Imide-treated flight muscle submitochondrial particles results in a restoration of the ATPase activity to the control value. A similar result has been obtained with beef heart submitochondrial particles. These results suggest that mechanism (3) satisfactorily explains the present observations.


The Use of a Fatty Acid Desaturase Mutant of *Saccharomyces cerevisiae* to Investigate the Role of Lipids in Mitochondrial Membrane Functions

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The yeast *Saccharomyces cerevisiae* is an ideal model for the study of the relationships between lipid composition and mitochondrial membrane functions. Both physiological and genetic situations can be employed to restrict the synthesis of lipid components by *S. cerevisiae*, and under these conditions the organism is also able to directly incorporate

Table 1. Effects of changed fatty acid composition on the energy-linked functions of mitochondria from *S. cerevisiae*, strain KD 115

The table summarizes data from Haslam *et al.* (1971, 1973). Additional determinations of ATPase activities (µmol min⁻¹ mg of protein⁻¹) were performed at 30°C by a modification of the spectrophotometric method of Pullman *et al.* (1960). The decrease in $E_{340}$ was measured in 3.0 ml containing: Tris-maleate (10 mM), KH$_2$PO$_4$ (8 mM) EDTA (1 mM), sorbitol (0.5 M), bovine serum albumin (10 mg), NADH (0.1 mM), MgCl$_2$ (3 mM), ATP (1 mM), phosphoenolpyruvate (1 mM), antimycin A (5 µg), pyruvate kinase (Sigma; EC 2.7.1.40; 6 units), lactate dehydrogenase (Sigma; EC 1.1.2.3; 12 units) and mitochondria (100–300 µg of protein), final pH 6.5. Inhibition by oligomycin was determined with 50 µg of inhibitor/mg of mitochondrial protein. The specific protein inhibitor of mitochondrial ATPase (F$_1$-inhibitor), was isolated from beef heart mitochondria and assayed as described by Monroy & Pullman (1967). 0, +, ++, ++++, +++++ indicate relative activities: 0, zero; +, <25%; ++, 25–50%; ++++, 50–75%; +++++, 75–100%.

Mitochondrial function

<table>
<thead>
<tr>
<th>Unsaturated fatty acid content (mol % of total fatty acids)</th>
<th>70–80</th>
<th>30–50</th>
<th>20</th>
<th>10–15</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP synthesis</td>
<td>+++++</td>
<td>++</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>P$_1$-ATP exchange</td>
<td>+++++</td>
<td>+++</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>Active uptake of K$^+$</td>
<td>+++++</td>
<td>+++</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Active ejection of H$^+$</td>
<td>+++++</td>
<td>++</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Passive uptake of H$^+$</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>+++++</td>
</tr>
<tr>
<td>ATPase activity</td>
<td>0.9–1.8</td>
<td>0.9–1.8</td>
<td>0.8–1.7</td>
<td>0.6–1.2</td>
</tr>
<tr>
<td>Inhibition by oligomycin</td>
<td>75–80%</td>
<td>75–80%</td>
<td>65–75%</td>
<td>30–60%</td>
</tr>
<tr>
<td>Inhibition by F$_1$-inhibitor</td>
<td>80–90%</td>
<td>80–90%</td>
<td>80–90%</td>
<td>65–80%</td>
</tr>
</tbody>
</table>

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into its membranes lipids that are added to the growth medium (Proudlock et al., 1968, 1971). Further, _S. cerevisiae_ is a facultative anaerobe that does not require oxidative metabolism to grow on fermentable substrates, and hence situations which result in gross alterations in mitochondrial functions can be experimentally investigated.

If a fatty acid desaturase mutant of _S. cerevisiae_ is grown in batch culture with defined supplements of Tween 80 as a source of unsaturated fatty acids, the proportion of unsaturated fatty acids in the membrane lipids can be manipulated to be as high as 85% or as low as 6% of total fatty acids. By using this system Proudlock et al. (1971) showed that cells whose fatty acids are less than 20% unsaturated cannot grow on non-fermentable substrates, and their growth on glucose is restricted to that which can be supported by fermentation alone, indicating a loss of mitochondrial function. Table 1 summarizes the properties of yeast mitochondria whose lipids contain different proportions of unsaturated fatty acids. The mitochondria are normal with respect to morphology, cytochrome content, respiratory activity and ATPase (adenosine triphosphatase) activity, but progressively lose the ability to couple respiration to ATP synthesis as the proportion of unsaturated fatty acids is lowered from 60 to 20% of the total fatty acids. Mitochondria whose fatty acids contain less than 20% unsaturated fatty acids lack coupled phosphorylation and F_0-ATP exchange activity (Haslam et al., 1971). The lesion is fully reversible _in vivo_ by the addition of Tween 80 to the growth medium. The depleted cells incorporate unsaturated fatty acids into their mitochondrial lipids, and after a lag of 6–8h this results in the recoupling of oxidative phosphorylation, and the restoration of the ability to grow on non-fermentable substrates. Recoupling is not prevented by the simultaneous addition of chloramphenicol and cycloheximide to completely inhibit protein synthesis, indicating that all the proteins necessary for oxidative phosphorylation are present in the lipid-depleted mitochondria, and that the loss of coupling ability is purely a lipid lesion (Haslam et al., 1971).

More recently Haslam et al. (1973) have shown that depletion of unsaturated fatty acids also causes the loss of the energy-dependent uptake of K^+ ions in the presence of valinomycin and abolishes the respiration-dependent ejection of protons by mito-

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![Fig. 1. Effect of changed fatty acid composition on Arrhenius plots of mitochondrial ATPase activity](image)

The portion of mitochondrial ATPase activity sensitive to F_1-ATPase inhibitor was assayed as in Table 1. Three different preparations are shown. The percentages of unsaturated fatty acids in the total fatty acids of the membrane lipids are as follows: △, 13%, transition temperature = 35°C; ●, 20%, transition temperature = 27°C; □, 83%, transition temperature = 8°C.
chondria. Since all these effects resemble the action of classical chemical uncouplers, which Mitchell (1966) has suggested act by increasing the permeability of the inner mitochondrial membrane to protons, the effects of changed membrane fatty acid composition on the passive permeability of the mitochondria to protons was investigated. Lowering the unsaturated fatty acid content of the mitochondria progressively increased the passive permeability of the organelles to protons, thus providing a satisfactory explanation for the loss of oxidative phosphorylation and active cation transport (Haslam et al., 1973).

The next part of the investigation has concerned the relationship between altered membrane lipid composition and the physical changes in the membrane that cause the increased permeability to protons. It has been shown that discontinuities in the Arrhenius plots of membrane-bound enzymes sometimes reflect changes in the physical organization of the membrane lipids (for review see Raison, 1973). Accordingly, we have investigated the temperature-dependence of mitochondrial ATPase as a parameter that might detect changes in membrane organization in response to altered lipid composition.

Manipulation of the unsaturated fatty acid content between 20 and 80% of fatty acids has no effect on the activity of the mitochondrial ATPase at 30°C, nor is there any change in the sensitivity of the enzyme to oligomycin or the protein ATPase inhibitor from beef heart mitochondria (Table 1). However, Arrhenius plots of the temperature-dependence of the ATPase reveal profound effects on the kinetics of the ATPase at lower temperatures. Fig. 1 shows the temperature-dependence of F, inhibitor-sensitive ATPase of mitochondria whose fatty acids contain 83%, 20% and 13%-unsaturated fatty acids. In all three experiments there is a discontinuity in the Arrhenius plot with activation energies of about 40kJ/mol above and about 90kJ/mol below the transition temperature. Depletion of unsaturated fatty acids causes a marked shift of the transition temperature from 8°C for fully supplemented mitochondria (83%-unsaturated) to 27°C for uncoupled depleted mitochondria (20%-unsaturated), and to 35°C for extensively depleted mitochondria (13%-unsaturated). Collaborative experiments with Dr. J. Raison with electron paramagnetic resonance-labelled molecules as membrane lipid probes, show that the mitochondrial membrane lipids in situ undergo a phase transition at temperatures close to the transition temperatures obtained for Arrhenius plots of ATPase activities. These results support the hypothesis that changes in the activation energy of mitochondrial ATPase reflect a phase change in the mitochondrial membrane lipids that induces a conformational change in the enzyme proteins.

It is noteworthy that cells of the mutant stop growing on non-fermentable substrates when the fatty acids of the mitochondrial membrane lipids are 20% unsaturated, and the temperature of the lipid phase change is just below that of the growth temperature (28°C). This may indicate that the increased proton permeability of the depleted mitochondria and the loss of oxidative phosphorylation are a consequence of the cells operating below the transition temperature of the mitochondrial membrane lipid phase change. Indeed, initial experiments indicate that cells require higher amounts of unsaturated fatty acids to grow on non-fermentable substrates at temperatures below 28°C, the effect of the increased lipid unsaturation being to lower the transition temperature of the ATPase Arrhenius plot (and by inference that of the membrane phase transition) to below that of the growth temperature.