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MITOCHONDRIAL ALCOHOL DEHYDROGENASE FROM *SACCHAROMYCES CEREVISIAE**

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SUMMARY

Aerobic yeast mitochondria contain a unique isozyme of alcohol dehydrogenase that accounts for the ability of yeast mitochondria to respire with ethanol as a substrate. This alcohol dehydrogenase has been isolated from intact yeast mitochondria, purified approximately 65-fold, and further studied. The mitochondrial isozyme represents about 5% of the total cellular alcohol dehydrogenase and differs from the classical, cytoplasmic isozyme by its slower electrophoretic mobility on polyacrylamide gel and its more alkaline pH optimum. Only minor differences between the mitochondrial and cytoplasmic isozymes were noted in the apparent K_m's for NAD^+, NADH, ethanol, and acetaldehyde.

INTRODUCTION

The complete understanding of carbohydrate metabolism in yeast requires a knowledge of the reactions involved in the utilization of ethanol as well as a knowledge of the more familiar reactions involved in the production of this substance. Ethanol formation is associated with the functioning of the classical alcohol dehydrogenase (alcohol:NAD^+ oxidoreductase; EC 1.1.1.1)[1], which is a cytoplasmic enzyme that operates during the anaerobic fermentation of sugars. In contrast, ethanol utilization is an aerobic process that appears to be linked to the tricarboxylic acid cycle and the respiratory activities of mitochondria.

The direct involvement of mitochondria in the utilization of ethanol stems from the early observation of NOSSAL[2] and NOSSAL *et al.*[3] that a particulate fraction from mechanically disrupted yeast was capable of oxidizing ethanol in addition to substrates such as succinate and isocitrate. Although ethanol is not a typical substrate for mitochondria from most sources, it is utilized by aerobic yeast mitochondria as efficiently as intermediates of the tricarboxylic acid cycle, and P/O ratios approaching

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a value of 2 have been demonstrated in many laboratories[4–11]. These findings strongly suggest that yeast mitochondria contain a constitutive alcohol dehydrogenase that is functionally linked to the respiratory chain. Because of the importance of ethanol metabolism to the yeast cell, the mitochondrial alcohol dehydrogenase is of considerable interest. However, this isozyme has not previously been prepared. It was the purpose of the present investigation to isolate the alcohol dehydrogenase from aerobic yeast mitochondria and to compare some of its properties with those of the classical, cytoplasmic enzyme.

MATERIALS AND METHODS

Growth of yeast cells

A strain of *Saccharomyces cerevisiae* isolated from commercial yeast (Red Star Yeast and Products Co., Milwaukee, Wisc.) was grown aerobically on glucose for 12 h as outlined by DUELL *et al.*[12]. The cells were harvested by centrifugation, suspended in water, and filtered and washed on Millipore Type RA-47 filters (Millipore Corp., Bedford, Mass.). The cells were stored as cakes at 4° until used the next day.

Mitochondria

Intact mitochondria were obtained by the osmotic shock of yeast spheroplasts essentially according to the two-step procedure of DUELL *et al.*[12]. The final yield was approx. 900 mg of mitochondrial protein per 150 g (wet weight) of yeast cells. The mitochondria were suspended at a concentration of about 20 mg of protein per ml of suspending medium (20% sucrose (w/v), 1 mM EDTA, and 50 mM K_2HPO_4–KH_2PO_4 (pH 6.8)), and aliquots were rapidly frozen with a methyl Cellosolve–Dry Ice mixture and kept at −25° until used.

Spectrophotometric assay

Soluble alcohol dehydrogenase was assayed with 0.5 M ethanol, 10 mM $Na_4P_2O_7$ (pH 8.8) and 0.83 mM NAD^+ in a final volume of 3.0 ml using cuvettes of 1.00 cm light path. All reactions were conducted at 30° and were initiated by the addition of enzyme. One unit of enzyme reduced 1 μmole of NAD^+ per min under these conditions. When necessary, the enzyme was diluted with 10 mM K_2HPO_4–KH_2PO_4 (pH 7.5) and 0.1% bovine serum albumin.

Changes in the initial concentration of NADH were followed by measuring the absorption at 340 nm, and a level of enzyme was chosen so that the changes in absorption would not be greater than 0.220/min. Under these conditions, the reaction rates were proportional to the concentration of enzyme and were linear for at least a minute. Absorption measurements were made with a Gilford Model 2000 recording spectrophotometer equipped with a temperature-controlled cell compartment. Unless otherwise noted, all assays of alcohol dehydrogenase activity were spectrophotometric.

Polarographic assay

An O_2 polarograph was used to determine alcohol dehydrogenase activity in mitochondria and whenever turbid suspensions were encountered. The principles underlying this assay and the apparatus used are discussed elsewhere[13]. The alcohol dehy-

drogenase assay mixture consisted of 0.5 M ethanol, 10 mM $Na_4P_2O_7$ (pH 8.8), 2 mM NAD^+, 0.1 M semicarbazide (pH 8.3), 1 mM KCN (neutralized with HCl before use), 0.2 mM 5-ethylphenazinium ethyl sulfate, and 0.3% bovine serum albumin. Reactions were conducted in a final volume of 1.5 ml in a plexiglas chamber maintained at 30°, and they were initiated by introducing the enzyme through a small side arm. KCN was omitted when purified preparations were assayed.

The polarographic assay was proportional to the amount of enzyme present up to 0.12 unit, and the rates of O_2 uptake were linear for about 3 min. A direct comparison between the spectrophotometric and polarographic assays showed that the latter values were consistently 88% of the former. All polarographic assays were therefore corrected so as to correspond with spectrophotometric values.

Electrophoresis

Polyacrylamide gels were prepared as described by DIETZ AND LUBRANO[14], except that 28 g of acrylamide and 0.74 g of *N,N'*-methylenebisacrylamide were used in the preparation of their Solution C. Fresh ammonium persulfate solution was used for each set of gels. Samples containing 30–50 μg of protein in 50 μl of 20% sucrose, 0.06 M Tris–HCl (pH 6.9) were layered on top of the gels. No stacking or sample gels were used.

Electrophoresis was performed at 4° using an apparatus from Shandon Scientific Co. (Sewickley, Pa.). The buffer reservoirs contained 0.19 M glycine and 0.025 M Tris (pH 8.3). The upper reservoir also contained 0.2 μg of bromophenol blue per ml of buffer. The current was maintained at 2 mA per tube for the first 15 min and then was increased to 4 mA per tube. Electrophoresis was continued until the bromophenol blue marker migrated to the bottom of the gel. The gels were removed from the tubes and sliced longitudinally. One slice was stained for protein with Coomassie blue[15], and the other was stained for alcohol dehydrogenase activity with 0.5 M ethanol, 0.06 M $Na_4P_2O_7$ (pH 8.8), 2.4 mM NAD^+, 0.1% nitroblue tetrazolium, and 0.075 mM 5-ethylphenazinium ethyl sulfate. The enzymatic reaction was carried out in the dark at room temperature and was completed in less than 1 h.

Chemicals

ADP, NAD^+, NADH, nitroblue tetrazolium, Tris, glycine, sodium α-ketoglutarate, and crystalline yeast alcohol dehydrogenase were obtained from Sigma Chemical Co. (St. Louis, Mo.). Sodium deoxycholate, cholic acid, diethylstilbesterol, and Coomassie brilliant blue R-250 were purchased from Mann Research Labs. (New York, N.Y.), and *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid and *N,N*-bis(2-hydroxyethyl)glycine were from Calbiochem (Los Angeles, Calif.). Acrylamide, *N,N'*-methylenebisacrylamide, *N,N,N',N'*-tetramethylenediamine, and acetaldehyde were supplied by Eastman Kodak Co. (Rochester, N.Y.). The acetaldehyde was distilled just prior to use. Ethanol was the product of U.S. Industrial Chemicals Co. (New York, N.Y.), and crystalline bovine serum albumin was obtained from Pentex, Inc. (Kankakee, Ill.). DEAE-cellulose (Whatman DE-52) was purchased from Reeve Angel (Clifton, N.J.), and prepared for use according to instructions supplied by the manufacturer. All other chemicals were reagent grade, and solutions were prepared with glass-distilled water which was previously deionized.

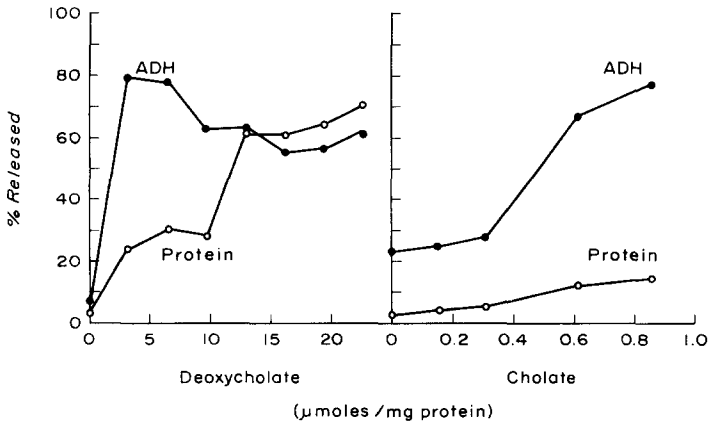


Fig. 1. Effect of bile salts on the release of mitochondrial alcohol dehydrogenase (ADH) and protein from yeast mitochondria. *Deoxycholate extraction*: Mitochondria were diluted in suspending medium (see text) to a final protein concentration of 0.31 mg/ml and incubated at 0° for 10 min in the presence of the indicated levels of sodium deoxycholate. The mixtures were centrifuged at 150 000 × *g* for 30 min, and the supernatant fractions were assayed spectrophotometrically for alcohol dehydrogenase activity. The percent released is based on the polarographic assay of the alcohol dehydrogenase activity in untreated mitochondria. *Cholate extraction*: Mitochondria were suspended in 0.1 M KCl and 10 mM Tris–HCl (pH 7.5) to a final protein concentration of 2.66 mg/ml and incubated at 0° for 15 min in the presence of the indicated levels of potassium cholate. The mixtures were centrifuged at 100 000 × *g* for 60 min, and the supernatant fractions were assayed spectrophotometrically for alcohol dehydrogenase activity. The percent released is based on the polarographic assay of the alcohol dehydrogenase activity in each reaction mixture prior to centrifugation.

Protein was determined by the method of LOWRY *et al.*[16], using crystalline bovine serum albumin as the standard.

RESULTS

Comparison of extraction methods

Bile salts readily disrupted the yeast mitochondria and caused the release of mitochondrial alcohol dehydrogenase (Fig. 1). At a ratio of 3–8 μmoles of deoxycholate per mg of mitochondrial protein, 80% of the alcohol dehydrogenase and 30% of the protein were solubilized. With cholate, 80% of the enzyme and 15% of the protein were released at a ratio of 0.9 μmole of cholate per mg of mitochondrial protein. Attempts to purify the mitochondrial alcohol dehydrogenase extracted by these methods were not successful because of interference from extracted phospholipid and residual bile salts.

More satisfactory results were obtained when mitochondrial alcohol dehydrogenase was extracted with the aid of diethylstilbesterol essentially according to the procedure described by BYINGTON *et al.*[17] and SMOLY *et al.*[18]. As seen in Fig. 2, up to 80% of the alcohol dehydrogenase and 20% of the protein were released at a ratio of 0.20 μmole of diethylstilbesterol per mg of mitochondrial protein. Although the amounts of enzyme and protein extracted are similar to those extracted with bile salts, the diethylstilbesterol procedure produces an extract that is relatively free from interfering phospholipid and residual diethylstilbesterol[17,18]. The optimum level of

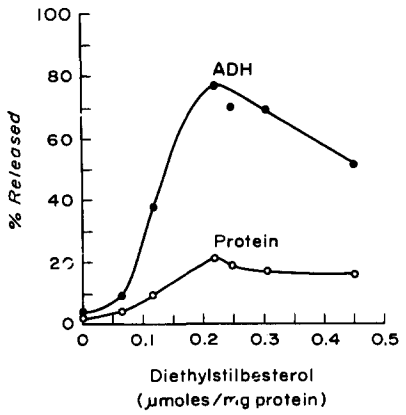


Fig. 2. Effect of diethylstilbesterol on the release of mitochondrial alcohol dehydrogenase (ADH) and protein from yeast mitochondria. Mitochondria were suspended in extraction buffer (see text) at a protein concentration of 4.0 mg/ml and then brought to 30°. Diethylstilbesterol (60 mM in ethanol) was added at the indicated levels to 2.0-ml aliquots, and the mixtures were vigorously stirred for 10 min. They were chilled and centrifuged at 150 000 × *g* for 20 min, and the supernatant fractions were decanted. The percent released is based on the assay of alcohol dehydrogenase in each reaction mixture prior to centrifugation. All fractions were assayed polarographically.

diethylstilbesterol varied from 0.10 to 0.20 μmole of diethylstilbesterol per mg of mitochondrial protein and seemed to depend upon the mitochondrial preparation.

Purification of mitochondrial alcohol dehydrogenase

A typical purification scheme is summarized in Table I.

Extraction. Mitochondria were suspended in extraction buffer (0.5 M sucrose, 1 mM EDTA, 10 mM Tris–HCl (pH 7.5) and 10 mM $MgCl_2$) at a final concentration of 9.5 mg of protein per ml, following a preliminary wash in the same buffer not containing the $MgCl_2$. The suspension was equilibrated at 30° for 5 min, and 60 mM diethylstilbesterol in ethanol was added with vigorous stirring to a final concentration of 0.15 μmole of diethylstilbesterol per mg of protein. After 10 min of stirring, the reaction mixture was chilled on ice and centrifuged at 20 000 × *g* for 15 min. The residue was washed twice with one-half the initial volume of extraction buffer, and the supernatant fractions were combined.

TABLE I

PURIFICATION OF MITOCHONDRIAL ALCOHOL DEHYDROGENASE

Step	*Vol. (ml)*	*Activity*		*Protein*		*Specific activity*
		Units	*Yield (%)*	*mg*	*Yield (%)*	
1. Washed mitochondria	35	553	100	334	100	1.7
2. Diethylstilbesterol-treated mitochondria.	36	553	100	334	100	1.7
3. Supernatant fraction	54	418	76	62	19	6.7
4. Heat treatment (58°)	48	275	50	17	5	16.5
5. Acetone precipitate (20–43%)	1.5	270	49	10	3	26.0
6. 0.075 M NaCl DEAE-cellulose eluate	3.5	44	8	0.4	0.12	110

Heat treatment. The combined supernatant fractions were heated to 58° in a water bath and maintained at that temperature with constant stirring for 3 min. The extract was chilled and centrifuged at 2000 × *g* for 15 min to remove denatured protein, and the supernatant fraction was filtered through glass wool to remove a small amount of floating precipitate. The pH was 6.5–7.0.

Acetone fractionation. Acetone, chilled to −25°, was added to the filtrate slowly and with constant stirring until its concentration was 20% of the final volume. The mixture was stirred in ice for 15 min, and the small amount of precipitate, which contained no alcohol dehydrogenase activity, was removed by centrifugation. The enzyme was precipitated by increasing the acetone concentration to 43% of the final volume. The precipitate was collected by centrifugation at 20 000 × *g* for 20 min and was thoroughly drained. The enzyme was allowed to dissolve slowly in a small volume of 10 mM Tris–HCl (pH 7.8) over a period of 12 h. This preparation was stable to storage at 4° for several days.

DEAE-cellulose chromatography. The enzyme from the acetone fractionation was diluted with an equal volume of water and applied to a 0.9 cm × 16.5 cm column of DEAE-cellulose equilibrated with 5 mM Tris–HCl (pH 7.8) at 4°. The column was first washed with 25 ml of 5 mM Tris–HCl (pH 7.8) and then with 20 ml of the same buffer containing 0.01 M NaCl. Mitochondrial alcohol dehydrogenase was eluted when the NaCl concentration in the buffer was raised to 0.075 M.

The active fractions were pooled and concentrated by centrifugation at 2000 × *g* for 20 min through Centriflo membrane ultrafilters (Amicon Corporation, Lexington, Mass.). The material collected on the ultrafilters was dissolved in a small volume of 20% sucrose and 0.06 M Tris–HCl (pH 6.9) and was either used immediately or frozen with a methyl Cellosolve–Dry Ice mixture and stored at −25°. The mitochondrial alcohol dehydrogenase which eluted from the DEAE-cellulose column was very

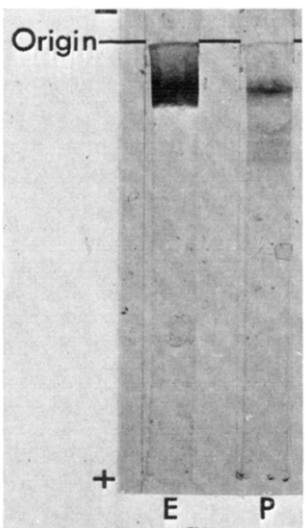


Fig. 3. Polyacrylamide gel electrophoresis of mitochondrial alcohol dehydrogenase after DEAE-cellulose chromatography. See MATERIALS AND METHODS for details of the electrophoretic procedure. E = enzyme; P = protein.

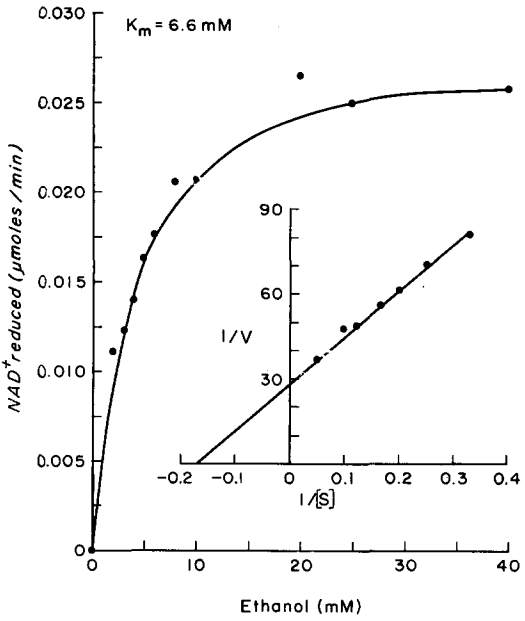


Fig. 4. Dependence of mitochondrial alcohol dehydrogenase activity on ethanol. The reaction mixtures contained 32 mM $Na_4P_2O_7$ (pH 8.8), 5 mM NAD^+, and ethanol as indicated. See text for further details.

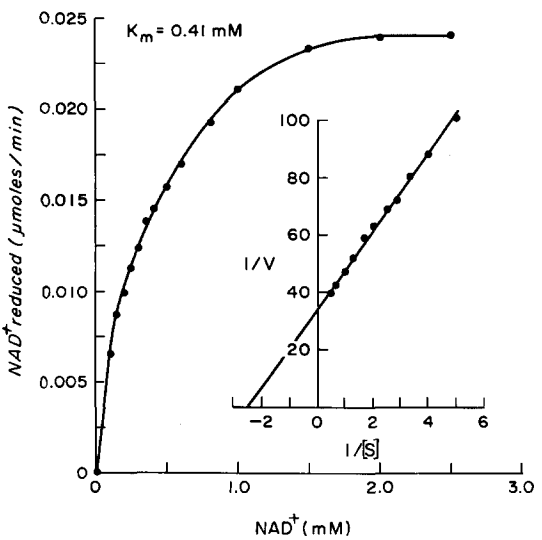


Fig. 5. Dependence of mitochondrial alcohol dehydrogenase activity on NAD^+. The reaction mixtures contained 32 mM $Na_4P_2O_7$ (pH 8.8), 0.2 M ethanol, and NAD^+ as indicated. See text for further details.

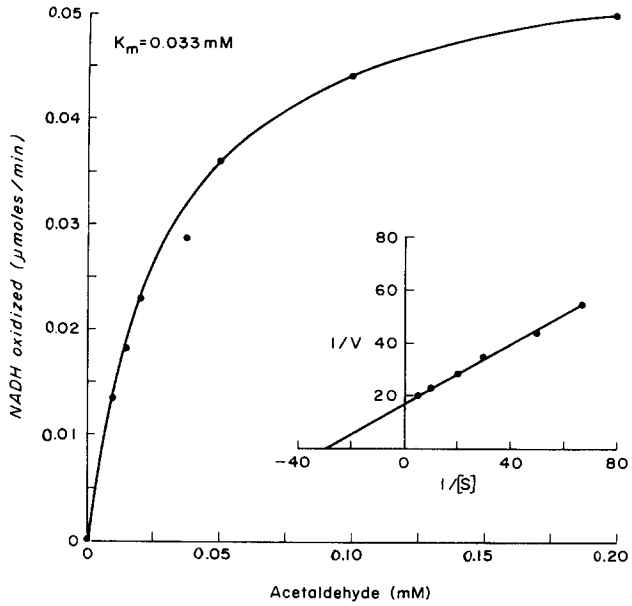


Fig. 6. Dependence of mitochondrial alcohol dehydrogenase on acetaldehyde. The reaction mixtures contained 20 mM K_2HPO_4–KH_2PO_4 (pH 7.15), 0.5 mM NADH, and acetaldehyde as indicated. See text for further details.

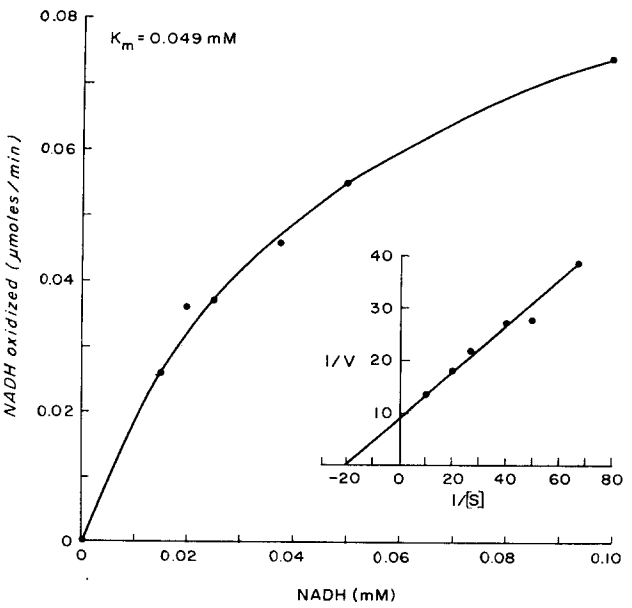


Fig. 7. Dependence of mitochondrial alcohol dehydrogenase on NADH. The reaction mixtures contained 20 mM K_2HPO_4–KH_2PO_4 (pH 7.15), 1 mM acetaldehyde, and NADH as indicated. See text for further details.

unstable. Inactivation could be minimized by concentrating the enzyme as soon as possible.

As shown in Table I, the mitochondrial alcohol dehydrogenase was purified nearly 65-fold to a specific activity of 110. Examination of this preparation by polyacrylamide gel electrophoresis (Fig. 3) showed that the major protein band coincided with the alcohol dehydrogenase activity, although several other minor protein bands were also present.

Kinetic studies

The apparent Michaelis constants of mitochondrial alcohol dehydrogenase were determined for ethanol, NAD^+, acetaldehyde, and NADH (Figs. 4–7, respectively). These studies were performed in 1.0-ml volumes using cuvettes of 1.00 cm light path, and each assay contained 0.025–0.031 unit of mitochondrial alcohol dehydrogenase.

TABLE II

APPARENT MICHAELIS CONSTANTS FOR VARIOUS YEAST ALCOHOL DEHYDROGENASES

Substrate	*Apparent K_m (mM)*		
	*This work**		NYGAARD AND THEORELL[19]**
	Mitochondrial enzyme (isolated)	*Crystalline enzyme (commercial)*	*Crystalline enzyme (commercial)*
NAD^+	0.41	0.29	0.20 (a)
NADH	0.049	0.13	0.038 (b)
Ethanol	6.6	5.5	18 (c)
Acetaldehyde	0.033	0.17	0.55 (d)

* See legends to Figs. 4–7 and text for details.

** Reaction conditions: K_2HPO_4–KH_2PO_4 (pH 7.15) (I = 0.1), 1 mM EDTA, and (a) 6.2 mM ethanol, (b) 5 mM acetaldehyde, (c) 0.80 mM NAD^+, (d) 0.15 mM NADH. Reactions were conducted at 23°.

The enzyme was derived from the 20–43% acetone fractionation and had a specific activity of 35. Crystalline commercial alcohol dehydrogenase from yeast was studied in the same manner, and a comparison of the apparent K_m's is presented in Table II together with the values obtained by NYGAARD AND THEORELL[19] for crystalline alcohol dehydrogenase from yeast.

In the present work, the mitochondrial and crystalline commercial alcohol dehydrogenases were found to have similar apparent K_m's in the direction of ethanol oxidation. Unexpectedly, the mitochondrial alcohol dehydrogenase had somewhat lower apparent K_m's for NADH and acetaldehyde. This result would seem to favor a reductive role for mitochondrial alcohol dehydrogenase. However, the apparent K_m values would not be expected to determine solely the direction of the alcohol dehydrogenase reaction in the mitochondrion. Other factors such as compartmentation of

substrates and the removal of products would also influence the direction in which the mitochondrial enzyme functions *in vivo*.

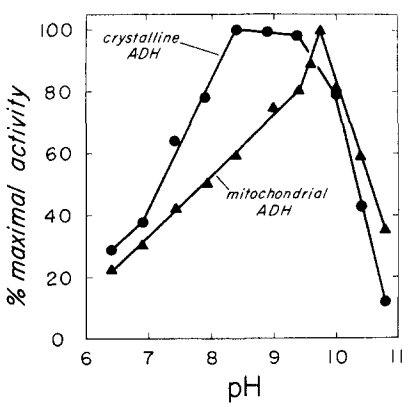
pH optimum

As shown in Fig. 8, mitochondrial alcohol dehydrogenase exhibited a sharp maximal activity at pH 9.8. Under the same conditions, the activity of crystalline commercial alcohol dehydrogenase from yeast had a broad optimum over the pH range of 8.4–9.5. The latter range is in general agreement with the pH optimum of 8.6 reported by WALLENFELS AND SUND[20] for crystalline alcohol dehydrogenase from yeast.

Intracellular distribution of alcohol dehydrogenase

Spheroplasts from 15 g of yeast were lysed[12], and the mixture was centrifuged at 1500 × *g* for 15 min to remove unbroken spheroplasts and large cellular fragments. The lysate was separated into supernatant and mitochondrial fractions by centrifugation at 12 000 × *g* for 15 min, and the mitochondria were washed twice with suspending medium. Electrophoretic analysis of this mitochondrial fraction (Fig. 9C) showed that it was free from contaminating cytoplasmic alcohol dehydrogenase. For electrophoresis, the mitochondria were disrupted with diethylstilbesterol and extracted as described above.

Polarographic assays showed that the supernatant and mitochondrial fractions accounted for 89% and 4.5%, respectively, of the alcohol dehydrogenase activity in the lysate. The value for the mitochondrial enzyme is uncorrected for recovery and may be low. JAGOW AND KLINGENBERG[21] recently reported that 6% of the alcohol



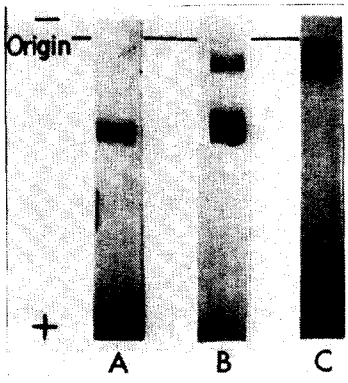


Fig. 8. Effect of pH on the activity of mitochondrial and crystalline commercial alcohol dehydrogenase (ADH). The reaction mixtures contained 0.2 M ethanol, 5 mM NAD^+, and a buffer system composed of 25 mM *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid, 25 mM *N,N*-bis(2-hydroxyethyl)glycine, and 25 mM glycine, previously adjusted to the appropriate pH with NaOH. The reactions were conducted in a final volume of 1.0 ml and were initiated by the addition of 0.04 unit of alcohol dehydrogenase. The mitochondrial enzyme was derived from the 20–43% acetone fractionation and had a specific activity of 35.

Fig. 9. Polyacrylamide gel electrophoresis of subcellular fractions. A, crystalline commercial alcohol dehydrogenase from yeast; B, supernatant fraction from lysed spheroplasts (see text); C, extract from diethylstilbesterol-treated mitochondria (see text). The gels were stained for alcohol dehydrogenase activity as described in MATERIALS AND METHODS.

dehydrogenase activity in *Saccharomyces carlsbergensis* is located in the mitochondria.

The alcohol dehydrogenase isozymes in the supernatant and mitochondrial fractions are compared electrophoretically in Fig. 9. The supernatant fraction (Fig. 9B) contained two types of alcohol dehydrogenase activity. The more rapidly migrating species was the predominant isozyme, and its electrophoretic mobility corresponded with that of the crystalline commercial enzyme (Fig. 9A). The electrophoretic mobility of the slowly migrating alcohol dehydrogenase in the supernatant fraction (Fig. 9B) corresponded with that of the mitochondrial enzyme (Fig. 9C). Some of the mitochondrial type of alcohol dehydrogenase was always found in the supernatant fraction from lysed spheroplasts despite the care taken during lysis. Separate experiments showed that small amounts ($< 1\%$) of the mitochondrial alcohol dehydrogenase could be extracted by the suspending medium.

DISCUSSION

The present work, together with the results of others[4–11], firmly establishes the presence of a unique mitochondrial alcohol dehydrogenase in yeast that is responsible in part for the coupled respiration of yeast mitochondria with ethanol as a substrate. As shown in the present study, this enzyme can be readily distinguished from the classical, cytoplasmic alcohol dehydrogenase by its electrophoretic mobility and pH optimum. Throughout the purification procedure, the mitochondrial alcohol dehydrogenase maintained its slow electrophoretic mobility on polyacrylamide gel. It is therefore unlikely that the electrophoretic difference between mitochondrial alcohol dehydrogenase and cytoplasmic alcohol dehydrogenase is an artifact of the purification procedure.

The strain of *Saccharomyces cerevisiae* used in the present study contained only two types of alcohol dehydrogenase when harvested in the early stationary phase of growth: mitochondrial alcohol dehydrogenase and cytoplasmic alcohol dehydrogenase. The electrophoretic pattern of these isozymes is similar to the pattern produced on starch gel by the A63 strain of LUTSDORF AND MEGNET[22] when those cells were harvested at a similar time in the growth cycle. HEICK *et al.*[23] have also indicated the presence of a mitochondrial alcohol dehydrogenase in yeast extracts.

Yeast has the ability to grow on ethanol, and the cell can apparently utilize this substance for the generation of ATP[4–11] and for the synthesis of carbon compounds of greater chain length. The formation of new carbon compounds from ethanol presumably proceeds by way of acetyl-CoA, and LYNEN *et al.*[24] have shown that acetyl-CoA is an intermediate of alcohol oxidation in yeast. Because the mitochondrial alcohol dehydrogenase described in the present paper is compartmented in the mitochondrion, the function of this enzyme might be limited to the generation of ATP and the production of acetyl-CoA within the mitochondrion. These processes could be important during mitochondrial biogenesis when there would be an enhanced need for phospholipid for membrane formation[25]. It is of interest that, similar to the behavior of the enzymes of the tricarboxylic acid cycle, mitochondrial alcohol dehydrogenase activity is repressed by glucose and appears again after glucose has been depleted from the medium[26]. This would indicate that the enzyme functions primarily under oxidative conditions.

In addition to the mitochondrial alcohol dehydrogenase described in this paper, other isozymes of alcohol dehydrogenase have been shown to appear when yeast cells are allowed to grow for long periods of time[22,27]. Although the significance of these additional cytoplasmic alcohol dehydrogenase isozymes is not clear, they appear to have an oxidative role[22,27], and may function in conjunction with the glyoxalate cycle[28–31]. It is tempting to speculate that the ability of yeast to produce carbohydrates and other compounds from ethanol in the extramitochondrial regions of the cell occurs after the development of functional mitochondria and corresponds with the appearance of the additional isozymes of alcohol dehydrogenase. This question could be clarified by a study which related the changing profile of alcohol dehydrogenase isozymes and other enzymatic activities to the physiological state of the cell.

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