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Purification and Properties of a Nicotinamide Adenine Dinucleotide-Linked Dehydrogenase that Serves an *Escherichia coli* Mutant for Glycerol Catabolism

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Glycerol:NAD^+ 2-oxidoreductase (EC 1.1.1.6) was purified to homogeneity from a mutant of *Escherichia coli* K-12 that uses this enzyme, instead of ATP:glycerol 3-phosphotransferase (EC 2.7.1.30), as the first enzyme for the dissimilation of glycerol. Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate shows a subunit of 39,000 daltons. During electrophoresis under nondenaturing conditions, the protein migrates as two bands. These two forms, both of which are enzymatically active, appear to be dimers and octomers of the same subunit. The optimal pH for the oxidation of glycerol is about 10, and that for the reduction of dihydroxyacetone is about 6. Glycerol dehydrogenation is highly activated by NH_4^+, K^+, or Rb^+, but strongly inhibited by *N*-ethylmaleimide, 8-hydroxyquinoline, 1,10-phenanthroline, Cu^{2+}, and Ca^{2+}. The enzyme exhibits a broad substrate specificity. In addition to glycerol, it acts on 1,2-propanediol and several of its analogs.

Wild-type *Escherichia coli* K-12 utilizes glycerol exclusively through the action of an ATP-dependent kinase. The product, *sn*-glycerol 3-phosphate (G3P), is then dehydrogenated by one of the two membrane-associated flavo-dehydrogenases, depending on the nature of the terminal electron acceptor available (5). A mutant was isolated in which the kinase was replaced by a NAD^+-linked dehydrogenase as the first enzyme for aerobic glycerol dissimilation. This mutant was selected from a parental strain with defects in both the gene for glycerol kinase and the gene for aerobic G3P dehydrogenase. The double mutation makes reversion to the original mode of glycerol metabolism highly improbable. In a preliminary study, it was shown that crude cell extracts of the mutant catalyzed the interconversion of glycerol and dihydroxyacetone (12). Thus, in this respect, the enzyme resembles the genuine anaerobic catabolic enzyme of *Klebsiella aerogenes* 1033 (6).

In the present report, we describe the purification and characterization of the glycerol dehydrogenase produced by the *E. coli* mutant, strain 424. The high constitutive level of the enzyme in this strain was the result of at least two mutations during prolonged selection in a glycerol medium (12).

MATERIALS AND METHODS

Chemicals. NAD^+ (grade III), $NADP^+$, NADH (grade III), NADPH (type 1), cyclohexylaminopropane sulfonic acid (CAPS), tris(hydroxymethyl)-methylaminopropane sulfonic acid (TAPS), *N*-tris-(hydroxymethyl)methyl-2-aminoethane sulfonic acid (TES), 2-(*N*-morpholino)ethane sulfonic acid (MES), 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyl tetrazolium bromide (MTT), Sepharose 6B, and NAD^+-agarose were obtained from Sigma Chemical Co. Tetraethylammonium hydroxide, tetraethylammonium chloride, DL-3-methoxy-1,2-propanediol, 1,2,3-propanetriol monoacetate, and DL-3-hydroxy-2-butanone (acetoin) were purchased from Eastman Kodak Co. Acetol, DL-3-mercapto-1,2-propanediol, D-2,3-butanediol, DL-3-amino-1,2-propanediol, and casein acid hydrolysate (vitamin free) were obtained from ICN/K & K Laboratories. Polyacrylamide gradient gels (Pharmacia PAA 4/30 gradient gels) and electrophoresis calibration kits for molecular weight determinations were purchased from Pharmacia Fine Chemicals. Glycerol and DL-1,2-propanediol were obtained from Fisher Scientific Co. Dihydroxyacetone and DL-3-chloro-1,2-propanediol were purchased from Calbiochem. DEAE-cellulose (DE52) was obtained from Whatman Inc., and DL-2,3-butanediol was obtained from L. Light & Co. Ltd. All other reagents used were commercial products of the highest grade available.

Bacterial strains and growth conditions. *E. coli* 424 is a derivative of K-12 strain 1 (12). The cells were grown in mineral medium (13) supplemented with 2% casein hydrolysate. Cultures of 250 ml were incubated at 38.5°C in 2-liter flasks on a rotary shaker at 240 cycles/min until the cells reached a density of about 4×10^9 cells per ml (late exponential phase).

Glycerol dehydrogenase assays. For purifica-

tion of the enzyme, the activity was measured at 30°C by the linear increase in absorbance at 340 nm produced by the addition of enzyme preparation to a reaction mixture containing 100 mM glycerol, 0.6 mM NAD^+, 30 mM ammonium sulfate, and 100 mM potassium carbonate buffer at pH 9.0 (10). Units of activity are expressed in micromoles per minute. In all of the procedures given below, the measurements were carried out with the enzyme purified to electrophoretic homogeneity.

For determination of pH optima of the enzyme, the activities in both forward and reverse directions were measured at 25°C. The reaction mixture contained 0.6 mM NAD^+ or 0.2 mM NADH, 100 mM substrate, 100 mM ammonium chloride, and 125 mM buffer. The buffers used included: CAPS, pH 9.5 to 11; TAPS, pH 8.0 to 9.0; TES, pH 7.0 to 7.5; MES, pH 5.5 to 6.5; and acetate, pH 4.0 to 5.0.

Unless otherwise stated for the other characterizations of the enzyme activity, the assays were performed at 25°C in the presence of 100 mM glycerol, 0.6 mM NAD^+, and 100 mM ammonium chloride (the cation as the activator) and buffered at the optimal pH of 10 with 125 mM CAPS.

Enzyme purification. Cells were harvested and washed twice with 25 ml of 0.1 M potassium phosphate (pH 7.0) at 4°C and resuspended in 5 ml of the same buffer. Cellular disruption was achieved by three 1-min sonic pulses in a 60-W disintegrator (Measuring and Scientific Equipment), during which time the suspension was chilled in an ethanol bath with dry ice. The debris was removed by centrifugation at 12,000 $\times g$ for 30 min at 4°C. Supernatant fractions from five individual cultures were pooled.

The combined extract was diluted to 20 mg of protein per ml with 0.1 M potassium phosphate, pH 7.0. Solid ammonium sulfate was added in small amounts to the extract, slowly stirred, and cooled in an ice bath, until the salt was brought to 35% saturation. After further stirring for 30 min, the precipitate was removed by centrifugation at 4°C. Ammonium sulfate was again gradually added to the supernatant fraction to 60% saturation, and the mixture was allowed to equilibrate for 60 min. The precipitated protein was collected by centrifugation and dissolved in 15 ml of 0.05 M potassium phosphate at pH 7.0 (buffer K).

The dissolved ammonium sulfate precipitate was equally divided into three fractions and applied to a column of Sepharose 6B (150 ml; 1.5 by 90 cm) equilibrated with buffer K. The buffer K eluate was collected in 1.5-ml fractions. Tubes containing significant glycerol dehydrogenase activity were pooled, and the combined fractions were applied to a column of DEAE-cellulose (DE52) equilibrated in buffer K. After washing with 100 ml of the equilibrating buffer, the column was eluted with 500 ml of KCl (0 to 1 M gradient) in the same buffer, and 5-ml fractions were collected. Tubes containing the highest specific activity were pooled and concentrated by pressure dialysis (PM-10 Amicon filter), and the concentrate was dialyzed overnight against 150 volumes of buffer K containing 20 mM glycerol.

Affinity chromatography was then initiated by mixing an equal volume of the dialyzed enzyme preparation with a suspension of excess of NAD^+-agarose already equilibrated with 20 mM glycerol in buffer K. The mixture was stirred slowly in an ice bath for 30 min and centrifuged. The supernatant fluid, containing no enzyme activity, was discarded. The sediment was washed five times with 4 volumes (40 ml) of buffer K containing 20 mM glycerol. The final pellet was resuspended in 10 ml of buffer K containing 2 mM NAD^+ in addition to 20 mM glycerol and stirred in an ice bath for 30 min. The mixture was centrifuged, and the pellet was extracted once more in the same manner. The pooled NAD^+ eluates of the enzyme were concentrated by pressure dialysis, a procedure which also removed the NAD^+ and the glycerol.

Protein concentrations were determined in these studies by the method of Lowry et al. (7).

Determination of molecular weights. Estimate of molecular weight of active glycerol dehydrogenase was made on the basis of the mobility of the protein during polyacrylamide gradient (4 to 30%) gel electrophoresis at pH 8.4 (Tris-borate buffer) by a modified method of Margolis and Wrigley (8) recommended by Pharmacia Fine Chemicals. Estimate of subunit molecular weight was made by using 10% acrylamide gels containing 0.1% sodium dodecyl sulfate buffered at pH 7.2 (15). Gels were stained for protein by Coomassie brilliant blue R and for enzymatic activity by incubating in 100 mM potassium carbonate (pH 9.0) containing 100 mM glycerol, 0.15 mM NAD^+, 30 mM ammonium sulfate, 250 μg of MTT per ml, and 25 μg of phenazine methosulfate per ml.

RESULTS

Purification of the enzyme. The four-step procedure devised for the purification of glycerol dehydrogenase yielded a 15-fold enrichment of the protein with a 4.3% recovery of the total initial activity in the crude extract (Table 1). The last step involved the elution of the enzyme from the NAD^+-agarose matrix by adding the coenzyme. All subsequent studies were carried out with the enzyme purified after this step.

Estimates of molecular weights. When the enzyme was electrophoresed in 7.5% polyacrylamide gels, two catalytically active bands appeared, a major one and a minor one (Fig. 1). (The presence of two enzymatically active bands after electrophoresis of crude cell extract was observed earlier by E. J. St. Martin in this laboratory.) To determine the molecular weights of these two species, their relative mobilities were compared with those of standard proteins by electrophoresis in a 4 to 30% polyacrylamide gradient gel. The major form of glycerol dehydrogenase behaved as a protein of 310,000 daltons, whereas the minor component behaved as a protein of 81,000 daltons (Fig. 2).

By carrying out polyacrylamide gel electrophoresis in the presence of sodium dodecyl sul-

TABLE 1. *Steps of purification of glycerol dehydrogenase from E. coli 424*[a]

Procedure	Total protein (mg)	Total activity (μmol/min)	Sp act (U/mg of protein)	Purification (fold)	Recovery (%)
Crude extract	1,100	5,100	4.6	1.0	100.0
Ammonium sulfate (35–60%)	540	3,200	5.9	1.3	63
Sepharose 6B	93	1,400	15.0	3.3	27
DE52	7.2	390	54.0	12.0	7.6
NAD^+-agarose	3.1	210	69.0	15.0	4.1

[a] Activity was assayed as described in the text.

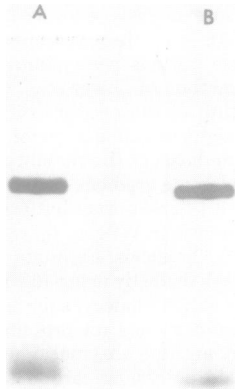

FIG. 1. *Polyacrylamide gel electrophoresis of glycerol dehydrogenase. Samples of the purified protein from the NAD^+-agarose step (Table 1) were electrophoresed in 7.5% polyacrylamide gels (3). (A) A 20-μg amount was initially added. The protein was stained with Coomassie brilliant blue R. (B) A 5 μg amount of protein was initially added. Staining for the enzyme activity was performed as described in the text.*

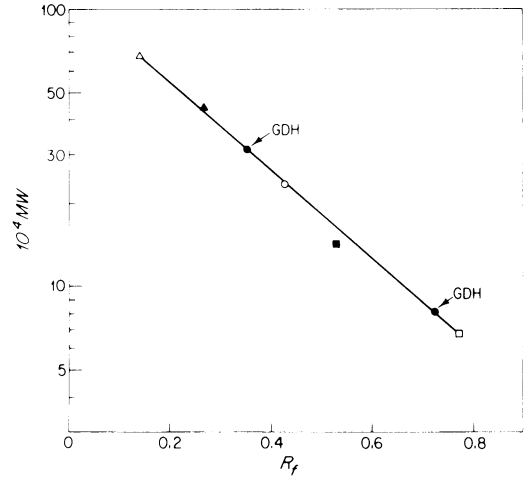

FIG. 2. *Molecular weight (MW) estimates of glycerol dehydrogenase by polyacrylamide gradient gel electrophoresis. See text for the conditions. The proteins compared included: bovine serum albumin, MW of 67,000 (□); lactate dehydrogenase, MW of 140,000 (■); catalase, MW of 232,000 (○); ferritin, MW of 440,000 (▲); thyroglobulin, MW of 669,000 (△); and glycerol dehydrogenase (GDH) (●).*

fate, however, only a single band of protein of 39,000 daltons appeared. Glycerol dehydrogenase thus appears to exist as a dimer and an octomer.

This interpretation was supported by recovering the two bands of enzymatically active proteins from the 7.5% polyacrylamide gel and by subjecting each molecular species to a new round of electrophoresis. The major component with the larger molecular weight again gave rise to two bands (Fig. 3). However, the minor component with the smaller molecular weight migrated as a single band without reforming a detectable amount of the larger molecular species.

The maintenance of the two discrete enzymatic bands during gel electrophoresis at pH 8.4 and the reformation of the dimers from the octomers after elution at pH 7 suggest that the octomer is stabilized at the higher pH. Otherwise, during electrophoresis one would expect the major band of enzyme to be preceded by a diffuse frontier. It is not clear which of the two forms is the predominant one in vivo.

pH optimum. The rate of catalysis of glycerol dehydrogenation and dihydroxyacetone reduction by the enzyme as a function of pH was measured in the presence of NH_4^+. The optimal pH for the oxidation of glycerol was in the range of 9.5 to 10, with a rapid decline of the activity below pH 8.5. The optimal pH for the reduction of dihydroxyacetone was in the range of pH 5.5 to 6.0, with a rapid decline in the activity above pH 7.5. Similar pH profiles were observed with K^+ as an activator. Because neither substrate has an ionizable group in the pH range studied, the difference in the optima probably reflects chiefly the participation of the proton in the interconversion of NAD^+ and NADH. A possible

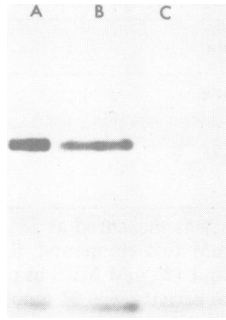

FIG. 3. *Polyacrylamide gel electrophoresis of glycerol dehydrogenase. Samples of enzyme preparation were electrophoresed in 7.5% polyacrylamide gels. (A) A 5-μg amount of purified protein from the NAD⁺-agarose step. (B) The high-molecular-weight protein recovered from a previous run of gel electrophoresis. (C) The low-molecular-weight protein recovered from a previous run of gel electrophoresis. The location of the two protein species to be eluted from the first gel was carried out by staining for glycerol dehydrogenase activity in a parallel strip of the same gel slab.*

role of ionizable group(s) on the enzyme surface, of course, is not ruled out.

Activation by monovalent cations. The effects of several monovalent cations on the activity of glycerol dehydrogenase are shown in Table 2. The assay buffer used was titrated to pH 10 with tetraethylammonium hydroxide because even up to 40 mM the cation of this compound has no apparent effect on the activity. At 2 and 40 mM, NH_4^+ was the most effective activator. At 40 mM, K^+ and Rb^+ activated the enzyme by about sixfold. Na^+ and Li^+ showed little or no effect. NH_4^+ and K^+ also behaved as activators when the enzyme was assayed as dihydroxyacetone reductase (Table 3).

In the presence of 100 mM NH_4^+, the apparent K_m for glycerol was 1.4 mM, and that for dihydroxyacetone was 1 mM. The V_{max} for glycerol oxidation is calculated to be 81 U per mg of protein at 30°C, and that for dihydroxyacetone reduction is 550 U. Assuming that each subunit has a catalytic site and that the rate of catalysis does not vary significantly with the state of polymerization of the subunit, the turnover number per catalytic site of this enzyme for glycerol dehydrogenation would be about 3,200 per min at 30°C.

Inhibitors. Effects of various divalent cations, chelating compounds, and sulfhydryl reagents on glycerol dehydrogenase activity were investigated. For measuring inhibition, the enzyme was first incubated for about 3 min at 30°C with the test compound in the assay mixture minus glycerol. The reaction was then started by adding the substrate.

The enzyme activity was 74% inhibited by 1 μM Cu^{2+} and 100% inhibited at 20 μM of this metal ion. At 1 mM, Ca^{2+} caused 50% inhibition, whereas Ba^{2+} and Mg^{2+} showed no effect at the same concentration.

Among the chelating agents examined, 8-hydroxyquinoline was the most powerful. It inhibited 77% of the activity at a concentration of 0.25 mM. A similar extent of inhibition was observed by 1 mM 1,10-phenanthroline. In contrast, 1 mM EDTA, α,α′-dipyridyl, or diethyldithiocarbamate, had little or no effect.

The requirement of sulfhydryl groups for enzyme activity is indicated by the effect of *N*-ethylmaleimide: at 0.1 mM 75% inhibition occurred, and complete inhibition occurred at 1 mM. The effect was not reversed by the addition of 2.0 mM dithiothreitol or 2-mercaptoethanol. The activity of uninhibited enzyme, however, was enhanced 20% by 2 mM glutathione.

Substrate specificity. Table 4 gives the ini-

TABLE 2. *Effects of monovalent cations on glycerol dehydrogenation activity*[a]

Cation added	Relative activity	
	2 mM	40 mM
NH_4^+	43	100[b]
K^+	25	62
Rb^+	27	62
Na^+	12	20
Li^+	9.8	9.6
$N(C_2H_5)_4^+$	10	10
None	8.0	

[a] Activity was measured as described in the text, except that the various monovalent cations were added at the concentrations indicated for testing activation. Chloride was used as the anion in all mixtures.

[b] Absolute activity was 19 nmol/min per ml of assay mixture.

TABLE 3. *Effects of monovalent cations on dihydroxyacetone reductase activity*[a]

Cation added	Relative activity	
	2 mM	40 mM
NH_4^+	43	100[b]
K^+	11	32
None	6	

[a] Activity was measured as described in the text, except that the various monovalent cations were added at the concentrations indicated for testing activation. Chloride was used as the anion in all mixtures.

[b] Absolute activity was 15 nmol/min per ml of assay mixture.

tial rates of NAD^+ reduction with glycerol and various substrate analogs. It might be noted that 5 mM 1,2-propanediol was more rapidly dehydrogenated than glycerol (Table 4). (A 1,2-propanediol oxidoreductase of *E. coli* was described previously. Although this enzyme also acts on glycerol, the attack of the substrate is at C1. Propanediol oxidoreductase plays a role in L-fucose fermentation [2, 11].) A number of compounds with similar structure also exhibited partial activity. A gem diol appears to be necessary but not sufficient for the enzyme to act on the internal carbon bearing the reactive hydroxyl group. Glycerol 3-phosphate was inactive. Perhaps the negative charge of the phosphoryl group is unacceptable. Several other substitutions of the third hydroxyl group, including that by an $-NH_3^+$ group, seem compatible with at least partial enzymatic activity. $NADP^+$ did not replace NAD^+.

The substrate specificity of the reverse reaction was studied by following the oxidation of NADH. Acetol was as active as dihydroxyacetone (Table 5). This activity can be expected from the fact that 1,2-propanediol is a good substrate in the forward reaction. A hydroxyl group on C1 cannot be replaced by a hydrogen because acetone was inactive. Again, this can be expected from the gem diol requirement of the dehydrogenation reaction. NADPH was partially active as a reducing cofactor, even though $NADP^+$ was totally inactive as an oxidizing cofactor.

TABLE 4. *Substrate specificity for dehydrogenation*[a]

Substrate	Relative activity
Glycerol	100[b]
1,2-Propanediol	165
3-Mercapto-1,2-propanediol	107
3-Chloro-1,2-propanediol	105
1,2,3-propanetriol monoacetate	57
3-Amino-1,2-propanediol	56
3-Methoxy-1,2-propanediol	38
DL-2,3-Butanediol	94
D-2,3-Butanediol	77
G3P	0
DL-Lactate	0
$NADP^+$	0[c]

[a] Activity was measured as described in the text, except that various compounds were tested at 5 mM as substrate.

[b] Absolute activity was 11 nmol/min per ml of assay mixture.

[c] In this assay, NAD^+ was replaced by $NADP^+$, and 5 mM glycerol was added as the substrate. By using NAD^+, no significant activity on the following compounds was observed at 100 mM: 1,3-propanediol, 2-amino-1-propanol, 2-amino-2-methyl-1-propanol, D-2-amino-1-butanol, L-2-amino-1-butanol, 3-amino-1-propanol, 2,4-pentanediol, DL-threitol, L-threitol, *i*-erythritol, D-arabitol, L-arabitol, ribitol, and xylitol.

TABLE 5. *Substrate specificity for reduction*[a]

Substrate	Relative activity
Dihydroxyacetone	100[b]
Acetol	97
Acetoin	8.3
Glyceraldehyde	0[c]
NADPH	26[d]

[a] The activity was measured at 25°C in a solution containing 0.4 mM test compound, 0.2 mM NADH, 100 mM NH_4^+, and 125 mM MES at pH 6.0.

[b] The absolute activity was 24 nmol/min per ml of assay mixture.

[c] The assay conditions were the same as above, except that the racemic substrate was tested at 5 mM. No activity was observed with 2 mM dihydroxyacetone phosphate, 2 mM pyruvate, and 5 mM acetone.

[d] In this assay, NADH was replaced by NADPH and 0.4 mM dihydroxyacetone was added as the substrate. Possible contamination of the NADPH sample (high-grade purity) by NADH could not account for the observed activity because in the presence of excess enzyme, the reaction proceeded rapidly to 80% oxidation.

DISCUSSION

Extracts of wild-type *E. coli* K-12 contain about 0.005 U of glycerol dehydrogenase activity per mg of protein. Extracts of strain 424 grown under favorable conditions (at 38.5°C on casein acid hydrolysate to late exponential phase) contain close to 1,000 times this activity. A 15-fold purification of the enzyme gives an apparent homogeneous preparation. This implies that the enzyme contributes almost 7% of the total soluble cell protein. It is not yet known whether this high level of enzyme synthesis reflects primarily gene duplication or enhanced gene expression resulting from alteration of the promoter or of a regulatory protein.

The hyperproduction of an enzyme by forced selection on a substrate under unusual circumstances has been observed in other cases. For example, the selection of mutants of *Klebsiella aerogenes* 1033 for growth on limiting concentrations of a novel substrate, xylitol, greatly elevated the constitutive synthesis of ribitol dehydrogenase. This enzyme fortuitously acts with low affinity on xylitol, converting it to D-xylulose, which happens to be a normal metabolite of the organism (4, 9). In a mutant selected under stringent xylitol limitation, the constitutively synthesized enzyme was found to comprise about 20% of the cellular protein (14).

A glycerol dehydrogenase with some resemblance to the enzyme reported in this study was previously purified 15- to 20-fold from *E. coli*

ECFS. The specific activity of the final preparation was only 1.5 U in sodium carbonate-bicarbonate buffer at 30°C. The properties found in common between the two enzymes were a pH optimum of about 10 and a high degree of sensitivity to Cu^{2+}. No substrate specificity was undertaken in the previous work, except that the enzyme was shown to catalyze the reduction of dihydroxyacetone with NADH, and therefore the attack was also at C2 (1). Unfortunately, strain ECFS seems no longer available.

The original role of the enzyme that assumed the new function of aerobic glycerol dissimilation in the mutant remains to be discovered. A supposition that the enzyme has a fermentative role in wild-type cells was not substantiated in a preliminary survey. Three independent mutants of strain 424 that lost the enzyme, and was therefore no longer able to grow on glycerol, showed no apparent defect when grown aerobically or anaerobically on a number of carbohydrates, such as glucose, L-arabinose, D-xylose, and pyruvate (E. St. Martin, unpublished data). The broad specificity of the enzyme makes it difficult to guess the physiological substrate on the basis of catalysis. Identification and mapping of the structural gene might be the best approach for solving this problem.

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LITERATURE CITED

1. **Asnis, R. E., and A. F. Brodie.** 1953. A glycerol dehydrogenase from *Escherichia coli*. J. Biol. Chem. **203:** 153-159.
2. **Cocks, G. T., J. Aguilar, and E. C. C. Lin.** 1964. Evolution of L-1,2-propanediol catabolism in *Escherichia coli* by recruitment of enzymes for L-fucose and L-lactate metabolism. J. Bacteriol. **118:**83-88.
3. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680-685.
4. **Lerner, S. A., T. T. Wu, and E. C. C. Lin.** 1964. Evolution of catabolic pathway in bacteria. Science **146:**1313-1315.
5. **Lin, E. C. C.** 1976. Glycerol dissimilation and its regulation in bacteria. Annu. Rev. Microbiol. **30:**535-578.
6. **Lin, E. C. C., and B. Magasanik.** 1960. The activation of glycerol dehydrogenase from *Aerobacter aerogenes* by monovalent cations. J. Biol. Chem. **235:**1820-1823.
7. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193:**265-275.
8. **Margolis, J., and C. W. Wrigley.** 1975. Improvement of pore gradient electrophoresis by increasing the degree of cross-linking at high acrylamide concentrations. J. Chromatogr. **106:**204-209.
9. **Mortlock, R. P., D. D. Fossitt, and W. A. Wood.** 1965. A basis for utilization of unnatural pentoses and pentitols by *Aerobacter aerogenes*. Proc. Natl. Acad. Sci. U.S.A. **54:**572-579.
10. **Ruch, F. E., J. Lengeler, and E. C. C. Lin.** 1974. Regulation of glycerol catabolism in *Klebsiella aerogenes*. J. Bacteriol. **119:**50-56.
11. **Sridhara, S., T. T. Wu, T. M. Chused, and E. C. C. Lin.** 1969. Ferrous-activated nicotinamide adenine dinucleotide-linked dehydrogenase from a mutant of *Escherichia coli* capable of growth on 1,2-propanediol. J. Bacteriol. **98:**87-95.
12. **St. Martin, E. J., W. B. Freedberg, and E. C. C. Lin.** 1975. Kinase replacement by a dehydrogenase for *Escherichia coli* glycerol utilization. J. Bacteriol. **131:**1026-1028.
13. **Tanaka, S., S. A. Lerner, and E. C. C. Lin.** 1967. Replacement of a phosphoenolpyruvate-dependent phosphotransferase by a nicotinamide adenine dinucleotide-linked dehydrogenase for the utilization of mannitol. J. Bacteriol. **93:**642-648.
14. **Taylor, S. S., P. W. J. Rigby, and B. S. Hartley.** 1974. Ribitol dehydrogenase from *Klebsiella aerogenes*. Biochem. J. **141:**693-700.
15. **Weber, K., and M. Osborn.** 1960. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. J. Biol. Chem. **244:** 4406-4412.