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**Identification of a mitochondrial alcohol dehydrogenase in *Schizosaccharomyces pombe* – new insights into energy metabolism**

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**Running title:** *S. pombe* mitochondrial alcohol dehydrogenase

**Abbreviations:** ADH, alcohol dehydrogenase; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; Q(H<sub>2</sub>), (reduced) ubiquinone

**Keywords:** *Schizosaccharomyces pombe* mitochondria, alcohol dehydrogenase isozymes, respiration, modular kinetics, NADH dehydrogenase, ubiquinone pool

## ABSTRACT

In this paper we show that mitochondria isolated from *Schizosaccharomyces pombe* exhibit antimycin A-sensitive oxygen uptake activity that is exclusively dependent on ethanol and is inhibited by trifluoroethanol, a potent inhibitor of alcohol dehydrogenase (ADH). Ethanol-dependent respiratory activity has, to our knowledge, not been reported in *S. pombe* mitochondria to date, and is indeed surprising as it has previously been concluded that only one ADH gene, encoding a cytosolic enzyme, occurs in this yeast. Spectrophotometric enzyme assays reveal that ADH activity in isolated mitochondria is increased ~ 16-fold by Triton X-100, which demonstrates the enzyme is located in the matrix. Using genetic knock-outs, we show conclusively that the novel mitochondrial ADH is encoded by *adh4* and, as such, is unrelated to ADH isozymes found in mitochondria of other yeasts. By performing a modular-kinetic analysis of mitochondrial electron transfer, we furthermore show how ethanol-dependent respiratory activity (which involves oxidation of matrix-located NADH) compares to that observed when succinate or externally added NADH are used as substrates. This analysis reveals distinct kinetic differences between substrates which fully explain the lack of respiratory control generally observed during ethanol oxidation in yeast mitochondria.

## INTRODUCTION

Over the last two decades, *S. pombe* has proved an invaluable model organism to study biological processes that are fundamental to higher eukaryotes. This has greatly improved the understanding of, most notably, mechanisms by which the cell cycle is controlled and damaged DNA is repaired (reviewed in ref. [1]). In our laboratory, *S. pombe* has been used for a different purpose, namely to functionally express the plant alternative oxidase [2]. This cyanide-insensitive mitochondrial respiratory enzyme is wasteful in terms of energy conservation, which is why considerable attention has been paid to the clarification of its physiological function [3]. Functional expression of the protein in *S. pombe* has provided valuable information on structure-function relationships of the oxidase [4-6] and, moreover, has resulted in this yeast becoming a useful model of plant mitochondrial electron transfer [7].

Although *S. pombe* is widely used as a model system, relatively little fundamental information is available on the mitochondrial energy metabolism of this yeast [8, 9]. From the few studies published to date it is clear that the respiratory chain of *S. pombe* contains at least two substrate dehydrogenases (oxidising succinate and external NADH, respectively) that both reduce ubiquinone, which is subsequently oxidised by the cytochrome pathway [8, 9].

In addition to NADH and succinate, mitochondria from other yeasts (*Saccharomyces carlsbergensis* and *Saccharomyces cerevisiae* for example) are able to use ethanol as a respiratory substrate [10-13]. Ethanol-dependent respiratory activity is generally attributed to the presence of mitochondrial alcohol dehydrogenase (ADH) isozymes [8-13]. In *S. pombe* only one ADH gene has been reported that relates to the mitochondrial ADH genes identified in other yeasts [14]. As this gene is believed to encode a cytosolic enzyme (e.g. [15]) it has been widely assumed that *S. pombe* does not contain a mitochondrial ADH isozyme and, as a result, differs from other yeast in how it metabolises ethanol [16].

In this paper we show unequivocally that *S. pombe* mitochondria exhibit ADH activity. Using genetic knock-out strains we confirm that the responsible enzyme is ADH4, the first protein of its type to be shown to play a role in mitochondrial function.

## EXPERIMENTAL

### Sample preparation

*S. pombe* cells were batch-cultured in glucose minimal medium [17] supplemented with 0.4 mM adenine, 1.1 mM leucine and 0.7 mM uracil. Mitochondria were isolated as described previously [6] from the following two strains: Sp.011 ( $h^-$ , *ade6-704*, *leu1-32*, *ura4-D18*), and JY743 carrying gene disruptions (see [18] for details) at either *adh1* ( $h^-$ , *leu1-32*, *ura4-D18*, *adh1Δ::ura4<sup>+</sup>*) or *adh4* ( $h^-$ , *leu1-32*, *ura4-D18*, *adh4Δ::leu1<sup>+</sup>*). Mitochondrial protein amounts were estimated using bicinchoninic acid [19] with bovine serum albumin as a standard.

### Respiratory analysis

Mitochondrial oxygen consumption was measured at room temperature using a Clark-type electrode (Rank Brothers, Cambridge, U.K.). Mitochondria (0.4-1.2 mg) were suspended in 2.4 mL reaction medium containing 0.65 M mannitol, 20 mM MOPS (pH adjusted to 6.8 with NaOH), 1 mM MgCl<sub>2</sub>, 5 mM Na<sub>2</sub>HPO<sub>4</sub> and 10 mM NaCl (medium A). For modular-kinetic experiments, the reduction level of the Q-pool was measured simultaneously with respiratory activity using a vessel adapted to accommodate glassy carbon, platinum and Ag/AgCl reference electrodes as described in [20]. For these experiments, 1 μM Q<sub>1</sub> was included in the reaction medium. Where necessary, Q-reduction measurements were corrected for NADH- or ethanol-induced artefacts by subtracting electrode responses observed in the absence of Q<sub>1</sub>.

### Alcohol dehydrogenase activity

ADH activity was followed spectrophotometrically as the production of NADH ( $\epsilon_{\text{NADH}, 340 \text{ nm}} = 6.22 \text{ mM}^{-1} \text{ cm}^{-1}$ ) using a lambda 16-Perkin Elmer spectrophotometer. Assays were performed with 17-24 μg mitochondrial protein at 24-25°C in medium A (pH 6.8) that included 1.7 μM antimycin A and 2 mM NAD<sup>+</sup>. Reactions were initiated by adding 100 mM ethanol.

## RESULTS

### Identification of a mitochondrial ADH

#### *S. pombe* exhibits mitochondrial ADH activity

Mitochondria isolated from *S. pombe* contain an active external NADH dehydrogenase [8, 9] that rapidly oxidises exogenous NADH (Table 1A). This respiratory activity ( $\sim 67 \text{ nmol min}^{-1} \text{ mg}^{-1}$ ) is stimulated 5-fold by the addition of the protonophore CCCP, indicating that electron transfer is tightly coupled to proton translocation and, hence, that the mitochondrial inner-membranes are relatively intact. Interestingly, when ethanol is added to *S. pombe* mitochondria, electron transfer rates are achieved that are comparable to those observed with NADH (Table 1A). This ethanol-dependent oxygen uptake activity is marginally increased by the addition of  $\text{NAD}^+$  (Table 1A) and appears to depend on the integrity of the mitochondrial inner-membrane, since it is significantly reduced in osmotically shocked mitochondria (data not shown).  $\text{NAD}^+$ -stimulated ethanol-dependent respiration rates, however, are similar in intact and disrupted mitochondria, implying that ethanol-dependent respiratory activity requires matrix  $\text{NAD}^+$ .  $\text{NAD}^+$ -stimulated, ethanol-dependent oxygen uptake is stimulated  $\sim 1.4$  fold by CCCP (Table 1A).

Ethanol-dependent respiratory activity is sensitive to antimycin A ( $\sim 95\%$  inhibition) and 2,2,2-trifluoroethanol ( $\sim 74\%$  inhibition), specific inhibitors of mitochondrial complex III [21] and ADH [22], respectively (Table 1A). These observations strongly suggest that ethanol-dependent oxygen consumption in *S. pombe* mitochondria, similar to other yeasts (e.g. refs [10-13]), is accounted for by a matrix-located ADH that oxidises ethanol resulting in reduction of endogenous  $\text{NAD}^+$ , which is subsequently re-oxidised by the respiratory chain. In *S. cerevisiae*, the acetaldehyde produced during this reaction can also be oxidised, which results in additional formation of NADH [23]. This appears not to occur in *S. pombe* mitochondria as acetaldehyde inhibits ethanol-dependent oxygen consumption ( $\sim 98\%$  at 8 mM), and does not sustain a respiratory activity when added separately (data not shown).

The presence of a matrix-located ADH in *S. pombe* is confirmed unambiguously by the spectrophotometric data presented in Table 1B, which show that oxidation of ethanol can be increased approximately 16 times by the addition of the detergent Triton X-100. This result clearly demonstrates that the major part of ADH activity in our mitochondrial sample is latent and only becomes apparent when membranes are disrupted by detergent. Such latency has been

observed in other yeasts (e.g. [10, 24]) and implies that nearly all NADH formed in the assay lacking Triton X-100 is shielded from detection by a membrane. This membrane is undoubtedly mitochondrial, since ADH activity is linked to antimycin A-sensitive oxygen consumption (Table 1A). Given that only the mitochondrial inner- and not the outer-membrane is impermeable to NADH [13], it can be concluded that the observed ethanol-dependent respiratory activity in *S. pombe* mitochondria involves a matrix-located ADH.

### **The molecular nature of mitochondrial ADH in *S. pombe***

The presence of a mitochondrial ADH in *S. pombe* is somewhat surprising given that one ADH gene only (*adh1*), encoding a cytoplasmic protein, has been reported to date [14]. Using *adh1* as a probe, Southern analysis did not reveal any homologous sequences in *S. pombe* [14], which is in contrast to other yeasts where multiple *adh1*-like genes are usually present [15]. Recently, however, a protein has been identified in *S. pombe* [18] that is unrelated to ADH1 but similar to ADH isozymes from the fermentative bacterium *Zymomonas mobilis* (ADH2) and *S. cerevisiae* (ADH4) [25, 26]. Using algorithms described in [27-29] we predict that, unlike ADH1, this novel ADH protein (now termed ADH4) is targeted to mitochondria, and may therefore account for the mitochondrial ADH activity reported in this paper. To test this notion, we measured respiratory activity in mitochondria isolated from *S. pombe* cell lines in which either ADH1 or ADH4 was ablated ( $\Delta adh1$  and  $\Delta adh4$ , respectively).

From the data presented in Table 2 it is clear that mitochondria isolated from  $\Delta adh1$  cells exhibit ethanol oxidation rates ( $60 \text{ nmol min}^{-1} \text{ mg}^{-1}$ ) that are comparable to wild-type activities (*cf.* Table 1). In striking contrast, no ethanol-dependent activity is detectable in mitochondria isolated from the  $\Delta adh4$  strain. This clear difference between  $\Delta adh1$  and  $\Delta adh4$  mitochondria confirms that the observed mitochondrial ADH activity in *S. pombe* can be attributed entirely to ADH4. Although quantitatively somewhat different from Sp.011, it is clear that mitochondria from JY743 cell lines oxidise NADH at appreciable rates that are stimulated significantly by CCCP (Table 2) confirming the respiratory competence of both the  $\Delta adh1$  and  $\Delta adh4$  strains.

## Modular-kinetic analysis of *S. pombe* respiration

### Respiratory control

The data shown in Table 1A reveal that mitochondrial ethanol-dependent oxygen consumption in *S. pombe* is not increased significantly by CCCP. This relative lack of respiratory control during ethanol oxidation has previously been observed in other yeast mitochondria, and has been attributed to a relatively high degree of proton leak in the presence of this respiratory substrate [30]. However, the coupled rates of ethanol oxidation are comparable to the equivalent rates observed with NADH, which would suggest that the proton conductivity of the mitochondrial inner-membrane in *S. pombe* is not affected significantly by 1% (v/v) ethanol.

From studies with plant and yeast mitochondria, it has become clear that respiratory control ratios are substrate-dependent, since they reflect the kinetic interaction between several components of the respiratory system, including substrate dehydrogenases [31, 32]. For example, oxygen consumption by *S. pombe* mitochondria is stimulated by CCCP more strongly when NADH is used as a substrate than when succinate is employed [7]. To assess if the lack of respiratory control during ethanol oxidation could be explained in a similar manner, we performed a modular-kinetic analysis to gain more detailed insights in the kinetics of the respiratory system in *S. pombe* mitochondria oxidising succinate, ethanol, or external NADH. In this analysis we simplified the respiratory system conceptually to comprise modules that either reduce or oxidise a common Q-pool (see reference [31] for details).

### Oxidation of succinate

From the simultaneous oxygen uptake and Q-reduction measurements shown in Fig. 1, it can be seen that succinate is oxidised in *S. pombe* mitochondria at  $\sim 28 \text{ nmol min}^{-1} \text{ mg}^{-1}$ , with a concomitant Q-reduction level of  $\sim 34\%$  (Fig. 1A). Subsequent sequential addition of ATP and glutamate leads to increases in both respiratory activity and Q-reduction since both compounds activate succinate dehydrogenase [7, 9]. Valuable information may be gained when the respective steady state respiratory rates are plotted as a function of Q-reduction (Fig. 1B). When interpreting such a Q-kinetic plot, it is important to realise that respiratory steady states result from the interplay between Q-reducing (in this case succinate dehydrogenase with the dicarboxylate carrier) and QH<sub>2</sub>-oxidising (cytochrome pathway) modules. Since ATP and

glutamate specifically affect Q-reduction, a single model representing coupled QH<sub>2</sub>-oxidation kinetics should suffice to describe the shown steady states. Indeed, the data in Fig. 1B are fitted readily to a single hyperbolic equation (*cf.* [33]) that accounts for the theoretically inevitable fact that cytochrome pathway activity is zero when the Q-pool is fully oxidised.

### Oxidation of ethanol

Ethanol-dependent respiratory rates are plotted as a function of Q-reduction in Fig. 2A, both in the absence and the cumulative presence of ADP and CCCP. Under coupled conditions, oxygen is consumed at  $\sim 52 \text{ nmol min}^{-1} \text{ mg}^{-1}$  at a Q-redox poise of  $\sim 78\%$ . Importantly, this steady state is modelled well by the curve that describes cytochrome pathway activity (Fig. 2A-curve 1). This implies that ethanol does not alter the kinetics of QH<sub>2</sub>-oxidation under coupled conditions, indicating that it neither affects the components of the cytochrome pathway nor the integrity of the mitochondrial inner-membrane. The rate of ethanol oxidation is increased only marginally by ADP and CCCP (to  $\sim 58$  and  $61 \text{ nmol O}_2 \text{ min}^{-1} \text{ mg}^{-1}$ , respectively), whereas sequential addition of these compounds decreases the Q-redox poise to 71 and 43%. Obviously, the resulting respiratory steady states do not fall on the curve describing coupled QH<sub>2</sub>-oxidation kinetics (Fig. 2A), since control by the protonmotive force is relieved by ADP and CCCP. During ethanol-dependent respiration, the Q-reducing module comprises mitochondrial ADH and a substrate dehydrogenase that oxidises matrix NADH. Since *S. pombe* does not contain a protonmotive complex I [34], it is unlikely that activity of this internal-NADH dehydrogenase is affected by ADP or CCCP. It is therefore reasonable to expect that all steady states shown in Fig. 2A are describable by a single Q-reduction model, which indeed appears the case (Fig. 2A-curve *ethanol*). Note that this model accounts for the theoretical inevitability that Q-reducing activity is zero when the Q-pool is fully reduced.

### Oxidation of external NADH

In Fig. 2B Q-reduction kinetics during ethanol-oxidation are compared directly with those observed during oxidation of externally added NADH. In the latter case, a Q-reduction level of  $\sim 95\%$  is achieved allowing a respiratory activity of  $\sim 65 \text{ nmol O}_2 \text{ min}^{-1} \text{ mg}^{-1}$  (see also Table 1A). This activity is stimulated significantly by ADP (1.5-fold) and CCCP (5-fold), whilst Q-reduction is hardly affected by either compound (Fig. 2B). This shows that the external-NADH

dehydrogenase exhibits a rather high elasticity towards the reduction level of the Q-pool. In contrast, Q-reduction kinetics during ethanol oxidation are such that activity ( $\sim 50$  to  $60 \text{ nmol O}_2 \text{ min}^{-1} \text{ mg}^{-1}$ ) is almost fully independent of the Q-redox poise.

**Apparent lack of respiratory control during ethanol oxidation is due to kinetic characteristics of the Q-reduction module**

Differences in the behaviour of Q-reducing modules cause clear system-kinetic differences between *S. pombe* mitochondria oxidising either NADH or ethanol. In Fig. 2C modelled data are shown that reflect both Q-reduction kinetics and cytochrome pathway behaviour under various energetic conditions. It is evident that lowering the protonmotive force with ADP or CCCP predominantly affects the oxygen consumption rate during NADH oxidation, whereas it has an exclusive effect on the Q-redox poise during ethanol oxidation (Fig. 2C, upper and lower case letters, respectively). These results explain clearly why NADH-dependent respiratory activity is stimulated considerably by CCCP, whereas ethanol-dependent activity is not (Table 1A).

## DISCUSSION

In this paper we have demonstrated that the fission yeast *S. pombe* expresses a functional ADH isozyme that is specifically located in the mitochondrial matrix (Table 1), and using genetic knock-out strains we have identified the responsible gene (Table 2). This gene (*adh4*) encodes for a novel ADH protein that is not homologous to any of the mitochondrial (ADH1-like) isozymes found in other yeasts, which would explain why it has not been discovered before. In this respect, our results are of significant interest as they convincingly challenge the apparent general acceptance in the literature that *S. pombe* does not contain a mitochondrial ADH. An exception to this general notion is a study by Tsai *et al.* [35], who reported gel-electrophoretic data that would indeed suggest the occurrence of a mitochondrial ADH in *S. pombe*. Although the results of these authors are often overlooked, probably because confirmation of the sub-cellular location of the various isozymes is lacking [35], they are clearly in agreement with our observations.

As is the case in other yeasts [10-13], mitochondrial ADH activity sustains considerable oxygen consumption when *S. pombe* mitochondria are incubated in the exclusive presence of ethanol (Table 1). This indicates that ADH reduces matrix  $\text{NAD}^+$ , which is subsequently re-oxidised by the respiratory chain. Although perhaps not surprising, our data therefore confirm that the mitochondrial electron transfer chain of *S. pombe* contains an active internal NADH dehydrogenase that couples oxidation of matrix NADH to reduction of ubiquinone. Importantly, however, it turns out that electron transfer is controlled differently during NADH- and ethanol-dependent respiration, such that control is exerted predominantly by  $\text{QH}_2$ -oxidation when NADH is used as substrate, and by Q-reduction when ethanol is employed (Fig. 2C). The apparent lack of respiratory control (Tables 1 and 2), often attributed to detrimental effects of ethanol on the integrity of mitochondrial membranes [30], can be explained fully by the particular distribution of control during ethanol oxidation.

In general, the physiological function of yeast mitochondrial ADH enzymes is unclear. It has been proposed, however, that they form an ethanol-acetaldehyde shuttle with their respective cytosolic counterparts, which facilitates equilibration of cytoplasmic and mitochondrial  $\text{NAD}^+/\text{NADH}$  ratios [13]. In *S. cerevisiae*, for example, this is thought to be of particular relevance during anaerobic growth, when the NADH produced in the matrix by assimilatory reactions cannot be oxidised by the respiratory chain. *S. cerevisiae* strains lacking mitochondrial

ADH3 display impaired anaerobic growth [10], which would agree with this notion. As *S. pombe* cells lacking ADH4 also display reduced rates of anaerobic growth [18], it is conceivable that ADH4 fulfils a function in this system similar to that of ADH3 in *S. cerevisiae*.

*S. pombe* ADH4 does not show significant amino acid sequence homology with ADH1 or any other member of the 'group I-type', NAD(P)<sup>+</sup>-dependent ADH enzyme family (as classified in [15]). Instead, it shares considerable sequence identity with ADH4 from *S. cerevisiae* (56%) and ADH2 from *Zymomonas mobilis* (52%), both of which belong to the 'group III-type', iron-activated, NAD(P)<sup>+</sup>-dependent ADH enzyme family [25, 26]. To our knowledge, *S. pombe* ADH4 is the first group III-type protein that has been shown to play a role in mitochondrial metabolism. This raises the possibility that these enzymes also function in mitochondria of other species. Interestingly in this respect, a *S. pombe* ADH4 homologue has recently also been discovered in human, where it is expressed during foetal development as well as in adult liver [36]. Inspection of the amino acid sequence of this enzyme reveals the presence of an ~ 40 amino acid N-terminal extension that we predict – using the algorithms published in [27-29] – to target the protein to mitochondria.

To summarise, we have identified a novel mitochondrial ADH enzyme in *S. pombe* and have demonstrated in detail how the activity of this enzyme is linked to *in vitro* respiration. With this information we believe that we have provided valuable new insights into the energy metabolism of this widely used model organism.

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## FIGURE LEGENDS

Figure 1 – The kinetic dependency of succinate-dependent oxygen consumption activity on the reduction level of the Q-pool. (A) Typical oxygen consumption (solid line) and Q-reduction (dotted line) traces are shown illustrating the stimulatory effect of ATP (0.2 mM) and glutamate (9 mM) on the oxidation of succinate (9 mM). The numbers adjacent to the traces represent specific O<sub>2</sub>-uptake rates (nmol min<sup>-1</sup> mg<sup>-1</sup>) and Q-reduction levels (fraction QH<sub>2</sub> in total Q-pool). (B) Steady state respiratory rates obtained with succinate alone (○) or in the cumulative presence of ATP (□) and glutamate (Δ) are shown as a function of the Q-redox poise. Data are averaged values from three independent mitochondrial preparations and were modelled with hyperbolic equations as described in [33].

Figure 2 – Kinetic interplay between QH<sub>2</sub>-oxidising and Q-reducing pathways in *S. pombe* mitochondria. Respiratory steady states were achieved upon the oxidation of ethanol (0.15 M, closed symbols) or NADH (1.8 mM, open symbols) in the absence (diamonds) or the cumulative presence of ADP (0.1 mM, circles) and CCCP (1 μM, triangles). Data are averaged values from two to three separate mitochondrial preparations and were modelled with hyperbolic equations as described in [33]. Curves representing QH<sub>2</sub>-oxidising kinetics under coupled conditions (labelled **1.** in panels **A** and **C**) are identical to the one presented in Figure 1B. Descriptions of these kinetics in the presence of ADP and CCCP (labelled **2.** and **3.** in panel **C**, respectively) are based on the data shown in panel **B** (circles and triangles, respectively) and include the origin, since cytochrome pathway kinetics with respect to the Q-redox poise should be substrate-independent and zero when the Q-pool is fully oxidised. The intersects of curves reflecting the kinetics of Q-reducing (labelled *NADH* and *ethanol*) and QH<sub>2</sub>-oxidising modules (panel **C**) indicate steady states attained during the oxidation of ethanol (lower case letters) or NADH (upper case letters) in the absence (**a**) or the cumulative presence of ADP (**b**) and CCCP (**c**).

Table 1 – Oxygen consumption (A) and ADH activity (B) in isolated *S. pombe* mitochondria. A) Concentration of substrates and effectors are: 1.8 mM NADH, 1  $\mu$ M CCCP (stock dissolved in propan-2-ol), 0.15 M ethanol, 0.5 mM NAD<sup>+</sup>, 1.7  $\mu$ M antimycin A (in propan-2-ol), 95 mM 2,2,2-trifluoroethanol. B) ADH activity was measured in the absence or presence of 0.1% (v/v) Triton X-100. All experiments were performed at pH 6.8 and presented data are average values ( $\pm$  standard deviation) obtained from three to nine separate mitochondrial preparations.

A.	Respiratory Rate (nmol O <sub>2</sub> min <sup>-1</sup> mg <sup>-1</sup> )
NADH	67 $\pm$ 13
NADH + CCCP	332 $\pm$ 54
Ethanol	53 $\pm$ 7
Ethanol + NAD <sup>+</sup>	65 $\pm$ 12
Ethanol + NAD <sup>+</sup> + CCCP	88 $\pm$ 8
Ethanol + NAD <sup>+</sup> + CCCP + Antimycin A	3 $\pm$ 0
Ethanol + NAD <sup>+</sup> + 2,2,2-trifluoroethanol	17 $\pm$ 6
B.	ADH activity (nmol NADH min <sup>-1</sup> mg <sup>-1</sup> )
- Triton X-100	44 $\pm$ 5
+ Triton X-100	716 $\pm$ 159

Table 2 – Ethanol-dependent respiratory activity in mitochondria from  $\Delta adh1$  and  $\Delta adh4$  mutant strains of *S. pombe*. Concentration of substrates and effectors are: 0.15 M ethanol, 1  $\mu$ M CCCP (stock dissolved in propan-2-ol), 1.8 mM NADH. All experiments were performed at pH 6.8 and presented data are average values ( $\pm$  standard deviation) obtained from three separate mitochondrial preparations.

	Respiratory Rate (nmol O <sub>2</sub> min <sup>-1</sup> mg <sup>-1</sup> )	
	$\Delta adh1$	$\Delta adh4$
Ethanol	60 $\pm$ 12	0 $\pm$ 0
Ethanol + CCCP	59 $\pm$ 16	-
NADH	94 $\pm$ 24	45 $\pm$ 12
NADH + CCCP	221 $\pm$ 31	135 $\pm$ 48

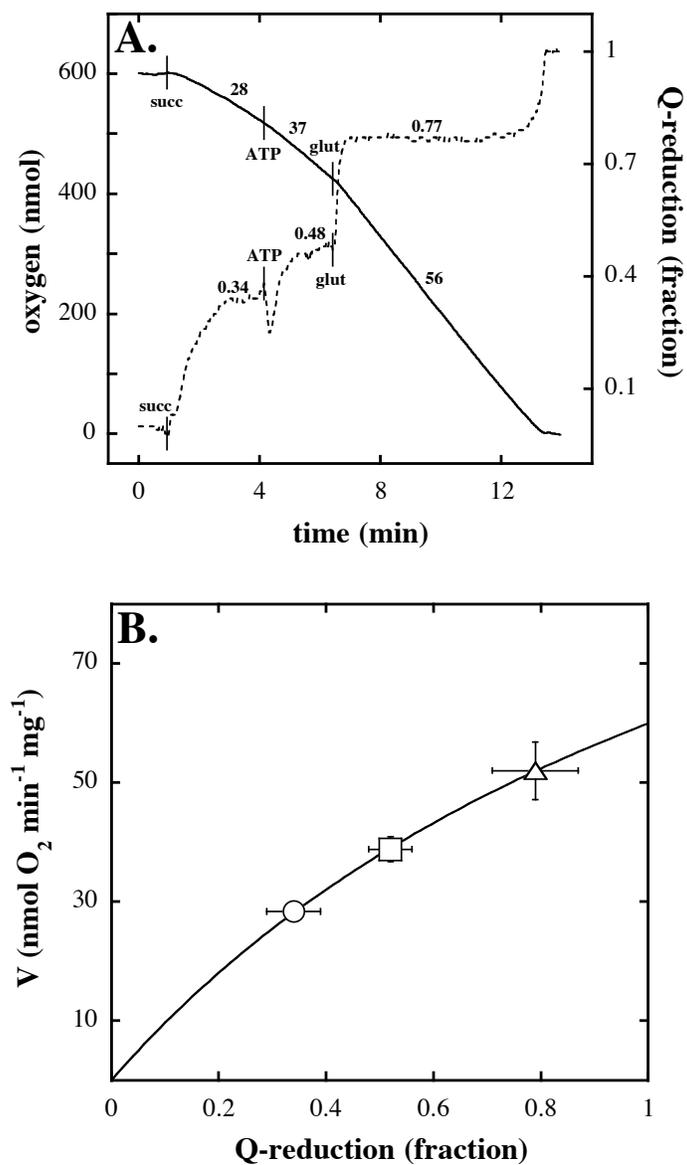


Figure 1

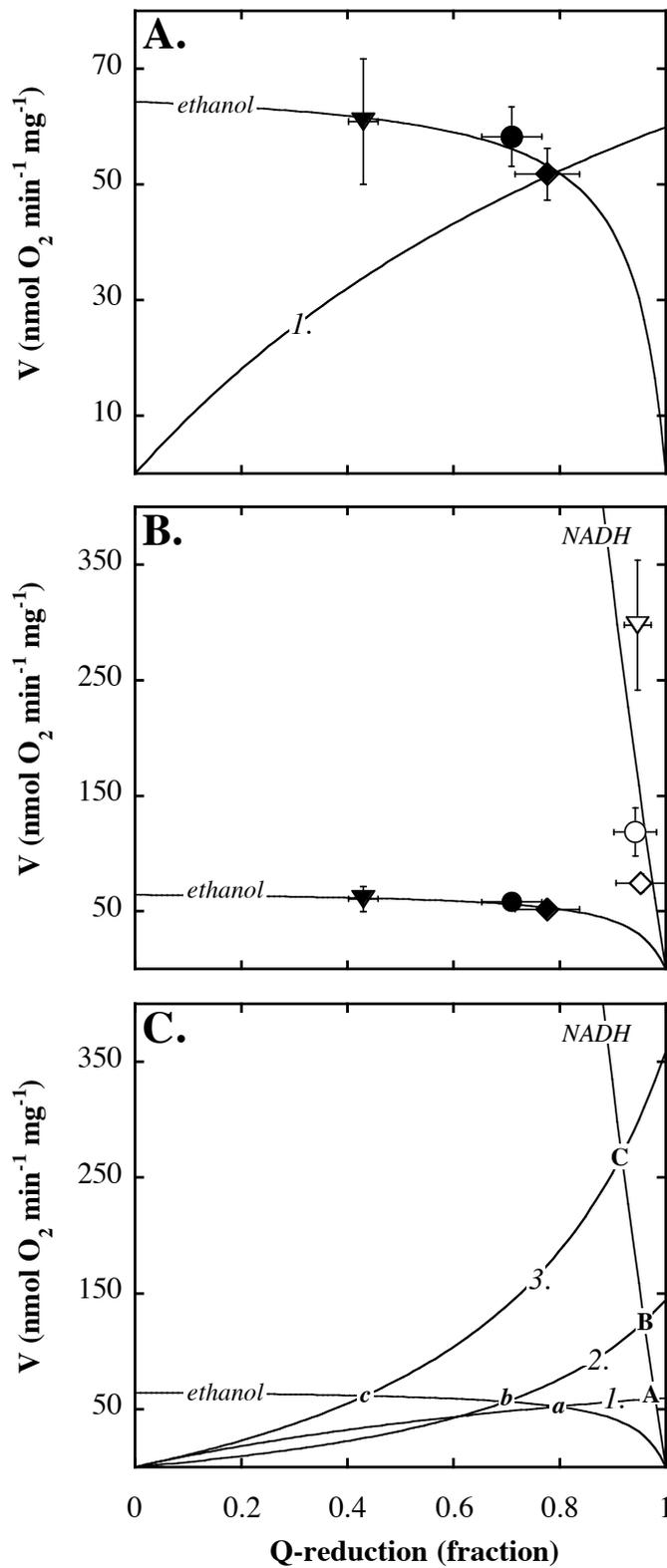


Figure 2