Search for a regulatory function of mitochondrial proteinases in yeast

I. Viard and A.J.C. Stahl

Laboratoire de Biochimie, Faculté de Pharmacie, Université Louis Pasteur, BP 10, 67048 Strasbourg-Cédex, France

Received 1 October 1984, revised version received 22 November 1984

Proteolytic activity in yeast mitochondrial membranes and soluble fractions was studied using a labeled substrate [^{14}C]dimethylcasein Optimal activity for the two fractions was at pH 6 in phosphate buffer at 37 C Activity was partially inhibited by adding 3 mmol/l ATP Activity was enhanced rapidly in both fractions when yeast cells were grown aerobically on a derepression medium and transfered on a repression medium in anaerobiosis Mitochondrial proteinase mediation in the mitochondrial breackdown observed during transition from aerobical derepression to anaerobical repression is discussed

Proteinase *Mitochondria* *Yeast* *Aerobiosis* *Anaerobiosis*

1. INTRODUCTION

Yeast mitochondria contain several proteinases. Some are located in the matrix [1,2], others are associated with the mitochondrial inner membrane [3,4]. These mitochondrial proteinases may play several functions. Some proteinases cleave the terminal extensions in polypeptidic precursors of cytoplasmically synthesized mitochondrial proteins [5,6]. On the other hand, when yeast cells grown aerobically in derepression medium are turned over to fermentation, mitochondrial proteins are catabolized to peptides, certainly by proteinases [7] either of mitochondrial or cytoplasmic origin. To obtain more information on this point, we studied changes in mitochondrial proteolytic activities of yeast cells grown in derepression medium, with low fermentable carbohydrate concentration, when transferred to a glucose-rich repression medium. We searched for an eventual variation of mitochondrial proteolytic activities depending on the physiological state of the cells.

Abbreviations [^{14}C]DMC, [^{14}C]dimethylcasein, DB-cAMP, dibutyryl 3′,5′-cyclic adenosine monophosphate

2 MATERIALS AND METHODS

Saccharomyces cerevisiae, strain ϱ^+ IL 8.8.C, was grown in a derepression medium as in [8]. When cells reached the exponential phase, part of them were harvested. Glucose to a concentration of 100 g/l was added to the medium and aeration stopped. Cells were harvested after 10, 30 and 120 min of culture at 28°C in anaerobiosis.

2.1. *Isolation of mitochondria*

This was performed according to [9], except that cytohelicase (I.B.F., Villeneuve-la-Garenne, France) was used [10]. The soluble fraction, i.e., mitosol and intermembrane space, and the membrane pellet were obtained after osmotic lysis of mitochondria and 105000 × *g* centrifugation as in [11].

Proteins were estimated according to the method of Lowry et al. [12] or of Bradford [13] with bovine serum albumin as a standard.

2.2. *Enzyme assay*

The reaction mixture contained 3 μg [^{14}C]DMC (7.8 μCi/mg protein, CEA, Saclay, France) and 5 μg protein of the enzymatic fraction. Volume was adjusted to 0.15 ml with phosphate buffer

Published by Elsevier Science Publishers B V (Biomedical Division)
00145793/85/$3 30

(50 mmol/l, pH 6.0). The incubation was performed at 37°C during 15 or 30 min; the reaction was stopped by addition of 0.15 ml trichloroacetic acid 10% (w/v). After 15 min centrifugation at 1200 × g, 0.15 ml supernatant was adsorbed on a 3 MM Whatman paper disc (∅ 25 mm), dried and counted in 5 ml liquid scintillation medium containing 4 g/l Omnifluor (NEN, Frankfurt) in toluene. Blanks were not higher than 150 cpm for 1 min incubation.

3. RESULTS

3.1. *Optimal pH for proteolytic activities in mitochondrial soluble and membrane fractions*

The effect of pH on activity was studied for the soluble fraction and the membrane pellet, using citrate-phosphate buffer (50 mmol/l from pH 4.5 to 7.0) and phosphate buffer (50 mmol/l from pH 6.0 to pH 8.0) for both fractions. Passing from pH 6.0 to 8.0 lowers the activity by nearly 30%.

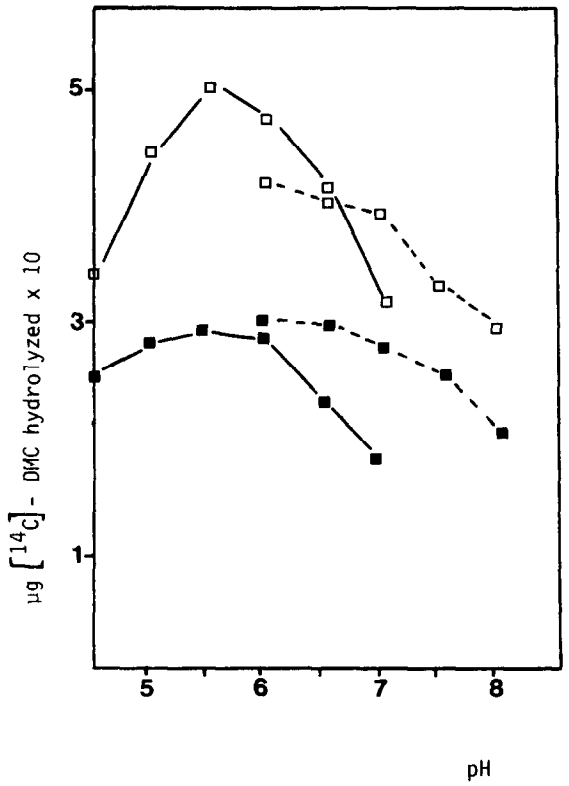

Fig.1 Proteolytic activities in mitochondrial fractions as a function of pH □, Soluble fraction; ■, membrane pellet; ——, citrate phosphate buffer (50 mmol/l), =--, phosphate buffer (50 mmol/l)

3.2. *Effect of adenosine nucleotides on mitochondrial proteinase activities*

ATP or DB-cAMP were added at a concentration of 3 mmol/l to the membrane and the soluble fractions (table 1). In experiment 3, ATP was added batchwise at 0, 5 and 10 min of incubation (3 × 2 mmol/l) to the medium already containing 0.1 mmol/l DB-cAMP. 3 mmol/l ATP induced a partial inhibition of mitochondrial proteinase activities, reaching 30–38%. Higher concentrations of ATP up to 10 mmol/l did not increase the inhibition (not shown). DB-cAMP had no effect on either activation or inhibition of the proteolytic activities. When ATP was added batchwise with DB-cAMP present the inhibition of the proteolytic activity did not rise significantly.

3.3. *Evolution of mitochondrial protein concentration as a function of the physiological state of the yeast cells*

Humid cells and mitochondrial purified pellets were weighted in the case of the four following types of cells: derepressed cells grown without glucose, repressed cells grown anaerobically in the presence of glucose and harvested after 10,30 and 120 min. In our conditions 85 ± 3% of the cells were converted to protoplasts.

Table 2 shows that, after glucose addition to the medium with oxygen cut off, the weight of the cells did not change much during 30 min; it increased after one generation time at 120 min. But the mitochondrial weight decreased in the cells with

Table 1

Effect of ATP and DB-cAMP addition on mitochondrial proteolytic activities

	Inhibition (% ± SE)	
	Membrane fraction	Soluble fraction
1 ATP (3 mmol/l)	30 ± 4	38 ± 5
2. DB-cAMP (0.1 mmol/l)	0	0
3 ATP (3 × 2 mmol/l) + DB-cAMP (0.1 mmol/l)	38 ± 4	44 ± 6

glucose repression: the decrease is small after 10 min repression but reached about 50% after 30 min. Mitochondrial protein concentration decreased during the repression; the membrane proteins were specifically reduced: 25% of membrane proteins were lost after 30 min. Proteins of the soluble fraction were decreased only by 15% during repression by glucose in anaerobiosis.

3.4 *Mitochondrial proteolytic activity variations as a function of the physiological state of the yeast cells*

Proteolytic activities measured in the two mitochondrial fractions are reported in table 3. The proteolytic specific activities increased in both mitochondrial fractions after a 10 min repression. They remained at a high level in the membrane

Table 2

Weight of cells and mitochondria, mitochondrial protein concentration, during the derepression and various repression states of yeasts

	Derepressed cells	Cells under repression by glucose		
		10 min	30 min	120 min
Weight of humid cells in g/l medium	20.5	18 2	19.5	27.0
Weight of humid mitochondria in g/100 g cells	8 0	7 5	4 2	4.8
Total mitochondrial proteins in mg/100 g cells	360	320	280	255
Membrane proteins	295	260	220	200
Soluble proteins	65	60	60	55

Results are the means of 4 experiments

Table 3

Mitochondrial proteolytic activities during derepression and repression by glucose in yeast cells

	Proteins (mg/100 g cells)	Specific proteolytic activities· μg [^{14}C]DMC hydrolyzed/min per mg protein	Enzymatic activity (%) compared to the activity in derepressed mitochondria	Proteolytic activities ratio of membranes *vs* total mitochondria
Derepressed mitochondria				
Membrane fraction	295	1 84	100	0.78
Soluble fraction	65	2 35	100	
10 min repressed mitochondria				
Membrane fraction	260	2.29	110	0.78
Soluble fraction	60	2.86	112	
30 min repressed mitochondria				
Membrane fraction	220	2 35	94	0.93
Soluble fraction	60	0.60	24	
120 min repressed mitochondria				
Membrane fraction	200	1 84	68	0.94
Soluble fraction	55	0 40	14	

fraction after 30 min repression but decreased to a greater extent in the soluble fraction between 10 and 30 min repression. After 120 min repression, the specific activity decreased in both fractions and the activity in the membrane fraction returned to its initial value of derepression conditions.

The percentage of the total proteolytic activity as compared to initial activity in derepressed cell mitochondria increased slightly within 10 min repression, but then decreased during longer repression in both mitochondrial fractions. At first the proteolytic activity was decreased in the soluble fraction already after 30 min, whereas the membrane activity decreased only after 120 min repression. Membranes which exhibited 78% of total mitochondrial proteolytic activity in derepressed cells, showed 94% of total activity after 120 min repression.

4. DISCUSSION

When *S. cerevisiae* cells are transferred from an aerobic derepression medium to an anaerobic repression medium mitochondria are transformed into promitochondria. During this transition, mitochondrial proteins could be transferred to the cytoplasm through mitochondrial membranes, or they could be proteolyzed to peptides and amino acids, which in turn could be transferred to the cytoplasm. To obtain more information on this mechanism we measured the mitochondrial proteolytic activities in aerobic derepression and during increasing time of anaerobic repression, in order to search for a regulation through mitochondrial proteolytic activity variations.

After a short time of repression (10 min), mitochondrial proteolytic activities indeed increased in both membrane and soluble fractions. With a more extended repression (30–120 min) these activities decreased extremely in the soluble fraction, more weakly in the membrane fraction. Thus, the proteinases of the membrane fraction behaved towards glucose repression as some yeast cytoplasmic proteinases, like endoproteinases A and B or carboxypeptidase Y [14,15]: these enzyme activities increase slightly during short time repression and decrease later on. Contamination of mitochondria with cytoplasmic enzymes is not likely because sensitive search for cytoplasmic constituents like cytoplasmic tRNA or cytoplasmic amino acid tRNA synthetases [8] was negative. On the other hand, lysosomal proteinase A is strongly inhibited by pepstatin, but mitochondrial proteinases are not [16].

Catalytic properties and chromatographic behaviour of proteinases from repressed or derepressed mitochondria are the same (to be published). The increase of proteolytic activities after short time repression by glucose in anaerobiosis, which we describe here, may partially explain the mitochondrial catabolism noticed during repression. The fact that mitochondrial proteolytic activities are optimal at acid pH and partially inhibited by ATP at 3 mmol/l concentration is also in favour of an increase of proteinase activities in mitochondria during repression. Indeed, intramitochondrial pH is about equal to 7.8–8.0 when yeast cells are derepressed; under those conditions mitochondrial proteinases could be partially inhibited. In repression conditions, the intramitochondrial pH may be decreased by acid production during fermentation and therefore the mitochondrial proteinases would be activated. During fermentation, ATP production is also reduced as compared to derepression conditions and this could be in favour of mitochondrial proteinase activation, as we showed that ATP inhibits mitochondrial proteolytic activities by 18–30% in both soluble and membrane fractions.

Yeast mitochondrial proteinases are able to participate in the degradation of mitochondria during repression. But up to now we cannot exclude a second mechanism, which perhaps adds to the first. Glucose repression also stops the synthesis of inner membrane proteins. Therefore, the mitochondrial membrane permeability towards protein is increased [17,18]. This higher permeability could explain the exit of mitochondrial proteins to the cytoplasm and the decrease of proteolytic activities noticed in the mitochondria after 30 min repression. Work is in progress to try to confirm the passing over of mitochondrial proteases into the cytoplasmic compartment during the repression by glucose in the yeast cells.

REFERENCES

[1] McAda, P H. and Douglas, M G. (1982) J Biol Chem. 257, 3177–3182
[2] Boehni, P.C., Daum, G. and Schatz, G (1983) J. Biol. Chem. 258, 4937–4943.

[3] Daum, G., Gasser, S.M and Schatz, G. (1982) J. Biol. Chem. 257, 13075–13080.
[4] Novikova, L.A , Zubatov, A.S. and Luzikov, U.N (1982) FEBS Lett. 135, 245–248
[5] Cerletti, N , Boehni, P.C and Suda, K (1983) J. Biol Chem 258, 4944–4949.
[6] Sevarino, K A. and Poyton, R.O. (1980) Proc. Natl. Acad Sci. USA 77, 142–146.
[7] Jayaraman, J., Cotman, C., Mahler, H R. and Sharp, C.W (1966) Arch. Biochem. Biophys. 116, 224–251.
[8] Schneller, J.M., Schneller, C., Martin, R. and Stahl, A.J.C. (1976) Nucleic Acids Res. 3, 1151–1165
[9] Faye, G., Kujawa, C and Fukuhara, H. (1974) J Mol. Biol 88, 185–203.
[10] Diatewa, M , Viard, I. and Stahl, A.J C. (1981) Biochimie 63, 67–69.
[11] Accoceberry, B., Schneller, J.M. and Stahl, A.J.C. (1973) Biochimie 55, 291–296.
[12] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[13] Bradford, M.M (1976) Anal. Biochem. 72, 248–254
[14] Hansen, R.J., Switzer, R.L., Hinze, H. and Holzer, H. (1977) Biochim. Biophys. Acta 496, 103–114.
[15] Frey, J. and Rohm, K.H. (1978) FEBS Lett. 100, 261–264.
[16] Viard, I and Stahl, A.J.C. (1984) Biochimie, submitted
[17] Linnane, A.W. (1964) in: Oxidases and Related Redox Systems (King, T.E. et al. eds) pp.1102–1128, John Wiley and Sons, New York.
[18] Perlman, P.S. and Mahler, H.R. (1974) Arch. Biochem. Biophys. 162, 248–271.