The Effect of Altered Membrane-Lipid Composition on Enzyme Activities of Outer and Inner Mitochondrial Membranes of *Saccharomyces cerevisiae*

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Summary

Enrichment of yeast mitochondrial membranes with specific unsaturated fatty acids was achieved by growing this organism in minimal medium supplemented with the desired unsaturated fatty acid. The alteration in the membrane lipid milieu resulted in changes in both catalytic and Arrhenius kineties of enzymes characteristic of the outer and inner mitochondrial membranes. The implication that lipids are involved in the expression of the activity of kyneurine hydroxylase, a marker enzyme of the outer mitochondrial membrane, is presented.

1. Introduction

Yeast mitochondria offer a useful system for the study of the biosynthesis of membranes and their structure-function relationship. Since the relative structure and hence the functions of these mitochondria can be altered either be manipulating their growth conditions or their metabolic environment, yeast lend themselves to this type of investigation [1-3]. A number of early workers using unsaturated fatty acid auxotrophs

of *S. cerevisiae* and *E. coli* have shown that alterations in the fatty acid moeity of membrane phospholipids lead to changes in many of the membrane-dependent functions [4-10]. However, a comparative study of changes in the activities of enzymes characteristic of outer and inner membranes of mitochondria due to such an alteration has not been made. In this present communication results are presented which show that manipulation of the membrane lipids leads to apparent differences in both catalytic function and Arrhenius kinetics of enzymes of the outer and inner membranes of yeast mitochondria (Kynurenine hydroxylase and oligomycin-sensitive ATPase respectively).

2. Materials and Methods

Saccharomyces cerevisiae, strain 77, were grown aerobically on either complete medium supplemented with ergosterol and Tween 80 [11] or minimal medium supplemented with ergosterol and 0.02% unsaturated fatty acids [12] and harvested as previously described [11]. Mitochondria were prepared by rupturing washed cells in a Braun Homogenizer followed by differential centrifugation using the procedure of Henson *et al.* [13]. The resulting mitochondria were suspended in 0.25M sucrose containing 10 mM Tris-HCl buffer, pH 7.4 (5-10 mg protein/ml). Oligomycin-sensitive ATPase and kynurenine hydroxylase activities were determined by the methods described by Tzagaloff [14] and Scholt *et al.* [15] respectively. A constant temperature refrigerated bath (± 0.1°) was used for all incubations. Lipids were extracted from the isolated mitochondria by the procedure of Folch *et al.* [16]. Methyl esters of the extracted fatty acids were prepared and resolved on a EGSS-X column using a Beckman GC-45 Chromatograph. Protein was determined by the procedure outlined by Lowry *et al.* [17].

3. Results and Discussions

Fatty Acid Composition of Mitochondrial Membranes

TABLE I

Percentage fatty acid composition of the mitochondrial membranes obtained from *S. cerevisiae* grown on minimal medium supplemented with different fatty acids.

Fatty acid Supplemented	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3
Tween 80	1	2	13	33	3	33	Trace	nil
Oleic	<1	4	19	18	6	40	Trace	nil
Linoleic	<1	2	20	14	8	9	42	nil
Linolenic	<1	3	21	10	7	7	Trace	44

% fatty acids were determined by gas chromatography as described under "Methods". Temperature was programmed from 160^{o}-190^{o} at a rate of 2^{o} per minute with nitrogen as carrier gas.

To determine whether supplementation of the minimal medium with various unsaturated fatty acids had any effect on the fatty acid composition of mitochondria, analysis of these mitochondrial lipids were undertaken. The results in Table I reveal that supplementing the growth medium with a specific fatty acid results in an enrichment of the resulting mitochondrial lipids with the fatty acid added. Such an enrichment of membranes from whole yeast have also been observed by Suomalainen and Keranan [18] and Keith *et al.* [19].

Effect of Mitochondrial Membrane Fatty Acid Composition on Kynurenine hydroxylase and ATPase activities

Having shown that the fatty acid composition of yeast mitochondrial membrane lipids can be manipulated, it was of interest to determine whether such changes in the lipid moieties reflect in any way differences in the specific activities of enzymes of the outer and inner mitochondrial

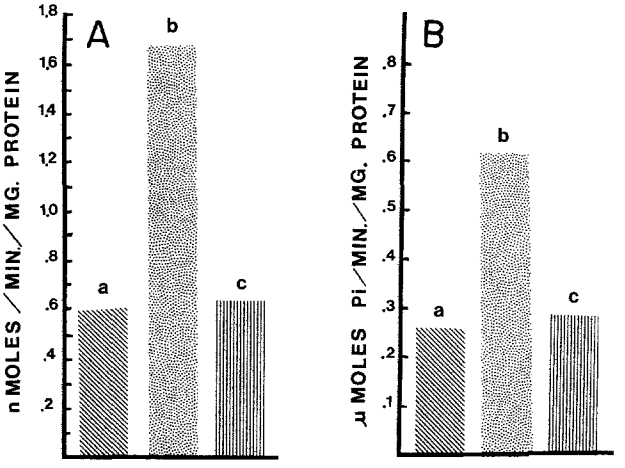

Figure 1. Effect of supplementation of minimal medium with different unsaturated fatty acids on the activities of Kynurenine hydroxylase and ATPase in yeast mitochondrial membranes.

Kynurenine hydroxylase (Figure 1A) and ATPase (Figure 1B) activities were determined by the method described by Schott *et al.* and Tzagoloff respectively. Mitochondria were obtained from cells grown on (a) complete medium supplemented with Tween 80 (b) minimal medium supplemented with oleate and (c) minimal medium supplemented with linoleate.

membranes. The results depicted in Figure 1A shows that the activity of the outer mitochondrial marker enzyme Kynurenine hydroxylase [20] is altered depending on the level of unsaturation of the fatty acid supplementing the membrane. Similar results were obtained for the inner mitochondrial marker enzyme ATPase [14] (Figure 1B). The results also show that linoleate enriched mitochondria show no significant effect on the activities of these enzymes when compared to the activities from mitochondria obtained from cells grown on complete medium. Oleate-enriched mitochondria however, shows an enhancement of these enzyme activities as measured as a function of their specific activity.

Effects of Fatty Acid Composition on the Temperature Characteristics of Kynurenine hydroxylase and ATPase

Earlier observations from this and other laboratories indicate

that the transition temperatures in Arrhenius plots of the enzymes of the inner membrane of mitochondria vary with the growth conditions and the lipid environment [2,12,21,22]. However, it is not known whether the enzymes characteristic of the outer membrane of mitochondria respond similarly. Consequently, studies were conducted to test this possibility using Kynurenine hydroxylase as a marker of the outer membrane and to compare its temperature characteristics with those of the inner membrane marker enzyme, ATPase. While detailed studies are underway, our initial data indicate that Kynurenine hydroxylase exhibits a temperature break which is dependent on the fatty acid enrichment of the membrane lipid (Figure 2A). Thus, while oleate-membrane shows a break around 12^{o} the linoleate-membrane shows a break point around 8^{o}. The activation energies above and below the transition temperature are 9.6 and 21.1 k.cal/mole respectively for oleate-mitochondria, while the corresponding values for linoleate-mitochondria are 11.2 and 30.8 k.cal/mole. This can be compared with the Arrhenius plots for ATPase (Figure 2B) which also exhibit similar temperature breaks. The oleate mitochondria shows a transition at 12^{o} with activation energies of 13.6 and 32.2 k.cal/mole above and below the break respectively, while the linoleate mitochondria shows a transition at 9^{o} with activation energies of 13.6 and 38.7 k.cal/mole above and below this break respectively. Recent observations made by Watson *et al* (2) that the transition temperature of mitochondrial ATPase varied with growth conditions which reflect in turn changes in fatty acid composition of the membrane support our present results.

Thus, alterations of the membrane lipids seem to influence the catalytic and physical properties of the enzymes characteristic of both outer and inner mitochondrial membranes. Since phospholipids are the major constituents of the membranes, it is reasonable to conclude that the fatty acid manipulation of the membrane lipids employed in the present studies, reflects an alteration of the membrane phospholipids that are

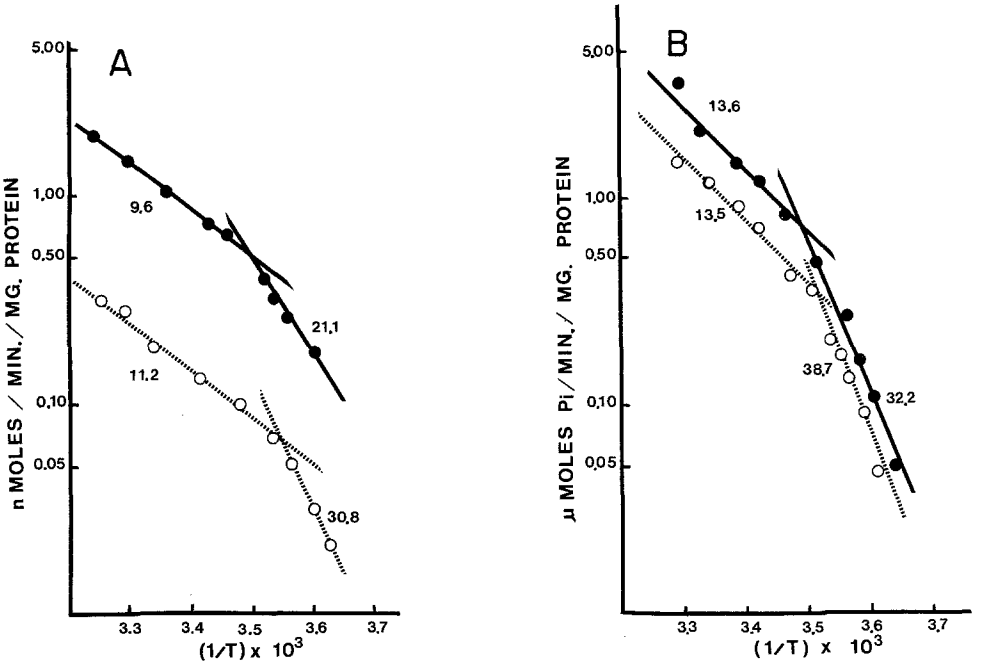

Figure 2. Arrhenius plots of yeast mitochondrial Kynurenine hydroxylase and ATPase.

Mitochondrial membranes were obtained from yeast grown on minimal medium supplemented either with oleate —●——● or linoleate -○- - - -○- . The numbers beside each lines denote the activation energies (k.cal/mole) calculated from the slope of the line.

A. Kynurenine hydroxylase

B. ATPase

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in the immediate vicinity of these enzymes. While the phospholipid requirement for the ATPase activity is well know, similar requirement for Kynurenine hydroxylase is not clearly understood. But, the results presented in this communication clearly demonstrates the involvement and the effect of lipid environment on the expression of this outer mitochondrial membrane enzyme. One explanation which could account for the enhancing effect observed in mitochondria from oleate grown cells is that it provides an environment which allows a spatially ideal arrangement of these enzymes

within the phospholipid milieu of the mitochondrial membranes (7). This conclusion is further supported by the observations of Parks and coworkers (23,24) who showed that oleate was necessary for the maintenance of respiratory competency in yeast. In addition to phospholipids, it has recently been reported that ergosterol also affects the physical characteristic of the membrane bound ATPase in yeast (25).

The implications of the altered lipid composition on enzymes of both the outer and inner mitochondrial membranes are immediately obvious in relation to the study of mitochondriogenesis. The current work in this laboratory is aimed at establishing the role of precursors in the formation of promitochondrial membranes and the ultimate fate of these structures during the differentiation into competent organelles.

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