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# Biochemical Basis of Mitochondrial Acetaldehyde Dismutation in *Saccharomyces cerevisiae*

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**As reported previously, *Saccharomyces cerevisiae* cells deficient in all four known genes coding for alcohol dehydrogenases (*ADH1* through *ADH4*) produce considerable amounts of ethanol during aerobic growth on glucose. It has been suggested that ethanol production in such *adh*$^0$ cells is a corollary of acetaldehyde dismutation in mitochondria. This could be substantiated further by showing that mitochondrial ethanol formation requires functional electron transport, while the proton gradient or oxidative phosphorylation does not interfere with reduction of acetaldehyde in isolated mitochondria. This acetaldehyde-reducing activity is different from classical alcohol dehydrogenases in that it is associated with the inner mitochondrial membrane and also is unable to carry out ethanol oxidation. The putative cofactor is NADH+H$^+$ generated by a soluble, matrix-located aldehyde dehydrogenase upon acetaldehyde oxidation to acetate. This enzyme has been purified from mitochondria of glucose-grown cells. It is clearly different from the known mitochondrial aldehyde dehydrogenase, which is absent in glucose-grown cells. Both acetaldehyde-reducing and acetaldehyde-oxidizing activities are also present in the mitochondrial fraction of fermentation-proficient (*ADH*$^+$) cells. Mitochondrial acetaldehyde dismutation may have some significance in the removal of surplus acetaldehyde and in the formation of acetate in mitochondria during aerobic glucose fermentation.**

The reduction of acetaldehyde to ethanol plays a key role in sugar metabolism of baker's yeast, *Saccharomyces cerevisiae*, by allowing regeneration of NAD$^+$ from glycolytic NADH+H$^+$. The in vivo significance of the reaction catalyzed by alcohol dehydrogenase (ADH) has been verified by mutants (*adh1*) deficient in ADH I, one of the four known ADH isozymes. Such mutant cells grow slowly on glucose, and growth is entirely dependent on mitochondrial functions (23). Despite this requirement for a functional respiratory chain in *adh1* mutants, glucose is largely converted to the major fermentation products, glycerol and ethanol, and to minor products such as acetaldehyde and acetate. We showed recently that the residual ethanol production in *adh1* mutant cells (approximately 30% of wild-type level) cannot be attributed to any of the known ADH isozymes (ADH II through ADH IV) by using mutant cells carrying null alleles at the four *ADH* loci (9). The existence of another cytoplasmic NAD$^+$-dependent ADH isozyme was also ruled out since *adh*$^0$ cells cannot grow in a medium containing ethanol as the sole source of carbon. Rather, acetaldehyde reduction in *adh*$^0$ cells occurs inside mitochondria. In fact, mitochondrial preparations from glucose-grown cells can quantitatively convert acetaldehyde to ethanol and acetate. This dismutation of acetaldehyde requires a functional respiratory chain, which is consistent with the finding that ethanol formation in *adh*$^0$ cells is blocked by antimycin A.

The dismutation of acetaldehyde by yeast cells has long been known from the studies of Neuberg and Hirsch (16). To our knowledge, there is no reliable explanation at the biochemical level for this type of yeast fermentative metabolism. In this report, we present data that elucidate the biochemistry of the dismutation reaction in yeast cells.

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## MATERIALS AND METHODS

**Yeast strains.** The following *S. cerevisiae* strains were used in this work: CD31-8C (*MATa* Δ*adh1 adh2* Δ*adh3 adh4*::*URA3*), MC65-2A (*MATα* Δ*adh1 adh2 adh3 adh4*::*URA3*), and MC66 (*MATα ADH1 adh2 adh3 adh4*::*URA3*).

**Media.** Yeast extract (1%)–Bacto Peptone (2%) supplemented with 1.8% glucose, with 2% ethanol, or with 3% glycerol served as standard media.

**Preparation of intact mitochondria.** The isolation of intact mitochondria as well as the estimation of purity and activity of the mitochondrial preparations have been described previously (9).

**Assay methods.** The activity of the mitochondrial acetaldehyde dehydrogenase (EC 1.2.1.5.) was determined by the method of Jacobson and Bernofsky (11) with some modifications (see Results). Enzyme assays were performed at 30°C by monitoring either NAD$^+$ reduction or NADH+H$^+$ oxidation spectrophotometrically at $\lambda$ = 340 nm. Enzyme activities are expressed in international units per milligram of protein (micromoles per minute per milligram of protein).

**Determination of metabolites.** Metabolites produced in acetaldehyde-incubated, isolated mitochondria were determined in the following manner. Acetaldehyde and acetate were determined by using test kits (Boehringer, Mannheim, Germany) in accordance with the protocols of Lundquist (14) and Bergmeyer and Möllering (1), respectively. Ethanol was determined by the method of Bernt and Gutmann (2).

**Isolation of a mitochondrial acetaldehyde dehydrogenase.** Mitochondria of glucose-grown cells (approximately 60 g, wet weight) were isolated as described before (9). The mitochondrial suspension was diluted with buffer (50 mM bicin, 50 mM KCl, 10 mM $KH_2PO_4$, 0.6 M mannitol) to obtain a protein concentration of 1 mg/ml. Subsequently, the mitochondria were sonicated (five 10-s periods; Branson Sonifier [Branson Sonic Power, Danbury, Conn.]). After centrifugation at 100,000 × *g* for 1 h, the supernatant (essentially the mitochondrial matrix fraction) was fraction-

ated further by affinity chromatography (Affi-Gel Blue; 100–200 mesh; 75 to 150 μm [Bio-Rad Laboratories, Richmond, Calif.]). Equilibration of the column material was done with a buffer containing 75 mM sodium pyrophosphate, 21 mM glycine, and 0.5 mM dithiothreitol, pH 8.6. Elution was carried out with a linear $NAD^+$ gradient from 0 to 8 mM.

**Chromatographic methods.** A Mono-Q column, HR5/5 (prepacked with Mono Beads; Pharmacia LKB, Uppsala, Sweden) was used in ion-exchange chromatography. Equilibration was performed with a buffer containing 10 mM sodium pyrophosphate and 2 mM glycine, pH 8.6. A linear gradient of KCl (0 to 1 M) was used for elution. Gel filtration was carried out with a TSK G4000 SW column (7.5 by 60 mm; LKB-Produkter AB, Bromma, Sweden). The column was equilibrated with a buffer containing 75 mM sodium pyrophosphate, 21 mM glycine, 100 mM KCl, and 1 mM dithiothreitol, pH 8.6.

Determination of molecular weights was based on calibration with a molecular weight standard, MW-G F-1000 (Sigma Chemical Co., St. Louis, Mo.).

**Other methods.** The concentration of proteins was determined as described by Lowry et al. (13). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out by the method of Laemmli (12). Protein bands were visualized by silver staining as described by Bluhm et al. (4).

**Chemicals.** Antimycin A, $NADH+H^+$, and $NADP^+$ were obtained from Boehringer, and $NAD^+$ was from Serva, Heidelberg, Germany. Decyl-Q was kindly provided by T. Friedrich, Institute of Biochemistry, University of Düsseldorf, Düsseldorf, Germany.

## RESULTS

**Acetaldehyde dismutation is dependent on mitochondrial electron transport.** We have shown previously that the mitochondrial fraction prepared from yeast cells deficient in all known ADH isozymes (*adh*$^0$) is capable of converting acetaldehyde to ethanol and acetate (9). We have reexamined this reaction by using various concentrations of exogenously added acetaldehyde. At concentrations of around 1 mM acetaldehyde, we observed a prototypic dismutation of acetaldehyde to ethanol and acetate within 30 min (Fig. 1). At higher acetaldehyde concentrations, formation of ethanol exceeded that of acetate significantly (9). We attribute this finding to relative inhibition of acetaldehyde oxidation versus reduction at higher acetaldehyde concentrations and/or to conversion of acetate to other metabolites.

Next, we examined in more detail the obvious dependence of this dismutation reaction on function of the respiratory chain. Addition of inhibitors of electron transport (antimycin A and potassium cyanide) resulted in a complete inhibition of ethanol formation (Fig. 2). This inhibition may reflect participation of the acetaldehyde reductase in the flow of electrons of the respiratory chain; alternatively, electron transport may indirectly be required if acetaldehyde reduction depends on the proton gradient across the inner mitochondrial membrane. The latter possibility could be ruled out by monitoring acetaldehyde reduction in the presence of the proton translocator carbonyl cyanide *m*-chlorophenylhydrazone, which, as shown in Fig. 2, even accelerated aldehyde reduction. Since removal of the proton gradient should result in an increased flow of electrons (10), this finding would be in agreement with the hypothesis that acetaldehyde reduction is directly dependent on electron transport.

When one compares the standard redox potentials $E_0'$ of

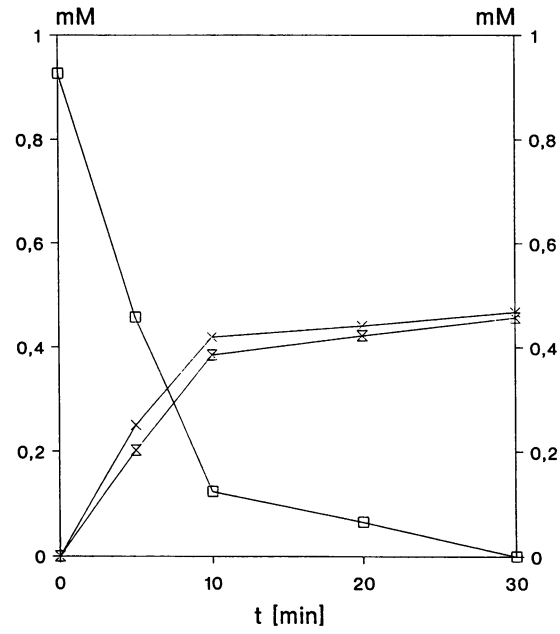


FIG. 1. Equimolar production of ethanol and acetate in mitochondria after incubation with 1 mM acetaldehyde. Mitochondria were prepared from glucose-grown *adh*$^0$ cells (strain CD31-8C). □, acetaldehyde; ×, ethanol; ×, acetate.

$NAD^+/NADH+H^+$ (−320 mV), ubiquinone/ubiquinol (+10 mV), and acetaldehyde/ethanol (−197 mV), it becomes obvious that acetaldehyde reductase would receive electrons from $NADH+H^+$ rather than from ubiquinol. Thus, the effect of inhibition of complex III (by antimycin A) or

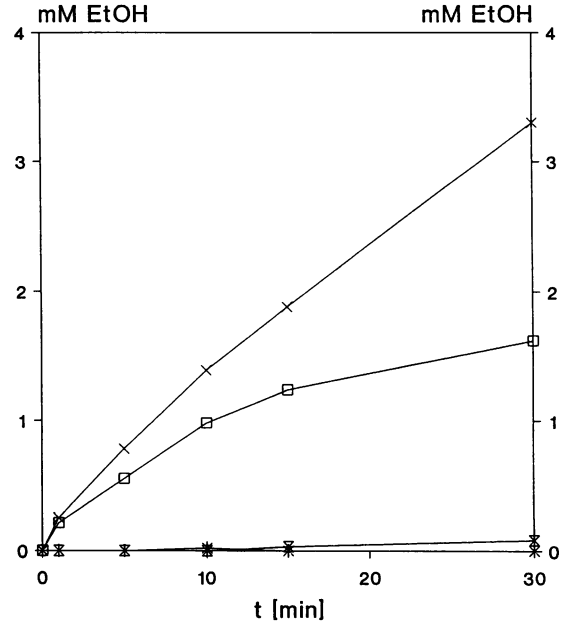


FIG. 2. Mitochondrial formation of ethanol from acetaldehyde in the presence of various inhibitors of the respiratory chain. The initial acetaldehyde concentration was 10 mM. □, control without inhibitor; *, 10 μM antimycin A; ×, 1 mM potassium cyanide; ×, 10 μM carbonyl cyanide *m*-chlorophenylhydrazone.

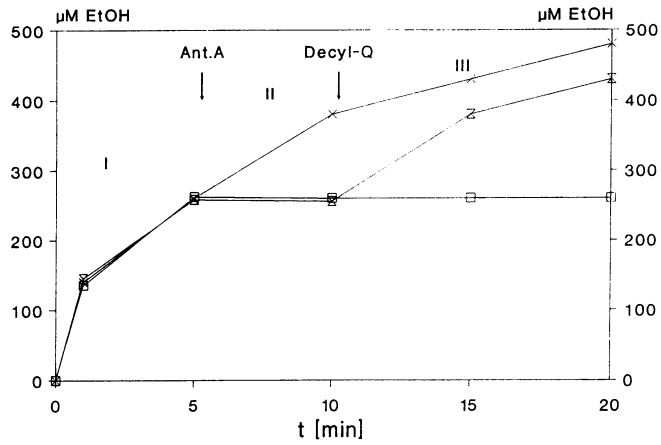


FIG. 3. Correlation of acetaldehyde reductase and NADH:ubiquinone-oxidoreductase of the respiratory chain. Mitochondria from glucose-grown *adh*$^0$ cells (CD31-8C) were incubated with 1 mM acetaldehyde. In two aliquots (× and □), 10 µM antimycin A was added after 5 min. After 10 min, 10 µM decyl-Q was added (×). ×, control without antimycin A and decyl-Q; □, addition of 10 µM antimycin A after 5 min; ×, addition of 10 µM antimycin A after 5 min and 10 µM decyl-Q after 10 min. For details, see text.

complex IV (by cyanide) on acetaldehyde reduction might be explained by a more indirect action, e.g., by inactivation of a complex I analog (NADH:ubiquinone oxidoreductase). Since *S. cerevisiae* mitochondria lack a classical piericidin- or rotenone-sensitive NADH:ubiquinone oxidoreductase (15, 17), the requirement of a functional complex I for the acetaldehyde reductase activity cannot be verified directly. An additional hint of an indirect requirement for such a complex I function was obtained after addition of an artificial ubiquinone analog, decyl-Q (24), to antimycin A-treated mitochondria. Acetaldehyde reduction was indeed restored upon addition of decyl-Q in large excess (Fig. 3). This finding can most easily be explained if one assumes that complex I has been reactivated by the presence of an electron acceptor. However, whether this is a direct corollary of the restoration of electron transport or rather is indirectly triggered by conformational changes of complex I and/or the acetaldehyde reductase cannot be determined.

Mitochondrial dismutation of acetaldehyde has been discovered in yeast cells deficient in ADHs (9). One might argue that this novel pathway of acetaldehyde metabolism is restricted to cells deficient in alcoholic fermentation. It is difficult to disprove this by analyzing the metabolism of ADH$^+$ cells. However, by isolation of mitochondria from an *ADH1* transformant of strain MC65-2A (*adh1 Δadh2 adh3 adh4::URA3*), we could demonstrate that acetaldehyde dismutation occurs with an activity similar to that of mitochondria from *adh*$^0$ cells (results not shown). Obviously, the expression of the dismutation activity does not depend on the absence of ADH I activity in *adh*$^0$ mutant cells.

**Acetaldehyde reductase and acetaldehyde oxidase are distinct activities.** The experiments described so far do not give clear evidence of the biochemical nature of the dismutation reaction. Thus, we have tried to find reliable assay systems for both activities. This would also allow examination of the intramitochondrial localization of acetaldehyde reductase and acetaldehyde oxidase. As reported previously (9), the acetaldehyde reductase activity is apparently different from classical ADHs in that ethanol oxidation coupled to NAD$^+$ reduction cannot be detected. Consistent with this is the finding that mitochondrial preparations from *adh*$^0$ cells are unable to oxidize ethanol (data not shown). We tried to monitor acetaldehyde reduction with NADH+H$^+$ as cofactor in Triton X-100-treated mitochondrial preparations. Although the relatively high NADH oxidase activity of yeast mitochondria (503 ± 27 mU/mg) turned out to be a serious problem in monitoring a NADH-specific acetaldehyde reduction, we could reproducibly find an acetaldehyde-specific NADH+H$^+$ oxidation of 183 ± 12 mU/mg, i.e., approximately one-third of the unspecific NADH oxidase activity. Both the unspecific NADH oxidase and the acetaldehyde-specific NADH oxidation were sensitive to antimycin A.

Acetaldehyde oxidation was monitored in a similar manner by using 0.6 mM NAD$^+$ as cofactor. To distinguish this activity from the classical aldehyde dehydrogenase (ALDH) isolated first in 1951 by Black (3), the assay was done in the presence of 50 mM $Mg^{2+}$ and in the absence of $K^+$. Under these conditions the latter enzyme is completely inactive (5, 11, 19; unpublished data). The NAD$^+$-specific aldehyde oxidation activity was 142 ± 11 mU/mg in mitochondrial preparations from glucose-grown cells. Thus, both reactions involved in the dismutation of acetaldehyde apparently require NAD$^+$ and NADH+H$^+$ as cofactors.

In a subsequent experiment, we examined whether acetaldehyde reductase and oxidase can be separated physically by fractionation of disintegrated mitochondria. For this purpose, a mitochondrial suspension was sonicated and fractionated by centrifugation. A 100,000 × *g* pellet of mitochondrial membranes contained the acetaldehyde reductase activity (102 ± 8 mU/mg, determined by monitoring NADH+H$^+$ oxidation), while a NAD$^+$-dependent acetaldehyde oxidase activity (115 ± 8 mU/mg) was obtained in the supernatant corresponding to the mitochondrial matrix fraction. Acetaldehyde reductase activity was <1 mU/mg in the matrix fraction, and acetaldehyde oxidase activity was <1 mU/mg in the membrane fraction. These findings clearly suggested that the two activities of the dismutation reaction can be separated. The dependence of the dismutation reaction on the function of the respiratory chain (see above) is consistent with the membrane localization of one of the dismutase components (acetaldehyde reductase). We have subsequently tried to purify both activities on a preparative scale. As expected, this turned out to be quite difficult for the membrane-located acetaldehyde reductase. Therefore, we have focussed first on characterization of the matrix-located acetaldehyde dehydrogenase involved in the dismutation reaction.

**Acetaldehyde oxidase is a novel, NAD$^+$-dependent acetaldehyde dehydrogenase.** Since acetaldehyde oxidase obviously is a soluble mitochondrial matrix enzyme, we examined whether it is identical to the known mitochondrial acetaldehyde dehydrogenase (EC 1.2.1.5.) first described by Black (3) and characterized by Jakoby and coworkers (6–8, 20, 21). Anticipating that the acetaldehyde dehydrogenase involved in the dismutation of acetaldehyde is distinct from the classical ALDH, we designate the latter enzyme ALDH I and the novel enzyme ALDH II. The first suggestion that these activities are distinct was obtained from the finding that ALDH I is strongly glucose repressed while ALDH II is present in glucose-grown cells (9).

To prove the existence of another mitochondrial acetaldehyde dehydrogenase, we have purified the protein on a preparative scale from mitochondria of glucose-grown cells. The purification procedure is summarized in Table 1. Using affinity chromatography (Affi-Gel Blue) and anion-exchange

TABLE 1. Purification protocol for a mitochondrial acetaldehyde dehydrogenase from glucose-grown *adh*$^0$ cells

| Step | Total protein (mg) | Total activity (U) | Yield (%) | Sp act (U/mg) | Purification factor |
|---|---|---|---|---|---|
| Crude extract | 903 | 85.3 | 100 | 0.095 | 1 |
| Mitochondrial suspension | 11.2 | 16.0 | 18.8 | 1.43 | 15 |
| Affinity chromatography | 0.65 | 5.8 | 6.8 | 8.92 | 94 |
| Ion-exchange chromatography | 0.22 | 4.3 | 5.0 | 19.60 | 206 |

chromatography, we obtained a preparation that consisted essentially of a single protein as determined by gel filtration of the native protein and by SDS-PAGE (Fig. 4, lane 1). The native enzyme has an apparent $M_r$ of 188,000, similar to that of the known ALDH I (208,000). However, the ALDH II subunit showed an unexpectedly low $M_r$ of 31,000. This protein is clearly distinct from commercial ALDH (Fig. 4, lane 5), which has been prepared from yeast mitochondria and therefore is very likely identical to ALDH I (according to references of the producer, Boehringer). In agreement with the previous finding (9, 11) that ALDH I is absent from glucose-grown cells, we could not detect an ALDH protein of the expected size (56 kDa, according to reference 7) in this preparation.

To verify that glucose-derepressed yeast mitochondria of the strain we used did contain ALDH I, we purified total ALDH activity from mitochondria of glycerol-grown cells by

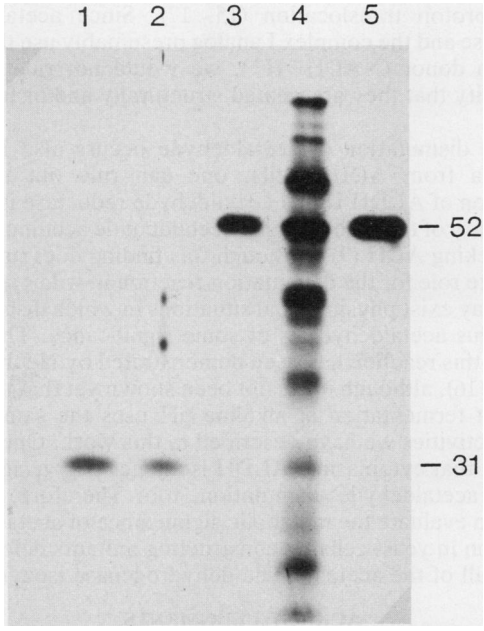


FIG. 4. SDS-PAGE (10%) of purified ALDH I and ALDH II. Proteins were visualized by silver staining. Positions of ALDH I and ALDH II are marked. Lane 1, ALDH II, purified from glucose-grown cells; lane 2, ALDH II, purified from glycerol-grown cells; lane 3, ALDH I, purified from glycerol-grown cells; lane 4, suspension of mitochondria, obtained from glycerol-grown cells (approximately 10 μg of protein); lane 5, ALDH I (500 ng), commercial product from Boehringer-Mannheim.

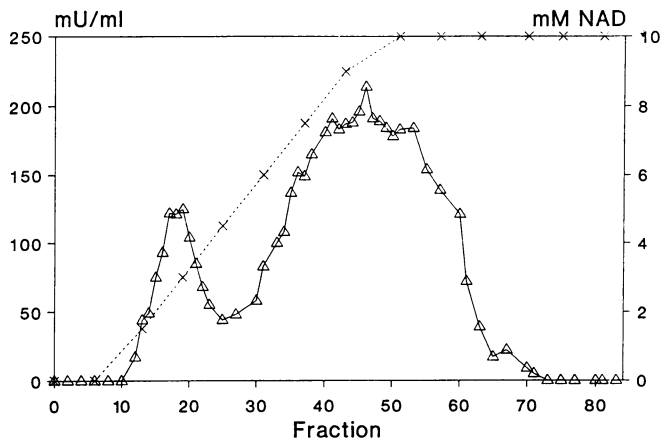


FIG. 5. Separation of ALDH I and ALDH II from glycerol-grown cells by affinity chromatography (Affi-Gel Blue). Acetaldehyde dehydrogenase activity was monitored in fractions eluted with a linear $NAD^+$ gradient (– – –). The major peak contains ALDH I, and the minor peak contains ALDH II (cf. Fig. 4).

using the scheme applied for ALDH II. By affinity chromatography (Affi-Gel Blue), total mitochondrial ALDH activity could clearly be separated into two fractions (Fig. 5). Subsequent gel filtration and SDS-PAGE showed that the major ALDH fraction consisted of a 52-kDa protein identical in size to commercial yeast ALDH (Fig. 4, lanes 3 and 5). Considering its size and its presence in glucose-derepressed mitochondria, we conclude that this major acetaldehyde dehydrogenase is identical to the enzyme described previously (7). As expected, the minor ALDH fraction consisted of ALDH II (Fig. 4, lane 2). This finding clearly suggests that yeast mitochondria from glucose-derepressed cells contain two distinct ALDH isozymes. ALDH I formation is glucose repressible, while ALDH II appears to be constitutively present. Because there is no mitochondrial protein which could serve as a standard for comparison of glucose-repressed and -derepressed mitochondria, it was difficult to compare the expression of ALDH II under each growth condition. We concluded from the elution profile of ALDH activity (Fig. 5) that ALDH I activity is at least 10-fold higher than ALDH II activity in mitochondria from glucose-derepressed cells. The pattern of total mitochondrial proteins (Fig. 4, lane 4) revealed that the purified ALDH I protein comigrates with a major mitochondrial protein. This band is absent in mitochondria from glucose-grown cells (data not shown). This finding coincides with the absence of ALDH I activity in such cells. Furthermore, a calculation of the amount of ALDH I protein, based on specific activity of the purified enzyme, revealed that total matrix protein contains >1% ALDH I. Thus, this isozyme is a major matrix protein.

We have compared the enzymatic parameters of purified ALDH I and ALDH II. The specific activity of the purified ALDH II is similar to that of ALDH I (approximately 20 U/mg of protein). ALDH II has higher $K_m$ values for both the substrate acetaldehyde and the cofactors (Table 2). ALDH II can also use $NADP^+$ instead of $NAD^+$ as a cofactor with equal efficiency. The initial failure to find a significant $NADP^+$-dependent acetaldehyde dehydrogenase in mitochondria from glucose-repressed cells (9) was due to the relatively low activity of ALDH II compared with ALDH I and to assay conditions used previously. Table 3

TABLE 2. Comparison of $K_m$ values of purified ALDH I and ALDH II[a]

| Substrate or cofactor | $K_m$ (μM) | |
|---|---|---|
| | ALDH I | ALDH II |
| Acetaldehyde | 9 | 40 |
| $NAD^+$ | 20 | 246 |
| $NADP^+$ | 50 | 202 |

[a] Determination of the $K_m$ for acetaldehyde was performed in the presence of 0.6 mM $NAD^+$, and that for $NAD^+$ and $NADP^+$ was done in the presence of 0.1 mM acetaldehyde.

shows the substrate specificities of ALDH I versus ALDH II. Most remarkably, ALDH II cannot oxidize formaldehyde. The enzyme is activated by potassium ions only threefold (ALDH I, eightfold). Other monovalent cations have no activating effect on ALDH II. Like ALDH I, ALDH II is inactivated by higher concentrations of acetaldehyde (data not shown). Both the 10-fold elevation of ALDH I versus ALDH II in catalytic activity and the 10-fold-lower $K_m$ for $NAD^+$ of ALDH I versus ALDH II would suggest that ALDH I is the major isozyme responsible for acetaldehyde oxidation in glucose-derepressed cells.

## DISCUSSION

We have analyzed some aspects of the enzymatic dismutation of acetaldehyde in *S. cerevisiae*. This phenomenon was discovered by Neuberg and Hirsch in 1919 in aerobic yeast cultures grown at alkaline pH (16). We have recently shown that such a dismutation of acetaldehyde is effective in the sugar metabolism of cells lacking all of the four known ADH isozymes (*adh*$^0$ cells [9]). Surprisingly, this reaction is apparently localized in the mitochondrion and is functionally associated with the respiratory chain. Enzymatic dismutation of acetaldehyde is remarkable, since chemically the disproportion of acetaldehyde, according to a Cannizzaro reaction, is not possible (22). Rather, a two-step mechanism is required for the biochemical dismutation of acetaldehyde. In *S. cerevisiae* mitochondria, this is apparently executed by the combined action of two distinct enzymes, acetaldehyde reductase and ALDH. Both activities use $NAD^+$ and $NADH+H^+$ as cofactors, although this feature has not yet been proven directly with a purified preparation of the acetaldehyde reductase. The latter enzyme is clearly different from the classical yeast ADH in several respects. First, it is not able to oxidize ethanol either in vitro or in vivo. Second, the enzyme seems to be tightly associated with the mitochondrial membrane and coupled functionally in some way to the respiratory chain. Obviously, acetaldehyde reduction is directly dependent on electron transport, while the proton gradient across the membrane is not required functionally. It is possible that the apparent irreversibility of the reductase reaction is a direct corollary of its tight association with the mitochondrial electron transport.

TABLE 3. Substrate specificities of ALDH I and ALDH II[a]

| Substrate | Specificity | |
|---|---|---|
| | ALDH I | ALDH II |
| Formaldehyde | 0.45 | <0.01 |
| Acetaldehyde | 1.00 | 1.00 |
| Propionaldehyde | 0.44 | 0.95 |
| Glutaraldehyde | 0.50 | 0.23 |
| Benzaldehyde | 0.09 | <0.01 |

[a] Concentrations of the aldehydes were 0.1 mM except for formaldehyde (1 mM). The concentration of $NAD^+$ was 0.6 mM in all cases. Activities were related to the specific activity with acetaldehyde as substrate (1.0).

The ALDH (ALDH II) participating in the mitochondrial dismutation reaction is also clearly distinct from the known yeast ALDH isozymes. Apart from the difference in regulation by glucose repression, ALDH II appears to be an unusually small (31-kDa) molecule compared with the known ALDHs from yeasts and other eukaryotic organisms (8, 18). Cloning of the genes coding for ALDH isoforms would allow more precise determination of the structural relationship between these isozymes. In this way, the construction of mutants deficient in ALDH II function would also be feasible. Such mutants would be quite useful in assessing the physiological role of ALDH II in wild-type yeast cells.

The dependence of the dismutation reaction on a functioning electron transport remains a puzzling feature. One might argue that the transfer of electrons from $NADH+H^+$ to acetaldehyde does not occur directly in a cyclic process but requires ATP from oxidative phosphorylation or the proton gradient across the mitochondrial membrane. This was disproven by demonstrating accelerated dismutation in the presence of a proton translocator (carbonyl cyanide *m*-chlorophenylhydrazone) which causes the disappearance of the proton gradient and, consequently, the cessation of ATP synthesis (10). Thus, it seems more plausible to assume that acetaldehyde reductase requires the respiratory chain complex(es) in an active conformation. This would imply that the reductase is itself closely associated with the respiratory chain. Unfortunately, only limited information on the NADH dehydrogenase complex in *S. cerevisiae* is available. This yeast complex I analog is unique in that it does not cause proton translocation (15, 17). Since acetaldehyde reductase and the complex I analog presumably use the same electron donor ($NADH+H^+$), we would not rule out the possibility that they are related structurally and/or functionally.

Since dismutation of acetaldehyde occurs also in mitochondria from $ADH^+$ cells, one can rule out that the formation of ALDH II and acetaldehyde reductase is simply a corollary of the high levels of acetaldehyde accumulating in cells lacking ADH (9). Although this finding does not prove a definite role for the dismutation reaction in wild-type cells, there may exist physiological situations in which dismutation of surplus acetaldehyde is of some significance. The existence of this reaction has been demonstrated by Neuberg and Hirsch (16), although it has not been shown yet that this type of yeast fermentation at alkaline pH uses the same enzymatic activities we have described in this work. One cannot rule out that cytoplasmic ALDH isozymes play a significant role in acetaldehyde dismutation, too. Therefore, we are trying to evaluate the metabolic significance of acetaldehyde oxidation in yeast cells by constructing mutants defective in one or all of the acetaldehyde dehydrogenase isozymes.

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### REFERENCES

1. **Bergmeyer, H. U., and H. Möllering.** 1974. Acetat: Bestimmung mit vorgeschalteter Indikatorreaktion, p. 1566–1574. *In* H. U.

Bergmeyer (ed.), Methoden der enzymatischen Analyse—1974. Verlag Chemie, Weinheim, Germany.
2. **Bernt, E., and I. Gutmann.** 1974. Äthanol: Bestimmung mit Alkoholdehydrogenase und NAD, p. 1545–1551. *In* H. U. Bergmeyer (ed.), Methoden der enzymatischen Analyse—1974. Verlag Chemie, Weinheim, Germany.
3. **Black, S.** 1951. Yeast aldehyde dehydrogenase. Arch. Biochem. Biophys. **34**:86–97.
4. **Bluhm, H., M. Beier, and M. J. Gross.** 1987. Improved silver staining of plant proteins, RNA and DNA in polyacrylamide gels. Electrophoresis **8**:93–99.
5. **Bostian, K. A., and G. F. Betts.** 1978. Rapid purification and properties of potassium-activated aldehyde dehydrogenase from *Saccharomyces cerevisiae*. Biochem. J. **173**:773–786.
6. **Bradbury, S. L., and W. B. Jakoby.** 1971. Ordered binding of substrates to yeast aldehyde dehydrogenase. J. Biol. Chem. **246**:1834–1840.
7. **Clark, J. F., and W. B. Jakoby.** 1970. Yeast aldehyde dehydrogenase. III. Preparation of three homogeneous species. J. Biol. Chem. **245**:6065–6071.
8. **Clark, J. F., and W. B. Jakoby.** 1970. Yeast aldehyde dehydrogenase. IV. Dissociation and reassociation of native and hybrid forms. J. Biol. Chem. **245**:6072–6077.
9. **Drewke, C., J. Thielen, and M. Ciriacy.** 1990. Ethanol formation in *adh*$^0$ mutants reveals the existence of a novel acetaldehyde-reducing activity in *Saccharomyces cerevisiae*. J. Bacteriol. **172**:3909–3917.
10. **Heytler, P. G.** 1979. Uncouplers of oxidative phosphorylation. Methods Enzymol. **55**:462–472.
11. **Jacobson, M. K., and C. Bernofsky.** 1974. Mitochondrial acetaldehyde dehydrogenase from *Saccharomyces cerevisiae*. Biochim. Biophys. Acta **350**:277–291.
12. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
13. **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.
14. **Lundquist, F.** 1974. Acetaldehyd: Bestimmung mit Aldehyd-Dehydrogenase, p. 1555–1559. *In* H. U. Bergmeyer (ed.), Methoden der enzymatischen Analyse—1974. Verlag Chemie, Weinheim, Germany.
15. **Mackler, B., and B. Haynes.** 1973. Studies of oxidative phosphorylation in *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis*. Biochim. Biophys. Acta **292**:88–91.
16. **Neuberg, C., and J. Hirsch.** 1919. Die dritte Vergärungsform des Zuckers. Biochem. Z. **92**:304–322.
17. **Ohnishi, T.** 1973. Mechanism of electron transport and energy conservation in the site I region of the respiratory chain. Biochim. Biophys. Acta **301**:105–128.
18. **Pickett, M., D. I. Gwynne, F. P. Buxton, R. Elliott, R. Davies, R. A. Lockington, C. Scazzocchio, and H. M. Sealy-Lewis.** 1987. Cloning and characterization of the *aldA* gene of *Aspergillus nidulans*. Gene **51**:217–226.
19. **Sorger, G. J., and H. J. Evans.** 1966. Effects of univalent cations on the properties of yeast $NAD^+$ acetaldehyde dehydrogenase. Biochim. Biophys. Acta **118**:1–8.
20. **Steinman, C. R., and W. B. Jakoby.** 1967. Yeast aldehyde dehydrogenase. I. Purification and crystallization. J. Biol. Chem. **242**:5019–5023.
21. **Steinman, C. R., and W. B. Jakoby.** 1968. Yeast aldehyde dehydrogenase. II. Properties of the homogeneous enzyme preparations. J. Biol. Chem. **243**:730–734.
22. **Streitwieser, A., Jr., and C. H. Heathcock (ed.).** 1986. Organische Chemie—1986, p. 474–476. Verlag Chemie, Weinheim, Germany.
23. **Wills, C., and J. Phelps.** 1975. A technique for the isolation of yeast alcohol dehydrogenase mutants with altered substrate specificity. Arch. Biochem. Biophys. **167**:627–637.
24. **Zweck, A., G. Bechmann, and H. Weiss.** 1989. The pathway of the quinol/quinone transhydrogenation reaction in ubichinol: cytochrome-c reductase of *Neurospora* mitochondria. Eur. J. Biochem. **183**:199–203.