The Effect of Changed Sterol Composition on the Kinetics of Mitochondrial Membrane Enzymes in the Yeast Saccharomyces cerevisiae

SAHAR AL-MAHDAWI and J. M. HASLAM

Department of Biochemistry, University of Liverpool, P.O. Box 147, Liverpool L69 3BX, U.K.

The effect of sterols on the kinetics of membrane-bound enzymes was examined. Two strains of the yeast Saccharomyces cerevisiae were used for this purpose: the wild-type haploid strain, S288C, and the lipid auxotroph mutant Ole-3 (Resnick & Mortimer, 1966). The primary lesion in the Ole-3 mutant is the loss of δ-aminolaevulinate synthetase (EC 2.3.1.37) (Woods et al., 1975). Consequently, the mutant lacks all cytochromes and the products of enzymes containing cytochromes, including unsaturated fatty acids and ergosterol (Bard, 1972; Bard et al., 1974). Astin et al. (1977) have shown that this mutant may be used specifically to manipulate the total sterol content of mitochondrial membranes by growing the organism in media containing different concentrations of δ-aminolaevulinate plus excess Tween 80 to supply unsaturated fatty acids.

Cells were grown aerobically in Saccharomyces salts media (Wallace et al., 1968) containing Difco yeast extract (1%, w/v), Oxoid bacteriological peptone (0.5%, w/v) and ethanol (1%, w/v) as carbon source. Varying the concentrations of δ-aminolaevulinate in the media from 10 to 200mg/litre increased the sterol and cytochrome content of the mitochondria, and the respiratory activity was enhanced (Astin et al., 1977). Mitochondria isolated from these cells were used for the measurement of respiratory activity, with pyruvate plus L-malate as substrate; the oxidation of these substrates involves the oxidation of NADH by the complete respiratory chain. The activity of oligomycin-sensitive ATPase* (EC 3.6.1.3) of the mitochondria was also measured.

As the concentration of δ-aminolaevulinate was increased from 10 to 200mg/litre, the sterol content of Ole-3 cells increased from 9.5 to 18.4μg/mg of mitochondrial protein. Arrhenius plots of oligomycin-sensitive ATPase activity and of pyruvate plus L-malate oxidation showed discontinuities; the temperature of the discontinuity decreased from 22 to 12°C for ATPase activity and from 19.8 to 11.8°C for respiratory activity as the sterol content doubled.

Plots of the transition temperature against sterol content indicated an inverse relationship between the sterol content of the mitochondrial membrane and the temperature of discontinuity in the Arrhenius plots of membrane-bound enzyme activities. A similar relationship was shown by Cobon & Haslam (1973) for the ATPase activity of mitochondria from anaerobically grown yeast cells.


* Abbreviation: ATPase, adenosine triphosphatase.