Table 1
The fatty acid composition of aerobic and lipid-depleted anaerobic yeast mitochondria.

Mitochondria	C_8	C_{10}	C_{12}	C_{14}	C_{16}	C_{18}	$C_{16:1}$	$C_{18:1}$	UFA[a] (%)
Aerobic	< 1	< 1	1	–	8	2	53	35	88
Anaerobic	2	15	15	14	37	8	5	4	9

Fatty acids expressed as percentage of the total fatty acid. The fatty acids are denoted by the convention, number of carbon atoms:number of double bonds.

[a] Unsaturated fatty acid. Mitochondrial fatty acids were determined by gas chromatography of the methyl esters. Methylation was carried out in methanolic HCl at 60° for 2 hr. A Perkin–Elmer F-11 gas chromatograph and a polyethylene glycol succinate column operating at 180° was used to separate the methyl esters.

However, some differences as compared to F_1-ATPase were observed. Firstly, the break points in the Arrhenius plots for aerobic mitochondria was in the range 13–15° which was significantly lower than that observed with F_1-ATPase, 17–19° (fig. 1). On the other hand, the break point for succinate dehydrogenase in promitochondria, 24–26°, was similar to that observed for promitochondrial F_1-ATPase (cf. fig. 1). The energies of activation above and below the transition temperatures for succinate dehydrogenase were similar to that observed for the F_1-ATPase.

3.3. *Fatty acid composition of mitochondria and promitochondria*

Temperature-induced changes in activation energy of mitochondrial membrane-bound enzymes have been suggested as resulting from phase transitions in the lipid components of the membrane [6, 7]. It has recently been reported that the lipid composition of mitochondria isolated from aerobically and anaerobically grown yeast cells are different [3, 12]. Table 1 shows the fatty acid composition of mitochondria isolated from ethanol grown aerobic cells and from glucose repressed, lipid-depleted anaerobic cells. Striking differences in the fatty acid composition were observed. The fatty acids of aerobic yeast mitochondria were almost exclusively *unsaturated*, consisting of 54% palmitoleic and 35% oleic acid. On the other hand, the fatty acids of promitochondria were almost exclusively *saturated*. The saturated fatty acids stearic and palmitic accounting for about one half and short chain fatty acids (C_8–C_{14}) for the other half of the total saturated fatty acids. We have recently proposed that a good criterion for the degree of anaerobiosis in yeast cells grown in the absence of lipid-supplements is the percentage of unsaturated fatty acids which for glucose grown cells is about 10% [5].

4. Discussion

The temperature dependent changes in activation energy of the mitochondrial membrane-bound enzymes succinate dehydrogenase and F_1-ATPase have been shown to be significantly different in aerobic yeast mitochondria. We have recently reported that respiratory enzymes, including cytochrome *c* oxidase, of yeast mitochondria all have a similar transition temperature in Arrhenius plots of about 13° [13]. Ainsworth et al. [14] have also recently reported that cytochrome *c* oxidase in yeast mitochondria has a transition temperature at 8–11° depending on the fatty acid composition of the cells. The transition temperature of about 18° for yeast F_1-ATPase appears, therefore, to be an exception and may reflect a different lipid or protein environment for this complex as compared to other membrane-bound enzymes in yeast mitochondria [13]. A transition temperature of about 18° for F_1-ATPase in beef heart mitochondria has also recently been reported [15].

The transition temperature for succinate dehydrogenase and other respiratory enzymes of yeast mitochondria [13] is about 8–10° lower than that

reported for mammalian mitochondria [6, 15] but are similar to that reported for chilling-sensitive plant mitochondria [16]. There is some correlation between the transition temperature and the fatty acid composition of yeast mitochondria (table 1) and chilling-sensitive plant mitochondria [17]. Aerobic yeast mitochondria have a high percentage of unsaturated fatty acid (85–90%) consisting of almost exclusively the monounsaturated fatty acids palmitoleic and oleic. Chilling-sensitive plant mitochondria, while having a less unsaturated fatty acid content (60–70%), are characterised by linoleic and linolenic acids, fatty acids with two and three double bonds, respectively. Hence, although the percentage of unsaturation is higher in yeast mitochondria, the presence of polyunsaturated fatty acids in plant mitochondria would compensate for this difference and thus the degree of unsaturation would be similar. The higher temperature breaks observed in mammalian mitochondria [6, 15] may similarly be correlated with the degree of unsaturation of the fatty acids [18].

It has been reported that promitochondria in anaerobic yeast cells are biochemically and morphologically distinct from that of aerobic mitochondria [3–5]. The present studies indicate that promitochondrial membranes in the lipid-depleted anaerobic cell are much less fluid than that of aerobic mitochondria as shown by the higher transition temperatures. The lower flexibility of the promitochondrial membranes may be a possible explanation for the difficulties encountered in the isolation of intact structures from the lipid-depleted anaerobic cell [3, 4]. Furthermore, the increase in activation energy at about 24–26° may also explain the inability of yeast cells to undergo more than a few cell divisions in anaerobic lipid-depleted growth conditions at temperatures of 20° or below (Watson, unpublished results).

The importance of unsaturated fatty acids in determining phase transitions in membranes has been recently emphasised by studies on *Mycoplasma laidlawii* and mutants of *Escherichia coli* [19–24]. These investigations have shown that the more saturated the fatty acid of the membrane lipids, the higher the phase transition temperature, presumably due to a change of the membrane lipids from a liquid to a more crystalline phase. By analogy, therefore, it is possible to explain the present results as due to the high degree of saturation of the fatty acids of the membrane lipids of promitochondria. However, the transition temperature in promitochondrial membranes is only a few degrees higher than that observed in rat liver mitochondria [6] which have a very high degree of unsaturated fatty acid [18]. In addition it would be difficult to explain the different transition temperatures observed, in the same membrane, for F_1-ATPase and succinate dehydrogenase in aerobic yeast mitochondria unless one postulates a heterogeneous distribution of lipids within the membrane or that some other factors, eg. lipid–protein interactions, may affect the transition temperatures. Several other observations, such as the disparity in the transition temperature in *E. coli* mutants as determined by X-ray diffraction and by Arrhenius plots [24] and the report that electrostatic lipid–protein interactions may play a role in determining phase transitions in *M. laidlawii* [25], would suggest some caution as to the interpretation of transition temperatures in membranes as being due exclusively to phase changes in the membrane lipids. We are currently investigating the role of other interactions, such as lipid–protein and lipid–lipid, which may contribute an important part in determining the nature of phase transitions in yeast mitochondrial membranes.

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