glucose as a carbon source, the synthesis of the enzyme was reduced in cultivations with organic acids as carbon sources, which were metabolized through a distant pathway from the G6P dehydrogenase reaction in the metabolic pathway. The addition of glucose to these carbon sources stimulated the synthesis of the enzyme, suggesting the occurrence of the induction by glucose or its metabolite(s). Wolf *et al.* also showed that the G6P dehydrogenase level decreased in cells grown on acetate as a carbon source in *Escherichia coli*, but they concluded that the carbon source affected its growth rate, which, in turn, affected the enzyme level.¹⁵⁾

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