THE EFFECT OF ALTERED STEROL COMPOSITION ON CYTOCHROME OXIDASE AND S-ADENOSYLMETHIONINE: Δ 24 STEROL METHYLTRANSFERASE ENZYMES OF YEAST MITOCHONDRIA

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Received February 25,1974

SUMMARY

Arrhenius kinetics of two mitochondrial enzymes, cytochrome oxidase and S-adenosylmethionine: Δ 24 sterol methyltransferase were analyzed in wild-type and sterol mutant strains of yeast. Temperature effects on the enzymes isolated from the ergosterol producing wild-type and nystatin resistant mutants (major sterol $\Delta^{8(9),22}$ ergostadiene-3-β-ol) were compared. Transition temperatures were lower in both mutant strains compared to wild-type. Lipid analysis shows a relationship between sterol content and the temperature dependent transition phases.

It is now widely accepted that the chemical composition of a membrane directly influences and modifies enzyme activities associated with these structures (for review see 1). Early work has shown that many membrane associated activities exhibit biphasic Arrhenius kinetics and that the temperature at which the transition occurs correlated well with the phase change of the membrane from a gel to liquid - crystalline state (2). It was subsequently demonstrated that the temperature of these transitions could be raised or lowered by altering the fatty acid composition of the membranes (3,4).

Many reports using in vitro bilayer systems have shown that sterol lipids increase the order and rigidity in these artificial systems (5,6,7). Darke et. al (7) also have shown that the sterol nucleus extends into the inner hydrocarbon layers of the membrane while the 3' hydroxy group is associated with the polar end of the phospholipid. This insertion of the essentially planar sterol lipid between the fatty acid chains causes an increase in rigidity of these membranes (7).

Cobon and Haslam (8) have recently demonstrated that changes in ergosterol levels in yeast produce concomitant changes in the phase transition temperature of mitochondrial ATPase. An inverse relationship between the sterol concentration in the mitochondria and the transition temperature was established.

The present communication presents evidence for the direct involvement of sterols in membrane associated activities. Mutant clones of Saccharomyces cerevisiae resistant to the polyene antibiotic nystatin produce $\Delta^{8(9),22}$ ergostadiene-3β-ol as their major sterol component in place of ergosterol (9,10). These mutants are respiratory competent enabling us to study the effects of two different sterols on the phase transition temperature for the mitochondrial enzymes cytochrome oxidase and S-adenosylmethionine: Δ 24 sterol methyltransferase.

MATERIALS AND METHODS

Saccharomyces cerevisiae, strains 3701B, a haploid uracil auxotroph; nys-3, a nystatin resistant mutant received from Dr. R.A. Woods; and 3701B-n3, a nystatin resistant mutant isolated from 3701B were used throughout this report. The sterol lipids of 3701B-n3 were analyzed as previously described (9,10) and the major constituent was shown to be $\Delta^{8(9),22}$ergostadiene-3β-ol as in nys-3. The organisms were grown in complete medium (11) with either 5% glucose (to repress mitochondria) or 2% ethanol, (to induce mitochondria) as carbon sources. All cultures were grown at 27 C.

Mitochondria and promitochondria isolation procedures have already been described (11,12). Cytochrome oxidase activity and lipids present in the mitochondria were determined as previously described (13). The sterol methyltransferase was assayed by measuring the incorporation of the [^{14}C] methyl group of S-adenosylmethionine (SAM) into the non-saponifiable sterol lipids (11). The isolation of zymosterol has been previously published and 4 μmoles were added to each reaction mixture (11).

The Arrhenius kinetics of cytochrome oxidase were measured using a

refrigerated water bath temperature block in a Zeiss PMQ spectrophotometer. The enzyme preparations were placed in small tubes in the water bath and allowed to equilibrate. The temperature was then increased at a rate of 10 C per hr, and the enzymes assayed at selected temperatures. Enzyme preparations from all three of the organisms were run simultaneously to minimize temperature differences between experiments.

The sterol methyltransferase enzyme was added to precooled buffer mixtures along with the zymosterol and allowed to equilibrate at each temperature for 15 min. The reaction was initiated by the addition of 0.25 μCi of [^{14}C] methyl-S-adenosylmethionine (2.5 nmoles). The reaction was incubated for 30 min with shaking and was terminated by the addition of 1.0 ml of cold 10% TCA. The protein was removed by trichloroacetic acid precipitation, the sterol released with DMSO, and extracted into n-hexane as reported by Monner and Parks (14).

RESULTS

Cytochrome oxidase was selected because of the vast amount of information accumulated on this enzyme (13,15). Fig. 1 shows Arrhenius kinetics of cytochrome oxidase from mitochondria isolated from 3701B, nys-3, and 3701B-n3. The transition temperature of 10 C for 3701B is in close agreement with that previously reported (15). Both nys-3 and 3701B-n3 show a distinct deviation from this temperature. We wish to emphasize the controls employed by using the isogenic strains 3701B and 3701B-n3 while comparing the nystatin resistant strains 3701B-n3 and nys-3. The fact that both nystatin resistant mutants have identical but lower transition temperatures than the ergosterol producer precludes the possibility of a protein difference between 3701B and nys-3. While Fig. 1 distinctly shows a difference in transition temperatures, it does not demonstrate that this difference is due to the sterol lipid present. Table I shows the composition of the lipids associated with cytochrome oxidase. The presence of oleic and palmitoleic acids as the predominant fatty acids previously has been documented (13,16). The sterol levels are similar quanti-

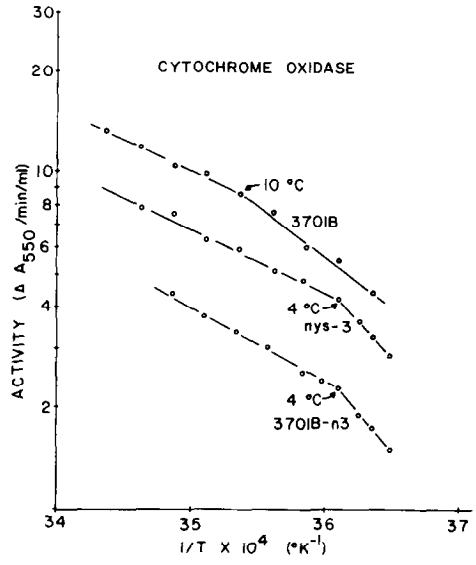

Fig. 1 Arrhenius kinetics of cytochrome oxidase from mitochondria of wild-type (3701B) and nystatin resistant mutants (3701B-n3 and nys-3) of Saccharomyces cerevisiae.

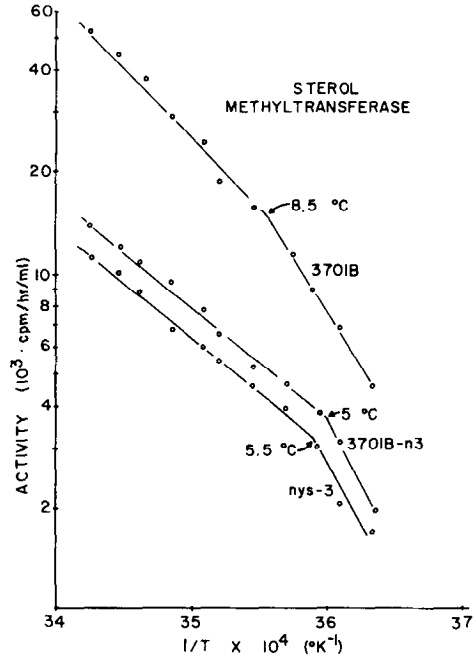

Fig. 2 Arrhenius kinetics of S-adenosylmethionine: Δ 24 sterol methyltransferase from wild-type (3701B) and nystatin resistant mutants (3701B-3n and nys-3) of Saccharomyces cerevisiae.

tatively, but differ by the predominance of ergosterol in 3701B and the $\Delta^{8(9),22}$ ergostadiene-3-β-ol in the nystatin resistant organisms.

We recently established that the sterol methyltransferase enzyme is a component of both mitochondria and promitochondria (11). Therefore, we decided to examine the effect of temperature on this enzyme. Figure 2 shows these results with the sterol methyltransferase from mitochondria of all three organisms. Again there is a distinct difference in the transition temperatures in 3701B and the two nystatin resistant mutants. Analysis of the fatty acid components and sterol levels associated with this enzyme yield essentially the same results shown in Table I.

TABLE I

The lipid composition of mitochondria and promitochondria isolated from Saccharomyces cerevisiae, strains 3701B (wild-type), 3701B-n3 (nystatin resistant mutant isolated from 3701B) and nys-3 (nonisogenic strain resistant to nystatin).

Lipid Component ug/mg protein	Organism	Organelle	
		Mitochondria	Promitochondria
Total Sterol[1]	3701B	175	28
	3701B-n3	150	33
	nys-3	200	32
Palmitoleic acid	3701B	150	125
	3701B-n3	180	140
	nys-3	175	170
Oleic acid	3701B	400	320
	3701B-n3	350	300
	nys-3	380	350

[1] Sterol is predominantly ergosterol in 3701B and $\Delta^{8(9),22}$ ergostadiene-3-β-ol in 3701B-n3 and nys-3.

In contrast, data collected on the methyltransferase from promitochondria isolated from all three strains showed a single distinct transition temperature around 7C. The lipid analysis of the promitochondria is shown in Table I. The fatty acids present are the same as those in mitochondria,

and the relative proportions of each with respect to protein is similar. The greatest difference between the promitochondria and mitochondria is in sterol levels. Promitochondria contain about 1/5 as much sterol lipid.

DISCUSSION

This report has presented evidence that sterol lipids provide an active function in membranes of yeast mitochondria. The substitution of ergosterol with the $\Delta^{8(9),22}$ergostadiene-3-β-ol produces a distinct change in the temperature characteristics of two mitochondrial enzymes. This change was not detected with the methyltransferase from promitochondria. Since the fatty acid constituents from the mitochondria and promitochondria essentially are the same, differences in the transition temperatures are most likely due to changes in packing of the phospholipid portions due to the sterols. Since ergosterol is more planar than the Δ 8 sterol, it would be expected to produce a more tightly packed structure. This is partially substantiated by reports demonstrating that ergosterol is more effective in phospholipid packing than the single unsaturated sterol species cholesterol (Δ 5) or lanosterol (Δ 8) (5,6).

The profound changes in temperature characteristics of these mitochondrial enzymes may have further manifestations in vivo. We may see temperature differences with respect to optimal growth or enzyme stabilities in these strains. Preliminary experiments already indicate the nystatin resistant mutants are much more sensitive to high growth temperatures.

ACKNOWLEDGMENTS

This research was supported by grants from the National Science Foundation (GB-31119) and the U.S. Public Health Service (AM-05190-11). The excellent technical assistance of Mrs. Elizabeth MacDonald is gratefully acknowledged. The Oregon Agricultural Experiment Station technical paper no. is 3772.

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