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THE OXIDASE SYSTEMS OF ASCARIS-MUSCLE MITOCHONDRIA

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SUMMARY

1. The oxidase systems of Ascaris-muscle mitochondria were investigated by polarographic and spectroscopic techniques.

2. Spectrophotometric studies at $-196°$ revealed that Ascaris-muscle mitochondria contained low levels of substrate-reducible *a*-, *b*- and *c*-type cytochromes. Cytochrome a_3, present in very low concentrations, was shown to be functional by photochemical action spectrum.

3. Ascaris-muscle mitochondria oxidized (in decreasing order of respiratory activity) succinate, malate, NADH and α-glycerophosphate with H_2O_2 as end-product.

4. The antimycin A-insensitive ascorbate–tetramethyl-*p*-phenylenediamine (TMPD) oxidase activity was inhibited 33 % by 1.0 mM CN^-, 58 % by CO and 100 % by CO *plus* 1.0 mM CN^-. CO inhibited 50 % of succinate oxidation.

5. Fluorescence spectrum confirms and further identifies the two flavin components involved with malate oxidation in Ascaris-muscle mitochondria.

INTRODUCTION

Conflicting results have been reported for the respiratory chain system of *Ascaris lumbricoides*, a roundworm from the small intestine of pigs. An electron transport system lacking functional cytochromes but with a flavoprotein-containing terminal oxidase[1] and one with several cytochromes in a branched respiratory chain system[2,3] have been proposed. This was further complicated by the recent findings[4,5] that Ascaris have *b* (557 nm) and *c* (549 nm) cytochromes with deficiency in *a*-type cytochrome.

This paper confirms that Ascaris-muscle mitochondria have cytochromes of types *b* and *c*. In addition, photochemical action spectra show cytochrome a_3 to function as a terminal oxidase and the CO difference spectrum shows the presence of CO-binding hemoprotein(s) as well.

METHODS

The mitochondria from Ascaris-muscle were isolated according to the method used for pigeon heart-muscle mitochondria preparation[6] with 75 mM sucrose con-

Abbreviation: TMPD, tetramethyl-*p*-phenylenediamine.

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taining 225 mM mannitol and 0.1 mM EDTA. Other experimental details are given in the legends to figures and tables.

RESULTS

Fig. 1 illustrates the difference spectra (— 196°) of Ascaris-muscle mitochondria obtained with dithionite (A) and various substrates (B–D). Both α-glycerophosphate (B) and malate (C) reduced the same *b*-(556 nm) and *c*-(548 nm)-type cytochromes, the latter component was also reduced by ascorbate (D). Ascorbate also reduced the *a*-type cytochrome which had a maximum at 443 nm instead of 446 nm as observed with α-glycerophosphate.

The most outstanding features observed with Ascaris-muscle mitochondria were the lack of any detectable *a*-type α-peak and the presence of the predominant maximum at about 480 nm with two corresponding minima at 460 and 502 nm in the substrate-reduced spectra.

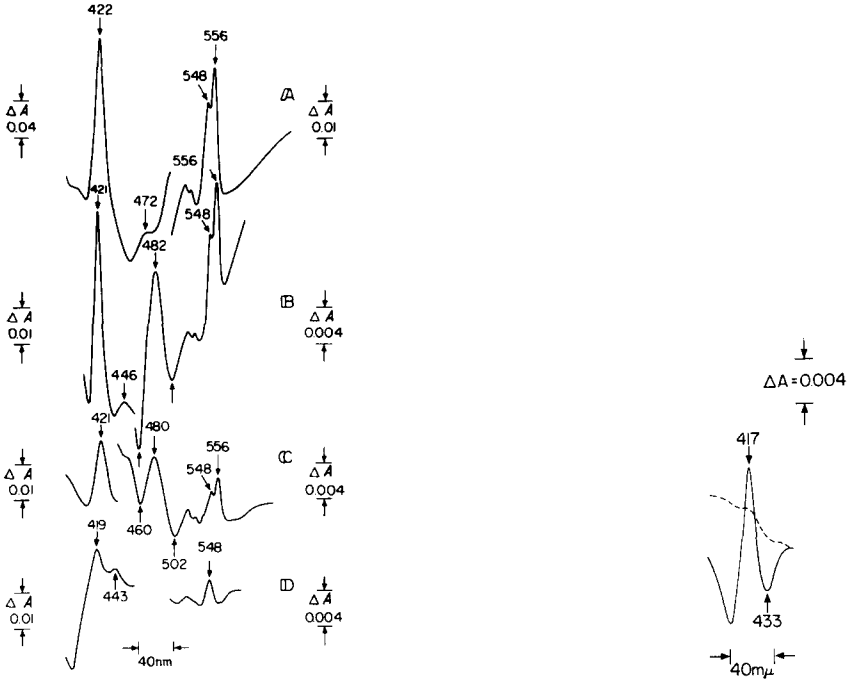

Fig. 1. Liquid-nitrogen temperature (— 196°) difference spectra of Ascaris-muscle mitochondria. These spectra were recorded with a split-beam spectrophotometer using the rapid freezing technique described by WILSON[7], CHANCE AND SPENCER[8] and CHANCE AND SCHOENER[9]. Both the sample and reference cuvettes (2.0-mm lightpath) contained 0.35 ml Ascaris-muscle mitochondria (7.0 mg protein per ml) suspended in 220 mM mannitol, 50 mM sucrose and 15 mM Tris–HCl (pH 7.4). A, dithionite-reduced *minus* oxidized; B, α-glycerophosphate-reduced *minus* oxidized; C, (malate + Mn^{2+} + P_i) *minus* oxidized; D, (ascorbate + CN^-) *minus* oxidized. Final concentration (mM): α-glycerophosphate, ascorbate, 5.0; malate, 10.0; Mn^{2+}, 1; CN^- and P_i, 2.0.

Fig. 2. CO difference spectrum (α-glycerophosphate + CO *minus* α-glycerophosphate) of Ascaris-muscle mitochondria at liquid-nitrogen temperature (— 196°). Both the sample and reference cells (2.0-mm lightpath) contained 0.35 ml Ascaris-muscle mitochondria (13.0 mg protein per ml). – – – –, baseline (α-glycerophosphate *minus* α-glycerophosphate); ———, CO difference spectrum.

The spectral evidence presented in Fig. 1 suggests that Ascaris-muscle mitochondria contained *b*- and *c*-type cytochromes with a very low level of an *a*-type cytochrome. This was supported by the reduced pyridine hemochromogen spectrum which showed maxima at 553, 524 and 417 nm. The α-peak at 553 nm was contributed by heme *c* and protoheme[10]; the ratio of heme *c* to protoheme was about 1 assuming that there was no decomposition of heme. No heme *a* band was detected because of the extremely low level of the *a*-type cytochrome in Ascaris mitochondria.

The CO-reactive hemoproteins in Ascaris-muscle mitochondria were detected by bubbling the anaerobic α-glycerophosphate-reduced system with CO for 2 min. The CO difference spectrum (− 196°) illustrated in Fig. 2 closely resembles an *o*-type cytochrome[11]. No cytochrome a_3–CO complex (γ-peak at 427–430 nm) could be detected but it was possible that the γ-peak of cytochrome a_3–CO complex was obscured by the strong absorption band at 417 nm. The CO-binding pigment could be due to either hemoglobin or myoglobin contamination or to cytochrome *o* and the problem was not further resolved.

The action spectrum of Ascaris-muscle mitochondria (Fig. 3A) with maxima at approx. 593 nm (α-peak) and 432 nm (γ-peak) is characteristic of cytochrome a_3 (ref. 11) and corresponds closely with that of Baker's yeast (Fig. 3B), the latter was

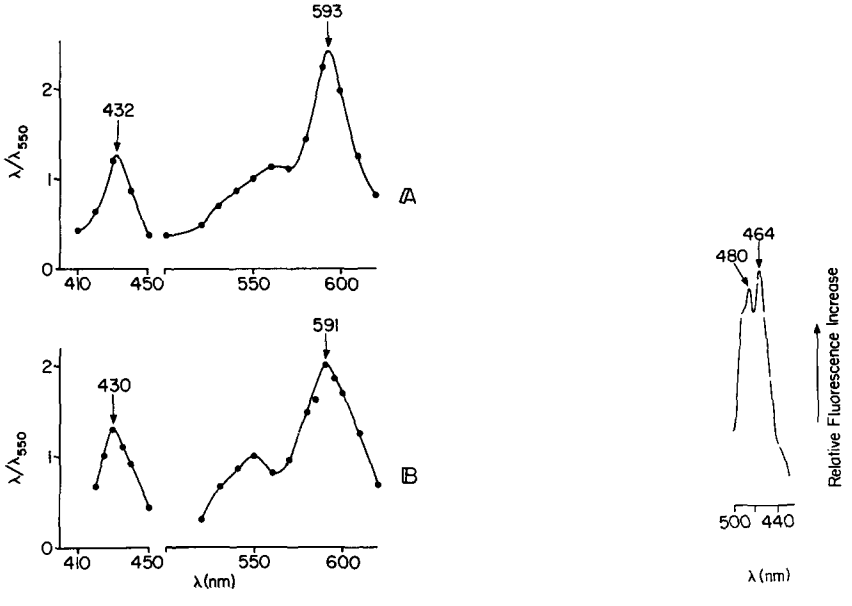

Fig. 3. Photochemical action spectra of CO-inhibited respiration of Ascaris-muscle mitochondria and Baker's yeast. Experimental procedure as described by CASTOR AND CHANCE[12], as improved by HYDE[13]. The ordinate represents the efficiency of light compared with 550-nm light in reversing the CO-inhibited respiration. Gas mixture, O_2:CO:N_2 (10:40:50, by vol.); slit, 2.0 mm. Ascaris-muscle mitochondria (A) (5.0 mg protein) were reduced with malate (5 mM) and succinate (5 mM) in 5 mM phosphate buffer (pH 7.2).

Fig. 4. Excitation difference spectrum of Ascaris-muscle mitochondria (20°). The excitation difference spectrum recorded as previously described[14] was obtained by treating 0.3 ml Ascaris-muscle mitochondria in 2.7 ml medium containing 220 mM mannitol, 50 mM sucrose, 15 mM Tris–HCl (pH 7.4), 1.0 mM $MgCl_2$, 1.5 mM ATP, 3.0 mM P_i and 10 mM malate. Protein concentration, 4.0 mg/ml. Lightpath, 10 mm; slit, 0.5 mm; voltage, 980; Wratten-21 filter.

used as a control. The data clearly indicates that respiration of Ascaris-muscle mitochondria involved a CO-sensitive and light-reversible heme pigment identified as cytochrome a_3. The *o*-type–CO complex previously observed (Fig. 2) was not light reversible under the experimental conditions employed.

The fluorescence spectrum (Fig. 4) confirms and further identifies the two flavin components associated with malate oxidation previously reported by LEE AND CHANCE[5]. The excitation difference spectrum reveals two maxima at about 480 and 464 nm with a corresponding trough at 475 nm.

Table I illustrates the approximate concentration of the respiratory components reduced by α-glycerophosphate calculated from the liquid-nitrogen difference spectrum. Assuming that all the cytochromes were equally enhanced, the *b*- and *c*-type cytochromes are about 5 times greater than the CO-binding hemoprotein, which in turn is about 5 times greater than the *a*-type pigment (including cytochrome a_3). The values for the *b*-, *c*- and *a*-type cytochromes are much lower than those reported for other animal tissues[16]. The cytochrome *o* concentration is approximately 1.5 times less than that reported in another parasitic worm, *Moniezia expansa*[17].

TABLE I

APPROXIMATE CONCENTRATION OF THE RESPIRATORY PIGMENTS IN ASCARIS-MUSCLE MITOCHONDRIA REDUCED BY α-GLYCEROPHOSPHATE

The approximate concentration of the respiratory components involved in α-glycerophosphate oxidation was estimated from difference spectrum recorded at −196° (Fig. 1B) using the millimolar extinction coefficient of CHANCE[11,15].

Respiratory components	*Concentration (nmole/mg protein)*
CO-binding hemoprotein	0.012
c-type	0.089
b-type (556 nm)	0.073
a-type	0.0027

TABLE II

RESPIRATORY ACTIVITIES OF ASCARIS-MUSCLE MITOCHONDRIA

The oxidation of various substrates was measured polarographically at 25° in a medium containing 220 mM mannitol, 50 mM sucrose and 15 mM Tris–HCl (pH 7.4). Mn^{2+} (1.0 mM) and P_i (3.0 mM) were included in the medium for malate oxidation, and Mg^{2+} (1.0 mM) for α-glycerophosphate oxidation. The turnover number was calculated using the following formula: Turnover number = [(O_2 uptake (nmoles O_2/sec per mg protein))/(concentration of cytochrome a_3 (nmoles/mg protein))] × 4. Final concentration of substrates: malate, succinate, α-glycerophosphate, 4.4 mM; NADH, 450 μM.

Substrate	*Rate (nmoles O_2/min per mg protein)*	*Approx. turnover number (electrons/cytochrome a_3 per sec)*
Malate	2.14	53
Succinate	3.69	91
NADH	0.83	21
α-Glycerophosphate	0.55	13

The respiratory activities of Ascaris-muscle mitochondria and their turnover number with respect to cytochrome a_3 are illustrated in Table II. Ascaris-muscle mitochondria could oxidise (in decreasing order of respiratory activity) succinate, malate, NADH, and α-glycerophosphate. Malate oxidation, stimulated by Mn^{2+} *plus* P_i, was inhibited 92 % by rotenone (5 μM). The oxidation of succinate was not effected by either Mn^{2+}, rotenone, Ca^{2+}, cytochrome *c* or purified beef-heart cytochrome oxidase (EC 1.9.3.1). The end-product of substrate oxidation was H_2O_2 (*cf.* KMETEC AND BUEDING[18]), determined polarographically with catalase.

The sensitivity of the terminal oxidase towards CN^- and CO and CN^- *plus* CO was investigated polarographically using ascorbate–TMPD and succinate as electron donors. 1.0 mM CN^- inhibited 33 % of the ascorbate–TMPD oxidase activity. When repeated in a CO-saturated reaction medium, the ascorbate–TMPD activity was inhibited 58 % by CO, with complete inhibition following the subsequent addition of 1.0 mM CN^-. CO also inhibited 50 % of succinate oxidation.

DISCUSSION

Ascaris-muscle mitochondria thus contained substrate-reducible *b*-(including cytochrome *o*)-, *c*- and *a*-type cytochromes with functional cytochrome a_3 acting as the terminal oxidase. The *a*-type cytochrome (including cytochrome a_3) concentration is very low, the *c*/*a* ratio was about 33/1. The results are complementary to those of BUEDING[1] and KMETEC AND BUEDING[18] who reported that Ascaris-muscle mitochondria have no cytochromes associated with electron transport and afford a biochemical explanation of our observations of the partially CO-sensitive respiration. Our data also supports the earlier postulation made by KIKUCHI *et al.*[2] derived from studies with crude particulate preparations and elaborates further by showing that Ascaris-muscle mitochondria have *a*-, *b*-(including an *o*-type pigment) and *c*-type cytochromes and minute amounts of functional cytochrome a_3 demonstrated by photochemical action spectra.

It is interesting to note that the existence of an *o*-type pigment and cytochrome a_3, the production of H_2O_2 from substrate oxidation and a mechanism for reducing fumarate to succinate[18,19] in Ascaris have also been reported for another parasitic worm, *M. expansa*[20], living in a similar type of environment. The results obtained from these two different parasitic worms tend to suggest that perhaps the above characteristic features occur among other intestinal parasites living in a similar type of environment.

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