Correlation between Oligomycin-Sensitivity and Ergosterol in a Purified Mitochondrial Adenosine Triphosphatase from *Saccharomyces cerevisiae*

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The mode of action of oligomycin, a widely used inhibitor of oxidative phosphorylation introduced by Lardy *et al.* (1958), is of obvious interest as a potential clue to the nature of the respiratory-chain-driven ATP synthesis.

To study this problem we have attempted to make use of mutants of *Saccharomyces cerevisiae* that, while maintaining the aerobic growth characteristics of the parent wild-type, show a resistance to oligomycin (Avner & Griffiths, 1970; Griffiths *et al.*, 1972). This resistance appears to be located in the ATP-synthesizing or -hydrolysing enzyme (ATPase†) of the inner mitochondrial membrane.

As a less complex material than entire mitochondrial membranes is desirable for investigation of the difference in oligomycin-sensitivity and its possible cause, we have purified and compared an ATPase from wild-type and mutant yeast mitochondria.

Materials and methods

The strain of *S. cerevisiae* used, designated D22, has been described by Anver & Griffiths (1970). The mutants were Class II-type, designated D22-A13, D22-A16 and D22-A21, showing resistance to oligomycin only (Griffiths *et al.*, 1972). The yeast was grown in a medium consisting of glucose (20g/l), bacteriological peptone (10g/l) and yeast extract (10g/l) at 30°C. The yeast was harvested at the end of the exponential growth phase.

Oligomycin was obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A., and bovine cardiolipin from Sylvana, Orange, N.J., U.S.A.

ATPase was assayed enzymically (Pullman *et al.*, 1960). Protein was measured with a biuret method (Gornall *et al.*, 1949). Ergosterol in whole yeast cells was measured by the method of Woods (1971). Ergosterol in enzyme preparations was determined by measuring the absorbance at the 294nm peak of an acid-butan-1-ol extract. The enzyme was extracted once with an equal volume of butan-1-ol-acetic acid-water (380:3:14, by vol.) followed by two

extractions with the same volume of butan-1-ol-water (19:1, v/v). The extracts were combined.

Total lipid was extracted and subjected to Folch partition as described by Rouser & Fleischer (1967).

Polyacrylamide-gel electrophoresis in sodium dodecyl sulphate was performed as described by Lenard (1970).

Yeast submitochondrial particles, prepared as described by Tzagoloff (1969), were used as a starting material for purification of the ATPase. Loosely adhering lipid was removed by flotation; particles were resuspended in 0.5M-sucrose and sedimented by centrifugation at 105000g for 20min at 0°C. This step was repeated once. Then 0.75g of KCl and 0.3ml of deoxycholic acid (100g/l), adjusted to pH8 with 5M-KOH, were added to the washed submitochondrial particles (200mg in 10ml of 0.5M-sucrose), followed by centrifugation at 105000g for 20min at 0°C. The supernatant was further purified by detergent gradient chromatography (Swanljung, 1971) on a Sepharose 6B (Pharmacia, Uppsala, Sweden) column (95cm × 2.5cm) with 200ml of a linear potassium cholate gradient (0–5g/l) in 10mm-tris adjusted to pH7.5 with acetic acid. A latent lipid-dependent ATPase was eluted with the first part of the void-volume peak (approx. 325–345ml of effluent, measured from the start of gradient application). Yields ranged between 20 and 30mg of protein.

Results and discussion

Polyacrylamide-gel electrophoresis of the purified mitochondrial ATPase showed eight clearly defined bands. This compares favourably with the oligomycin-sensitive ATPase preparation described by Tzagoloff & Meagher (1971). No clear-cut differences were detected in the gel-electrophoretic patterns between preparations from wild-type and mutant yeasts.

The purified enzyme, although containing 0.3–0.4mg of chloroform-methanol (2:1, v/v)-extractable material/mg of protein, required added exogenous lipid (e.g. bovine cardiolipin, 1.5mg/mg of protein) for full activity. This lipid-dependence is similar to that observed with ox heart mitochondrial ATPase (Swanljung & Ernster, 1971). The specific

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† Abbreviation: ATPase, adenosine triphosphatase.

Table 1. *Specific activity, oligomycin-sensitivity and ergosterol content of purified mitochondrial ATPase from wild-type and oligomycin-resistant mutant yeast*

Specific activity and effect of oligomycin were assayed in the presence of bovine cardiolipin (1.5–2.0 mg/mg of protein). Results are expressed as mean values obtained with four different preparations of each type, with the observed ranges in parentheses.

Yeast ...	Wild-type (D22)	Mutants (D22-A16 and D22-A21)
Specific activity of ATPase ($\mu\text{mol}/\text{min}$ per mg of protein)	2.1 (0.8–7.0)	1.1 (0.8–3.0)
Oligomycin required for 50% inhibition of ATPase ($\mu\text{g}/\text{mg}$ of protein)	4.0 (1.5–5.0)	80 (50–200)
Ergosterol content (nmol/mg of protein)	74 (62–81)	28 (18–40)

activities of the enzyme preparations, activated with bovine cardiolipin, are shown in Table 1. The large variations observed are probably due to the high instability of the enzyme.

Titration with oligomycin revealed that the enzymes from the mutant yeasts required ten times or more oligomycin for 50% inhibition than did the enzyme from the wild-type. As shown in Table 1, this resistance was consistently correlated with a decrease in ergosterol associated with the enzyme. There was no difference between the yeasts in the amount of proteolipid, total lipid or lipid phosphate associated with the purified enzyme, nor in the phospholipid composition and fatty acid distribution of lipids from submitochondrial particles.

The decrease in ergosterol associated with the enzymes is paralleled by a decrease in total ergosterol in the mutant cells. The ergosterol content of the wild-type (D22) yeast was $1.28 \pm 0.12 \mu\text{mol}/\text{g}$ dry wt. (mean $\pm$ s.d. of eight determinations), whereas that of three mutants (D22-A13, D22-A16 and D22-A21) averaged $0.25 \pm 0.11 \mu\text{mol}/\text{g}$ dry wt. (mean $\pm$ s.d. of 12 determinations).

This finding implies that the mutation is affecting a gene that directly or indirectly controls the synthesis of ergosterol. Since the mutants studied in this work are thought to acquire oligomycin-resistance through a one-point mutation, coincident changes in sensitivity and sterol content can hardly be completely unrelated. At the moment, however, there are no unequivocal answers as to the nature of this relationship. Reconstitution experiments, where ergosterol is added to mitochondria or the purified enzyme from a mutant, are technically difficult, as added lipid appears to bind oligomycin also at other sites than that of ATPase inhibition. No increase in ergosterol content of mitochondria was obtained by growing the mutants in the presence of ergosterol.

In summary, a mitochondrial ATPase has been purified from a strain of *S. cerevisiae* and from

oligomycin-resistant mutants of this strain. Apart from a low oligomycin-sensitivity, a striking feature of the ATPase from the mutants is a lower content of associated ergosterol. Regardless of whether the correlation between oligomycin-sensitivity and ergosterol is due to a direct interaction or, e.g., to a common binding protein, the decrease in the content of ergosterol observed means that the mutation leading to oligomycin-resistance is accompanied by a change in the hydrophobic environment of the membrane.

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