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OXIDATION-REDUCTION POTENTIAL OF CYTOCHROME c OXIDASE IN MITOCHONDRIA OF YEAST GROWN UNDER VARIOUS COPPER CONCENTRATIONS.

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SUMMARY

Growth of copper deficient yeast cells in copper supplemented medium led to an increase in the amount of cytochrome c oxidase and cytochromes b and c. Potentiometric titration of cytochrome c oxidase showed that the oxidation-reduction midpoint potentials of cytochromes a and a_3 were, respectively, for cells grown at 56 μg copper/liter : 205 ± 10, 325 ± 10 ; for cells grown at 140 μg copper/liter, 215 ± 10, 360 ± 10 ; and for cells grown at 330 μg copper/liter, 215 ± 10, and 345 ± 10. Data showed that the midpoint potential of cytochromes a and a_3 in yeast mitochondria was close to the value reported for rat liver and beef heart mitochondria. In addition the stimulation of cytochrome c oxidase biosynthesis in yeast by copper does not alter significantly the redox properties of the enzyme.

INTRODUCTION

Copper is an essential trace element for life in all aerobic eucaryotic and many aerobic procaryotic organisms. It plays a fundamental role in four-electron-transfer cytochrome c oxidase and participates in the only pathway for the biological reduction of molecular oxygen to water. A critical deficiency of cytochrome c oxidase as a result of copper deficiency was demonstrated in yeast almost five decades ago (1). The function of this metal in numerous enzymes has been documented in many studies since then (for review see ref. 2). In mammals, dietary copper deficiency results in the absence of lysyl oxidase (3) and an increase fragility of vascular

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tissues which results in the spontaneous rupture of major vessels (4). Excessive uptake of copper leads to an accumulation of the metal in the liver and in the locus niger in the brain, and results in hepato-lenticular degeneration or Wilson's disease (5).

The oxidation-reduction properties of cytochrome c oxidase have been determined for isolated mitochondria and the purified enzyme from mammalian systems (6-9). In beef heart mitochondria the mid-point potential of cytochromes a and a_3 were respectively 205 and 360 mV (7) compared to 210 and 360 mV for isolated cytochrome c oxidase (8). However the redox state of cytochrome c oxidase in yeast has not been reported previously ; such a study is reported in this paper.

MATERIALS AND METHODS

Chemicals : Zinc dibenzyldithiocarbamate and phenazine methosulfate were obtained from K & K Laboratories, Plainview, N.Y. N,N,N',N'-tetramethyl-p-phenylenediamine (TMPD) and potassium ferricyanide were purchased respectively from Sigma Chemical Co., St. Louis, Mo. and Baker Chemical Co., Philadelphia, Pa. Argon (ultra high purity : $O_2 < 1$ ppm) was purchased from Matheson Co., and glusulase (snail gut juice), from Endo Laboratories, Garden City, N.Y. All other reagents were of enzyme grade and were used without further purification.

Isolation of mitochondria : Yeast cells (Candida utilis, 100 g wet weight) were suspended in 200 ml 0.05 M Tris, 0.05 M EDTA, pH 8.2 medium. The cell suspension was incubated for 15 minutes at 30°C in mercaptoethanolamine-HCl 0.01 M, and centrifuged at 6 000 RPM for 5 minutes. To remove the cell wall, the pellet was carefully suspended in 200 ml of 0.02 M Tris-maleate, 2 mM EDTA buffer, pH 5.7, supplemented with 1 mg/ml glusulase (final concentration) and incubated at 30°C. An aliquot of cell suspension was removed at 15 minute intervals, and optical density was followed using a Klett spectrophotometer, until no change in turbidity was observed. The cells were centrifuged and washed carefully in Tris-maleate buffer medium. The washed pellet was suspended in 500 ml 0.6 M mannitol, 500 μM EDTA and blended in a Waring blender at top speed for 30 seconds. The mitochondria were isolated by differential centrifugation according to reference (10). The growth conditions and preparation of copper deficient medium were according to reference (10).

Spectrophotometry : Room temperature difference spectra of cytochromes were recorded with a Perkin-Elmer 356 dual wavelengths spectrophotometer. Dithionite was used as reductant. The concentration of cytochromes was determined, using an extinction coefficient of 16 for the α band of cytochrome c oxidase (11), 19 and 18 for the α bands of cytochrome c (11) and cytochrome b (12), respectively.

Potentiometric titration : The oxidation-reduction potentials of

cytochrome c oxidase were measured according to Dutton's procedure (13). This method enables a simultaneous determination of oxidation-reduction potentials by means of platinum and calomel electrodes, and of absorbance changes using a dual-wavelengths spectrophotometer under strictly anaerobic conditions. The contents (6 ml) of the reaction vessel (1 cm path-length) were stirred continuously and maintained under an atmosphere of argon. The oxidation-reduction mediators used to act between the membrane-bound cytochrome c oxidase and the platinum electrode were TMPD (Em = + 260 mV), phenazine methosulfate (Em = + 80 mV) and potassium ferricyanide (Em = + 430 mV). After addition of antimycin A (10 μg) the anaerobiosis was established by addition of ascorbate until the oxidation-reduction potential reading was less than + 200 mV. Oxidation-reduction potentials were made more positive with 100 mM potassium ferricyanide and more negative with ascorbate (5-20 mM). The determinations were carried out at room temperature (24°C).

RESULTS

Fig. 1 shows the effect of copper on the growth of Candida utilis. Copper deficiency severely impairs the growth of yeast cells. The minimum amount of copper for optimal growth was 10 μg copper/liter. Between 10 and 330 μg copper/liter, no change in the cell yield was observed. High copper concentrations (> 330 μg/liter) also inhibited the growth of yeast cells.

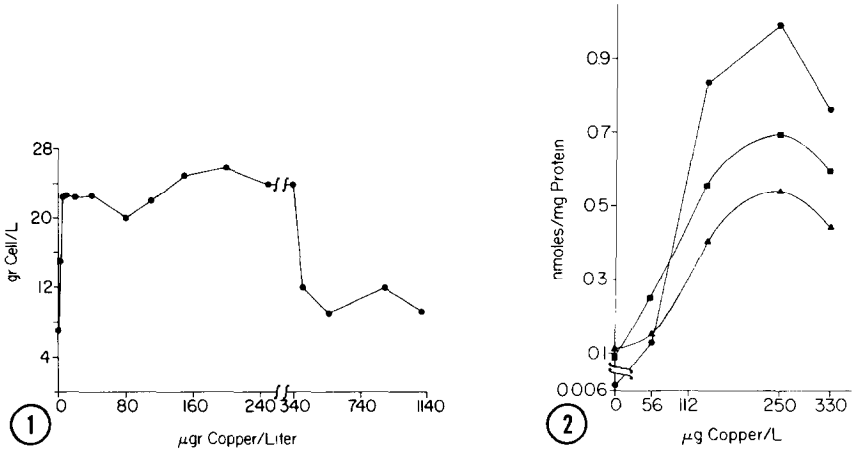

Fig. 1 - Yield of yeast cells grown for 24 hrs in media containing increasing copper concentrations.

Fig. 2 - The amount of cytochrome c oxidase and cytochromes b and c in mitochondria isolated from yeast cells grown in the presence of increasing concentrations of copper. Cytochrome c oxidase : ●——●; cytochrome b : ▲——▲ ; cytochrome c : ■——■.

Fig. 2 shows the amounts of cytochrome c oxidase and cytochromes b and c from isolated mitochondria of yeast grown at various copper concentrations. Copper stimulates the biosynthesis of cytochromes up to 250 µg copper/liter ; the level then drops off

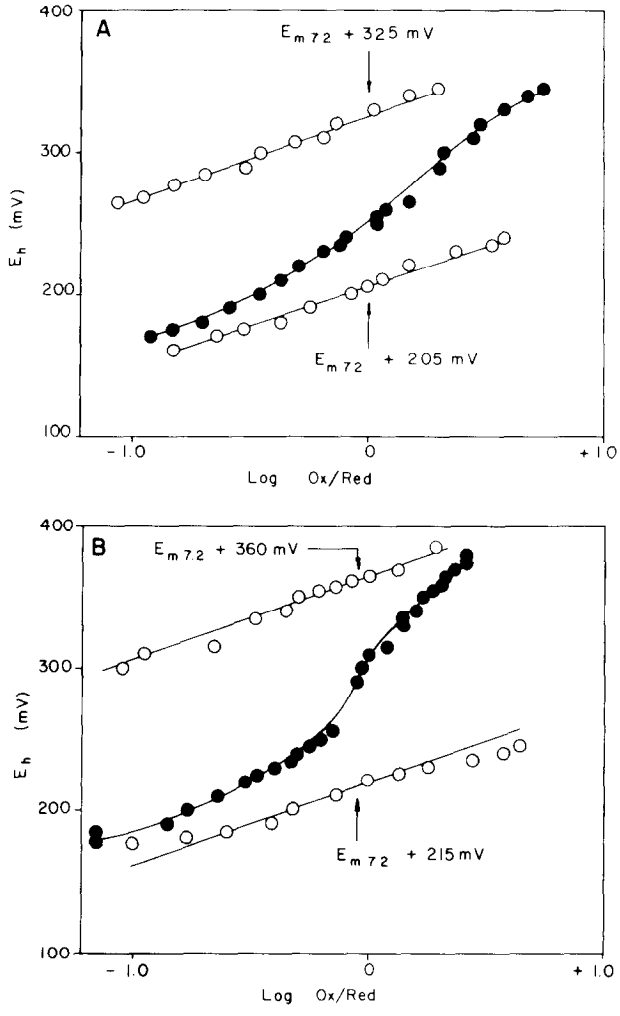

Fig. 3 - The course of oxidation-reduction titration of cytochromes a and a3 and the resolution of the sigmoidal curve into its component parts in isolated mitochondria of Candida utilis grown in the presence of A : 56 µg copper/liter; B : 140 µg copper/liter; C : 330 µg copper/liter. Approximately 20 mg protein/ml of mitochondria were suspended in 0.15 M mannitol, 0.05 M sucrose and 0.04 M morpholinopropanesulfonate, pH 7.2. 20 µM TMPD and 20 µM phenazinemethosulfate were added as redox mediator. An excess of ascorbate was added until reductive phase was obtained. The oxidative phase was obtained by addition of small amounts of potassium ferricyanide. The wavelengths measured were 445-455 nm.

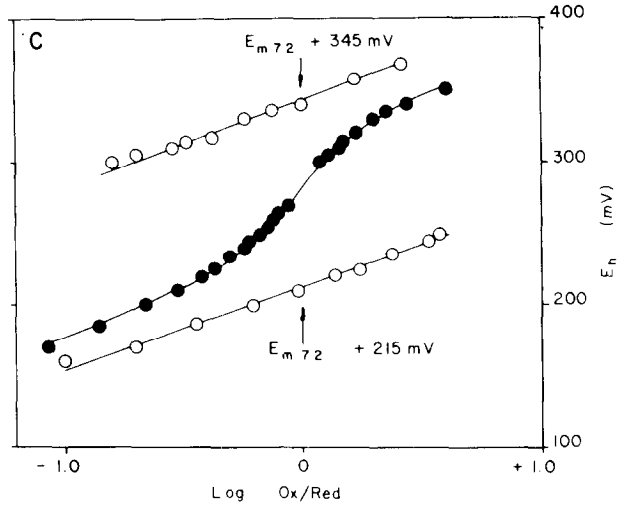

Fig. 3 (continued)

slightly for yeast cells grown at 330μg copper/liter. This observation suggests that copper stimulates the biosynthesis of all cytochromes by a complex mechanism of interactions between copper and iron, a mechanism which remains to be elucidated.

The increase in the biosynthesis of cytochrome c oxidase in the presence of copper opens the question of whether the properties of this cytochrome c oxidase are similar to cytochrome c oxidase in normal cells. The absorption spectrum of cytochrome c oxidase is seen to be unchanged regardless of the copper concentration in the medium (14). The redox state of hemes in cytochrome c oxidase should provide additional information regarding the functional integrity of the molecule. Fig. 3 shows the oxidation-reduction potential of cytochromes a and a_3 in the isolated mitochondria from yeast grown at 56, 140 and 330μg copper/liter. The sigmoid curve describes the course of oxidation-reduction of cytochromes a and a_3. Resolution of the sigmoid curve into its components produces points with n = 1, showing the Em at pH 7.2 of cytochromes a and a_3.

Table I shows the oxidation-reduction curve of cytochromes a and a_3 in isolated mitochondria of yeast grown at various copper

TABLE I

Oxidation-reduction potential of cytochromes a and a_3 in isolated mitochondria of yeast grown in media containing various copper concentrations.

Copper[a]	Cytochrome c oxidase[b]	Cytochrome a Em 7.2	Cytochrome a_3 Em 7.2
56	0.12	205	325
140	0.85	215	360
330	0.76	215	345

(a) The values are expressed as the amount of copper (μg/liter) available in the growth medium.

(b) The values represent the amount of heme a/mg mitochondrial protein for yeast grown at various copper concentrations.

concentrations. The midpoint potentials of cytochromes a and a_3 were : 205 $\pm$ 10, 325 $\pm$ 10 for 56μg copper/liter in growth medium ; 215 $\pm$ 10, 360 $\pm$ 10 for 140μg copper/liter in growth medium and 215 $\pm$ 10, 345 $\pm$ 10 for 330μg copper/liter in growth medium. This observation suggests that the midpoint potential of cytochromes a and a_3 in yeast mitochondria is close to the value reported for cytochrome a (+ 205 mV) and cytochrome a_3 (+ 360 mV) in beef heart mitochondria (6).

DISCUSSION

In this work we have studied the redox state of cytochrome c oxidase in isolated mitochondria of the aerobic yeast Candida utilis. The anaerobic potentiometric titration yielded information about the absolute potential of hemes a and a_3. Titration of the hemes should be characterized by potentials differing by more than 100 mV (15) ; the oxidation-reduction midpoint potential of cytochromes a and a_3 in isolated mitochondria from the yeast Candida utilis showed a value close to those reported for oxidase from other tissues (6-8).

The role of copper in the biosynthesis of cytochrome c oxidase is still subject to speculation. The dependence of the cytochrome

c oxidase level on the amount of copper in the growth medium suggests that copper plays a key role in the assembly of active cytochrome c oxidase. In copper-deficient cells, exhibiting an absence of cytochrome c oxidase, both apo-cytochrome c oxidase and the porphyrin precursor of heme a are nevertheless synthesized (10,16). It has been proposed that copper is required at the last step of the assembly of cytochrome c oxidase, to facilitate the incorporation of iron into porphyrin a (17). The increase in the biosynthesis of cytochrome c oxidase as well as of cytochromes b and c by copper might also be related in mechanism to the similar stimulation of apo-ferritin synthesis by iron (18) and of apo-hemoglobin synthesis by heme and iron (19,20). Thus the stimulation of cytochrome c oxidase synthesis by copper leads to the formation of cytochrome oxidase, which exhibits redox properties similar to those of cytochrome c oxidase from other tissues (6-8). Our observations suggest that mitochondrial alterations as seen in the cells grown in high copper concentrations (21) or in Wilson's disease (22), are due to the overloading toxic effects of copper on mitochondria, rather than the biosynthesis of non-functional oxidase.

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