THE EFFECT OF ALTERED MEMBRANE STEROL COMPOSITION ON THE TEMPERATURE DEPENDENCE OF YEAST MITOCHONDRIAL ATPase

Gary S. Cobon and J.M. Haslam

Department of Biochemistry
Monash University
Clayton, Victoria 3168, Australia

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SUMMARY. The sterol content of cells of Saccharomyces cerevisiae was manipulated by growing the organism anaerobically in a medium containing excess supplements of unsaturated fatty acids and a range of supplements of ergosterol. Anaerobic mitochondrial precursor structures were isolated whose membrane lipids contain the same fatty acid composition but whose sterol content varies from 7 to 105 mg/g mitochondrial protein. Arrhenius plots of the mitochondrial ATPase activity of the different preparations show a discontinuity with Arrhenius activation energies of about +40 and +80 KJ/mole, respectively, above and below the transition temperature. However, the temperature of the transition is markedly dependent on sterol composition, and increases by up to 17° as the sterol content of the mitochondria is progressively decreased. These results support the concepts that membrane lipid composition influences the activity of membrane-bound enzymes, and that sterols promote the gel to liquid phase transition in biological membranes.

INTRODUCTION. A large body of evidence now indicates that the composition of membrane lipids modifies the activities of membrane functions (for reviews see 1, 2). Arrhenius plots of many membrane-bound enzymes and transport systems show discontinuities with an increase in the activation energies at lower temperatures, and physical studies indicate that the discontinuities approximately coincide to a change in the phase of the membrane lipid from gel to liquid crystalline (2). In order to probe the role of membrane lipids in membrane functions, a number of investigators have used mutant organisms, that are auxotrophic for fatty acids, to manipulate the fatty acid composition of the membrane lipids of Escherichia coli (3-6), Mycoplasma laidlawii (7) and Saccharomyces cerevisiae (8-12). These studies indicate that the temperature of the discontinuities in Arrhenius plots of membrane functions depends on the

Footnote: All correspondence to Dr. J.M. Haslam, present address Department of Biochemistry, Liverpool University, P.O. Box 147, Liverpool, L69 3BX, England.

proportion of unsaturated fatty acids in the membrane lipids. The role of sterols in biological membranes is less clear, but physicochemical studies show that the addition of cholesterol to membranes lowers the temperature of the gel to liquid crystalline transition, and at the same time inhibits the motion of hydrocarbon chains in the liquid crystalline phase (1, 13-16).

The sterol composition of S. cerevisiae can be manipulated, as the organism cannot synthesize unsaturated fatty acids or sterols under anaerobic conditions (17, 18) but can incorporate into its membrane lipids a wide variety of added fatty acids and sterols (19, 20). In the present study, cells are grown anaerobically with excess supplements of Tween 80 as a source of unsaturated fatty acids, and the content of ergosterol in the cells is varied over wide limits by adding different levels of ergosterol to the medium. The effects of changed sterol composition on the temperature dependence of mitochondrial ATPase activity is investigated, as this membrane-bound enzyme is known to exhibit a discontinuity in the Arrhenius plot (2, 10, 21).

METHODS. A haploid strain of S. cerevisiae, L410 (α ura his), is grown anaerobically in a 1% yeast extract-salts medium as described by Wallace et al. (22). Excess Tween 80 (0.5%) and supplements of ergosterol in the range 0 to 40 mg/l are added to the medium. Ergosterol is dissolved in ethanol and then added slowly to the stirred medium; after autoclaving it is present as a fine emulsion due to the detergent action of the Tween 80. Cultures are inoculated with 50 mg dry weight of cells/l and grown anaerobically at 28° for 16-20 hours. Cells are harvested when they reach a density of 0.9 to 1.5 mg dry weight per ml of medium, and a sample of cells is taken for lipid analysis. Mitochondrial precursor structures are isolated by the method of Watson et al. (23). Great care is taken to exclude air during the preparation of the mitochondria, and all solutions are continually flushed with oxygen-free nitrogen, as ergosterol is rapidly synthesized by the cells in the presence of molecular oxygen (24). The lipids of the cells and isolated mitochondria are analysed as described previously (8). Mitochondrial ATPase is assayed by a modification of the method of Pullman et al. (25); the coupled oxidation of NADH, mediated by pyruvate kinase and lactate dehydrogenase, is followed spectrophotometrically

at 340 nm. The cuvette is placed in the thermostatted cuvette holder of a Zeiss spectrophotometer and contains sorbitol (0.5 M); tris-maleate, pH 6.5 (10 mM); EDTA (1 mM); K-PO_4 (8 mM); bovine serum albumin (1 mg/ml); NADH (0.15 mM); antimycin A (2 μg/ml); $MgCl_2$ (3 mM); ATP (1 mM); phosphoenol pyruvate (1 mM); Sigma pyruvate kinase, EC 2.7.1.40 (2 units/ml); Sigma lactate dehydrogenase, EC 1.1.2.3 (4 units/ml); and yeast mitochondria (30 to 70 μg protein/ml). F_1 inhibitor is prepared from beef heart mitochondria, and its effect on yeast mitochondrial ATPase is assayed as described by Monroy and Pullman (26). The ATPase activity of twice-washed mitochondria is inhibited about 50% by F_1 inhibitor, suggesting considerable contamination by non-mitochondrial ATPase. However, purification of the mitochondria on a continuous density gradient of sorbitol (30-80% w/v) results in the ATPase activity of the organelles being inhibited up to 80% by both F_1 inhibitor and oligomycin, indicating that the gradient purified organelles are suitable for the study of the properties of mitochondrial ATPase.

RESULTS AND DISCUSSION

The ergosterol content of whole cells and of isolated mitochondrial precursor structures from cells grown anaerobically on media containing various supplements of ergosterol are shown in Table 1. The ergosterol content of the cells varies from 0.53 to 3.25 mg/g dry weight, and that of the mitochondria ranges from 7.3 to 105 mg/g protein. Although cells grown with higher supplements of ergosterol tend to contain higher levels of sterol, there is no direct correlation between the sterol level of the cells and that present in the medium. This is not surprising, as ergosterol is highly insoluble in water and in the presence of Tween 80 is present as a fine emulsion. The ability of cells to utilize ergosterol in this form probably varies because the properties of the emulsion differ in each incubation. The mitochondrial concentration of sterol also increases in cells with higher sterol content, but the relationship is again not direct. This variability probably occurs because the cells are not all harvested at exactly the same stage of growth and the rate of growth of the cells in the different preparations decreases as the ergosterol is exhausted. Such factors may influence the distribution of sterol within the cell.

TABLE 1

The effect of different supplements of ergosterol on the ergosterol content of whole cells and isolated mitochondrial precursor structures of S. cerevisiae.

The preparation of cells and mitochondria and the assay of sterols are described in Methods. Results are for ten individual experiments. Cellular fatty acids of all preparations contain 70-80% unsaturated fatty acids (C16:1 + C18:1).

Ergosterol Supplement (mg/l medium)	Yield of cells (g dry weight/l)	Cellular Ergosterol (mg/g dry weight)	Mitochondrial Ergosterol (mg/g protein)
0	0.95	0.74	7.3
5	0.95	1.15	35.8
10	1.25	2.20	22.1
10	1.25	0.53	39.5
10	1.34	1.80	47.0
15	1.34	2.20	105.0
20	1.45	1.95	32.6
40	1.34	2.20	60.0
40	1.45	3.25	40.7
40	1.45	3.10	47.0

Samples of mitochondria with widely differing sterol composition have very similar total ATPase activities. The mitochondrial identity of the ATPase measured in all preparations is confirmed, as the enzyme is inhibited by either F_1 inhibitor or oligomycin. However, the ATPase activity of organelles that are most extensively depleted in ergosterol is inhibited by only 60 to 65%, indicating that there is some contamination by non-mitochondrial ATPases. Nevertheless, the ATPase activity of even the most extensively depleted preparations is predominantly mitochondrial, and reflects the properties of the mitochondrial enzyme.

When Arrhenius plots of the mitochondrial ATPase activity of organelles

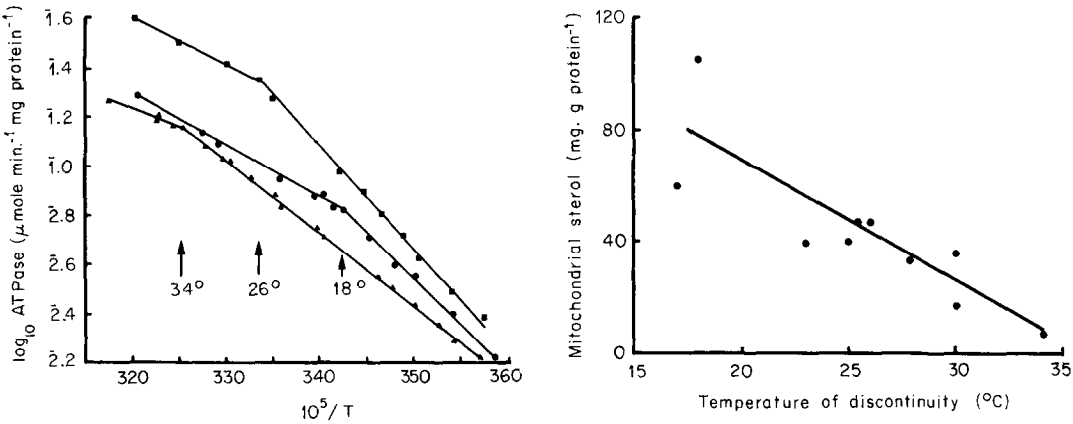

FIGURE 1

The effect of changed sterol content on Arrhenius plots of mitochondrial ATPase.

Mitochondria were isolated, and ATPase activity determined as described in Methods. Three different preparations are shown: ▲——▲ 7.3 mg ergosterol/g protein, transition temperature 34°, Arrhenius activation energies +29 and +71 KJ/mole; ■——■ 47.0 mg ergosterol/g protein, transition temperature 26°. Arrhenius activation energies +38 and +86 KJ/mole; ●——● 105 mg ergosterol/g protein, transition temperature 18°, Arrhenius activation energies +39 and +80 KJ/mole.

FIGURE 2

The effect of sterol composition on the temperature of the discontinuity in the Arrhenius plots of ATPase activity.

The temperature dependence of the ATPase activity of the ten preparations of mitochondria in Table 1 was determined as described in Methods. The temperatures of the discontinuities were determined graphically as shown in Figure 1.

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containing different levels of ergosterol are compared, profound differences are observed (Figure 1). In each preparation there is a discontinuity in the Arrhenius plot, but the temperature of the transition decreases as the sterol content of the mitochondria is increased. The activation energies are approximately the same for mitochondria containing 47 and 105 mg ergosterol/g protein, being about 40 KJ and 85 KJ/mole respectively above and below transition temperature, but the activation energies of the extensively depleted organelles (7.3 mg ergosterol/g protein) are somewhat lower (+29 and +71 KJ/mole). However, since the changes in activation energies of the extensively sterol-depleted organelles may be due to the higher proportion of non-mitochondrial ATPases, no great significance is presently attached to the observation. The results of ten individual experiments are summarized in Figure 2, which indicates that

there is a clear relationship between the sterol content of the mitochondrial membranes and the temperature of the discontinuity in the Arrhenius plots for ATPase activity. The temperature of the transition in the Arrhenius plot is inversely proportional to sterol content of the mitochondrial, and decreases by up to 17° as the sterol content of the membranes is decreased from 105 to 7.3 mg/g protein.

The present report adds to the large body of evidence that membrane lipid composition influences the activity of membrane-bound enzymes. It has previously been shown that anaerobic yeast cells, grown in the presence of Tween 80 but with limiting supplements of ergosterol, have a normal total lipid, fatty acid, glyceride and phospholipid composition, but the amount of cellular squalene increases as the sterol content decreases (27). Thus, it still remains to be established whether the large changes in the transition temperature of Arrhenius plots for mitochondrial ATPase are purely due to changes in sterol composition, or are also influenced by changes in mitochondrial squalene content. We consider it likely that the change in sterol composition is the main factor, as a lowering of the transition temperature for the gel to liquid transition is produced by the addition of cholesterol to a number of artificial phospholipid systems (1, 13-16). It is anticipated that further information on the role of sterols in biological membranes will be provided by the substitution of different sterols in the lipids of anaerobically grown yeast cells, and the use of sterol mutants of yeast to manipulate membrane sterol composition.

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REFERENCES

1. Oldfield, E. and Chapman, D. FEBS Letters 23, 285 (1972).
2. Raison, J.K. J. Bioenergetics 4, 285 (1973).
3. Esfahani, M., Limbrick, A.R., Knutton, S., Oka, T. and Wakil, S.J. Proc. Nat. Acad. Sci. U.S. 68, 3180 (1971).
4. Wilson, G. and Fox, C.F. J. Mol. Biol. 55, 49 (1971)
5. Mavis, R.D. and Vagelos, P.R. J. Biol. Chem. 247, 652 (1972).
6. Schairer, H.U. and Overath, P. J. Mol. Biol. 44, 209 (1969).
7. Steim, J.M., Tourtellotte, M.E., Reinert, R.N., McElhaney, R.N. and Rader, R.L. Proc. Nat. Acad. Sci. U.S. 63, 104 (1969).

8. Proudlock, J.W., Haslam, J.M. and Linnane, A.W. J. Bioenergetics 2, 327 (1971).
9. Haslam, J.M., Proudlock, J.W. and Linnane, A.W. J. Bioenergetics 2, 351 (1971).
10. Raison, J.K., Haslam, J.M., Cobon, G.S., McMurchie, E.J. and Linnane, A.W. In preparation.
11. Ainsworth, P.J. and Tustanoff, E.R. Biochem. Biophys. Res. Commun. 47, 1299 (1972).
12. Electr, S. and Keith, A.D. Proc. Nat. Acad. Sci. U.S. 69, 1353 (1972).
13. Demel, R.S., Kinsky, S.C., Kinsky, C.B. and van Deenen, L.L.M. Biochim. Biophys. Acta 150, 655 (1968).
14. de Gier, J., Mandersloot, J.G. and van Deenen, L.L.M. Biochim. Biophys. Acta 173, 143 (1969).
15. Ladbrooke, B.D., Williams, R.M. and Chapman, D. Biochim. Biophys. Acta 150, 333 (1968).
16. Oldfield, E. and Chapman, D. FEBS Letters 21, 302 (1972).
17. Andreason, A.A. and Stier, T.J.B. J. Cell. Comp. Physiol. 41, 23, (1953).
18. Bloomfield, D.K. and Bloch, K. J. Biol. Chem. 235, 337 (1960).
19. Bloch, K., Baronowsky, B., Goldfine, H., Lennarz, W.J., Light, R., Norris, A.T. and Scheuerbrandt, G. Fed. Proc. 20, 921 (1961).
20. Proudlock, J.W., Wheeldon, L.W., Jollow, D.J. and Linnane, A.W. Biochim. Biophys. Acta 152, 434 (1968).
21. Kemp, A., Groot, G.S.P. and Reitsma, H.J. Biochim. Biophys. Acta 180, 28 (1969).
22. Wallace, P.G., Huang, M. and Linnane, A.W. J. Cell. Biol. 37, 207 (1968).
23. Watson, K., Haslam, J.M. and Linnane, A.W. J. Cell. Biol. 46, 88 (1968).
24. Klein, H.P. J. Bacteriol. 69, 620 (1955).
25. Pullman, M.E., Penefsky, H.S., Datta, A. and Racker, E. J. Biol. Chem. 235, 3322 (1960).
26. Monroy, G.C. and Pullman, M.E. (1967). In Methods in Enzymology (eds Estabrook, R.W. and Pullman, M.E.), 10, 510. Acad. Press, N.Y.
27. Jollow, D., Kellerman, G.M. and Linnane, A.W. J. Cell. Biol. 37, 221 (1968).