Journal of Bioenergetics and Biomembranes, Vol. 19, No. 5, 1987

The Cytochrome *c* Oxidase from the Yeast *Candida parapsilosis*

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Received January 27, 1987

Abstract

In the yeast *Candida parapsilosis*, the proteins encoded by mitochondrial DNA are different in number and size from those of *Saccharomyces cerevisiae*. Nevertheless, the purified cytochrome *c* oxidase from *Candida parapsilosis* shows kinetic properties similar to those of *Saccharomyces cerevisiae*.

Key Words: Cytochrome *c* oxidase; *C. parapsilosis*; subunit composition; apparent molecular weight.

Introduction

Mitochondria biogenesis requires the active and collaborative participation of both cytoplasmic and mitochondrial protein synthesizing systems. Thanks to the use of selective inhibitors of protein synthesis, eukaryotic microorganisms, such as yeasts, are a readily available material for these studies. The products of mitochondrial protein synthesis can be labeled specifically in the presence of cycloheximide which is known to be a powerful inhibitor of cytoplasmic protein synthesis (Siegel and Sisler, 1965; Lamb *et al.*, 1968). It is known that, in yeast, at least three enzymatic complexes of the inner mitochondrial membrane are cooperatively synthesized by the mitochondrial and cytoribosomal protein translation machineries (Weiss *et al.*, 1971; Tzagoloff and Meagher, 1971; Rubin and Tzagoloff 1973; Mason and Schatz, 1973): OSATPase, cytochrome *c* oxidase, and cytochrome bc_1 complex.

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0145-479X/87/1000-0495$05.00/0

Cytochrome *c* oxidase (EC 1.9.3.1) is the terminal enzyme of the respiratory chain of most aerobic organisms, composed of a variable number of subunits depending on the evolutionary stage of the species. In the yeast *Saccharomyces cerevisiae*, the enzyme contains nine subunits (Power *et al.*, 1984) whereas it contains 13 different subunits in mammalian tissues (Kadenbach *et al.*, 1983; Kuhn-Nentwig and Kadenbach, 1984). The three largest subunits, I, II, and III, are encoded by mitochondrial DNA (mt DNA), and the other subunits by cytoplasmic DNA. The mitochondrially encoded polypeptides represent the catalytic subunits, and the nuclear-encoded subunits are suggested to have a regulatory function (Kadenbach and Merle, 1981, Kadenbach, 1983).

The results described in this paper deal with the electrophoretic pattern of the mitochondrially encoded polypeptides of the yeast *Candida parapsilosis* CBS 7154, namely, with a study of the cytochrome *c* oxidase.

Materials and Methods

Strains

Saccharomyces cerevisiae AB1-4A/8 (*a*, his_4) was from the collection of P. P. Slonimski (Gif sur Yvette, France). *Candida parapsilosis* CBS 7154 (SP1) was previously described by Guérin *et al.* (1982) and Guérin and Camougrand (1986).

Culture

Cells were grown on a 2% glycerol complete medium.

Labeling of Mitochondria

Cells were labeled *in vivo* from small-scale experiments by using $^{35}SO_4$ in the presence of cycloheximide (Douglas and Butow, 1973; Velours *et al.*, 1980). Mitochondria were prepared with the manual shaking method in the presence of 1 mM PMSF (Lang *et al.*, 1977).

Cytochrome c Oxidase Isolation Procedure

Cytochrome *c* oxidase was isolated from yeast mitochondria, according to a procedure derived from that of Kadenbach for mammalian mitochondria (Kadenbach *et al.*, 1986). All the steps were performed at 4°C. The mitochondrial proteins were adjusted to a concentration of 20–25 mg/ml. After addition of 1 M potassium phosphate, pH 7.2, to a final concentration of 200 mM, a 20% solution (v/v) of Triton X-114 was added under stirring

up to 0.05 mg per mg protein. The mixture was stirred for 30 min, centrifuged for 30 min at 250,000 *g*, and the supernatant discarded. The pellet was homogenized in a Potter homogenizer in 200 mM potassium phosphate buffer and centrifuged for 30 min at 250,000 *g*. Two washes with the same buffer containing, in addition, first 0.1% Triton X-100 and then 1% Triton X-100 were performed for complete removal of cytochrome bc_1. The pellet was dissolved in 10–20 ml 5% Triton X-100 (v/v) and 200 mM potassium phosphate buffer, pH 7.2, and centrifuged for 30 min at 250,000 *g*. The green supernatant was diluted 1:4 with water, layered on a DEAE-Sephacel column (1.5 × 20 cm) equilibrated with 50 mM potassium phosphate buffer and 0.05% Triton X-100, and washed with the same buffer. Cytochrome *c* oxidase was eluted with 250 mM potassium phosphate buffer, pH 7.2, and 0.05% Triton X-100. Green fractions were combined and adjusted to 1% with solid sodium cholate (w/v), and a saturated ammonium sulfate solution was added to 20% saturation. The solution was stirred overnight. Two other precipitations with 28% and 35% ammonium sulfate saturation, respectively, were performed. Cytochrome *c* oxidase was precipitated at 35% ammonium sulfate saturation. Two different dialysis procedures described in Kadenbach *et al.* (1986) were carried out, depending on the subsequent use of the enzyme.

Cytochrome c *Oxidase Activity*

Enzymatic activity was measured polarographically at 25°C as described in Kuhn-Nentwig and Kadenbach (1985).

Electrophoresis

A modification of the discontinuous SDS system of Laemmli (1970) described in Esparza *et al.* (1981) was used for slab electrophoresis. The gel slab was washed with methanol/H_2O/acetic acid (5:5:1) buffer and stained with the nitrate silver method (Ansorge, 1982).

Results

Products of the mitochondrial protein synthesis encoded by the mt DNA of *C. parapsilosis* were compared to those of *S. cerevisiae*. When *S. cerevisiae* cells were labeled *in vivo* with ^{35}S-SO_4 in the presence of cycloheximide, 8–10 prominant radioactive bands were seen in polyacrylamide gels of mitochondria solubilized with sodium dodecyl sulfate: the three largest subunits I, II, and III of the cytochrome *c* oxidase, the cytochrome *b*, the subunits 6, 8, and 9 of ATPase, and the protein var 1. Figure 1 shows that the radioactive pattern of these proteins is quite different in *C. parapsilosis* (lane b) when

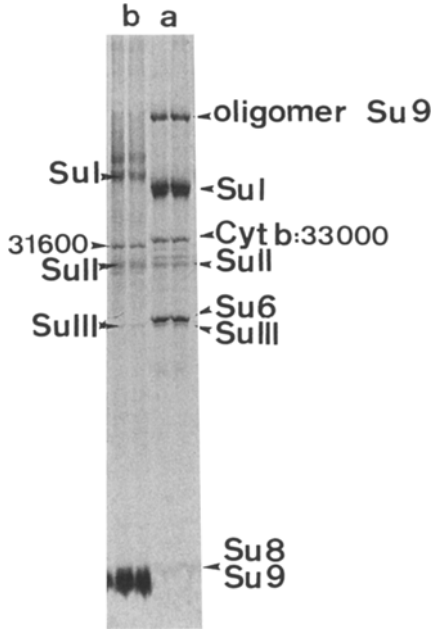

Fig. 1. Autoradiography of electrophoretic analysis (15% acrylamide, 15% glycerol) of mitochondrially translated products labelled *in vivo* with [^{35}S] sulfate in the presence of cycloheximide. Su I, Su II, and Su III represent the subunits of cytochrome oxidase; Su 6, Su 8, and Su 9 represent the subunits of ATPase. Lane a: *S. cerevisiae*; lane b: *C. parapsilosis*.

compared to that of *S. cerevisiae* (lane a). Radioactivity was mainly found at the level of cytochrome *b*-subunit II of the cytochrome *c* oxidase and subunits 8–9 of ATPase. Subunit I of the cytochrome oxidase was higher, and cytochrome *b* was lighter, than those of *S. cerevisiae*. Protein var 1 and subunit 6 of ATPase are not detectable.

These results can be related to the peculiar organization of the mt DNA of *C. parapsilosis*. Indeed, studies performed on the strain CBS 7157 (incorrectly called *C. raghii* in Kovac *et al.*, 1984) demonstrated that the mt DNA of *C. parapsilosis* was linear and unique in its gene order (Kovac *et al.*, 1984). A comparative analysis of the mt DNA of CBS 604 (reference strain of *C. parapsilosis* from CBS), CBS 7154, and CBS 7157 with different restriction enzymes showed that all these strains exhibited similar patterns of restriction fragments (M. Guérin *et al.*, unpublished results).

This result, combined with the fact that *C. parapsilosis* exhibited some capacity for respiration, even when 0.1 mM cyanide (the adequate inhibitory concentration of the cytochrome *c* oxidase) was added, prompted us to study the pure enzyme.

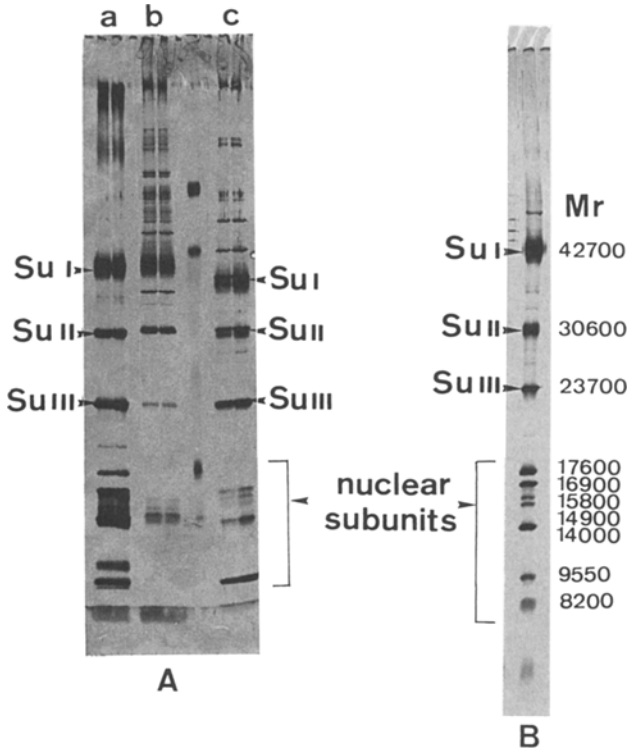

Fig. 2. Polypeptide pattern of isolated cytochrome oxidase after SDS polyacrylamide gel electrophoresis. (A) 12.5% acrylamide, 15% glycerol. a: *C. parapsilosis*; b: *C. raghii*; c: *S. cerevisiae*. (B) 15% acrylamide, 15% glycerol represented the *C. parapsilosis* enzyme. 1 μg proteins was layered. Gels were stained with the silver method.

The isolation procedure of the cytochrome *c* oxidase from yeast mitochondria was based on a method for mammalian enzymes, described previously and slightly modified (see Materials and Methods). This purification procedure has the advantage of giving rapidly a pure enzyme with a ratio mole heme per mg of protein equal to 7–9 for both yeasts. From Fig. 2, it can be seen that the enzyme of *C. parapsilosis* was very pure as compared to that obtained from *S. cerevisiae*. Polypeptide patterns of the isolated enzyme were studied for both yeasts with a SDS polyacrylamide gel electrophoretic system (Fig. 2). Differences were found between the two enzymes for both mitoribosomal and cytoribosomal subunits. Under our experimental conditions, 10 polypeptides were detectable in *C. parapsilosis* instead of 8 in *S. cerevisiae*. The mitochondrially encoded polypeptides were identical in number, but their apparent molecular weights were slightly different (average of five experiments), namely for subunit I. Differences

Table I. Comparative Analysis of the Subunits of the Cytochrome *c* Oxidase Complexes from Beef Heart, *S. cerevisiae*, and *C. parapsilosis*[a]

	Beef heart[b]	*S. cerevisiae*	*C. parapsilosis*
I	57000	40700	42700
II	30000	31600	30600
III	26000	23400	23700
Nuclear subunits	17150		17600
		16800	16900
		15700	15800
		14950	14900
		14000	14000
	12400		
	10700		
	10000		
	9400		9550
	8500	8800	8200
	6200		
	5500		
	4960		

[a]Gels were run with the following marker proteins: bovine albumin (M_r = 66,000), egg albumin (M_r = 45,000), pepsin (M_r = 34,700), trypsinogen (M_r = 24,000), lactoglobulin (M_r = 18,400), myoglobin (M_r = 16,950), myoglobin I and II (M_r = 14,400), lysozyme (M_r = 14,300), mygolobin I (M_r = 8160), and myoglobin II (M_r = 6210).
[b]According to Kadenbach *et al.*, 1983.

in number and apparent molecular weights at the level of nuclear-encoded subunits also appeared between the two yeasts. In Table I, we compared the apparent molecular weights of cytochrome *c* oxidase subunits from *C. parapsilosis*, *S. cerevisiae* and beef heart mitochondria (Kadenbach *et al.*, 1983). It can be seen that in *C. parapsilosis* five of the nuclear subunits show the same apparent molecular weight as those of *S. cerevisiae*. The two supplementary subunits found in *C. parapsilosis* had similar apparent molecular weights as compared to the two subunits from beef heart mitochondria.

In a second set of experiments, the kinetic properties of enzymes isolated from *C. parapsilosis* and *S. cerevisiae* were studied. Enzyme pellets were dissolved in an adequate lauryl maltoside buffer keeping the activity up (Kadenbach *et al.*, 1986). The kinetic parameters were determined polarographically at various cytochrome *c* concentrations with ascorbate + TMPD[4] as electron donor. As shown in the Eadie–Hofstee plot from Fig. 3, some minor differences in cytochrome *c* affinity were found between both enzymes. The apparent K_{m_1} was equal to 7×10^{-8} and 29×10^{-8} M for *S. cerevisiae* and *C. parapsilosis*, respectively. The apparent K_{m_2} was the same for both enzymes. Both enzymes were inhibited by 0.1 M cyanide.

[4]Abbreviations: PMSF: phenylmethylsulfonylfluoride; TMPD: *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine.

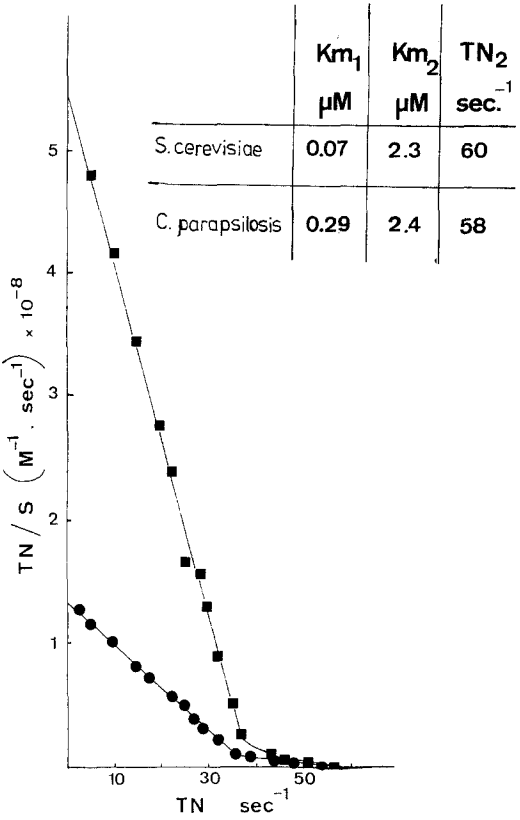

Fig. 3. Comparison of the kinetic properties of the cytochrome *c* oxidases from *S. cerevisiae* (■) and *C. parapsilosis* (●). The activities, expressed as turnover number (TN, mol cytochrome *c*/sec/mol cytochrome aa_3), were measured polarographically at 25°C in 25 mM Tris, 30 mM sodium phosphate, 0.014 mM EDTA, 1 mM lauryl maltoside, 0.7 mM TMPD, 0.02–40 μM cytochrome *c*, and 20 nM cytochrome oxidase. K_{m_1} and K_{m_2} represent the Michaelis constants for the high- and low-affinity binding sites for cytochrome *c*, respectively, and TN_2 the activity at infinite cytochrome *c* concentrations. The curves represent the average of seven experiments.

Discussion

Data presented in this paper demonstrated that the mitochondrial DNA from *C. parapsilosis* encoded as well for polypeptides which were different in number and size from those of other eukaryotes, such as *S. cerevisiae* or mammalian mitochondria.

The peculiar behavior of the respiratory metabolism of *C. parapsilosis* prompted us to inquire about the cytochrome *c* oxidase. The enzyme of

C. parapsilosis exhibited different electrophoretic patterns, both for nuclear and mitochondrial DNA-encoded subunits; however, the enzyme showed the same kinetic properties as those from *S. cerevisiae* and was inhibited by 0.1 M cyanide. Since it was demonstrated that the secondary mitochondrial pathway of *C. parapsilosis*, sensitive to high cyanide concentrations, was branched at the cytochrome *c* level, the cytochrome *c* oxidase cannot be the terminal oxidase of this pathway (Guérin *et al.*, 1986).

In numerous organisms, the number of functional subunits of the cytochrome *c* oxidase complex is still debated due to the difficulty of separating all the polypeptides on the SDS-dependent gel electrophoretic system. In all eukaryotic cytochrome *c* oxidases studied, the subunits I, II, and III, the catalytic ones, are encoded by the mitochondrial genome, and their sequences exhibit a homology of about 40% among the various organisms (Power *et al.*, 1984). Differences in the number of nuclear subunits account for the overall differences reported for various eukaryotes. These nuclear-encoded subunits are assumed to be the regulatory ones, and their number and size could reflect the regulatory adaptations of organisms to environment.

Further investigations on the regulation of the cytochrome *c* oxidase of *C. parapsilosis* should be carried out, which would explain its peculiar respiratory metabolism.

Acknowledgments

This work was supported by grants from the Forschungsmeinshaft (SFB 103 A2), the University of Bordeaux II, and the Centre National de la Recherche Scientifique.

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