JOURNAL OF BACTERIOLOGY, Mar. 1980, p. 1115-1121
0021-9193/80/03-1115/07$02.00/0

Vol. 141, No. 3

Genetic and Physiological Characterization of *Escherichia coli* Mutants Deficient in Phosphoenolpyruvate Carboxykinase Activity

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Mutants doubly deficient in phosphoenolpyruvate carboxykinase (*pck*) and phosphoenolpyruvate synthetase (*pps*) were unable to grow with succinate as the sole carbon source. A number of *pck* mutations isolated from *pps* strains by penicillin selection mapped at 74 min on the *Escherichia coli* chromosome, between *glpD* and *aroB*. Several of the strains containing these mutations had a protein antigenically related to phosphoenolpyruvate carboxykinase, and, therefore, the mutations probably represented mutations in the structural gene for this enzyme. Phosphoenolpyruvate carboxykinase was regulated at the level of transcription by catabolite repression. Enzyme levels also increased in stationary-phase cultures by a mechanism independent of cyclic adenosine monophosphate or the product of the *relA* gene.

Previous results from this laboratory (Goldie and Sanwal, Proc. Can. Fed. Biol. Soc. **18**:43, 1975) and elsewhere (10, 11) have shown that two pathways exist for the synthesis of phosphoenolpyruvate (PEP) from four-carbon intermediates in *Escherichia coli* (Fig. 1). Double mutants deficient in PEP carboxykinase (EC 4.1.1.49) and PEP synthetase (EC 2.7.1. dd) (4) and double mutants deficient in PEP carboxykinase and the NAD-dependent malic enzyme (EC 1.1.1.38) are unable to grow on succinate or other four-carbon intermediates, whereas single mutants (deficient in any of these enzymes) do so readily.

This paper reports the isolation of a number of mutants deficient in PEP carboxykinase (*pck*) from two different *pps* strains (deficient in PEP synthetase) and the genetic characterization of the *pck pps* strains. In addition, the genetic regulation of PEP carboxykinase was investigated by using mutants defective in adenyl cyclase (*cya*) or cyclic AMP receptor protein (*crp*) and mutants lacking the stringent response (*relA*).

MATERIALS AND METHODS

Bacterial strains and bacteriophage. *E. coli* K-12 strains are described in Table 1. Bacteriophage P1 *CAM clr*100 (16) was supplied by J. L. Rosner.

Media. LB medium (15) was supplemented with 2.5 mM $CaCl_2$. LB plus glucose contained 0.1% glucose in addition. Medium A (15) was prepared without citrate and supplemented with 0.4% carbon source and 10 μg of thiamine per ml. Auxotrophs were supplemented with 100 μg of amino acids per ml or 20 μg of nucleosides per ml, as required. *asd* mutants (12) were supplemented with 50 μg of *rac*-2,6-diaminopimelic acid per ml in enriched media and L-threonine, L-methionine, L-lysine, and *rac*-2,6,-diaminopimelic acid (100 μg of each per ml) in medium A. Strains completely deficient in inorganic phosphate transport (*pit pst*) (20) were supplemented with 40 μg of *rac*-glycerol-3-phosphate per ml in all media, and *pit*$^+$ transductants were scored on low-phosphate medium 56LP (20). MacConkey agar contained (per liter of medium) 17 g of peptone, 3 g of proteose peptone, 1.5 g of bile salts no. 3, 5 g of NaCl, 30 mg of neutral red, 1 mg of crystal violet, and 15 g of agar. Various carbohydrates were sterilized separately and added to the medium to give final concentrations of 1%. Bacteriological media were obtained from Difco Laboratories.

Chemicals and reagents. *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine was from Aldrich Laboratories. Penicillin G (potassium salt) was from Connaught Medical Research Laboratories. Oxaloacetic acid was from Boehringer Mannheim Corp., and tetrapropylammonium hydroxide was from Eastman Kodak Co.

General methods. Mutants were selected after *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine mutagenesis (1) by penicillin selection (8) with 4×10^4 IU of penicillin per ml. Bacteriological matings in LB plus glucose and transduction with P1 *CAM clr*100 were performed as described by Miller (15).

Selection of mutants defective in PEP carboxykinase. Cells with *pps* genotype were mutagenized, allowed to recover in medium A containing 0.4% glucose, subjected to penicillin selection in medium A containing 0.4% succinate, and allowed to recover in medium A containing 0.4% glucose. Cultures were diluted and plated onto medium A containing glucose and replica plated on succinate and glucose plates. Colonies which grew on glucose but not succinate were picked and purified.

Succinate$^-$ isolates were grown to maximum density

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in 5 ml of LB medium, treated with toluene (15), suspended in 1 ml of 0.1 M Tris (pH 7.5), and assayed for PEP carboxykinase activity.

Preparation of cell extracts and protein determinations. Large amounts of cell extracts for determination of enzyme specific activities or PEP carboxykinase antigen were prepared at 4°C from cells grown to early stationary phase in 500 ml of LB medium. Cells were centrifuged at 9,000 × *g*, washed in 50 mM sodium phosphate buffer (pH 7.5), and suspended in 0.1 M Tris–1 mM EDTA–0.1 mM dithiothreitol, pH 7.5 (dithiothreitol was omitted for immunoassay with no effect on PEP carboxykinase activity). Protein was determined by a modification of the method of Lowry et al. (6), using bovine serum albumin as a standard.

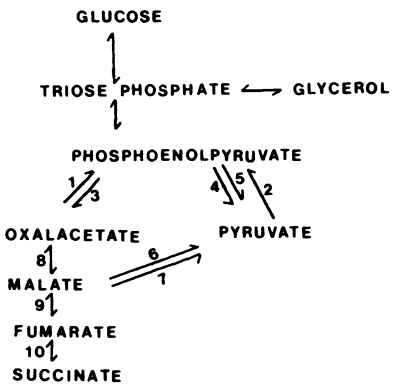

FIG. 1. *Outline of the pathways of gluconeogenesis from four-carbon metabolites in E. coli. Reactions thought to be irreversible in vivo are represented by monodirectional arrows. Duplicate arrows for malic enzyme and pyruvate kinase reactions indicate the presence of two independent enzymes catalyzing these steps. The numbers next to the arrows denote the following: 1, PEP carboxykinase; 2, PEP synthetase; 3, PEP carboxylase; 4, AMP-activated pyruvate kinase; 5, fructose diphosphate-activated pyruvate kinase; 6, NAD-dependent malic enzyme; 7, NADP-dependent malic enzyme; 8, malate dehydrogenase; 9, fumarase; 10, succinate dehydrogenase.*

Enzyme assays. PEP carboxykinase was assayed by the $^{14}CO_2$ exchange assay described elsewhere (24), except that the concentration of ATP used was 1 mM. Oxaloacetate was titrated to pH 6.1 with tetrapropylammonium hydroxide and immediately used to start PEP carboxykinase assays. NAD-dependent malic enzyme (18), NADP-dependent malic enzyme (EC 1.1.1.40) (19), succinate dehydrogenase (EC 1.3.99.1) (13), malate dehydrogenase (EC 1.1.1.37) (17), fumarase (EC 4.2.1.2) (14), PEP synthetase (EC 2.7.1. dd) (4), malate oxidase (EC 1.1.3.3) (7), and fructose diphosphate-activatable and AMP-activatable pyruvate kinases (EC 2.7.1.40) (23) were assayed by published methods. One unit of enzyme activity was defined in each case as the amount producing 1 mol of product per min.

Immunological methods. Sera with specific anti-PEP carboxykinase activities were obtained from rabbits injected with homogeneously purified enzyme (manuscript in preparation), which was obtained from strain KLF41/HG45. Each of two 1-kg New Zealand rabbits received subcutaneous injections of 2 ml of an emulsion containing 1 ml (400 μg of protein) of enzyme

TABLE 1. *Bacterial strains*

Strain	Genotype	Derivation[a]
DF1651	F⁻ *thi pps lac pyrD edd-1 his tyrA rpsL*	Institut Pasteur
HG2	F⁻ *pck-1 pps* (otherwise as for DG1651)	This work; NNG mutagenesis of DF1651
HG4	F⁻ *pck-2 pps* (otherwise as for DF1651)	NNG mutagenesis of DF1651
HG5	F⁻ *pck-3 pps* (otherwise as for DF1651)	NNG mutagenesis of DF1651
HG7	F⁻ *pck-4 pps* (otherwise as for DF1651)	NNG mutagenesis of DF1651
HfrH	Hfr 3000 *thi-1 rel-1* λ⁻ (wild type)	Institut Pasteur
Hfr "C" U482	HfrH *asd* (otherwise as for HfrH)	Institut Pasteur
HG29	HfrH *pps leu argG rpsL asd*	This work; *asd* allele from Hfr "C" U482
HG34	HfrH *pck-5 pps* (otherwise as for HG29)	
HG49	HfrH *pps asd*	NNG mutagenesis of Hfr "C" U482
F′ strains	See Fig. 2	CGSC collection
AB2834	F⁻ *aroE353 mal-352 tsx-352*	CGSC collection
HG46	*pps aroE353* (otherwise as for AB2834)	NNC mutagenesis of AB2834
AB2847	F⁻ *aroB351 mal-354 tsx-354*	CGSC collection
HG47	*pps aroB351* (otherwise as for AB2847)	NNG mutagenesis of AB2847
10B5	HfrC *glpD-3 pit-1 pst-2*	CGSC collection
HG54	*pck-5 pps glpD-3 pit-1 pst-2 leu*	10B5 × HG34 (phenocopied HG34 and selected *asd*⁺ *rpsL*)
MD3	HfrG6 Δ(*mal-glpD-asd*)	CGSC collection
GP1	HfrH *cya*	Institut Pasteur
CA8445	HfrH Δ*cya* Δ*crp*	J. Beckwith
CP78	W3110 F⁻ *relA*⁺ *thi-1 thr-1 leu-6 his-65 arg-46*	J. D. Friesen
CP79	F⁻ *relA2* (otherwise as for CP78)	J. D. Friesen

[a] NNG, *N*-Methyl-*N*′-nitro-*N*-nitrosoguanidine; CGSC, Coli Genetic Stock Center, Yale University, New Haven, Conn.

in 0.1 M Tris–1 mM EDTA (pH 7.5) and 1 ml of Freund complete adjuvant (Difco). Three injections were given at 14-day intervals. Sera of both animals obtained 10 days after the third bleeding were used without further purification.

The anti-PEP carboxykinase activities of the sera were determined by the quantitative method of Hamers and Hamers-Casterman (9), as described by Deleo and Magasanik (5). Various volumes (25 to 150 μl) of extract diluted 10-fold in buffer (0.1 M Tris–1 mM EDTA, pH 7.5) were added to a series of tubes containing a fixed amount (50 to 100 μl) of 10-fold-diluted serum. Total volume was brought to 260 μl with Tris buffer. The tubes were incubated at 37°C for 1 h and at 40°C for 21 h and assayed for PEP carboxykinase activity. At the equivalence point, 1 ml of the serum precipitated 560 ± 60 U (nmol of $^{14}CO_2$ exchanged per min) of PEP carboxykinase activity, and titers remained stable in antisera stored for 12 months at −20°C.

Antigen was measured in extracts by the same procedure which was used for titrating antiserum. To tubes containing appropriate amounts of 10-fold-diluted serum, a fixed amount of 10-fold-diluted mutant extract was added and adjusted to a final volume of 200 μl. After 1 h at 37°C and 21 h at 4°C, various amounts of titrated wild-type extract were added to the tubes (final volume, 260 ml). After 2.5 h at 37°C and 21 h at 4°C, tubes were centrifuged at 8,000 × *g*, and supernatants were assayed for PEP carboxykinase activity. Cross-reacting material (CRM) was expressed as units of enzyme activity remaining in the extract (above controls with no mutant extract) per milligram of mutant extract.

RESULTS

Characteristics of mutants deficient in PEP carboxykinase. The *pps* strains used to isolate PEP carboxykinase mutants (DF16511 and HG29) were not able to grow on pyruvate or lactate as sole carbon source due to deficiencies in PEP synthetase (3), but grew well on other carbon sources.

About 20% of the succinate$^-$ isolates obtained from these *pps* strains by penicillin selection had reduced activity of PEP carboxykinase (Table 2) and were designated *pck* mutants. For each of these mutants it was ascertained that the cell-free extract did not inhibit PEP carboxykinase activity of wild-type extracts. The *pck pps* strains (such as strain HG4 [Table 3]) were defective for growth on succinate, fumarate, malate, acetate, and pyruvate but grew well on glucose or glycerol as sole carbon source. Spontaneous revertants were obtained from all *pck pps* strains on pyruvate plates and succinate plates. Revertants obtained on pyruvate plates were uniformly succinate$^+$ and pyruvate$^+$ and had regained activity of PEP synthetase (e.g., HG4-1, *pck pps*$^+$ [Tables 2 and 3]). Revertants obtained on succinate plates fell into two classes: succinate$^+$ pyruvate$^-$ revertants, which had regained activity of PEP carboxykinase (e.g., HG-4-2, *pck*$^+$ *pps* [Tables 2 and 3]) and succinate$^+$ pyruvate$^+$ revertants, which had regained activity of PEP synthetase (e.g., HG4-3, *pck pps*$^+$ [Tables 2 and 3]). It was evident that the succinate$^-$ phenotype was dependent on the presence of both *pck* and *pps* lesions.

TABLE 2. *Specific activities of PEP carboxykinase, PEP carboxykinase antigen (CRM), and PEP synthetase in mutant strains of E. coli K-12*

Strain	Relevant genotype	PEP carboxykinase activity (U/mg)[a]	PEP carboxykinase antigen (CRM) (U/mg)[b]	PEP synthetase activity (U/mg)[a]
HfrH	*pck*$^+$ *pps*$^+$	32	ND[c]	9.0
DF1651	*pck*$^+$ *pps*	33	ND	0.8
HG2	*pck-1 pps*	0.2		ND
HG4	*pck-2 pps*	0.2	33	0.8
HG5	*pck-3 pps*	11	9.0	ND
HG7	*pck-4 pps*	0.6	43	ND
HG29	*pck*$^+$ *pps*	32	ND	ND
HG34	*pck-5 pps*	1.1	1.4	ND
H64-1[d]	*pck-2 pps*$^+$	<0.5	ND	11
H64-2[d]	*pck*$^+$ *pps*	22	ND	0.9
H64-3[d]	*pck-2 pps*$^+$	<0.5	ND	8.2

[a] Enzyme activity units are nanomoles per minute.

[b] Units of CRM are equivalent to units of enzyme activity (enzyme precipitating units removed from antiserum per milligram of mutant protein added to the immunoassay).

[c] ND, Not determined.

[d] Revertant strains made from HG4. HG4-1 was selected on a pyruvate plate, and HG4-2 and HG4-3 were selected on succinate plates.

TABLE 3. *Growth of PEP carboxykinase mutants on different carbon sources*

Strain	Relevant genotype	Growth on carbon sources[a]		
		Glucose	Pyruvate	Succinate[b]
HfrH	*pck*$^+$ *pps*$^+$	+++	++	++
DF1651	*pck*$^+$ *pps*	+++	−	++
HG4	*pck pps*	+++	−	−
HG4-1[c]	*pck pps*$^+$	+++	++	++
HG4-2[c]	*pck*$^+$ *pps*	+++	−	++
HG4-3[c]	*pck*$^+$ *pss*	+++	++	++

[a] Growth was scored as described in the text.

[b] For the strains listed, growth on acetate, furmarate, and malate carbon sources was similar to growth on succinate. All strains grew well on glycerol as carbon source.

[c] Revertant strains made from HG4. HG4-1 was selected on a pyruvate plate, and HG4-2 and HG4-3 were selected on succinate plates.

Preliminary mapping of *pck* mutations. The succinate$^-$ phenotype of *pck pps* strains was used to map *pck* in crosses where both the Hfr donor and the F$^-$ recipient were *pps* (pyruvate$^-$). Linkage of *pck* was observed with *metB*, *ilv*, and *argG* (68 to 87 min).

A number of F′ strains (Fig. 2) were then tested with *pck pps* recipients in spot matings

on succinate plates. F′ factors MAF1 and KLF41 produced confluent spots on succinate plates, whereas other F′ factors (KLF2, KLF11, KLF12, and KLF17) did not, indicating that the *pck* mutations were probably located between 70 and 76 min on the *E. coli* genetic map (Fig. 2). All 15 independently isolated *pck* mutants tested (some of which are listed in Table 2) were complemented by KLF41 but not KLF2. Control matings showed that MAF1 and KLF41 did not complement the *pps* mutation (spot tests on pyruvate) and that all F′ strains complemented suitable markers, which was consistent with Fig. 2 and previously published data.

Mapping of *pck* by P1-mediated transduction. A number of strains were constructed with *pps* and an auxotrophic marker mapping in the region of 70 to 76 min. These strains were then crossed with *pck pps* strains by using P1-mediated transduction (Table 4). The *pck* mutations were linked with *asd* (29 to 39%), *glpD* (45%), and *aroB* (23 to 26%). Linkages were 0 to 0.5% with *rpsL* and 0% with *aroE* and *pit*.

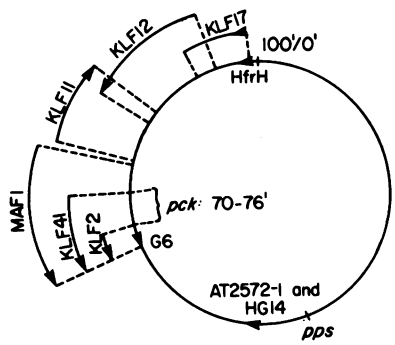

FIG. 2. *E. coli genetic map showing F′ strains used to map PEP carboxykinase (pck) locus. The circular E. coli genetic map is calibrated in units of 0 to 100 min. F′ factors are shown outside the circle, containing the fertility factors (arrowheads) and small portions of the E. coli chromosome (indicated by the tails of the arrows).*

For crosses with *glpD* and *asd* strains, *pck* was usually scored as the unselected marker due to the problem of background growth of *pck pps* strains on plates containing the supplements for these mutants. Reciprocal crosses were unsuccessful since some of the strains were P1 resistant and could be used only as donors (lysates were prepared by selecting chloramphenicol-resistant lysogens with P1 *CAM clr*100).

Three-factor crosses with *pck-5* (Table 5) were consistent with the orders *pck-5–glpD–asd* (Fig. 3) and *aroB–pck-5–glpD*. Crosses of other alleles (*pck-1*, *pck-2*, and *pck-4*) and *pck-5* showed that these mutations were very closely linked to *pck-5*, since less than 1.3% recombination occurred (0 of 921 recombinants for *pck-7*). Regardless of the relative order of the different *pck* alleles, these mutations mapped very near *pck-5*, which was positioned between *aroB* and *glpD*.

Strain MD3, which has a chromosomal deletion extending from *asd* through *glpD* to *malA* (12) (the region of *malT* and *mal IPO*), had normal activity of PEP carboxykinase (data not shown). This confirms the order and indicates that *pck* maps between *mal* and *aroB* at about 74 min on the *E. coli* chromosome.

Assay of PEP carboxykinase antigen in mutant strains. Typical titrations of mutant extracts with PEP carboxykinase antisera are

TABLE 4. *Results of transductional mapping of PEP carboxykinase mutations*

Donor	Recipient	Selected marker	Unselected marker	% Linkage
HG46	HG4 (*pck-2*)	*pck*$^+$ (215)[a]	*aroE*	<0.5
HG47	HG4 (*pck-2*)	*pck*$^+$ (190)	*aroB*	23
			rpsL$^+$	0.5
HG49	HG4 (*pck-2*)	*pck*$^+$ (157)	*asd*	18[b]
			rpsL$^+$	<0.6
HG4 (*pck-2*)	HG49	*asd*$^+$ (163)	*pck-2*	28
			rpsL	<0.6
HG2 (*pck-1*)	HG49	*asd*$^+$ (136)	*pck-1*	25
HG47	HG2 (*pck-1*)	*pck*$^+$ (214)	*aroB*	26
HG5 (*pck-3*)	HG49	*asd*$^+$ (375)	*pck-3*	39
HG7 (*pck-4*)	HG49	*asd*$^+$ (187)	*pck-4*	37
HG47	HG7 (*pck-4*)	*pck*$^+$ (227)	*aroB*	23
HG54 (*pck-5*)	HG49	*asd*$^+$ (503)	*pck-5*	38
			glpD	59
			pit$^+$	0.6
HG47	HG54 (*pck-5*)	*glpD*$^+$ (223)	*pck*$^+$	45
			aroB	16
		pit$^+$ (or *pst*$^+$) (356)	*pck*$^+$	<0.3

[a] Values in parentheses are numbers of colonies scored.
[b] Linkages with *asd* were generally lower when *pck*$^+$ was the selected marker.

TABLE 5. *Three-factor crosses used to map PEP carboxykinase mutations*

Cross	Selected marker	Recombinant class	%
HG54 (*pck-5 pps glpD*) × HG49 (*pps asd*)	*asd*$^+$ (503)[a]	*pck*$^+$ *glpD*$^+$	39
		pck-5 glpD	35
		pck$^+$ *glpD*	24
		pck-5 glpD$^+$	3
HG47 (*pps aroB*) × HG54 (*pck-5 pps glpD*)	*glpD*$^+$ (223)	*aroB*$^+$ *pck-5*	52
		aroB pck$^+$	13
		aroB$^+$ *pck*$^+$	32
		aroB pck-5	3

[a] Values in parentheses are numbers of colonies scored.

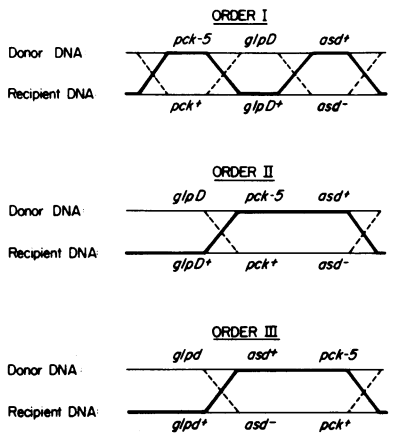

FIG. 3. *Genetic cross-over figures for three-factor transductions. The three possible gene orders for the cross of HG54 with HG49 are shown. The heavy lines show the DNA required to form the low frequency class of recombinants (pck-5, glpD*$^+$*, asd*$^+$*). Order I (pck-5–glpD–asd), but not order II or III, is compatible with the low frequency of these recombinants since a quadruple genetic cross-over (an infrequent event) is required.*

shown in Fig. 4. The data in Table 2 show that *pck-1*, *pck-2*, and *pck-4* strains were CRM$^+$ mutants containing significant amounts of inactive PEP carboxykinase antigen, whereas *pck-3* and *pck-5* mutant strains had levels of antigen corresponding to the reduced levels of active enzyme; inactive enzyme was not detected in these strains.

Genetic induction of PEP carboxykinase. The specific activities of PEP carboxykinase in strain HfrH (wild type) and other strains were very similar (about 30 nmol/min per mg) when cells were grown on carbon sources which did not exert catabolite repression (medium A containing 0.4% succinate, malate, glycerol, α-ketoglutarate, acetate, or Casamino Acids) or on LB medium (without glucose). Maximum specific activities were obtained in stationary-phase cultures; therefore, assays were performed in extracts from freshly harvested, stationary-phase cells (after 90% of maximum growth).

PEP carboxykinase levels were lower in cells grown on medium A containing 0.4% glucose or LB medium containing 0.4% glucose, but normal specific activities were restored by the addition of 5 mM cyclic AMP to these media (Table 6).

Strain GP1 (HfrH *cya crp*$^+$) had lower levels of PEP carboxykinase activity, even in the absence of glucose, but normal levels were restored by cyclic AMP (Table 6). The lower levels obtained with GP1 on minimal medium (even with cyclic AMP) were probably due to the slow growth of this strain on minimal media (causing less staionary-phase induction). Strain CA8445 (HfrH Δ*cya* Δ*crp*) had lower levels of PEP carboxykinase on all media, even in the presence of 5 mM cyclic AMP (Table 6). These findings indicate that PEP carboxykinase is regulated at the transcriptional level by cyclic AMP and the cyclic AMP receptor protein.

Induction of PEP carboxykinase was also investigated by using isogenic *relA* and rel$^+$ strains (CP78 and CP79) since the *relA* gene appears to

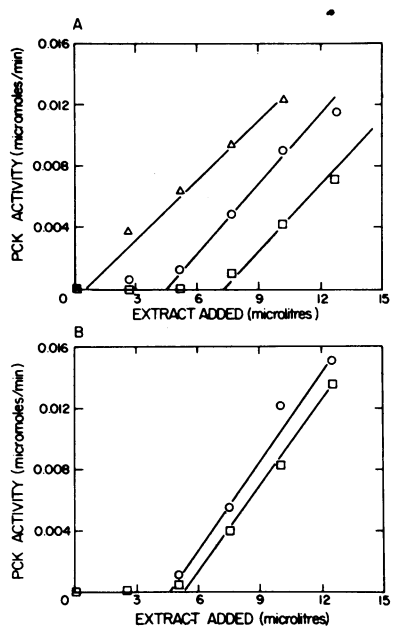

FIG. 4. *Immunoassays of PEP carboxykinase antigen in mutant extracts. Antisera (10 μl) were incubated with and without 5 μl of pck cell extract and then incubated with varying amounts of pck*$^+$ *extract, as described in the text. (A) Typical CRM*$^+$ *mutants possessing inactive enzyme. Symbols: □, control without mutant extract; ○, extract from HG2 (pck-1); △, extract from HG7 (pck-4). (B) Typical CRM*$^+$ *mutants possessing reduced levels of active enzyme. Symbols: □, control; ○, HG5 (pck-3). Antisera of different titers were used for (A) and (B).*

control stationary-phase induction of glycogen synthesis (2). No difference was found in these strains during stationary phase (Table 7), during amino acid starvation, or after glucose to succinate shiftdown (data not shown). These strains had the expected phenotypes, i.e. stringent (CP78) or relaxed (CP79) control of [^{3}H]uridine incorporation into trichloroacetic acid-soluble material (data not shown). As Table 7 shows, stationary-phase induction of PEP carboxykinase occurs in the presence or absence of cyclic AMP, glucose, or the *relA* gene product and must therefore occur by some other mechanism.

DISCUSSION

The structural gene for PEP carboxykinase was mapped on the *E. coli* chromosome at 74 min, based on cotransduction frequencies with *aroB*, *glp*, and *asd*. One of these mutations (*pck-5*) was used to deduce the order *aroB-pck-glpD-asd* (which probably also holds for all other CRM$^+$ mutants). These results are consistent with the finding that a strain deleted for *asd*, *glpD*, and *malA* (MD3) had normal PEP carboxykinase activity, placing *pck* between *aroB* and *malA*.

TABLE 6. *Catabolite repression of PEP carboxykinase activity*

Strain	Medium	PEP carboxykinase act (U/mg)[a]
HfrH (*cya*$^+$ *crp*$^+$)	LB	34
	LB + cyclic AMP	34
	LB + glucose	15
	LB + glucose + cyclic AMP	32
	A + glycerol	30
	A + glycerol + cyclic AMP	48
	A + glucose	4.5
	A + glucose + cyclic AMP	29
GP1 (HfrH *cya crp*$^+$)	LB	16
	LB + cyclic AMP	40
	LB + glucose	15
	LB + glucose + cyclic AMP	36
	A + glycerol	1.5
	A + glycerol + cyclic AMP	8.6
	A + glucose	1.3
	A + glucose + cyclic AMP	11
CA8445 (HfrH Δ*cya* Δ*crp*)	LB	6.4
	LB + cyclic AMP	6.2
	LB + glucose	9.1
	LB + glucose + cyclic AMP	9.5
	A + glycerol	1.6
	A + glycerol + cyclic AMP	0.9
	A + glucose	1.4
	A + glucose + cyclic AMP	1.4

[a] Lower specific activities for GP1 and CA845 grown on minimal media were possibly due to poor growth (lack of stationary-phase induction).

TABLE 7. *Stationary-phase induction of PEP carboxykinase in the presence and absence of glucose, cyclic AMP, or the relA gene product*

Strain	Medium	Growth Phase	PEP carboxykinase (nmol/min per mg)
HfrH (wild type)	A + glycerol	Log[a]	6.3
		Stationary[b]	32
	A + glycerol + cyclic AMP	Log	7.0
		Stationary	35
	A + glucose	Log	0.8
		Stationary	6.2
CP78 (*relA*$^+$)	A + glycerol	Log	7.1
		Stationary	34
CP (*relA*)	A + glycerol	Log	5.6
		Stationary	31

[a] Cells were harvested during logarithmic growth at a Klett reading of 100 U.

[b] Cells were harvested after 90% of maximum growth (measured in Klett units) was obtained.

The specific activities of PEP carboxykinase in different strains (wild type, *cya*, and *crp*) in the presence and absence of glucose and cyclic AMP indicate that synthesis of this enzyme is regulated by catabolite repression, which would cause enzyme activity (and gluconeogenesis) to vary inversely with the availability of glucose and other catabolites. Stationary-phase induction of PEP carboxykinase was independent of cyclic AMP induction since maximum specific activity was obtained in stationary phase, even in the presence of cyclic AMP.

Stationary-phase induction has been observed for the enzymes of glycogen biosynthesis (2, 21), for proteolysis (22), and for certain Krebs cycle enzymes (7). The induction of glycogen biosynthesis and the induction of proteolysis were found to be controlled by the *relA* gene product (2, 22). When stationary phase was caused by means other than nitrogen limitation (carbon, phosphate, potassium, or energy depletion), however, induction of proteolysis was independent of the *relA* gene (22). We have found that PEP carboxykinase of *E. coli* is also induced during stationary phase by mechanisms which are independent of *relA*. The mechanism could involve depletion of nutrients, depletion of oxygen, or even accumulation of certain metabolites.

ACKNOWLEDGMENTS

This work was supported by a grant from the Medical Research Council of Canada. A.H.G. was a recipient of a Medical Research Council studentship from 1973 to 1976.

LITERATURE CITED

1. **Adelberg, E. A., M. Mandel, and G. C. C. Chen.** 1965. Optimal conditions for mutagenesis by N-methyl-N'-nitro-nitrosoguanidine in *Escherichia coli* K12. Biochem. Biophys. Res. Commun. **18**:788–795.
2. **Bridger, W. A., and W. Paranchych.** 1978. *relA* control of bacterial glycogen synthesis. Can. J. Biochem. **56**: 403–406.
3. **Cooper, R. A., and H. L. Kornberg.** 1965. Net formation of phosphoenolpyruvate from pyruvate by *Escherichia coli*. Biochim. Biophys. Acta **104**:618–620.
4. **Cooper, R. A., and H. L. Kornberg.** 1967. The direct synthesis of phosphoenolpyruvate from pyruvate by *Escherichia coli*. Proc. R. Soc. London Ser. B **168**:263–280.
5. **Deleo, A. B., and B. Magasanik.** 1975. Identification of the structural gene for glutamine synthetase in *Klebsiella aerogenes*. J. Bacteriol. **121**:313–319.
6. **Geiger, P. J., and S. P. Bessman.** 1972. Protein determination by Lowry's method in the presence of sulfhydryl reagents. Anal. Biochem. **49**:467–473.
7. **Goldie, A. H., S. Narindrasorasak, and B. D. Sanwal.** 1978. An unusual type of regulation of malate oxidase synthesis in *Escherichia coli*. Biochem. Biophys. Res. Commun. **83**:421–425.
8. **Gorini, L., and H. Kaufman.** 1960. Selecting bacterial mutants by the penicillin method. Science **131**:604–605.
9. **Hamers, R., and C. Hamers-Costerman.** 1961. Synthesis by *Escherichia coli* of an abnormal β-galactosidase in the presence of thiouracil. J. Mol. Biol. **7**:166–174.
10. **Hansen, E. J., and E. Juni.** 1974. Two routes for synthesis of phosphoenolpyruvate from C4-dicarboxylic acids in *Escherichia coli*. Biochem. Biophys. Res. Commun. **59**:1204–1210.
11. **Hansen, E. J., and E. Juni.** 1975. Isolation of mutants of *Escherichia coli* lacking NAD- and NADP-linked malic enzyme activities. Biochem. Biophys. Res. Commun. **65**:559–566.
12. **Hofnung, M., M. Schwartz, and D. Hatfield.** 1971. Complementation studies in the maltose A region of the *Escherichia coli* K12 genetic map. J. Mol. Biol. **61**:681–694.
13. **King, T. E.** 1963. Reconstitution of respiratory chain enzyme systems. XI. Use of artificial electron acceptors in the assay of succinate dehydrogenase enzymes. J. Biol. Chem. **238**:4032–4036.
14. **Massey, V.** 1963. Fumarase. Methods Enzymol. **1**:729–735.
15. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
16. **Rosner, J. L.** 1972. Formation, induction and curing of bacteriophage P1 lysogens. Virology **49**:679–689.
17. **Sanwal, B. D.** 1969. Regulatory mechanisms involving nicotinamide adenine dinucleotides as allosteric effectors. I. Control characteristics of malate dehydrogenase. J. Biol. Chem. **244**:1831–1837.
18. **Sanwal, B. D.** 1970. Regulatory characteristics of the diphosphopyridine nucleotide-specific malic enzyme of *Escherichia coli*. J. Biol. Chem. **245**:1212–1216.
19. **Sanwal, B. D., and R. Smando.** 1969. Malic enzyme of *Escherichia coli*. Possible mechanisms for allosteric effects. J. Biol. Chem. **214**:1824–1830.
20. **Sprague, G. F., R. M. Bell, and J. E. Cronan.** 1975. A mutant of *Escherichia coli* auxotrophic for organic phosphates: evidence for two defects in inorganic phosphate transport. Mol. Gen. Genet. **143**:71–77.
21. **Steiner, K. E., and J. Preiss.** 1977. Biosynthesis of bacterial glycogen: genetic and allosteric regulation of glycogen biosynthesis in *Salmonella typhimurium* LT-2. J. Bacteriol. **129**:246–253.
22. **St. John, A. C., K. Conklin, E. Rosenthal, and A. L. Goldberg.** 1978. Further evidence for the involvement of charged tRNA and guanosine tetraphosphate in the control of protein degradation in *Escherichia coli*. J. Biol. Chem. **253**:3945–3951.
23. **Waygood, E. B., and B. D. Sanwal.** 1974. The control of the pyruvate kinases of *Escherichia coli*. I. Physiological and regulatory properties of the enzyme activated by fructose 1,6-diphosphate. J. Biol. Chem. **249**: 265–274.
24. **Wright, J. A., and B. D. Sanwal.** 1969. Regulatory mechanisms involving nicotinamide adenine dinucleotides as allosteric effectors. II. Control of phosphoenolpyruvate carboxykinase. J. Biol. Chem. **244**:1838–1845.