Bioenergetics (1971) **2**, 135–140.

Effect of Phospholipids from Saccharomyces Cerevisiae at Different Stages of Development on Restoration of Succinooxidase Activity in Lipid-Depleted Mitochondria

Enrico Bertoli, Giuliano Barbaresi and Adriano Castelli

Istituto di Chimica Biologica—Università Cattolica, Roma (Italy)

and Giorgio Lenaz

Istituto di Chimica Biologica, Università di Bologna (Italy)

Received 10 March 1971

Abstract

Phospholipids extracted from *Saccharomyces cerevisiae* at different stages of development after glucose repression contain three major fatty acids: palmitic, palmitoleic and oleic. The ratio palmitic : palmitoleic strongly decreases beginning at the 6th hour of growth.

To test the effect of fatty acid composition and in particular of unsaturation on succinoxidase activity, all these phospholipids, phospholipids from commercial yeast, and Asolectin were incubated with lipid-depleted yeast mitochondria. The amount of P bound was not much different for the various phospholipids; succinoxidase activity was restored best by Asolectin; the least effective reactivation was given by phospholipids from yeast at the middle stages of growth. There are not great differences between the various phospholipids and there is no correlation with unsaturation. If we compare the pattern of appearance of respiration during morphogenesis of yeast mitochondria with the pattern of the capability of the phospholipids from cells at different stages of mitochondrial morphogenesis to restore activity of lipid-depleted yeast mitochondria, we find no correlation. The results of this investigation are consistent with the idea that changes in phospholipids and changes in enzyme activities are not linked by a causal relation.

Introduction

Glucose repression in *Saccharomyces cerevisiae* is accompanied by lack of respiratory activity and absence of microscopically demonstrable normal mitochondria, although certain mitochondrial activities such as the oligomycin-sensitive ATPase are present.[1,2] In the repressed condition and also in anaerobiosis (where are found more drastic reductions of the mitochondrial structures and functions)[3,4] the lipid composition is changed with a tendency to a higher saturation of the lipids.[5–7] The concomitance of the high saturation of the lipids with scarce or no respiratory activity raises the question whether the two observations are in a cause-to-effect relationship. Up to date only general correlations between phospholipid content and respiration in yeast have been invoked.[9,10]

The hypothesis that lipid unsaturation may be correlated to respiratory activity in developing yeast may receive theoretical and experimental support from the recognized

importance of the hydrophobic moiety of the phospholipids in the bonds with the proteins of the inner mitochondrial membrane, such as the respiratory proteins.[8, 11–13] Unsaturated fatty acids in the phospholipids appear to favor the formation of hydrophobic interactions with the respiratory chain since it was found that myelin phospholipids, which have lower unsaturation and longer carbon chains than the physiological mitochondrial phospholipids, are much less effective in restoring succinoxidase and DPNH oxidase activities in beef heart mitochondria.[14] The specific fatty acid compositions of different membranes, with more unsaturated fatty acids in mitochondria than in myelin are in accord with this idea.[15]

It is clear that the respiratory activity in the repressed stage of yeast is absent for the lack of (complete) respiratory proteins, since in such condition the typical cytochrome spectra are lacking.[3] The hypothesis therefore can be dismissed *a priori* that absence of respiratory activity depends directly upon lack of reactivation by a "wrong" type of phospholipids in the membrane. On the other hand the possibility has to be explored that respiratory proteins, although synthesized in an inactive form are not organized in a functional inner mitochondrial membrane for the lack of phospholipids having the correct structure for the assembly of the membrane.

To test this hypothesis, in this investigation we have tried the effect of mixed phospholipids, extracted from yeast cells at different stages of de-repression and analyzed for fatty acid composition, on the restoration of the physiological phospholipid content and of succinoxidase activity of lipid-depleted mitochondria of mature cells of *Saccharomyces cerevisiae*.

Methods

Culture Conditions and Preparation of the Mitochondria

Saccharomyces cerevisiae, strain ATCC7754 was grown in the identical experimental conditions previously described[1] and aliquots were withdrawn at different times of growth in order to extract the lipids.

Commercial yeast cells were used to prepare the mitochondria with the method of Schatz.[16]

Lipid-depleted mitochondria. Acetone extraction (90% aqueous acetone) was employed to extract the phospholipids as described by Fleischer and Fleischer.[17]

Phospholipids. Yeast cells at different stages of growth and cells from commercial yeast were homogenized with a Braun cell disruptor and the homogenate was centrifuged at 2000 × g. The supernatant was centrifuged at 105,000 × *g* in the No. 40 rotor of the Spinco model L ultracentrifuge in order to collect a membranous fraction comprising large particles (mitochondria) and small particles together. This fraction was homogenized in a mixture of chloroform and methanol (2:1 v/v) and lipids were extracted according to Folch *et al.*[18] The phospholipids were separated with the silicic acid batch procedure of Marks *et al.*[19]

The phospholipids obtained in this way and commercial soybean phospholipids (Asolectin) were dispersed by ultrasonic irradiation with a Branson sonifier, as described by Fleischer and Fleischer.[17]

Gas-liquid chromatography. Fatty acids from the phospholipid vesicles were methylated and gas-liquid chromatography was accomplished as described previously.[9, cf. 20]

Succinoxidase activity. The lipid-deficient mitochondria (LDM) were assayed for succinoxidase activity in presence of different additions as described by Lester and Fleischer[21] with a Braun respirometer. To the succinoxidase medium phospholipid dispersions of different origin were added at different concentrations, and CoQ and cytochrome *c* additions were necessary to observe activity.

Phospholipid binding. The binding was determined directly on the mitochondria employed for succinoxidase assays. The content of each Warburg flask was centrifuged at 20,000 rpm for 10 min in the No. 40 rotor of the Spinco and washed once in 0·25 M sucrose, 0·01 M Tris Cl. The residue resuspended in sucrose-Tris was assayed for protein and phosphorus.

Analytical determinations. Protein was determined with the biuret method of Gornall *et al.*[22] and phosphorus with the method of Marinetti.[23]

Results

Fatty acid composition of the phospholipids. In Table I is reported the fatty acid composition of Asolectin, of the mixed phospholipids extracted from commercial yeast, and of the

TABLE I. Fatty acid composition of different phospholipids used in this investigation. Only the major fatty acids are reported. The values are expressed as per cent of total fatty acids

		Phospholipids from yeast					
Fatty acid*	Asolectin	commercial	4th hr	6th hr	8th hr	10th hr	24th hr
16:0	17·1	11·4	37·8	8·6	13·0	7·3	6·3
16:1	3·2	41·1	28·7	59·3	50·6	60·0	61·7
18:0	3·1	3·5	traces	traces	traces	traces	traces
18:1	7·1	32·7	33·5	32·1	36·4	32·7	32·0
18:2	58·6	—	—	—	—	—	—
18:3	8·7	—	—	—	—	—	—

* Number of carbon atoms: number of double bonds.

mixed phospholipids extracted from yeast at different stages of de-repression (4th, 6th, 8th, 10th and 24th hour of growth). Asolectin has a high content of unsaturated fatty acids (mainly linoleic acid); yeast phospholipids are high in saturated and monounsatured acids. There is a striking change of fatty acid composition during de-repression; the content of palmitic acid sharply decreases after the 4th hour of growth and the content of palmitoleic acid increases. The ratio saturated : unsaturated fatty acids falls after the 4th hour of growth.

Effect of yeast phospholipids on Succinoxidase. Table II shows a summary of the effect of all the different phospholipids studied at different levels on the restoration of succinoxidase activity. At all levels studied, Asolectin appeared more effective for restoration of activity than the more physiological yeast phospholipids. The phospholipids extracted from the commercial yeast were almost as effective as Asolectin. The phospholipids from yeast cells at different stages of growth had a somewhat lower activity. The least active were those prepared from yeast at the 8th hour of de-repression.

TABLE II. Effect of mixed phospholipids on restoration of succinoxidase activity of LDM from S. cerevisiae

Mixed phospholipids	5 μg	10 μg	15 μg	20 μg	25 μg	45 μg
4*	0·049†	0·068	0·084	0·094	0·104	0·129
6*	0·068	0·088	0·102	0·114	0·117	0·124
8*	0·039	0·051	0·062	0·073	0·083	0·097
10*	0·051	0·070	0·083	0·012	0·097	0·111
24*	0·065	0·088	0·098	0·098	0·104	0·115
Commercial Yeast	0·066	0·096	0·104	0·115	0·136	0·163
Asolectin	0·079	0·091	0·115	0·134	0·154	0·177

* Phospholipids extracted from *S. cerevisiae* at different hours of cell growth.
† Specific activity (μatoms O_2/min/mg protein).

Binding of the phospholipids. The amounts of phospholipids which were bound to the LDM during the incubation experiments are shown in Fig. 1. There seems to be a rough correlation between amount of phospholipid which is taken up by the particle and restoration of succinoxidase activity. Less P was found in the mitochondria incubated with phospholipids of yeast at the 8th hour of de-repression, and more P in those incubated with Asolectin or phospholipids from commercial yeast.

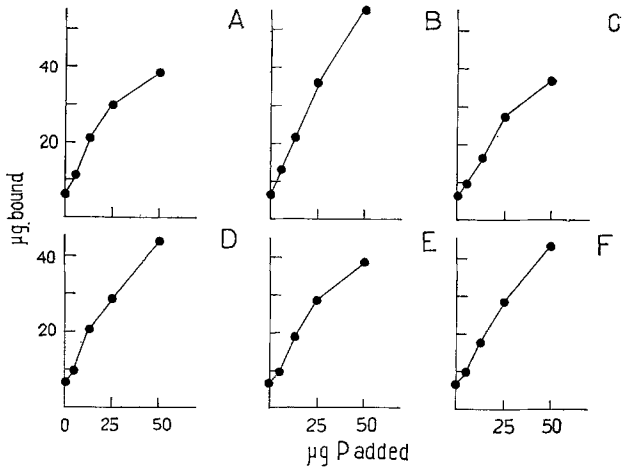

Figure 1. Phospholipid binding to LDM from S. cerevisiae. Phospholipids from yeast (A) 4th hour, (B) 6th hour, (C) 8th hour, (D) 10th hour, (E) 24th hour, (F) commercial.

Discussion

There is little doubt that fatty acid composition of phospholipids may affect restoration of respiratory activity in lipid-depleted mitochondria, although the mitochondrial proteins have great tolerance of the type of phospholipids which are bound to the membrane.[14, 24]

In this investigation we have found that phospholipids having a complex composition

and high unsaturation (Asolectin) are best in restoring succinoxidase activity in lipid-deficient mitochondria from yeast. On the other hand, little differences were found between phospholipids obtained from yeast at different stages of de-repression from glucose, in spite of the differences in fatty acid composition. As for the fatty acids, these phospholipids have a simple composition with only three major fatty acids: palmitic, palmitoleic and oleic. It appeared therefore possible to easily compare fatty acid composition and respiratory activity. Changes in fatty acid composition concern the 16-C fatty acids; during de-repression palmitic acid decreases and palmitoleic acid rises, with a significant decrease of the ratio saturated:unsaturated acids. In spite of the difference in unsaturation, the phospholipids (e.g. those obtained from yeast at the 4th hour and at the 24th hour of growth) show little difference in restoration of succino-oxidase activity. There is a lower activity of the phospholipids at the 8th hour, but the

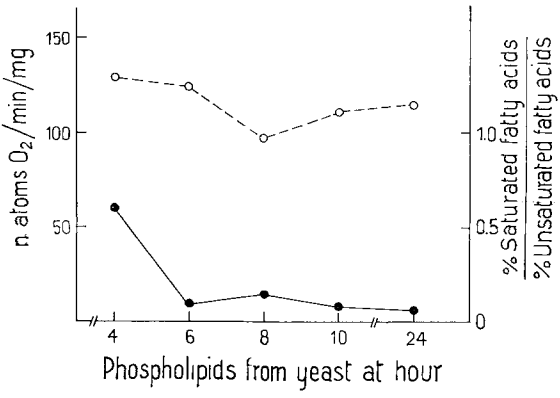

Figure 2. Comparison between the maximal restoration of succinoxidase activity by different mixed phospholipids and their fatty-acid composition. ●——●, ratio saturated: unsaturated fatty acids of the phospholipids; ○ - - - - ○, succinoxidase activity restored by the same phospholipids (45 μg of P for each assay).

pattern of restoration of activity has no relation with the pattern of fatty acid composition (Fig. 2). The differences in this case must therefore be related to the types of phospholipids present, in other words, to the hydrophilic moiety of the phospholipids.

If we compare the pattern of appearance of the respiration during the morphogenesis of yeast mitochondria with the pattern of the capability of the phospholipids from cells at different stages of mitochondrial morphogenesis to restore activity of lipid-depleted mature yeast mitochondria, we find no correlation at all (Fig. 3). On the basis of these results we should therefore exclude that specific phospholipids have a main role during the biogenesis of yeast mitochondria in the appearance of respiratory activity. We must be very cautious however in transposing these *in vitro* results to what happens *in vivo* during the assembly and organization of the mitochondrial membrane, where many factors should be operative at the same time; moreover succinoxidase is one activity of the mitochondria, and more activities should be examined before reaching a definite conclusion. The results of this investigation appear consistent with the idea that changes

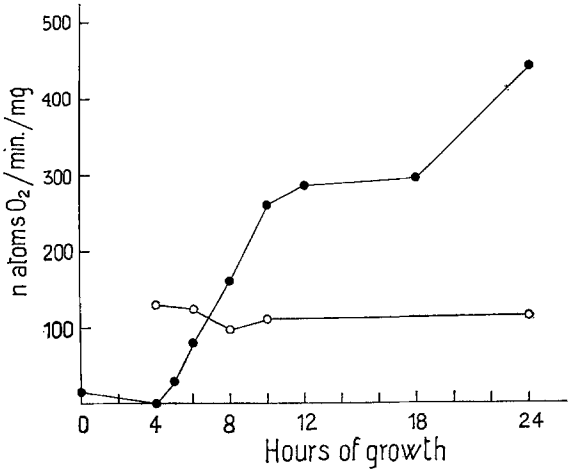

Figure 3. Comparison between succinoxidase activity of Yeast mitochondria during the growth phases and succinoxidase activity of lipid-deficient yeast mitochondria supplemented with phospholipids extracted from yeast at different hours of growth. ●——●, succinoxidase activity of intact mitochondria during growth (ref. 1); ○——○, succinoxidase activity restored by phospholipids extracted from yeast at different hours of growth.

in phospholipids and changes in enzyme activities are parallel and concomitant consequences of the same stimulus during mitochondrial biogenesis, but they appear independent from each other and not linked by a causal relationship.

References

1. A. Castelli, G. Parenti-Castelli, E. Bertoli, and G. Lenaz, *Ital. J. Biochem.*, **18** (1969) 35.
2. G. Lenaz, G. P. Littarru, and A. Castelli, *FEBS Letters*, **2** (1969) 198.
3. P. G. Wallace and A. W. Linnane, *Nature*, 201 (1964) 1191.
4. P. G. Wallace, M. Huang, and A. W. Linnane, *J. Cell Biol.*, **37** (1968) 207.
5. D. Jollow, G. M. Kellerman, and A. W. Linnane, *J. Cell Biol.* **37** (1968) 221.
6. H. B. Lukins, D. Jollow, P. G. Wallace, and A. W. Linnane, *Aust. J. exp. Biol. med. Sci.*, **46** (1968) 651.
7. F. Paltauf and G. Schatz, *Biochemistry*, **8** (1969) 335.
8. G. Lenaz, A. M. Sechi, G. Parenti-Castelli, L. Masotti, A. Castelli, G. P. Littarru, and E. Bertoli, in *Autonomy and Biogenesis of Mitochondria and Chloroplasts*, N. K. Boardman, A. W. Linnane, and R. M. Smillie (eds.), p. 119, 1970.
9. A. Castelli, G. Barbaresi, and E. Bertoli, *Ital. J. Biochem.*, **18** (1969) 91.
10. L. Kovac, J. Subik, G. Russ, and K. Kollark, *Biochim. Biophys. Acta*, **144** (1967) 94.
11. D. E. Green and A. Tzagoloff, *J. Lipid Res.*, **7** (1966) 587.
12. G. Lenaz, A. M. Sechi, L. Masotti, and G. Parenti-Castelli, *Arch. Biochem. Biophys.*, **141** (1970) 79.
13. G. Lenaz, A. M. Sechi, G. Parenti-Castelli, and L. Masotti, *Arch. Biochem. Biophys.*, **141** (1970) 89.
14. J. Cabo-Soler, A. M. Sechi, G. Parenti-Castelli, and G. Lenaz, *J. Bioenergetics*, in press.
15. J. S. O'Brien, *Science*, **147** (1965) 1099.
16. G. Schatz, *Methods Enzymol.*, **10** (1967) 199.
17. S. Fleischer and B. Fleischer, *Methods Enzymol.*, **10** (1967) 406.
18. J. Folch, M. Lees, and G. H. Sloane-Stanley, *J. Biol. Chem.*, **226** (1957) 497.
19. P. A. Marks, A. Gellhorn, and C. Kidson, *J. Biol. Chem.*, **235** (1960) 2579.
20. A. T. James, *Methods Biochem. Anal.*, **8** (1960) 1.
21. R. L. Lester and S. Fleischer, *Biochim. Biophys. Acta*, **47** (1961) 358.
22. A. G. Gornall, C. J. Bardawill, and M. M. David, *J. Biol. Chem.*, **177** (1949) 751.
23. P. V. Marinetti, *J. Lipid Res.*, **3** (1962) 1.
24. S. Fleischer, G. Brierley, H. Klouwen, and D. B. Slautterback, *J. Biol. Chem.*, **237** (1962) 3264.