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BIBLIOGRAPHIE

1 R. ACHER, J. CHAUVET ET G. OLIVRY, *Biochim. Biophys. Acta*, 22 (1956) 421.
2 R. ACHER, A. LIGHT ET V. DU VIGNEAUD, *J. Biol. Chem.*, 233 (1958) 116.
3 R. ACHER, J. CHAUVET ET M. T. LENCI, *Bull. soc. chim. biol.*, 40 (1958) 2005.
4 R. ACHER, J. CHAUVET ET M. T. LENCI, *Compt. rend.*, 248 (1959) 1435.
5 A. LIGHT ET V. DU VIGNEAUD, *Proc. Soc. Exptl. Biol. Med.*, 98 (1958) 692.
6 P. HOLTON, *Brit. J. Pharmacol.*, 3 (1948) 328.
7 F. N. LANDGREBE, M. H. F. MACAULAY ET H. WARING, *Proc. Roy. Soc. Edinburgh. B*, 62 (1946) 202.
8 E. A. PETERSON ET H. A. SOBER, *J. Am. Chem. Soc.*, 78 (1956) 751.
9 C. H. W. HIRS, S. MOORE ET W. H. STEIN, *J. Biol. Chem.*, 200 (1953) 493.
10 R. ACHER ET C. FROMAGEOT, *Ergeb. Physiol. u. exptl. Pharmakol.*, 48 (1955) 287.
11 O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR ET R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 265.
12 G. W. SCHWERT, *J. Am. Chem. Soc.*, 79 (1957) 139.

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A COMPARISON OF SOME *VIC* GLYCOL DEHYDROGENASE SYSTEMS FOUND IN *AEROBACTER AEROGENES**

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SUMMARY

Two DPN-requiring *vic* glycol dehydrogenases can be isolated from extracts of a variant of *A. aerogenes*. Deamino DPN can replace the DPN requirement of enzyme A (obtained from cells grown on a glycerol-salts medium) and the 3-acetylpyridine analogue of DPN can replace the DPN requirement of enzyme B (obtained from cells grown on a glucose-salts media). Deamino DPN cannot replace the DPN requirement of enzyme B nor can the 3-acetylpyridine analogue substitute for DPN with enzyme A.

Enzymes A and B can be distinguished by properties other than pyridine nucleotide specificity. For example, they can be identified by differences in stability and in pH optima. Finally, enzyme A was found to be inducible.

Though enzymes A and B are physically inseparable, they are immunologically unrelated since no cross reactions take place.

Several closely related strains of bacteria were found to differ widely in their ability to carry out DPN (and DPN analogue) mediated oxidations of *vic* glycols.

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INTRODUCTION

The properties of a partially purified glycerol dehydrogenase obtained from *Aerobacter aerogenes* (American Type Culture Collection 8724) have been described by BURTON AND KAPLAN[1,2]. This DPN* specific enzyme catalyzes the oxidation of glycerol to dihydroxyacetone. Other *vic* glycols, for example, 1,2-propanediol and 2,3-butanediol are oxidized to the corresponding ketone. STRECKER AND HARARY[3] have studied a butanediol dehydrogenase from *Escherichia coli*, and pyridine nucleotide linked glycol dehydrogenases from *Acetobacter suboxydans* have been described by GOLDSCHMIDT AND KRAMPITZ[4] and VIRTANEN AND NORDLUND[5].

A variant of *A. aerogenes* (ATCC No. 8724) has been isolated which contains an enzyme having a general *vic* glycol specificity and a requirement for DPN. Of particular interest is the observation that *A. aerogenes* (var.) grown on glycerol as the sole carbon source forms a *vic* glycol dehydrogenase capable of substituting deamino DPN for DPN. When the organism is grown in a medium containing glucose as the sole carbon source, deamino DPN is no longer capable of substituting for DPN when assayed with the *vic* glycol dehydrogenase. This paper describes these glycol dehydrogenases and compares some of the properties of these enzymes.

MATERIALS AND METHODS

Glycerol was purchased from Merck and Company, dihydroxyacetone from Nutritional Biochemicals Corporation, and 1,2-propanediol (propylene glycol) and 2,3-butanediol (2,3-butylene glycol) from Fischer Chemical Company. Acetol (hydroxyacetone) was synthesized according to the method of LEVENE AND WALTI[6]. Lactaldehyde was synthesized according to the method of DWORZAK AND PRODINGER[7]. The 3-acetylpyridine and the 3-pyridine aldehyde analogues of DPN were prepared by the exchange reaction with the pig brain DPNase as described by KAPLAN AND STOLZENBACH[8] and deamino DPN by the action of nitrous acid on DPN[9]. *A. aerogenes* (ATCC No. 8724) was grown on a medium according to DAGLEY *et al.*[10] consisting of 0.54 % KH_2PO_4, 0.12 % $(NH_4)_2SO_4$, 0.04 % $MgSO_4 \cdot 7H_2O$ made up to volume with distilled water. The carbon source was 1.5 % glucose or glycerol (as indicated), and the final pH 7.1 (30°). A 25-ml actively growing inoculum (of approx. $1 \cdot 10^8$ cells/ml) was used per 20 l when the bacteria were grown on glucose, and a 50-ml inoculum per 20 l was used when the bacteria were grown on glycerol.

Enzyme unit of activity

One unit of enzyme is defined as that amount of protein which causes an initial rate of change in O.D. (at 340 mμ) of 1.0 per min under the conditions employed. The specific activity is expressed as units/mg of protein.

Enzyme assay

0.5 mmole of 1,2-propanediol or glycerol and 1.2 μmoles of DPN are diluted with 2.7 ml of sodium pyrophosphate solution (0.1 *M*, pH 9.2). After initially deter-

* The following abbreviations are used: DPN, diphosphopyridine nucleotide; DPNH, the reduced form of diphosphopyridine nucleotide; deamino DPN, the deaminated analogue of diphosphopyridine nucleotide.

mining the O.D. at 340 mμ, the reaction is started by the addition of approximately 0.2 unit of enzyme.

Acetol was measured fluorometrically according to the method of MILLER AND HUGGINS[11].

Because of the multiplicity of enzyme activities measured we shall refer to glycol dehydrogenase enzyme systems A and B (or simply enzymes A and B). These enzymes will be operationally defined in terms of their enzymic activities and source as shown in Table I.

TABLE I

DEFINITION OF ENZYME SYSTEMS USED IN THE TEXT

	Growth medium	*Substrate and pyridine nucleotide requirements for enzymic activity*
Enzyme A	glycerol plus salts	1,2-propanediol plus deamino DPN 2,3-butanediol plus deamino DPN Glycerol plus DPN 1,2-propanediol plus DPN 2,3-butanediol plus DPN
Enzyme B	glucose plus salts	Glycerol plus DPN 1,2-propanediol plus DPN 2,3-butanediol plus DPN

EXPERIMENTAL

Purification procedure for enzyme A

Step 1: 50 g of wet cells grown in the glycerol-salts media (obtained from 20 l) were suspended in 150 ml of cold distilled water and disrupted in a Raytheon sonic oscillator (10 kc) for 30 min. The cell debris was removed by centrifugation in a refrigerated Servall centrifuge (3°) for 15 min at maximum speed. The supernatant solution was poured off and diluted to 490 ml. Since the enzymic activity of the extract was found to be extremely labile (complete loss of activity in 24 h), it was necessary to proceed with step 2 immediately.

Step 2: Solid $(NH_4)_2SO_4$ was slowly added to the stirred protein solution (5°, pH maintained at 7.0–7.2 with concentrated NH_4OH) to give a final saturation of 50 % with respect to $(NH_4)_2SO_4$. After a 15-min equilibration period, the small amount of precipitate was removed by centrifugation (3°) and discarded. Ammonium sulfate was added to the supernatant solution to a final concentration of 85 %. After equilibration and centrifugation, this large precipitate was extracted 3 times with cold TRIS buffer (tris(hydroxymethyl)aminomethane 0.03 *M*, pH 8.0), and the insoluble material was removed by high speed centrifugation. The clear, yellowish supernatant solution contained the enzymic activity (total volume 35 ml). The preparation was stored at —20°.

It has been found that crude extracts contain a very active DPNH oxidase. Since the enzymic activity is defined in terms of the rate of formation of DPNH, the enzymic activity observed in step 1 is low because the DPNH formed in the dehydrogenase reaction is reoxidized by the contaminating oxidase. Separation of this DPNH oxidase activity by ammonium sulfate treatment caused the apparent increase in total units of activity recoverable as shown in step 2 of Table II.

TABLE II

PURIFICATION OF ENZYME SYSTEM A*

	Protein (mg)	*Units/ml*	*Total units*	*Specific activity (units/mg protein)*	*Purification*	*% recovery*	*a/c***	*b/c****
1. Crude extract								
a. 1,2-propanediol plus DPN**	7.14	0.68	338	0.10				
b. 1,2-propanediol plus deamino DPN**	7.14	0.22	109	0.03	—	—	2.0	0.6
c. glycerol plus DPN**	7.14	0.38	189	0.05				
2. $(NH_4)_2SO_4$ precipitate								
a. 1,2-propanediol plus DPN	15.5	23.0	805	1.48	14.8	238§		
b. 1,2-propanediol plus deamino DPN	15.5	10.0	350	0.65	21.7	321§	1.4	0.6
c. glycerol plus DPN	15.5	16.5	578	1.07	21.7	305§		
3. $MnCl_2$ supernatant								
a. 1,2-propanediol plus DPN	5.5	6.8	585	1.24	12.4	173		
b. 1,2-propanediol plus deamino DPN	5.5	2.6	224	0.47	15.3	205	1.3	0.5
c. glycerol plus DPN	5.5	5.3	456	0.96	19.2	241		
4. Combined $Ca_3(PO_4)_2$ washings								
a. 1,2-propanediol plus DPN	1.04	3.7	259	3.56	35.6	77		
b. 1,2-propanediol plus deamino DPN	1.04	1.6	114	1.54	51.3	104	1.1	0.5
c. glycerol plus DPN	1.04	3.4	238	3.27	65.4	126		
5. Supernatant after heating								
a. 1,2-propanediol plus DPN	0.47	2.8	185	5.96	59.6	55		
b. 1,2-propanediol plus deamino DPN	0.47	0.7	46	1.49	49.6	42	1.3	0.3
c. glycerol plus DPN	0.47	2.1	139	4.47	89.4	74		

* Details are described in the text.

** a, b and c (1,2-propanediol plus DPN, etc.) are the substrate–coenzyme combinations used in assaying enzymic activity.

*** a/c and b/c are ratios of the specific activities of 1,2-propanediol plus DPN to glycerol plus DPN, and of 1,2-propanediol plus deamino DPN to glycerol plus DPN.

§ The apparent recovery of greater than 100% enzymic activity is due to removal of a DPNH oxidase activity present in the crude extract (see text for details).

Step 3: Nucleic acid can be removed from extracts of *A. aerogenes* by employing manganese chloride according to the method of KORKES *et al.*[12]. The protein solution was diluted to a concentration of 6 mg/ml with cold TRIS buffer (0.2 *M*, pH 7.9 total volume 86 ml), placed in an ice bath and stirred continuously while 3.5 ml of manganese chloride (1.0 *M*) was added dropwise. After a 10-min equilibration period, the precipitate was removed by centrifugation. The supernatant (86 ml, 5.5 mg protein/ml) was found to contain most of the enzymic activity.

Step 4: Calcium phosphate gel was added to the protein solution to give a final gel-protein ratio of 2:1. After equilibration, the gel was removed by centrifugation and washed 3 times with cold potassium phosphate buffer (0.05 *M*, pH 7.5, 70 ml total volume of the combined washes). The washings were found to contain most of the activity.

Step 5: 10-ml portions of the enzymically active solution were placed in a 50-ml Erlenmeyer flask and heated in a 70° water bath for 2 min then rapidly cooled in an ice bath. The denatured protein was removed by centrifugation and the supernatant solution (60 ml) was lyophilized. The powder was dissolved in cold TRIS buffer (0.05 *M*, pH 7.5, 6.6 ml) and centrifuged at high speed to remove insoluble material. The protein solution was divided into two parts for subsequent dialysis and storage. 1,2-propanediol was added to one portion to a final concentration of 20 % (v/v) while the remaining solution was diluted with glycerol to a final concentration of 20 % (v/v). The former solution was dialyzed for 24 h against TRIS-20 % 1,2-propanediol (pH 7.5), while the latter was dialyzed against TRIS-20 % glycerol (pH 7.5) to remove salt, and other dialysable impurities. The solutions were stored frozen for several months without loss of enzymic activity.

It was suspected that the more general diol dehydrogenase enzymic specificity of glycerol grown bacteria might be due to the combined effect of several enzyme systems. The purification of enzymes derived from glycerol grown bacteria offers no support for this hypothesis. It can be seen in the last column of Table II that during the course of purification, there is no appreciable change in the ratio of specific activities. However, one cannot conclude from the results of the purification that there is only one enzyme present, for all proteins with similar physical properties can be expected to behave similarly on purification. Experiments dealing with the number of enzymes in this system, their origin and synthesis, will be described later.

Purification procedure for enzyme B

This enzyme was partially purified according to the method of BURTON[2], and a summary of the purification achieved is shown in Table III. As previously stated, the enzyme differs from enzyme A in that it is incapable of substituting deamino DPN for DPN (see Table VI for coenzyme specificity). The glycerol dehydrogenase which is present in the parent strain of *A. aerogenes* No. 8724 has not been identified in the variant isolated and studied herein. It should be noted that enzyme B was partially purified by the same procedure described by BURTON for the purification of the glycerol dehydrogenase identified and studied in the parent strain. Table III lists the specific activities calculated for both glycerol and 1,2-propanediol. The ratio of these specific activities suggests that there may be some slight contamination of the enzyme B by the glycerol dehydrogenase (originally purified by BURTON) or of some other *vic* glycol dehydrogenases. In addition to the enzymes described, there

TABLE III

PURIFICATION OF ENZYME SYSTEM B*

Fraction	*Specific activity (units/mg)***		*a/b****
	a *Glycerol*	*b* *1,2-propanediol*	
1. Cell-free extract	0.4	0.4	1.0
2. 0–40% saturated ammonium sulfate supernatant	2.1	3.2	0.65
3. Eluate of 0–40% saturated ammonium sulfate precipitate	16.1	19.2	0.84
4. Calcium phosphate gel (20 mg/ml) eluate from step 3	14.2	23.6	0.60
5. Heating at 60° for 5 min	22.5	29.5	0.75
Overall purification	57 ×	74 ×	—

* Obtained from cells grown on a glucose–salts medium.

** Purification and assay were conducted according to the method of BURTON[2]. One unit of enzyme activity is defined as that amount of enzyme which causes an initial rate of change in O.D. at 340 mμ of 1.0/min under the conditions of the assay. The specific activity is expressed as units of activity/mg of protein. Assay conditions are described in MATERIALS AND METHODS.

*** a/b is the ratio of the specific activities of glycerol plus DPN to that of 1,2-propanediol plus DPN.

TABLE IV

STABILITY OF ENZYME SYSTEMS A AND B

Enzyme A was obtained from bacteria grown in a glycerol-salts media and enzyme B was obtained from bacteria grown in a glucose-salts media.

	*Activity**			
	Enzyme A		*Enzyme B*	
	Units	*% activity*	*Units*	*% activity*
Crude extract control	0.685	100	0.590	100
Storage (24 h, —15°)**	0.034	5	0.431	73
Storage (14 days, —15°)	0	0	0.283	48
Storage (3 months, —15°), in 0.1 *M* TRIS, 20% 1,2-propanediol	0.630	92	0.607	103
Dialysis (24 h, 3°)***	0.010	1	0.212	36

* The assay is the same as defined previously under "enzyme assay". The activities are recorded in terms of change in O.D. (at 340 mμ) per min. The activities are recorded for 1,2-propanediol plus DPN. Other substrate-pyridine nucleotide combinations normally assayed with enzyme A give the same results.

** The enzyme was diluted to a concentration of 10 mg/ml with sodium pyrophosphate (0.1 *M*, pH 9.3), sodium phosphate (0.05 *M*, pH 7.5) and TRIS (0.1 *M*, pH 7.0).

*** Dialysis was carried out against distilled water, potassium phosphate (0.05 *M*, pH 7.5) and Versene ($1 \cdot 10^{-3}$ *M*).

is present in extracts of *A. aerogenes* No. 8724 a *vic* glycol dehydrogenase specific for 2,3-butanediol and DPN. The 2,3-butanediol dehydrogenase can be separated from the other *vic* glycol dehydrogenases by ammonium sulfate fractionation[13].

Enzyme stability

As seen in Table IV crude extracts containing enzyme A prepared from glycerol grown cells were found to have an enzymic half-life of approximately 1 day or less regardless of the temperature of storage (one preparation was found to be completely

inactive after 12 h storage at —15°). In contrast, extracts of enzyme B prepared from glucose grown cells retained 50 % of their enzymic activity after two weeks storage at —15°. The partially purified enzyme A which is stabilized with 1,2-propanediol or glycerol was found to retain complete activity after 3 months storage at —15° despite the fact that the preparation had been repeatedly frozen and thawed. Enzyme A, when not protected by the addition of glycols, completely lost enzyme activity after a 24-h dialysis against distilled water or dilute phosphate buffer (0.1 *M*, pH 7.5). Enzyme B retained 36 % of its original activity under the same conditions.

Product of 1,2-propanediol oxidation

BURTON found that *A. aerogenes* oxidized glycerol according to eqn. (1).

$$CH_2OHCHOHCH_2OH + DPN^+ \rightleftharpoons HOCH_2COCH_2OH + DPNH + H^+ \quad (1)$$

It has been stated that chemically synthesized acetol (hydroxyacetone) but not lactaldehyde could participate in the reverse reaction. Utilizing the method of HUGGINS AND MILLER[11], it is possible to show that the product of the oxidation of 1,2-propanediol is acetol and that acetol and DPNH are produced in equimolar amounts. The reaction can be formulated according to eqn. (2).

$$CH_3CHOHCH_2OH + DPN^+ \rightleftharpoons CH_3COCH_2OH + DPNH + H^+ \quad (2)$$

The results of this analysis are presented in Table V. The relationship between DPN reduction and acetol formation was demonstrated by measuring DPNH spectrophotometrically, then the reaction was stopped with trichloroacetic acid (10 % final concentration), and the acetol was measured fluorometrically. From the controls one can see that substrate, pyridine nucleotide and enzyme are all required for the reaction.

TABLE V

OXIDATION OF 1,2-PROPANEDIOL

Constituents	*Acetol formed* (μmoles)*	*DPNH formed** (μmoles)*
Complete***	0.23	0.21
Minus 1,2-propanediol	0.035	0
Minus enzyme	0	0
Minus DPN	0.015	0

* Measured according to the method of HUGGINS AND MILLER[11].

** Determined spectrophotometrically at 340 mμ assuming a molar absorbancy index (α_M) of[16] $6.22 \cdot 10^3$. α_M is the molar absorbancy index (nomenclature recommended by the Bureau of Standards, circular LC 857, May 19, 1947) and is defined as the absorbancy ($-\log_{10}$ transmission) divided by the concentration (moles/l).

*** Complete system described in enzyme assay.

Characteristics of the partially purified enzymes A and B

Activity of analogues of pyridine nucleotides: Several pyridine nucleotide analogues of DPN are capable of substituting for DPN in the partially purified *vic* glycol dehydrogenase systems (see Table VI). The pyridine-3-aldehyde analogue of DPN is active with both enzymes A and B and seems to have some specificity for 1,2-propanediol as substrate. The 3-acetylpyridine analogue of DPN is active only with enzyme B

TABLE VI

ENZYMIC ACTIVITY OF PYRIDINE NUCLEOTIDE ANALOGUES

500 μmoles of glycerol or 1,2-propanediol and pyridine nucleotide as indicated were diluted to a final volume of 3.0 ml with sodium pyrophosphate buffer (0.1 *M*, pH 9.3). The reaction was started with 1 unit of the appropriate enzyme, and the rate of reduction of the pyridine nucleotide was measured every 15 sec at the following wavelengths: DPN, 340 mμ; deamino DPN, 340 mμ; 3-acetylpyridine analogue of DPN, 365 mμ; deamino-3-acetylpyridine DPN, 365 mμ; pyridine-3-aldehyde, 355 mμ; deamino-pyridine-3-aldehyde DPN, 355 mμ; nicotinic acid DPN, 330 mμ.

	*Activity (%)**			
	Enzyme B		*Enzyme A*	
	1,2-propanediol	*Glycerol*	*1,2-propanediol*	*Glycerol*
DPN (1 μmole/ml)	100*	15	100*	67
Deamino DPN (2 μmoles/ml)	5(12)**	3(10)**	16(252)**	4(98)**
3-acetylpyridine analogue of DPN (1 μmole/ml)	73	3	4	1
Deamino-3-acetylpyridine analogue of DPN (2 μmoles/ml)	1	1	0	0
Pyridine-3-aldehyde DPN (1 μmole/ml)	55	6	20	13
Deamino-pyridine-3-aldehyde (2 μmoles/ml)	3	0	0	0
Nicotinic acid analogue (2 μmoles/ml)	0	0	0	0

* The enzymic activity of the various analogues is reported in % activity relative to the activity measured with DPN and 1,2-propanediol.

** The values given in the parentheses were obtained when the concentration of deamino DPN was increased to 9 μmoles/ml.

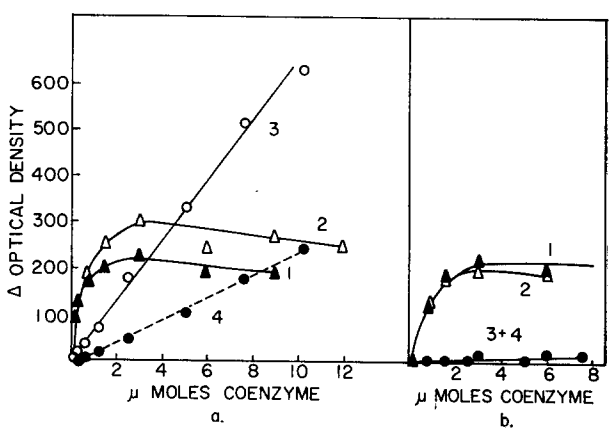

Fig. 1. Rate studies of glycerol and glucose grown *A. aerogenes* extracts. (a): glycerol grown cells; (b): glucose grown cells. 100 μmoles of glycerol or 1,2-propandediol, 0.1 *M* potassium pyrophosphate (pH 9.3) and the glucose or glycerol grown crude bacterial extract (approximately 0.5 units) was added and the reaction started by the addition of coenzyme (final volume, 3.0 ml; 25°). The change in O.D. at 340 mμ in the first 2 min was observed as a function of coenzyme concentration. The substrate and pyridine nucleotide combinations used are as follows: curve 1, glycerol plus DPN; curve 2, 1,2-propanediol plus DPN; curve 3, 1,2-propanediol plus deamino DPN; curve 4, glycerol plus deamino DPN.

and will not substitute for DPN in the presence of enzyme A. Deamino DPN is active only with enzyme A and not enzyme B. Though the deamino DPN activity is only 16% of the DPN activity in this particular experiment, high concentrations of deamino DPN have considerably higher activity than the natural coenzyme with enzyme A, but very little activity with enzyme B (Table VI). The acetyl pyridine

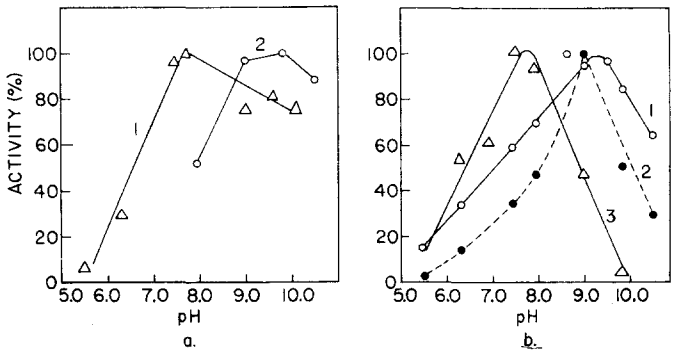

Fig. 2. pH optima of partially purified enzyme systems. 500 μmoles of 1,2-propanediol or glycerol and 0.63 μmole of DPN or deamino DPN were diluted with sodium phosphate (0.2 *M*, 25°, final volume, 3.0 ml) which was adjusted to the pH value shown on the abscissa. The reaction was started with 100 μg of enzyme protein and the reduction of pyridine nucleotide (at 340 mμ) was measured for the first 2 min of reaction. Maximal activity for each substrate–coenzyme combination is arbitrarily set at 100, other activities of this combination being expressed as % of the maximum. pH optima are plotted for 1,2-propanediol plus DPN (curve 1), glycerol plus DPN (curve 2), and 1,2-propanediol plus deamino DPN (curve 3). (a): enzymes from glucose grown cells. (b): enzymes from glycerol grown cells.

TABLE VII

A SURVEY OF SOME GLYCOL DEHYDROGENASES

Bacteria	*Carbon compound used in growth media*	*Enzymic activity (units/ml)**			
		1,2-propanediol plus DPN	*1,2-propanediol plus deamino DPN*	*Glycerol plus DPN*	*Glycerol plus deamino DPN*
A. aerogenes No. 8724	Glucose	1.34	0	0.86	0
	Glycerol	5.46	1.00	4.60	0.08
A. aerogenes No. 884	Glucose	0.76	1.20	0.80	0.90
	Glycerol	0.28	0.18	0.36	0.12
A. aerogenes No. 8329	Glucose	0	0	0	0
	Glycerol	35.0	39.0	12.2	4.30
E. coli K-12	Glucose	0.20	0.10	0.12	0.08
	Glycerol	0.48	0.28	0.40	0.24
Acetobacter suboxydans	Glucose	1.00	1.58	0.12	0.22
	Glycerol	2.48	3.82	0.78	0.64

* 500 μmoles of 1,2-propanediol or glycerol and DPN (1,2 μmoles) or deamino DPN (6.1 μmoles) were diluted with sodium pyrophosphate (0.1 *M*, pH 9.3, 25°) to give a final volume of 3.0 ml. The reaction was started with approximately 1 mg of enzyme protein, the rate of reaction being followed by the reduction of pyridine nucleotide at 340 mμ. The various combinations of substrate and pyridine nucleotide assayed are listed at the top of each column. Activity measurements are recorded in terms of units/ml of crude sonic extract which contained approximately 10 mg of protein/ml estimated by the method of WARBURG AND CHRISTIAN.

analogues of DPN and deamino DPN have a substrate specificity largely for 1,2-propanediol.

Fig. 1 shows that the K_m for deamino DPN with enzyme A is infinity in the concentration range from 0.1 to 10 μmoles/ml (using 1,2-propanediol as substrate) and that within this concentration range, the rate of reduction of deamino DPN

increases with increasing concentration of this analogue. At high concentrations (10 μmoles/ml), the initial rate of reaction is 3 to 5 times greater than the rate observed with DPN. It would therefore seem that deamino DPN and the 3-acetylpyridine analogue of DPN can be used as "chemical tracers" in identifying enzymes A and B since deamino DPN can substitute for DPN only with enzyme A while the 3-acetylpyridine analogue of DPN can substitute for DPN only with enzyme B.

pH optimum: The diol dehydrogenases (enzymes A and B) differ not only in coenzyme specificity but also in the pH optimal enzyme activity as can be seen in Figs. 2a and 2b. For example, enzyme B, assayed with glycerol and DPN (Fig. 2a) exhibits a broad pH optimum (from 9–10) compared with enzyme A which has a sharp maximum at pH 9. This difference in the pH optimum (for the two systems studied) reflects changes in the physical structure of the two diol dehydrogenases. Preliminary information, to be presented later in this paper, indicates that these diol dehydrogenases are immunochemically different.

Strains of bacteria: Table VII summarizes the results of a study made to determine the ubiquity of these enzyme systems in some related strains of bacteria. When the strains of *A. aerogenes* are grown on a glucose medium, marked differences in enzymic activity are noted. Strain No. 8724 (var.) (the strain which we have studied in detail) cannot substitute deamino DPN for DPN and extracts of this bacterium are most active when 1,2-propanediol is the substrate. Strain No. 884 has a general specificity with regard to both substrate and pyridine nucleotide and strain No. 8329 has no *vic* glycol dehydrogenase activity at all when grown on glucose. When these same organisms are grown on a glycerol medium a considerable increase in enzyme activity is noted with strains No. 8724 (var.) and No. 8329, whereas a decrease in activity is noted with strain No. 884. In addition, it is noted that the extract of glycerol grown strain No. 8329 has a marked specificity for 1,2-propanediol while extracts of strains No. 884 and No. 8724 (var.) have a more general substrate specificity when

TABLE VIII

ANTIGEN-ANTIBODY REACTIONS OF ENZYMES A AND B

	DPN		*Deamino DPN*	
	Units	*% activity*	*Units**	*% activity*
A. Enzyme A				
Alone	0.271	100	0.160	100
+ control γ-globulin	0.278	103	0.173	108
+ anti-enzyme A γ-globulin	0.107	39	0.043	27
+ anti-enzyme B γ-globulin	0.260	96	0.160	100
B. Enzyme B**				
Alone	0.142	100	—	—
+ control γ-globulin	0.142	103	—	—
+ anti-enzyme A γ-globulin	0.159	112	—	—
+ anti-enzyme B γ-globulin	0.153	108	—	—

* 0.2 mg of enzyme protein and 0.1 ml of the appropriate γ-globulin (except for the enzyme controls) were preincubated for 15 min at 37°. 500 μmoles of 1,2-propanediol and DPN (1.2 μmoles) or deamino DPN (6.1 μmoles) were diluted with sodium pyrophosphate (0.1 *M*, pH 9.3, 25°) to give a final volume of 3.0 ml. The reaction was started with the preincubated enzyme, γ-globulin mixture and the change in O.D. (at 340 mμ) was recorded for the first 2 min of reaction.

** Enzyme B does not catalyse the reaction between deamino DPN and 1,2-propanediol.

grown in a glycerol medium. The K-12 strain of *E. coli* has a general low level of *vic* glycol dehydrogenase activity and *Acetobacter suboxydans* shows a greater specificity for 1,2-propanediol as substrate regardless of the carbon source used in the growth medium. The results are of interest since there seems to be no common enzyme system in these organisms for the direct metabolism of *vic* glycols. The fact that several strains of the same organism differ widely in their capacity to metabolize *vic* glycols is noteworthy.

Immunochemical studies of enzymes A and B: Antisera to enzymes A and B were produced in rabbits by intravenous injection of alum-precipitated proteins. Nine injections, one every other day, were given. The doses were from 0.2 to 0.7 mg of protein. The rabbits were rested 1 month and a second course of injections were administered. The rabbits were exsanguinated 6 days after the last injection. Sera were cleared by centrifugation, heated for 30 min at 56° to inactivate complement and fractionated with ammonium sulfate to obtain the γ-globulin according to the method of STERNBERGER AND PETERMAN[14]. 0.1 ml of the γ-globulin fractions were preincubated with enzymes A and B for 15 min at 37°. The results are presented in Table VIII. Enzyme B does not appear to be antigenic, since no inhibition of enzyme activity is observed and no enzyme activity was precipitated on standing in the cold (3°) for 24 h. Anti-enzyme A selectively inhibits enzyme A but does not inhibit enzyme B activity. It is of interest to note that the antisera inhibit both the DPN and deamino DPN activities of enzyme A.

DISCUSSION

Crude extracts of *A. aerogenes* grown in a medium containing both glucose and glycerol exhibited *vic* glycol dehydrogenase activity which we believed to be due to multiple enzyme systems. Extracts of cells grown in a medium containing glycerol as the sole carbon source exhibited a general *vic* glycol activity. When grown in this carbon source deamino DPN was not only active in the system but at high concentrations exceeded the rate of reaction with DPN. In contrast, extracts of cells grown on glucose as the sole carbon source showed a specificity for DPN. We have not been able to find deamino DPN in extracts of glycerol grown bacteria.

Based on the enzymes elicted when bacteria were grown on glucose or glycerol, it was expected that cells initially grown in a glucose medium and subsequently transferred to a glycerol medium would contain both enzymes A and B. Kinetic studies (mixed substrate at saturation) have not revealed the presence of both enzymes. We have had no success in separating enzymes A and B found in extracts of *A. aerogenes* grown on the mixed carbon source medium, nor can the partially purified enzymes be separated when artificially mixed. However, a few differences have been noted in extracts (and partially purified enzymes) of bacteria grown on either glycerol or glucose. For example, the enzyme obtained from glycerol grown bacteria is much more unstable during storage and dialysis. Other differences noted relate to pyridine nucleotide specificity and pH optima for enzymic activity. When glucose grown bacteria are induced by the presence of glycerol (and in the absence of glucose), the enzyme activities produced are indistinguishable from the system obtained by growing the bacteria solely on glycerol. It is suspected that these two glycol dehydrogenases are different proteins having sufficiently similar properties to

make physical separation difficult. The fact that there is no immunochemical cross reaction indicates that these two proteins are not structurally identical. The properties of the inducible *vic* glycol dehydrogenase are described in the next article.

PULLMAN *et al.*[15] have compared the activity of DPN with deamino DPN in a number of enzyme systems and concluded that differences in rates reflect a difference in affinities of some of the reactants. We have concluded that deamino DPN had a much lower affinity for glycol dehydrogenase A than DPN. At high concentrations of deamino DPN the rate which is observed is 3 times faster than with DPN when 1,2-propanediol is the substrate. When deamino DPNH or DPNH were assayed with acetol (hydroxyacetone), the reduced deamino DPN rate again exceeded that of DPNH. Lactaldehyde was inactive in this system.

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REFERENCES

1 R. M. BURTON AND N. O. KAPLAN, *J. Am. Chem. Soc.*, 75 (1953) 1005.
2 R. M. BURTON, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. I, Academic Press Inc., New York, 1955, p. 397.
3 H. J. STRECKER AND I. HARARY, *J. Biol. Chem.*, 211 (1954) 263.
4 E. P. GOLDSCHMIDT AND L. O. KRAMPITZ, *Bacteriol. Proc.*, (1954) 96.
5 A. I. VIRTANEN AND M. NORDLUND, *Biochem. J.*, 27 (1933) 442.
6 P. A. LEVENE AND A. WALTI, *Organic Syntheses*, John Wiley & Sons, Inc., New York, 1930.
7 R. DWORZAK UND W. PRODINGER, *Monatsh. Chem.*, 50 (1928) 467.
8 N. O. KAPLAN AND F. E. STOLZENBACH, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. III, Academic Press Inc., New York, 1957, p. 902.
9 N. O. KAPLAN, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. III, Academic Press Inc., New York, 1957, p. 874.
10 S. DAGLEY, E. A. DAWES AND G. A. MORRISON, *J. Bacteriol.*, 60 (1950) 369.
11 C. G. HUGGINS AND O. N. MILLER, *J. Biol. Chem.*, 221 (1956) 711.
12 S. KORKES, A. DEL CAMPILLO, I. C. GUNSALUS AND S. OCHOA, *J. Biol. Chem.*, 193 (1951) 721.
13 R. M. BURTON, unpublished.
14 L. A. STERNBERGER AND M. L. PETERMAN, *J. Immunol.*, 67 (1951) 207.
15 M. E. PULLMAN, S. P. COLOWICK AND N. O. KAPLAN, *J. Biol. Chem.*, 194 (1952) 593.
16 A. KORNBERG AND B. L. HORECKER, in SNELL, *Biochemical Preparations*, Vol. III, John Wiley and Sons, New York, 1953, p. 23.