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ANNA SZKOPIŃSKA

FACTORS AFFECTING OXYGEN-INDUCED CHANGES IN THE ACTIVITY OF CTP-DEPENDENT LIPID KINASES IN YEAST

Institute of Biochemistry and Biophysics, Polish Academy of Sciences. Rakowiecka 36; 02-532 Warszawa, Poland

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Previous work from this Laboratory (Szkopińska *et al.*, 1988, *Arch. Biochem. Biophys.*, 266, 124-131) indicated that CTP is a phosphate donor for the synthesis of phosphatidic acid and dolichyl phosphates. The elucidation of the role of mitochondrial membranes and mitochondrial proteins (isolation of rho$^-$ mutant) as well as specific detergents and sterols has been the aim of this work.

Previous work from this laboratory [1] indicated that CTP plays a specific role as a γ phosphate donor for the synthesis of phosphatidic acid (PA) and dolichyl phosphates (DolP) in yeast.

The activity of the phosphorylating enzymes present in membrane fractions could be modified by the yeast growth conditions. The enzyme(s) from yeast grown aerobically favoured the synthesis of PA over DolP in the ratio 3:1, while membrane fraction from anaerobically grown yeast synthesized both compounds in the ratio 0.5:1, respectively. After oxygen adaptation membranes from yeast grown anaerobically reverted the synthesis of phospholipids to the ratio characteristic for aerobic membranes, see [1]. It seemed reasonable to think of mitochondria as organelles having the decisive influence on the regulation of synthesis of the discussed phospholipids. To verify this idea rho$^-$ mitochondrial mutant has been isolated.

The attempt to elucidate the reasons for different levels of synthesis of lipids depending on growth conditions was the aim of this work.

MATERIALS AND METHODS

Materials. [γ-^{32}P]CTP was synthesized from CTP and [^{32}P] orthophosphate by the modified method of Johnson & Walseth [2].

Enzyme preparation. The harvested cells of *Saccharomyces cerevisiae* strain

X-28-1A were broken with glass beads in a Brown homogenizer. The membrane fraction was prepared as described by Lehle & Tanner [3].

Mitochondria preparation. Yeast mitochondria were isolated in the presence of 0.6 M sorbitol and 0.5% bovine serum albumin as described in [4].

Induction of rho⁻ mutant. Yeast cells were grown in the YPglu medium containing 30 μg/ml of ethidium bromide for 24 h at 28°C in a flask wrapped in aluminium foil.

Biosynthesis of lipids. To 3 nmol of C_{95}dolichol dried under nitrogen, other components of the reaction mixture were added to a total volume of 0.1 ml. The incubation mixture contained 30 mM Tris/HCl (pH 7.5), 0.5 mM EDTA -Na, 5 mM mercaptoethanol, 30 mM $CaCl_2$, Nonidet P-40 or Tween 80 as indicated, 1 μM CTP, 1×10^6 cpm of [γ-^{32}P]CTP (5000 Ci/nmol) and appropriate amounts of protein. After incubation for 20 min at 30°C the reaction was stopped with 2 ml of chloroform:methanol (3:2, by vol.).

Lipid analysis. T.l.c. of the ^{32}P-labeled lipids was performed on Silica Gel G precoated plates in the solvent system chloroform:methanol:water (65:25:4, by vol.).

RESULTS

Mitochondrial enzyme. The dependence of the activity of the enzymes synthesizing PA and DolP on the aerobicity of the yeast growth media could suggest the participation of mitochondria in this synthesis. However, the specific activity of the enzymes present in the purified mitochondrial membranes was not as high as that of the crude membrane fraction containing mitochondrial as well as microsomal membranes. Nevertheless the ratio of both synthesized compounds (PA and DolP) remained the same as in crude membranes. The influence of phospholipids on the activity of membrane bound enzymes is well known. Our previous experiments [1] show that phosphatidylcholine (PC) may be the lipid cofactor of the investigated enzymes. Enrichment of mitochondrial membranes with PC resulted in a small (10-20%) but consistent stimulation of the maximal velocity of the reaction (Fig. 1), which however still did not account for the activity present in the total membrane fraction.

Effect of Tween and ergosterol. Yeast grown in the absence of oxygen are unable to synthesize ergosterol which is a predominant sterol (90% of total sterol lipid pool) affecting all vital functions of the cell. Routinely, the medium for cultivation of yeast under anaerobiosis is enriched with ergosterol suspended in Tween 80. It seems possible that unsaturated fatty acid esters present in the above detergent could alter the fluidity of yeast membranes which, in turn, could influence the ratio of the lipids synthesized. To check this possibility, increasing amounts of Tween 80 were added to the incubation mixture. As a result the total amount of incorporation of labeled phosphate

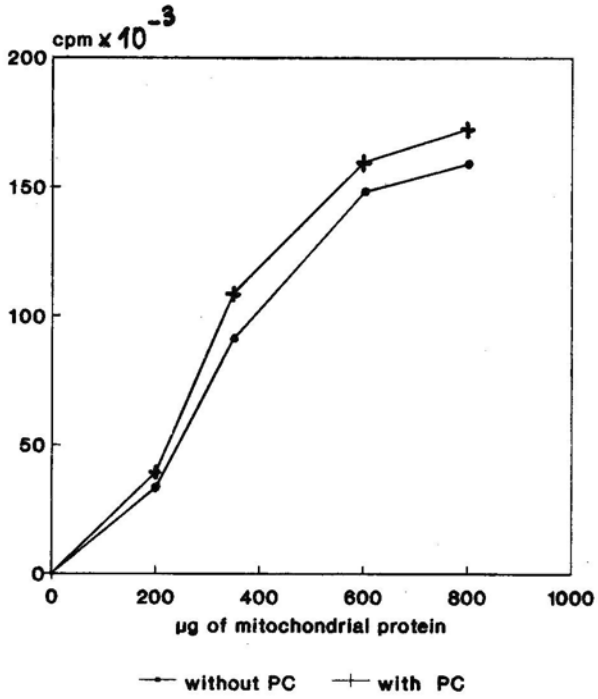

Fig. 1. Stimulation of the mitochondrial enzyme phosphorylating activity by phosphatidylcholine; +, with PC; ●, without PC

into lipid fraction was increased by 32% but the ratio of phosphatidic acid to dolichyl phosphate remained characteristic for aerobical membranes i.e. 3:1. The possibility that the increase of the incorporation of ^{32}P into lipids in the presence of Tween 80 was due to the partial solubilization of enzymes from membranes did not find confirmation in the experiments (data not shown). Ergosterol added to the reaction mixture had practically no effect.

Mitochondrial rho⁻ mutant. The other approach to elucidate the influence of aerobicity on phospholipid synthesis was induction and isolation of rho⁻ mutant. Mutation of mitochondrial DNA gives respiratory deficient cells (RD). Surprisingly, the enzyme prepared from the membranes of rho⁻ mutant synthesized PA and DolP at the ratio typical for aerobically grown yeast.

DISCUSSION

The regulation of phospholipid synthesis is a very complex process. In the previous paper [1] it was shown that growth conditions may dramatically change the amounts of individual phospholipids which are synthesized by CTP dependent phosphorylation in the yeast. However, at present we are unable to offer a satisfactory explanation of the previously observed facts.

Although Tween 80 substiantially raised phospholipid synthesis it did not change PA/DolP ratio. Preferential synthesis of PA and a 6-fold increase in the PA/DolP ratio took place on shifting from anaerobiosis to aerobic conditions of growth. These changes, however, were not mediated by mitochondria since PA synthesis was more efficient by the crude membrane fraction than by the purified mitochondrial preparation. Moreover, addition of PC stimulated the reaction by not more than 17% while in the crude membrane fraction the stimulation was as much as 4-fold, see [1]. It seems that the block of synthesis of mitochondrial proteins (by the destruction of mtDNA) did not influence the synthesis of phospholipids. Moreover PA/DolP ratio was identical in rho$^-$ mutant and in the wild yeast strain. The results of experiments with purified mitochondria from normal cells and rho$^-$ mutants support the idea that differentiated synthesis of phospholipids is more dependent on the oxygen-induced changes in the membranes then on inactivated mitochondria. The synthesis of lipids comprises multistep regulation processes which cannot be explained by straightforward correlations.

Further works will comprise investigations of the relations between oxygen-dependent changes in yeast membranes and the amount of accessible diacylglycerol serving as a substrate for the synthesis of PA.

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