RESPIRATORY ACTIVITY OF BEEF THYROID MITOCHONDRIA *

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No study of the respiratory chain components and the coupled phosphorylation reactions of isolated thyroid mitochondria has been described previously. Several observations have been made which suggest that the thyroid gland contains mitochondria possessing similar properties to those isolated from other tissues. First, electron microscope studies of thyroid gland sections have revealed the presence of particles having the characteristic shape, size and structure of mitochondria (1). Secondly, the uncoupling agent 2,4-dinitrophenol (DNP) has been found to stimulate the oxygen uptake of thyroid slices and to inhibit the energy-dependent uptake of iodide (2). Thirdly, citric acid cycle enzymes have been detected in thyroid extracts (3). Recently a study of some oxidase and reductase activities of thyroid fractions has been reported (4). The 'mitochondria' used in the latter studies had evidently become damaged during the course of preparation since they were fully permeable to exogenous DPNH. Such studies are of limited value compared to those reported here, which employ preparations characterized by electron microscope and spectroscopic data and which exhibit the physiologically significant properties of respiratory control and impermeability to pyridine nucleotides.

METHODS

Fresh beef thyroid glands (about 120 g. after the removal of fat and connective tissue) were obtained at the slaughter-house and placed in an

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ice-cold solution containing 0.225 M mannitol, 0.075 M sucrose and 0.05 mM EDTA. Mitochondria were isolated from the glands by a slight modification of the proteinase method (5). The particles sedimented from the homogenate by centrifugation between 700 g for 10 minutes and 8,000 g for 10 minutes were washed twice and finally resuspended to yield 3 ml. of a stock suspension of mitochondria. Electron microscope studies of the mitochondrial fraction by Dr. D. F. Parsons revealed that the mitochondria were well preserved and contained contaminating lysosomes, colloid particles and a few microsomes. Oxidative phosphorylation was assayed polarographically with a Clark oxygen electrode (6). Low temperature difference spectra were recorded in a wavelength scanning spectrophotometer (7). Protein was estimated by a biuret method (8).

RESULTS

Figure 1 presents a typical oxygen electrode tracing obtained during the assay of oxidative phosphorylation reactions in isolated thyroid mitochondria. A negligible oxygen uptake was recorded before the addition of substrate, whereupon a 'resting' or State 4 rate (9) of respiration was established. On the addition of ADP, there was an accelerated phase of respiration ('active' or State 3 condition) followed by a decrease after the ADP was consumed. Subsequent State 3-State 4 cycles of respiration, characteristic of mitochondrial suspensions in a condition of respiratory control, were observed upon the addition of further aliquots of ADP. When the uncoupling agent DNP was added, a prolonged stimulation of respiration was recorded, which continued until all the oxygen present had been consumed, and respiratory control was abolished. The rates of oxidation of some other substrates are listed in Table 1. Among the physiological substrates, succinate gave the highest oxidation rate. The oxidation rate of ascorbate-TMPD gave an estimate of the cytochrome oxidase activity of the preparations, and was found to be in twenty-fold excess of the succinate oxidase activity. Fatty acids were oxidized at a slow rate, whereas no significant oxygen uptake was observed upon the addition of either β-hydroxybutyrate, choline, proline, DPNH

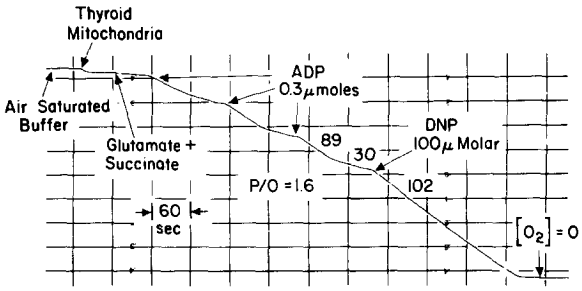

Fig.1 An example of the polarographic assay of oxidative and phosphorylative activities of beef thyroid mitochondria.The buffer (3 ml) contained 0.15 M sucrose;20 mM KCl;8 mM $MgCl_2$;1 mM EDTA;10 mM K phosphate buffer, pH 7.4;and bovine serum albumin (0.5 mg/ml).Thyroid mitochondria (1.5 mg protein),glutamate plus succinate (5 mM each),ADP and DNP were added at the times indicated.Figures above the trace denote rates of oxygen uptake (μl oxygen consumed/mg. protein/hour).Temperature,22°C.

or TPNH.After the mitochondria were preincubated with swelling agents (phosphate or calcium ions) an antimycin A-sensitive oxygen uptake was observed with DPNH as substrate.Adequately prepared thyroid mitochondria therefore appear to be impermeable to pyridine nucleotides.We have detected the

Table 1. Respiratory activity of beef thyroid mitochondria

Expt	Substrate	Rate of oxygen uptake (μ litres O_2 consumed/mg protein/hr)
1	None	4
	Succinate	60
	α-glycerophosphate	29
	α-ketoglutarate	16
	Malate	16
	Pyruvate + Malate	24
	Glutamate + Malate + Malonate	45
2	Ascorbate + tetramethyl-p-phenylene diamine (TMPD)	1,220

In Expt.1,the reaction mixture was the same as that specified in the legend to Fig.1.The concentration of each substrate used was 5 mM,and that of malonate,3 mM.Rates were measured in the presence of ADP,0.5 mM.In Expt.2,the mixture contained 50 mM K phosphate,pH 7.4;15 mM ascorbate; 0.15 mM TMPD;0.05 mM cytochrome c;0.2% w/v sodium cholate;and thyroid mitochondria (0.1 mg protein/ml).Temperature in both experiments,22°C.

presence of an extra-mitochondrial DPN-linked α-glycerophosphate dehydrogenase and a mitochondrial flavoprotein-linked α-glycerophosphate dehydrogenase in the thyroid gland. Thus both the enzymes required for the postulated glycerol phosphate cycle (10) are present and may provide a pathway for the mitochondrial oxidation of extra-mitochondrial DPNH. Ferricyanide reductase activities were observed with DPNH, succinate and α-glycerophosphate as substrate, but not with choline or β-hydroxybutyrate (plus DPN). These results suggest that no significant choline or β-hydroxybutyrate dehydrogenase activity is present in thyroid mitochondria.

The values of P:O ratios obtained were in the range 2.2 to 2.5 with glutamate-malate as substrate and 1.3 to 1.5 with succinate. Equal State 3 rates were obtained with either ADP or AMP as phosphate acceptor, since the mitochondria contained an active adenylate kinase. Hexokinase was also present in the preparation.

Antimycin A (0.5 μg/mg protein) or potassium cyanide (1 mM) reduced oxygen uptake to a negligible rate with each substrate used, except for the oxidation of ascorbate-TMPD, which was antimycin-insensitive. The oxidation of DPN-linked substrates was prevented by rotenone (3 μM) and amytal (2 mM) and succinate oxidation was inhibited 90% by trifluorothienylbutanedione (0.1 mM). Oligomycin (1.5 μg/ml) inhibited State 3 oxidation rates to a level similar to the State 4 rate, but had no influence on the oxidation rates with DNP-treated mitochondria. Kanaya (11) found that 1 mM thiocyanate increased oxygen uptake and decreased phosphorylation and iodide uptake in beef thyroid slices, and concluded that thiocyanate acted by depressing the energy supplied by high-energy phosphate bonds. In the present study, no uncoupling effect of thiocyanate was detected, since respiratory control and P:O ratios were unaffected by the presence of 1 to 10 mM thiocyanate. It is concluded that the anti-thyroid actions of thiocyanate are not due to a rapidly induced uncoupling action on respiratory chain phosphorylation.

Figure 2 shows a difference spectrum of the cytochromes of a

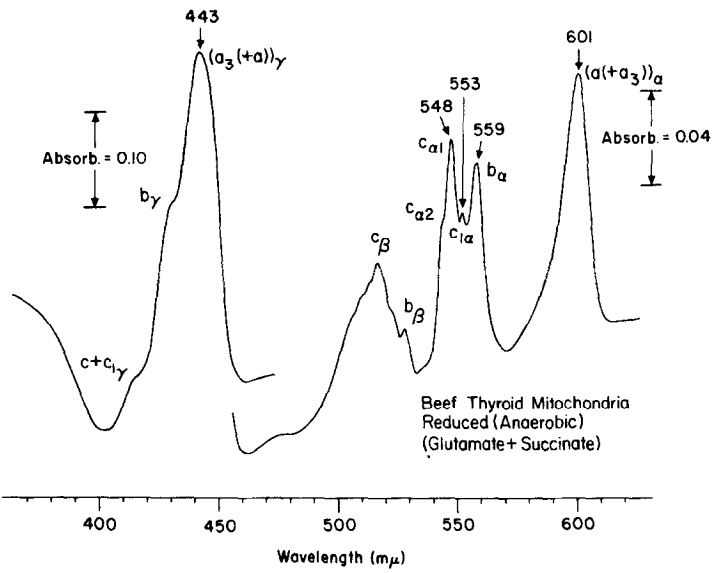

Fig.2 Low temperature spectrum of the enzymatically reduced pigments of beef thyroid mitochondria. Both sample and reference cuvettes contained 0.6 ml of mitochondria (14.3 mg protein/ml) in 50 mM K Phosphate buffer, pH 7.4. The sample cuvette was treated with 6 μl of glutamate-succinate solution (0.5 M each substrate), both cuvettes were cooled to liquid N_2 temperature, and the spectrum recorded. Light path, 1 mm.

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suspension of thyroid mitochondria, in which the α bands of cytochromes a + a_3, b, c_1 and c and the β bands of cytochromes b and c are clearly defined. In conjunction with other spectral studies, it may be concluded that the components and organization of the respiratory pigments in thyroid mitochondria closely resemble those of other types of mammalian mitochondria described previously (7).

It may be noted that the degree of stimulation of oxygen uptake of thyroid mitochondria by either ADP and phosphate, or by uncoupling agents, was at least as large as the stimulation of oxygen uptake of thyroid slices treated with thyroid stimulating hormone (12) or with DNP (2). The significance of the respiratory control property of isolated mitochondria has been discussed by Lardy (13) and by Chance (14). In accordance with this concept, it seems resonable to assume that the respiratory control mechanism present in isolated thyroid mitochondria is operative in the thyroid <u>in vivo</u>, and represents a fundamental control

mechanism during the intermediary metabolism of the thyroid cells.

In summary, our observations leave little doubt that the respiratory chain pathway and the oxidative phosphorylation reactions of thyroid mitochondria are very similar to those occurring in mitochondria isolated from other mammalian tissues. A full account of our experiments will be presented elsewhere.

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