SPECIFIC EFFECT OF UNSATURATED FATTY ACID DEPLETION ON MITOCHONDRIAL OXIDATIVE PHOSPHORYLATION IN SACCHAROMYCES CEREVISIAE

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SUMMARY: A mutant of Saccharomyces cerevisiae which cannot synthesise unsaturated fatty acid (UFA) was used to investigate the role of UFA in yeast metabolism. UFA depleted cells contained cytochromes and respired at normal rates, but could not grow on non-fermentable substrate, and their growth on glucose was restricted to that which could be supported by fermentation alone. Isolated mitochondria lacked respiratory control and had negligible phosphorylation. Thus UFA depletion leads to the specific loss of mitochondrial oxidative phosphorylation by S. cerevisiae both in vivo and in vitro.

Anaerobic S. cerevisiae cannot synthesise ergosterol and unsaturated fatty acid (UFA), and supplements of these lipids are required for growth. In the presence of excess lipid supplements well-formed mitochondrial profiles are present in anaerobic yeast, but if UFA is present only in growth-limiting amounts the yeast cells contain only primitive, poorly defined mitochondrial structures (1). However, the anaerobic system is of limited usefulness in investigating the role of UFA in the formation of mitochondria, as anaerobically grown cells lack cytochromes and cannot respire (2). To overcome this problem we have obtained a mutant of S. cerevisiae which requires a source of UFA for aerobic growth (3). The organism thus offers the opportunity to study aerobically the relationship of changes in fatty acid composition to the formation of functional mitochondria. This communication reports that UFA is required for the development of phosphorylative but not oxidative ability in yeast mitochondria.

METHODS

S. cerevisiae strain KD115, which cannot synthesise UFA, was obtained from Dr. M.A. Resnick, and the revertant strain

KD115-1 was obtained by selecting a prototrophic colony after ultraviolet irradiation of KD115. Cells were grown aerobically at 28° on a 0.5% Difco yeast extract-salts medium supplemented where indicated with glucose, glycerol and Tween-80 as a source of UFA. Dry weight and absorption spectrum determinations were as described previously (4). Mitochondria were prepared essentially as described by Lamb et al. (5), except that in order to obtain mitochondria with maximum respiratory control protoplasts were broken by passing through a French press at 500 p.s.i. in a medium containing 0.5M sorbitol, 0.5mM EDTA, 10mM tris-HCl, pH 7.4. Respiratory control ratios were determined in a polarograph, and P:O ratios were measured by conventional manometric techniques

TABLE 1

FATTY ACID COMPOSITION OF WHOLE CELLS OF KD115 AND KD115-1

Cell Type	Saturated Fatty Acid		UFA
	$C_8 - C_{14}$	$C_{16} + C_{18}$	$C_{14} - C_{18}$
Aerobic KD115 3mg. UFA/ml	3	30	67
Aerobic KD115 500 µg UFA/ml	8	64	28
Aerobic KD115 50 µg UFA/ml	16	77	7
Aerobic KD115-1	2	25	73
Anaerobic KD115-1 50 µg UFA/ml	29	64	7

Cells were grown aerobically in media containing 1% glucose or anaerobically in 5% glucose media. Fatty acid composition is expressed as weight percentage, and was determined as described by Jollow et al. (7).

Fatty acid composition was determined by gas chromatography of the methyl esters of the fatty acids. Inorganic phosphate was assayed by the method of Gomori (6).

RESULTS AND DISCUSSION

The fatty acid composition of mutant and revertant yeast cells grown under different conditions are compared in Table 1. When the mutant was grown aerobically with full supplements of UFA (3mg/ml) the lipid composition was similar to that of the aerobic revertant strain, whereas when grown aerobically with strictly limiting supplies of UFA (50µg/ml) its lipid composition was grossly changed and resembled that of the revertant cell grown anaerobically.

In order to investigate the effects of UFA depletion on mitochondrial metabolism the growth of yeast on fermentable and non-fermentable substrates was compared. Growth of yeast on a non-fermentable substrate such as glycerol requires mitochondria capable of oxidative phosphorylation, whereas growth on a fermentable substrate such as glucose can be supported by glycolysis alone (8). The mutant was found to grow aerobically in the pres-

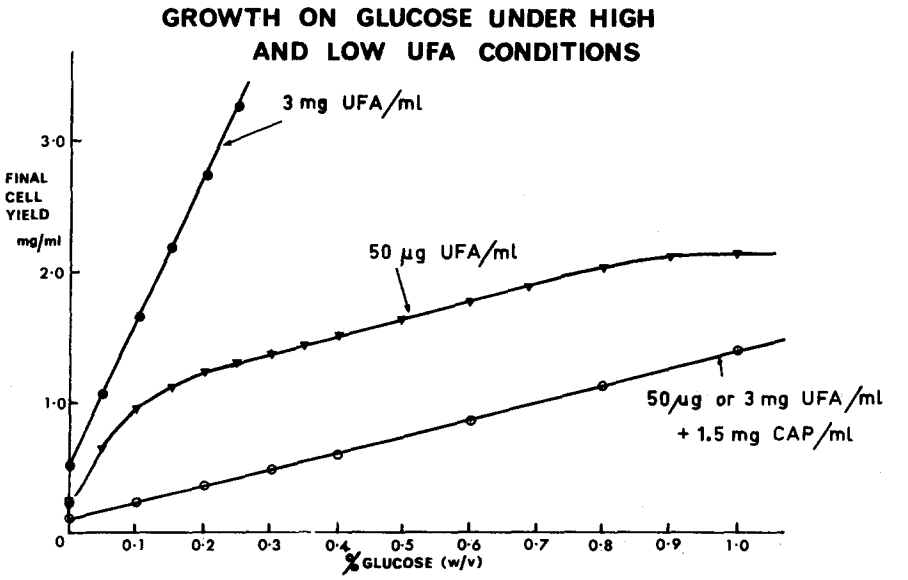

Fig.1

Cells were grown as described in Methods. Further additions where indicated were UFA, 50µg/ml or 3 mg/ml; glucose 0-1.0%; and chloramphenicol 1.5mg/ml.

ence of strictly limiting UFA (50µg/ml) in glucose medium but not in glycerol medium, whereas growth in the presence of excess UFA (3mg/ml) was very similar in both media. These results indicated that mitochondrial metabolism was specifically affected by the depletion of UFA. Fig.1 further substantiates this conclusion. Here the mutant was grown in media containing growth-limiting concentrations of glucose at two different levels of UFA. At high levels of UFA (3mg/ml) cell growth was proportional to glucose concentration and the yield of cells in the medium employed was 1.10mg dry weight/mg glucose indicating a full utilization of glucose by both fermentation and mitochondrial oxidative metabolism. In the presence of chloramphenicol, a specific inhibitor of mitochondrial cytochrome formation (8,9), the growth increment was 0.12mg dry weight/mg glucose and represents glucose utilization by fermentation alone. When the mutant was grown on strictly limiting UFA a biphasic growth response was obtained. At low extents of growth where the cells were only slightly depleted in UFA the cells obtained their energy for growth by both fermentative and oxidative metabolic pathways and the growth increment approached that of cells grown on excess UFA. However, at high growth levels, where the UFA was depleted, the growth increment approximated to that of the purely fermentative cell. The fermentative and oxidative growth yield is similar to that recently reported by Kormancikova *et al*. (10).

The nature of the mitochondrial lesion was investigated in the following way. Yeast cells were grown on low concentrations of glucose, and harvested after the glucose had been completely converted to fermentation products. By varying the initial concentrations of UFA in the medium, cells were finally obtained containing 15-70% UFA. In all cases the cells contained similar amounts of cytochromes and respired at rates of 0.2-0.3µg atoms oxygen/min/mg dry weight with glucose as substrate. Mitochondria isolated from the UFA depleted cells had a similar fatty acid composition to that of the whole cells, respired normally and had normal cytochrome spectra. Table 2 shows the UFA content, P:O ratios and respiratory control ratios of the isolated mitochondria. The P:O ratios of mitochondria containing high levels of UFA (67-74%) were in the normal range for yeast mitochondria (12), and respiratory control ratios (3-5) were high. In mito-

TABLE 2

EFFECTS OF UFA DEPLETION ON OXIDATIVE PHOSPHORYLATION

Mitochondrial UFA (weight % total fatty acid)	Number of Expts.	P:O Ratios				Respiratory control ratio (α OG)
		Pyruvate + malate	Succinate	α oxoglutarate	α oxoglutarate + DNP	
67 - 74	6	1.1-1.3	1.1-1.3	1.6-2.5	0.8-1.1	3-5
60	1	0.6	0.9	1.3	0.6	2.2
39	1	0.4	0.4	1.5	0.7	2.0
15 - 28	6	<0.2	<0.2	0.6-0.8	0.6-0.8	1.0-1.3

P:O ratios were determined manometrically as described by Haslam (11). The main compartment of the Warburg flasks contained in 2.62 ml : 0.48M mannitol, 1.1mM EDTA, 10mM tris-maleate, 10mM K-PO_4, 5mM $MgCl_2$, 20mM NaF, 0.4mM ATP, 25mM glucose, 1mg hexokinase, 4mg bovine serum albumin, and yeast mitochondria (2-4mg protein), final pH=6.4. Further additions where indicated were 0.1mM 2:4 dinitrophenol (DNP), and substrates: 4mM pyruvate plus 4mM L-malate, 4mM succinate, 4mM α oxoglutarate. Respiratory control ratios were assayed in a polarograph cell in a medium containing 0.65M mannitol, 5mM K-PO_4, 0.5mM EDTA, 10mM tris-maleate, 4mM α oxoglutarate, yeast mitochondria (1-2mg protein) final pH=6.5. State 3 respiration was initiated by addition of 0.2mM ADP.

chondria containing low levels of UFA (15-28%), P:O ratios with pyruvate plus malate and succinate as substrate approached zero and respiratory control was absent. Intermediate P:O ratios were obtained with mitochondria partially depleted in UFA. P:O ratios with α oxoglutarate as substrate fell to 0.6 - 0.8 in the UFA depleted mitochondria, but as the ratio was unaffected by 2:4 dinitrophenol it could be attributed to the substrate level phosphorylation catalysed by succinate thiokinase. Thus the *in vitro* results agree with the *in vivo* observations that the energy of respiration is unavailable for growth. Further studies are in progress to define the precise lesion affecting oxidative phosphorylation in mitochondria depleted in UFA.

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