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The respiratory components of *Moniezia expansa* (Cestoda)

One of the most interesting features of the carbohydrate metabolism of intestinal parasites is that a great majority of these organisms excrete succinate (see ref. 1). This observation suggests that such organisms, apparently living in a partially anaerobic environment, probably possess a special kind of electron transport system. Investigations with *Moniezia expansa*[2], a tapeworm, demonstrated that it has a major pathway linking to an *o*-type cytochrome, and is responsible for the reduction of fumarate to succinate. There is an indication, however, that the classical cytochrome oxidase (EC 1.9.3.1) might also function as a secondary terminal oxidase. This report presents further evidence for the existence of a branched respiratory chain in *M. expansa*[2], one pathway linking to an *o*-type terminal oxidase, the other to cytochrome oxidase.

The mitochondria-containing particulate fraction and spectrophotometric studies at room and liquid nitrogen temperature with a Cary (Model 14R) spectrophotometer were carried out as previously described[2]. Nitrogen was determined by the method of JOHNSON[3], and converted to protein by multiplying by 6.25. The concentrations of cytochromes *a* and a_3 and cytochrome *o* were determined using the extinction coefficients of VAN GELDER[4] and TABER AND MORRISON[5], respectively.

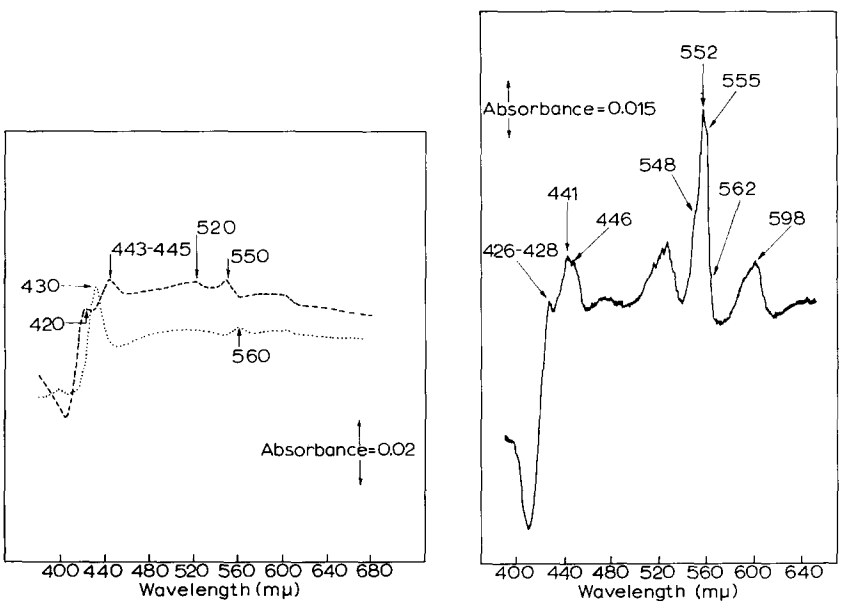

Fig. 1. Room-temperature (20°) difference spectra of the reduced respiratory pigments of *M. expansa*. Both sample and reference cuvettes (4.0 mm light-path) contained 0.48 ml preparation (4.7 mg protein per ml) in 50 mM phosphate buffer (pH 7.6). ·····, antimycin A (1.0 μM) *minus* O_2; -------, *p*-phenylenediamine (mM) + CN^- (mM) *minus* O_2.

Fig. 2. Low-temperature (77 °K) difference spectrum of the reduced respiratory components of *M. expansa*. Both sample and reference cuvettes (2.0 mm light-path) contained 0.24 ml preparation (2.4 mg protein per ml) in 50 mM phosphate buffer (pH 7.6). The preparation in the sample was treated with succinate (10 mM) and cyanide (mM), after which both cuvettes were oxygenated.

Concentrations of cytochromes *b* and *c* were calculated using the millimolar extinction coefficients of CHANCE AND WILLIAMS[6].

Fig. 1 demonstrates the presence of cytochromes *b*, *c*, and $a + a_3$, in *M. expansa*. Cytochrome *b* with its α (560 mμ) and γ (430 mμ) peaks was detected in the presence of antimycin A (1.0 μM). Cytochromes $a + a_3$ (443–445 mμ) and cytochrome *c* (550 mμ) were observed with *p*-phenylenediamine *plus* cyanide.

Fig. 2 illustrates the difference absorption spectrum (77 °K) obtained following addition of cyanide and oxygen to the preparation previously reduced with succinate. The autoxidizable *o*-type terminal oxidase having a split peak (552, 556 mμ) at liquid nitrogen temperature and a Soret peak at 425 mμ (refs. 2, 7) was not observed. Cytochromes *b* (562 mμ), c_1 (552 mμ), *c* (548 mμ), and $a + a_3$ (598, 446 mμ) were detected. The slight shoulder component at 555 mμ might be the *b*-type pigment in the major pathway which is only sensitive to high antimycin A concentration (0.1 mM)[2].

Fig. 3 shows the difference spectra of the CO complexes of the two terminal oxidases. The difference spectrum recorded at 3.0 min with peaks at 433–436 and 410 mμ and corresponding troughs at 444–445 and 425 mμ represents the CO complexes of cytochrome a_3 and the *o*-type pigment. The difference spectra recorded at 8.0 and 11.0 min clearly illustrate the predominant *o*–CO complex superimposed on that of cytochrome a_3, and the overall spectral change was due to the sum of these two pigments. The above data suggest that CO binds with cytochrome a_3 more readily than with cytochrome *o*. A similar type of observation was recently reported by BROBERG AND SMITH[8] in *Bacillus megaterium*. This bacterium has cytochrome a_3 and cytochrome *o*, and that the predominant *o* pigment appears not to bind maximally even after 15 min treatment with CO.

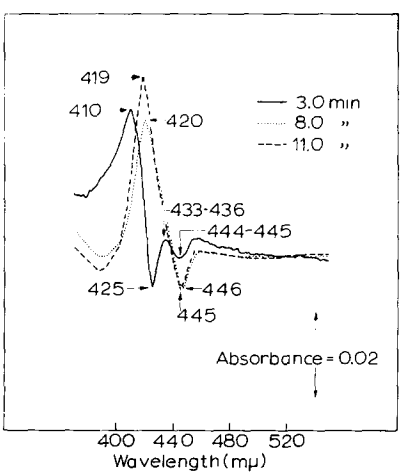

Fig. 3. CO difference spectra of *M. expansa* (20°). The preparation was similar to that described in Fig. 1. Both cells were treated with α-glycerophosphate (10 mM), and CO bubbled through the sample.

Table I shows the concentrations of the respiratory components in *M. expansa*. The cytochrome *c* content probably includes that of cytochrome c_1 (Fig. 2), which was also previously demonstrated to be present[2]. Concentrations of cytochromes *a* and a_3 (expressed as mμmoles/mg protein) are 7 times lower than those in rat liver

mitochondria[9]. Table I also illustrates that cytochrome *o* is the major terminal oxidase in *M. expansa*.

The possibility that the *o*-type pigment could be due to either haemoglobin or myoglobin is most unlikely as neither the oxy nor the carboxy form of either of these pigments was detected in the preparation. Furthermore, this *o*-type pigment was reducible with various substrates (succinate, α-glycerophosphate, NADH) and the reduced form of this pigment could be re-oxidized with oxygen and fumarate[2].

TABLE I

CONCENTRATIONS OF THE RESPIRATORY COMPONENTS OF *M. expansa*

Concentrations of cytochromes *b*, *c*, and *a*, are calculated from Fig. 1, and cytochromes a_3, and *o* from the CO complexes in Fig. 3.

Respiratory components	*Concn. (mμmole/mg protein)*
b	0.120
c	0.139
a	0.032
a_3	0.032
o	0.331

The above evidence strongly supports the conclusion that *M. expansa* has cytochrome a_3 and an *o*-type pigment as terminal oxidases as well as the existence of a small amount of the classical cytochrome chain in its branched respiratory chain system[2]. Such a respiratory chain system with more than one terminal oxidase could perhaps be a common feature among intestinal parasites living in a partially anaerobic environment. The major electron pathway having an *o*-type cytochrome and probably a functional *b*-type cytochrome, which is sensitive to only high antimycin A concentration[2], could perhaps also be the pathway for other succinate-excreting intestinal parasites[1].

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