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Low-temperature spectroscopy of ascites cells and mitochondria

Difference spectroscopy of mouse ascites tumor cells and mitochondria has indicated that the respiratory-chain cytochromes are present in nearly equimolar amounts[1,2]. Using an improved method for the isolation of mitochondria from ascites cells we have found that this stoichiometry holds true in both hyperdiploid and tetraploid cell strains. The cytochrome *c* concentration in these mitochondria, a sensitive test of mitochondria integrity, is about the same as that which has been found in rat-liver mitochondria[3].

EL_2 (tetraploid) and ELD (hyperdiploid)[4,5] mouse ascites tumor cells were grown in the peritoneal cavities of 5-week-old female Hauschka/ICR mice 7–10 days. About 5 g of cells (wet wt.) were used for the preparation of mitochondria. The cells were washed free of trace amounts of red cells by 1–3 rapid (3 min) distilled water washes, and then suspended in an equal volume of 0.4 M mannitol made to pH 7.0 with saturated sodium bicarbonate, containing 1 mM disodium EDTA. The cells were incubated in 15 mg Nagase per 50 ml medium per 5 g of cells for 15 min at 0° (ref. 6), and homogenized 6 times in a glass homogenizer with a tight-fitting Teflon motor-driven pestle (1150 rev./min). The homogenate was centrifuged at 755 × *g* for 5 min. The supernatant was saved. The precipitate composed mostly of unbroken cells was rehomogenized 6 times, filtered through a double layer of cheesecloth and recentrifuged at 755 × *g*. The combined supernatants were centrifuged at 11000 × *g* for 10 min. The mitochondrial pellet was washed in 0.4 M mannitol, and finally suspended in 4–5 ml of 0.4 M mannitol.

0.5 ml of aerated mitochondria was quickly taken in a syringe and injected into a copper-walled cuvette precooled in liquid N_2 (ref. 7). 0.1 ml of this suspension was used for biuret protein estimation. A few crystals of sodium dithionite were added to a third aliquot of mitochondria and the reduced suspension was injected into the sample cuvette. The wavelength scanning spectrophotometer of YANG AND LEGALLAIS[8] was used to record the reduced—oxidized difference spectra. Cytochrome concentrations were calculated using the millimolar extinction coefficients of CHANCE AND WILLIAMS[1]. Each absorption increment was divided by an experimentally determined intensification factor of 5, to compensate for the enhanced absorption at liquid N_2 temperature[9].

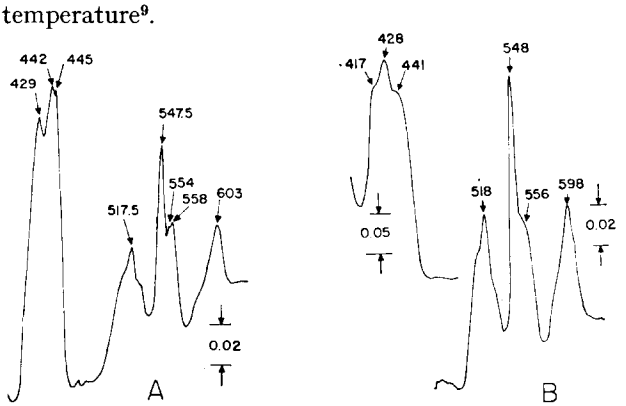


Fig. 1.

TABLE I

CYTOCHROME CONCENTRATIONS IN ELD AND EL_2 ASCITES TUMOR CELLS AND MITOCHONDRIA

Cells and mitochondria in the reference cuvette were treated either with amytal (2.0 mM), or with rotenone (1 μg/mg protein). The material in the sample cuvette was treated with either sodium hydrosulfite or with sodium sulfide (3 mM).

Material	*Expt.*	*Treatments*	*Cytochrome concentration (μM/g protein)*			
			a	*b*	*c*	a_3
ELD cells	(4.27,3)	$S_2O_4^{2-}$ — amytal	0.083	0.107	0.187	0.065
ELD cells	(4.27,2)	S^{2-} — amytal	0.031	0.013	0.060	0.010
ELD mitochondria (3 preparations)		$S_2O_4^{2-}$ — amytal	0.198	0.176	0.391	0.076
EL_2 cells	(8.31,1)	$S_2O_4^{2-}$ — rotenone	0.060	0.069	0.140	0.029
EL_2 mitochondria (3 preparations)		$S_2O_4^{2-}$ — amytal	0.218	0.203	0.372	0.101

Fig. 1A shows a difference spectrum between aerated, amytal-treated ELD cells and $Na_2S_2O_4$-treated cells. These cells contained about twice the cytochrome concentration previously reported (Table I). The high cytochrome content was seen in one out of every 3 or 4 preparations and is not considered to be an artifact. Also shown in Table I are cytochrome concentrations calculated from a difference spectrum obtained by treating ELD cells with sodium sulfide. The concentrations of cytochromes seen after sulfide treatment are 3 times less than those resulting from the dithionite treatment. Either sufficient sulfide was not used to fully inhibit electron transport, or most of the intracellular cytochrome was not being used in electron transport. In the spectrum resulting from dithionite treatment there is a 554 mμ peak which is not present in the spectrum resulting from sulfide treatment. In the spectrum resulting from sulfide treatment there is a peak at 552 mμ. It seems likely that the 552 mμ peak is due to cytochrome c_1 (refs. 10, 11), and that at 554 mμ is due to cytochrome b_5 (ref. 12).

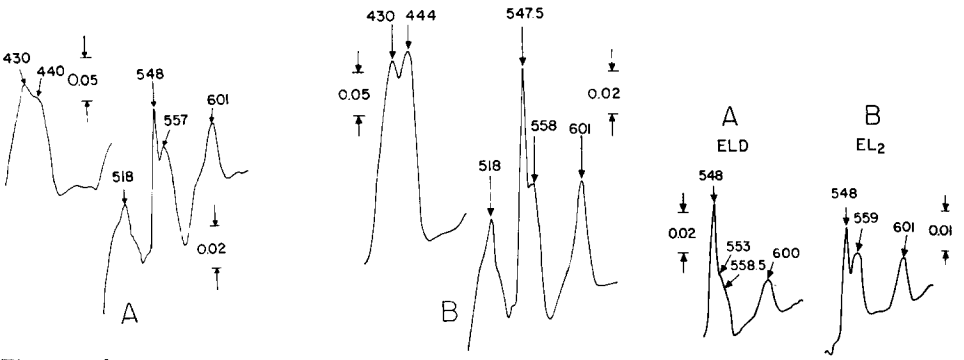
Fig. 1B shows an oxidized—reduced difference spectrum of mitochondria isolated from ELD cells. The 5 mμ shift of the cytochrome $a+a_3$ peak, from 603 to 598 mμ is a common finding. The 554 mμ absorption seen in the intact cell spectrum (Fig. 1A) is missing. This may indicate that the 554 mμ peak was due to cytochrome b_5, and that this endoplasmic reticulum-associated cytochrome is present only in a very small traces, if at all, in the ELD mitochondria. The average concentrations of cytochromes in three preparations of ELD mitochondria are shown in Table I.

Fig. 2A shows an oxidized—reduced difference spectrum of EL_2 cells. This spectrum shows that there is an extra increment of absorption at 557 mμ in the EL_2 cells (compared with the ELD cells) (Fig. 1). This 557 mμ absorption is probably largely due to the presence of *b*-type cytochrome.

Fig. 2B shows the difference spectrum of EL_2 mitochondria (amytal—$Na_2S_2O_4$). The EL_2 mitochondria show a more pronounced absorption in the 558–552 mμ region than do the ELD mitochondria.

Fig. 3 (A and B) shows reduced—oxidized spectra over the 630–530 mμ range of glass-grown ELD and EL_2 cells. The cytochrome complement of these two cell

types differs in the amount of *b*-type cytochrome. The glass-grown and mouse-grown EL_2 cell cytochromes (Fig. 2A) are essentially identical. The glass-grown ELD cells have less absorption in the 554–560 mμ region than mouse-grown ELD cells (Fig. 1A). The spectrum of glass-grown ELD cells closely resembles that of mitochondria isolated from mouse-grown ELD cells (Fig. 1B). It may be that in the EL_2 cells the endoplasmic reticulum is more closely associated with the outer mitochondrial membrane than is the case in the ELD cells. The EL_2 mitochondrial-endoplasmic reticulum association may be similar to that which exists in rat liver[13].



Figs. 2 and 3.

In the ELD mitochondria the cytochrome *a* and *b* concentrations are about one-half of the $c+c_1$ concentration. The apparent deficit of cytochrome a_3 in both types of mitochondria is probably due to the fact that the reaction with dithionite was carried out for only a short time (2–3 min). In the EL_2 mitochondria a similar proportionality between $a:b:(c+c_1)$ exists when account is taken of the presence of traces of b_5. This substantiates the findings of CHANCE AND HESS[2]. Combined with the findings of BORST[14,15] and of KOOBS AND MCKEE[16], that similar mitochondria have normal respiratory control and substrate utilization characteristics, these mitochondria seem very competent to perform the tasks of aerobic metabolism. The chief advantage of the proteinase method over the methods used by BORST[15] and by CHANCE AND HESS[17] is its consistency and the large yield of mitochondria.

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