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Properties of an *Escherichia coli* Mutant Deficient in Phosphoenolpyruvate Carboxylase Catalytic Activity

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A mutant *Escherichia coli* (Ppc^{c-}) which was unable to grow on glucose as a sole carbon source was isolated. This mutant had very low levels of phosphoenolpyruvate carboxylase activity (approximately 5% of the wild type). Goat immunoglobulin G prepared against wild-type phosphoenolypyruvate carboxylase cross-reacted with the Ppc^{c-} enzyme. The amount of enzyme protein in the mutant cells was similar to that found in wild-type cells, but it had greatly diminished specific activity. The catalytically less active mutant enzyme retained the ability to interact with fructose 1,6-bisphosphate, but did not exhibit stabilization of the tetrameric form by aspartate. The pI of the mutant protein was lower (4.9) than that of the wild-type protein (5.1). After electrophoresis and immunoblotting of the partially purified protein, several immunostaining bands were seen in addition to the main enzyme band. A novel method for showing that these bands represented proteolytic fragments of phosphoenolpyruvate carboxylase was developed.

Phosphoenolpyruvate carboxylase (EC 4.1.1.31) of *Escherichia coli* is a tetrameric protein composed of four 100,000-dalton subunits (25). The enzyme has a rather complex regulatory mechanism. It has at least two functionally well-defined allosteric activator sites (3, 9, 21, 22, 26), one allosteric inhibitor site (4), and binding sites for substrates. The enzyme is catalytically active both as a dimer and as a tetramer; however, the normal active form is believed to be the tetramer (25; Y.-B. Chiao and D. T. Chuang, Fed. Proc. 36:3170p, 1977).

We have described an altered phosphoenolpyruvate carboxylase, obtained from a mutant (PpcI), that is active primarily as the dimer (15). This mutant is unique in that it does not form kinetically productive complexes with fructose 1,6-bisphosphate, a site I activator (27), and cells containing this form of the enzyme grow very poorly on glucose as the sole carbon source.

In an attempt to characterize more completely the regulatory and catalytic functions of this enzyme and to relate these functions to structure, other mutants have also been isolated. Achievement of the goal of relating structure to function will be facilitated since the primary amino acid sequence of the enzyme, as derived from the DNA sequence, is available (7).

The purification of mutant enzymes with altered regulatory properties, but otherwise normal catalytic properties, may be monitored by measuring enzyme activity. Those mutants with altered or essentially nonexistent catalytic activity present a problem, however, since detection and quantification of the specific protein are not easily achieved. By using immunological techniques we have carried out the partial purification and characterization of a phosphoenolpyruvate carboxylase from a mutant, Ppc^{c-}, which is essentially inactive under normal assay conditions.

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MATERIALS AND METHODS

Cell culture. *E. coli* K-12 strain W-1485 (equivalent to ATCC 15153 of the American Type Culture Collection) (2) was maintained on agar slants made in Luria broth containing 10 g of tryptone, 10 g of NaCl, 5 g of yeast extract, and 1 g of glucose per liter (13). Since synthesis of phosphoenolpyruvate carboxylase is stimulated in cells grown on defined medium with glucose as the sole carbon source, cells used for enzyme purification were grown on a defined glucose-salts medium which contained (per liter) 15 g of KH_2PO_4, 0.0132 g of $CaCl_2 \cdot 2H_2O$, 0.2 g of $MgSO_4 \cdot 7H_2O$, 2 g of $(NH_4)_2SO_4$, 24.1 g of $Na_2HPO_4 \cdot 7H_2O$, 5 g of NaCl, and 5 g of glucose. For growth of the Ppc^{c-} mutant cells, 0.5 g of aspartate was also added per liter.

Enzyme purification. Cells were harvested after overnight growth, pelleted by centrifugation, washed once with 0.15 M KCl, suspended in twice the volume of PEA buffer (10 mM phosphate, 0.1 mM EDTA, 5 mM aspartate) and disrupted in a French pressure cell. Both wild-type and mutant enzymes were partially purified as described earlier (25).

Mutant selection. Cells were mutagenized by treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine as previously described (1, 20). The Ppc^{c-} mutant described here was selected as one of the colonies which did not grow on glucose as a sole carbon source, but showed growth when the medium was supplemented with succinate.

Enzyme activity. Phosphoenolpyruvate carboxylase activity was measured spectrophotometrically by monitoring the oxidation of NADH in a coupled assay with malate dehydrogenase.

The assay medium contained 6 mM phosphoenolpyruvate, 10 mM $KHCO_3$, 5 mM $MgSO_4$, 0.4 mM acetyl coenzyme A, 10 μg of malic dehydrogenase, 0.15 mM NADH, and 100 mM Tris hydrochloride (pH 8.5) in a total volume of 1 ml (24).

Protein assay. Protein concentration was determined spectrophotometrically by the ratio of absorbance at 280 nm to that at 260 nm (11) or colorimetrically by the method of Lowry et al. (12).

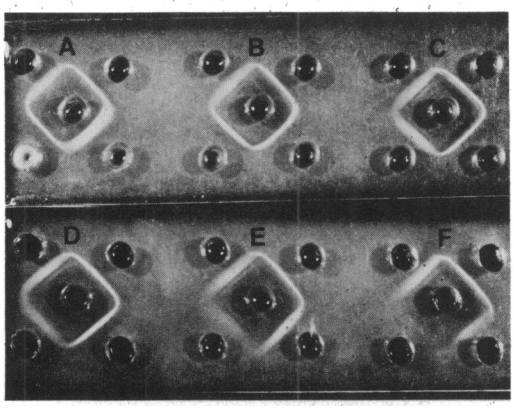

FIG. 1. Ouchterlony double diffusion showing cross-reactivity of wild-type and Ppc^{c-} mutant phosphoenolpyruvate carboxylase with antibody. Wild-type enzyme (124.6 U/ml, 59.1 mg/ml) and mutant enzyme (30.55 U/ml, 66.66 mg/ml) were placed in the outer wells, and antibody (128 mg/ml) was placed in the center well. The contents of the upper wells of both plates were kept constant with a 1:5 dilution of wild-type and mutant enzyme in the left and right wells, respectively. The lower wells (wild-type on the left and mutant on the right) received the following dilutions of enzyme: A, undiluted; B, 1:2; C, 1:5; D, 1:10; E, 1:20; F, 1:50.

Electrophoresis. Protein was heated with sodium dodecyl sulfate (SDS) and 2-mercaptoethanol and subjected to electrophoresis on 9% polyacrylamide gels in the presence of 0.1% SDS (10). Native enzyme was resolved on 5% polyacrylamide gels without SDS or 2-mercaptoethanol. The electrode buffers were 0.05 M Tris–0.38 M glycine (pH 8.3) with or without 0.1% SDS. Slab gels were stained with silver (17), and tube gels were stained with Coomassie blue.

Isoelectric focusing. The isoelectric point of phosphoenolpyruvate carboxylase was determined on a 110-ml column (LKB 8101; LKB Instruments, Inc., Rockville, Md.) as described by Vesterberg (31) and in the LKB technical manual (32). The 1% ampholite solution (Pharmacia Fine Chemicals, Piscataway, N.J.) covered a pH range of 3.5 to 10. Focusing was performed overnight at 5°C at 5 W.

Sucrose density gradient centrifugation. Linear gradients of 5 to 20% (wt/wt) sucrose (14) in 0.01 M Tris hydrochloride–0.1 mM EDTA (pH 7.8) were centrifuged in a Beckman model L-65 centrifuge in a SW50.1 rotor at 35,000 rpm for 16 h at 5°C. Sedimentation coefficients were calculated as described by McEwen (16).

Elicitation of antibodies. A homogeneous preparation of purified wild-type phosphoenolpyruvate carboxylase (500 μg) was injected into a goat subcutaneously into 10 sites. This was followed 2 weeks later by an injection of 350 μg into six sites in neck lymph glands. The first blood was drawn 4 weeks after the initial injection. Serum from the goat, containing anti-phosphoenolpyruvate carboxylase activity, was partially purified by fractionation with sodium sulfate (8). The immunoglobulin G (IgG) fraction thus obtained was tested for antigenicity by Ouchterlony immunodiffusion against *E. coli* homogenate and the purified enzyme. This IgG appeared to react only with phosphoenolpyruvate carboxylase and not with other *E. coli* proteins. Preinjection serum also showed no reactivity with *E. coli* proteins.

Two-dimensional immunoelectrophoresis. Samples of partially purified enzyme were mixed with barbital buffer (0.19 M Tris, 0.32 M glycine, 0.04 M barbital, pH 8.6), SDS, and bromphenol blue and heated in a boiling water bath. This was electrophoresed in 0.1% agarose in barbital buffer on a glass plate. Antibody containing agarose was then poured over the first-dimension gel and allowed to solidify, and electrophoresis was carried out in the second dimension. The plate was then pressed, washed twice with 0.1 M NaCl, rinsed with distilled water, and dried at 70°C. The two-dimensional gel was then stained with Coomassie blue and compared with a one-dimensional gel (31).

Western blotting. Proteins from SDS-polyacrylamide gels were transferred to nitrocellulose electrophoretically at 150 A overnight in 20 mM Tris–150 mM glycine–20% methanol (30). Proteins from native gels were transferred to nitrocellulose in 0.05 M Tris–0.39 M glycine–0.1% SDS–20% methanol. A piece of Whatman no. 41 filter paper was placed between the gel and the nitrocellulose during transfer of the native gel. This prevented the gel from adhering to the nitrocellulose and giving artificial spots upon drying of the nitrocellulose. No significant spreading of the enzyme bands was detectable. For direct immunoanalysis, samples of protein were dotted onto nitrocellulose with a Bio-Dot apparatus (Bio-Rad Laboratories, Richmond, Calif.). The nitrocellulose filters were stained by the method of Domin et al. (6) with rabbit anti-goat IgG (Cappel Laboratories, Cochranville, Pa.) and goat peroxidase-anti-peroxidase (Miles Laboratories Inc., Elkhart, Ind.).

Materials. All other chemicals were obtained commercially and were of the highest purity available.

RESULTS

Mutant phosphoenolpyruvate carboxylase reacts with antibody to wild-type enzyme. A mutant of *E. coli* (Ppc^{c-}) which was unable to grow on glucose as a sole carbon source was isolated. The doubling time of the mutant on glucose plus a citric acid cycle intermediate as carbon sources was 82 min, which may be compared with the 52- to 68-min doubling time of wild-type cells grown on glucose as a sole carbon source. Mutants with these characteristics lack a functional phosphoenolpyruvate carboxylase (29; J. M. Ashworth, H. L. Kornberg, and R. L. Ward, Biochem. J. **94**:28p, 1965); indeed, only low rates of phosphoenolpyruvate carboxylation (5% of the wild-type activity measured in a crude homogenate) were found for this mutant. No other major defects were found in this mutant by using a standard phenotyping scheme (5). Growth of the cells, both wild-type and mutant, could be improved somewhat, however, by the addition of uracil, threonine, methionine, lysine, and diaminopimelic acid to the culture medium. When wild-type cells were grown on glucose as a sole carbon source they contained a higher phosphoenolpyruvate carboxylase activity than cells grown on enriched medium (4.8-fold increase). The mutant cells, when grown on glucose plus a citric acid cycle intermediate, show a similar response (3.3-fold increase). Thus it may be inferred that the mutation is in the structural rather than the regulatory part of the gene.

Although low in activity, the mutant bacterium did contain a protein which cross-reacted with serum IgG fractions obtained from goats that had been immunized with wild-type phosphoenolpyruvate carboxylase (Fig. 1). Apparently identical reactions are seen between the IgG fraction and extracts from wild-type and mutant cells at the higher concentrations of antigens. This suggested that the antibody to the wild-type enzyme might be used in a productive manner to identify and quantify this phosphoenolpyruvate carboxylase protein during purification.

The antigenicity of the mutant enzyme was also shown by

precipitation of the enzyme from crude extracts by the antibody. After repeated washing of the precipitate, mostly antibody and enzyme bands were visible after SDS-polyacrylamide gel electrophoresis (Fig. 2). The homogeneous proteins could be eluted from the gels, but only in small quantities and in an inactive form.

Purification. Table 1 shows a summary of the purification steps. The concentration of the buffer required for elution from the anion-exchange column for the mutant enzyme covered a broader range (150 to 190 mM sodium-potassium phosphate, pH 7.8) than that required for elution of the wild-type enzyme (170 to 180 mM). Since the purification of the wild-type enzyme is normally monitored by activity, a wild-type fraction was carried through the same purification steps as the mutant, and the activity and blotting profiles were compared. The two detection methods for the wild-type enzyme paralleled each other, which indicated that the peak broadening in the chromatographic profile of the mutant enzyme was real and not a reflection of the different detection methods. The mutant enzyme from the anion-exchange column was pooled in two fractions; that eluting between 150 and 170 mM phosphate and that eluting between 170 and 190 mM. In case there was some difference between the earlier- and later-eluting material, the two fractions were applied separately to a gel filtration column. The elution profiles for the two fractions were essentially the same, suggesting that there were no differences in size of the phosphoenolpyruvate carboxylase-like protein. The later fraction from the DE-52 column had a much higher specific activity than the earlier fraction, which suggested that it was of greater purity. This latter fraction was used for most of the characterization studies of the enzyme.

Specificity of the antibody. Samples of various degrees of purity obtained from the DE-52 column (anion exchanger) and from the AcA 34 column (gel filtration), together with samples of the wild-type enzyme, were subjected to SDS-polyacrylamide gel electrophoresis, electroblotted, and immunostained. Some of the crude mutant and wild-type protein samples from the immunoblots show stained bands (Fig. 3) at lower molecular weights than the main phosphoenolpyruvate carboxylase monomer band (100,000 molecular weight). Either these faster-moving bands could represent peptides derived by hydrolysis of the enzyme, or they could indicate that the antibody preparation contains antibodies against some other *E. coli* proteins, even though initial tests with the antibody did not predict this.

The specificity of the antibody was confirmed by the following method. Duplicate concentrations of mutant and wild-type enzymes were subjected to electrophoresis in the presence of SDS and electroblotted onto nitrocellulose, and the nitrocellulose was incubated with anti-phosphoenolpyruvate carboxylase. The nitrocellulose was then divided into two parts. One part was reacted with the other antibodies and stained in the normal way to visualize the antigenic bands. The other half was incubated with a solution of phosphoenolpyruvate carboxylase (0.21 U/ml) in a 3% solution of bovine serum albumin in PEA buffer (pH 7.8) for 1 h. After washing, the nitrocellulose was cut into strips corresponding to the colored areas on the immunostained nitrocellulose. Pieces of equivalent size were also cut from other areas of nitrocellulose to serve as control. Each piece was incubated with assay mixture overnight, and the decrease in NADH was measured spectrophotometrically (Table 2). Phosphoenolpyruvate carboxylase reacted specifically with the strips of antibody-pretreated nitrocellulose, which corresponded to the areas which showed staining after development by the usual immunostaining method. This indicated that these bands are indeed derived from the carboxylase. The presence of additional immunoreactive bands was also suggested by the presence of at least two precipitin arcs in addition to the major peak when the protein was analyzed by two-dimensional immunoelectrophoresis (data not shown).

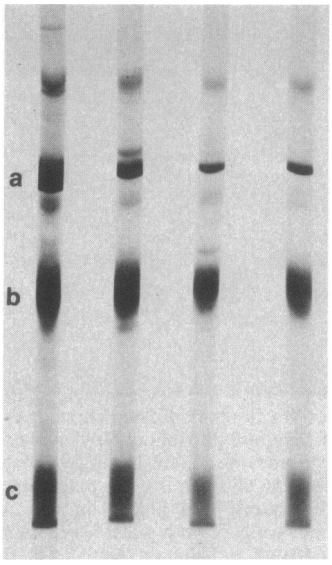

FIG. 2. Electrophoresis of immunoprecipitated phosphoenolpyruvate carboxylase. A precipitate between antiphosphoenolpyruvate carboxylase IgG and Ppc^{c-} enzyme was washed repeatedly (left to right) and separated on SDS-polyacrylamide gels by electrophoresis. Band a is the enzyme; bands b and c are the heavy and light IgG chains, respectively.

Differences in electrophoretic properties. The mutant enzyme exhibits a difference in charge from the wild-type enzyme, as indicated by its isoelectric point (pH 4.8; range,

TABLE 1. Summary of the purification steps for the phosphoenolpyruvate carboxylase-deficient *E. coli* mutant Ppc^{c-}

Purification step	U[a] recovered	Protein (mg)	Sp act (U/mg)	Yield (%)	Purification (fold)
Streptomycin sulfate	734 (7,036)[b]	17,784 (11,250)	0.041 (0.625)	100	1.0
Ammonium sulfate (35 to 50 %)	446 (4,874)	7,263 (7,058)	0.061 (0.69)	60 (69)	1.5 (1.1)
DEAE-cellulose chromatography	95 (4,007)	621 (426)	0.15 (9.4)	13 (57)	3.7 (15)
Gel filtration (AcA 34)	58 (1,793)	127 (41)	0.46 (44)	8 (26)	11.2 (70)

[a] Units are international units (micromoles of substrate metabolized per minute).
[b] Data from purification of phosphoenolpyruvate carboxylase from wild-type bacteria are presented within parentheses for comparison.

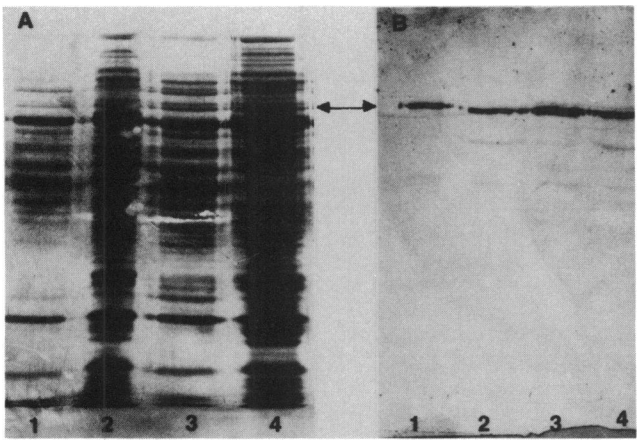

FIG. 3. Immunoblot of phosphoenolpyruvate carboxylase showing the main enzyme and smaller immunostaining peptides. Protein was electrophoresed in a 9% SDS–polyacrylamide gel and stained in the gel with silver (A) or transferred to nitrocellulose and immunostained (B). Lanes: 1, wild-type enzyme, 10 mU, 5 μg of total protein; 2, mutant enzyme, 0.4 mU, 28 μg of protein; 3, wild-type enzyme, 25 mU, 12 μg of protein; 4, mutant enzyme, 1 mU, 70 μg of protein.

pH 4.6 to 5.0) (Fig. 4). This is slightly lower than the pI of the wild-type enzyme determined under identical conditions (pH 5.2; range, pH 5.1 to 5.3). A difference in electrophoretic mobility of the two enzymes on a native gel at pH 6.5 and 8.5 was also observed (results not shown). The mutant enzyme migrated somewhat faster than the wild-type enzyme at pH 6.5. At pH 8.5, however, the wild-type enzyme migrated further into the gel than the mutant enzyme. This is probably due to a difference in conformation of the mutant enzyme relative to the wild-type enzyme at the different pH values.

TABLE 2. Activity of phosphoenolpyruvate carboxylase with anti-phosphoenolpyruvate carboxylase on a nitrocellulose filter

Protein (mU)	Change in absorbance at 340 nm[a]			
	Main band	Second band	Third band	Control
Wild-type (25)[b]	0.8	0.2	0.7	0
Wild-type (50)	1.6	0	0.7	0.2
Ppc^{c-} (1)	0.7	0.9	0	0.1
Ppc^{c-} (2)	0.6	1.9	0	0.2

[a] Oxidation of NADH overnight in the presence of nitrocellulose strips as described in the text.
[b] Amount of enzyme applied to polyacrylamide gel.

Association-dissociation properties. Table 3 shows the results of sedimentation analysis of the two enzymes by sucrose density gradient centrifugation. The presence of 5 mM aspartate causes aggregation or stabilization (or both) of the wild-type enzyme in its tetrameric form, but has no significant effect on the mutant enzyme. Fructose 1,6-bisphosphate, however, increased the average sedimentation coefficient of both wild-type and mutant enzymes. This effect on the mutant enzyme was potentiated by the addition of Mg^{2+}.

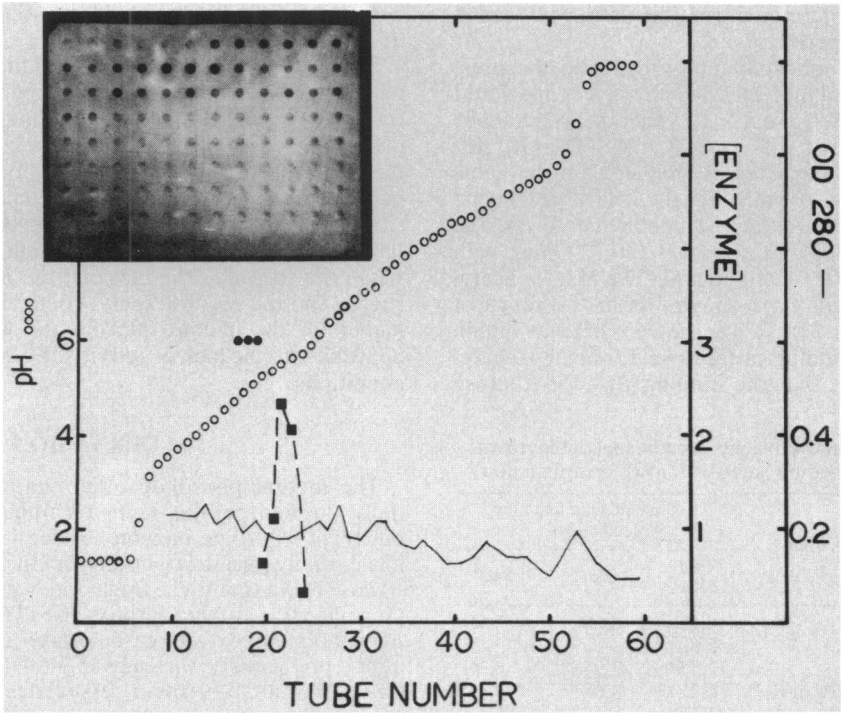

FIG. 4. Isoelectric point of the Ppc^{c-} mutant form of phosphoenolpyruvate carboxylase. Partially purified enzyme (303 mU, 0.66 mg) was applied to the approximate middle of the column and pH (○), optical density at 280 nm (——), and enzyme activity of wild-type enzyme which had been electrofocused under identical conditions (■) were determined. The inset shows immunostaining of fractions. The three most heavily stained dots are from material at pH 4.63, 4.83, and 5.00 (●). Immunostaining of wild-type enzyme fractions indicated a pI identical to that obtained from activity assays.

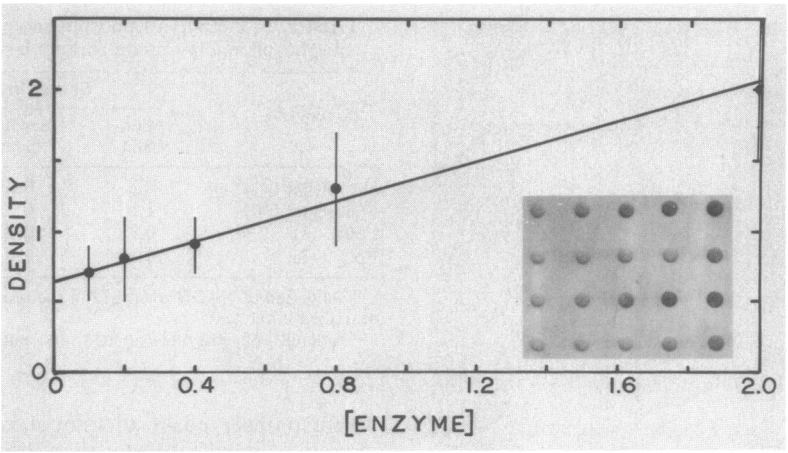

FIG. 5. Relationship between immunoblot staining and units of enzyme activity. Various amounts of wild-type enzyme were immunodotted on nitrocellulose and stained. Bars represent the standard deviation; $n = 5$, slope = 0.063; intercept = 0.066; $r = 0.995$.

Catalytic constant. Since it is known that the wild-type enzyme contains 0.1 U/μg (25), the microgram equivalents of mutant enzyme could be estimated. After immunodotting and staining, the nitrocellulose was photographed, and the intensity of the dots on the Polaroid 665 negative was evaluated with a densitometer. A linear relationship was obtained for dot intensity and the numbers of wild-type phosphoenolpyruvate carboxylase units between 0.1 and 2 U per sample, corresponding to 1 to 20 μg of enzyme protein (Fig. 5). A similar relationship was observed between units and dot density by directly measuring the density of the dots on the nitrocellulose strips with a laser densitometer as described by Domin et al. (6). A value of 8.5 mg of enzyme equivalents per ml, as determined from the data, was used in the following kinetic experiments.

Saturation curves for phosphoenolpyruvate in the presence of approximately 0.4 mM acetyl coenzyme A and 5 mM fructose 1,6-bisphosphate gave a V_{max} value of 1.82 ± 0.005 U/mg and a K_m value of 4.5 ± 0.03 mM. This K_m for phosphoenolpyruvate is considerably higher than the value of 0.6 mM (range, 0.55 to 0.77 mM) for the wild-type enzyme (27). Similar experiments for acetyl coenzyme A and the mutant enzyme gave a V_{max} value of 2.9 ± 0.32 U/mg, with a $K_{0.5}$ (K_m equivalent) of 0.66 ± 0.18 mM. The $K_{0.5}$ for acetyl coenzyme A and the wild-type enzyme is in the range of 0.006 to 0.011 mM (15, 27). It was not possible to obtain definitive data for the binding of fructose 1,6-bisphosphate. It did appear, however, that the limiting $K_{0.5}$ for fructose 1,6-bisphosphate at high concentrations of acetyl coenzyme A was in the vicinity of 0.25 mM, whereas the wild-type enzyme has a $K_{0.5}$ of 0.61 mM for this activator (unpublished results). It is clear from the kinetic behavior of this mutant enzyme that complete homotropic activation by phosphoenolpyruvate does not occur. The pattern of activation by acetyl coenzyme A at various concentrations of fructose 1,6-bisphosphate suggest that the more correct V_{max} is obtained from the latter experiments. In addition, the same V_{max} was obtained in the converse experiments with fructose 1,6-bisphosphate at each of the different concentrations of acetyl coenzyme A. This mutant enzyme, unlike the wild-type enzyme, lost activity rather rapidly. This also complicated the kinetic analyses, so the values given in any case are best estimates.

Those reservations notwithstanding and from the V_{max} data for the acetyl coenzyme A experiment and the enzyme (protein) equivalents calculated above, a catalytic constant of 4.6×10^3/min (based on a molecular weight of 400,000 for the enzyme) could be calculated for the mutant enzyme. The equivalent value for the wild-type enzyme is 3.4×10^4/min (25). This value of 4.6×10^3/min, or 10 U/mg, shows that the limiting specific activity of the mutant is only 1/10 that of the wild-type enzyme. The fact that the K_m for phosphoenolpyruvate and the $K_{0.5}$ for acetyl coenzyme A are considerably higher for the mutant than for the wild-type enzyme may contribute to the lack of activity of this enzyme under in vivo conditions.

TABLE 3. Sedimentation coefficients for phosphoenolpyruvate carboxylase as determined by sucrose density centrifugation

Addition to centrifugation medium	Estimated sedimentation coefficients		
	Wild type	PpcI	Ppc^{c-}
None	8.1S	6.6S[b]	7.9S
Urea (1 M)	5.8S[a]		
Aspartate (5 mM)	12.5S[a]	7.95S[b]	8.3S
Fructose 1,6-bisphosphate (6 mM)	12.2S[a]	8.0S[b]	9.4S
Mg^{2+} (5 mM)	12.2S[a]	7.6S[b]	
Fructose 1,6-bisphosphate (6 mM) and Mg^{2+} (5 mM)	12.7S[a]	8.1S[b]	11.2S

[a] Data from reference 23.
[b] Data from reference 15.

DISCUSSION

The mutant phosphoenolpyruvate carboxylase was partially purified by the same techniques used routinely for purifying wild-type enzyme. Several fragments were noted in a partially purified fraction from the ion-exchange column. It was shown that these fragments were indeed derived from phosphoenolpyruvate carboxylase (Table 2). It is known that phosphoenolpyruvate carboxylase can undergo denaturation, presumably through an initial stage of dissociation (28). The mutant protein dissociates more easily than the wild-type enzyme (Fig. 1), which may make it more susceptible to proteolysis. In addition, the data presented here show that the mutant enzyme cannot be stabilized in the tetrameric form by modifiers to the same extent as the wild-type enzyme. These and other factors are consistent

with the observation that multiple small-molecular-weight bands may be the results of proteolysis.

It has been stated earlier that no single mutation of phosphoenolpyruvate carboxylase has been found which alters only one characteristic of the enzyme (15, 18, 19). Therefore, it is important to describe each mutant as fully as possible and then compare structural differences which are characteristic of mutants expressing similar phenotypes. These characteristics can be divided into two types: regulatory and catalytic. The enzyme described here differs from that obtained from a mutant (PpcI) described earlier by McAlister et al. (15) in both respects. The mutation which is the subject of this paper affected the regulatory properties of the enzyme to a lesser degree, and the activity to a greater degree, than was apparent in the PpcI mutant. Since the substrate concentration normally used to measure activity (6 mM) is not much higher than the K_m for the mutant enzyme (approximately 4.9 mM), only about half-maximal activity is measured in in vitro assays, which have indicated only 5% as much activity in mutant cells as in wild-type cells. If, indeed, the true maximal activity is 10% of the wild type, and the catalytic constant is also only 10% of the wild type, it may be calculated that there is at least as much enzyme protein in the mutant cell as in the wild-type cell. This mutant thus synthesizes a phosphoenolpyruvate carboxylase which is defective in activity rather than deficient in quantity.

The changes in function described above are clearly accompanied by a change in structure. This is manifested by a change in isoelectric point and by a change in migration on polyacrylamide gels. The amino acid sequence for the wild-type phosphoenolpyruvate carboxylase has been reported (7). Since we have shown that immunochemical techniques can be used to follow the purification of this mutant protein, we are now in a better position to purify the enzyme to homogeneity and to relate structural changes to function. Experiments along that line are in progress.

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