The Mitochondrial Electron-Transport System of the Cellular Slime Mould *Dictyostelium discoideum*

By S. K. ERICKSON* and J. M. ASHWORTH

Department of Biochemistry, School of Biology, University of Leicester, LE1 7RH

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Recently there have been reports of the isolation of intact mitochondria from several protozoa, particularly the ciliate *Tetrahymena pyriformis* (Kobayashi, 1965) and the soil amoeba *Hartmanella castellanii* (Lloyd & Griffiths, 1968). We report the isolation and partial characterization of the mitochondria from the myxamoebae of the cellular slime mould *Dictyostelium discoideum*. The electron-transport system of this organism resembles that of yeast (Ohnishi, Kroger, Heldt, Pfaff & Klingenberg, 1967), *Tetrahymena* and *Hartmanella* in having a low cytochrome *a*/cytochrome *c* ratio. Otherwise the mitochondrial preparations from *D. discoideum* resemble mammalian systems in containing cytochromes *a*, *b* and *c*, ubiquinone, and NADH oxidase (rotenone-sensitive) and succinate oxidase (cyanide- and antimycin A-sensitive) systems. There is cytological evidence, however, that the mitochondria (Duncan, Rowe & Ashworth, 1969) and some mitochondrial enzymes (Mine & Takeuchi, 1967) change during the cell differentiation characteristic of the life cycle of this organism (for review see Bonner, 1968).

The myxamoebae were grown in liquid shake culture containing, in a total volume of 700 ml., 10 g. of bacteriological peptone (Oxoid), 5 g. of yeast extract (Oxoid), 0.895 g. of $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 0.34 g. of KH_2PO_4 and 10.8 g. of glucose at 23°. The myxamoebae were harvested when in the late exponential phase at 5×10^6 myxamoebae/ml., washed and suspended in 0.25 M-mannitol–5 mM-tris-HCl buffer, pH 7.4. The cell suspension was homogenized in a Dounce homogenizer until 95% of the cells had broken and the homogenate was centrifuged for 10 min. at 500g. The pellet of unbroken cells was discarded and the supernatant was recentrifuged at 4500g for 10 min. The pellet was washed twice in the original suspension medium by centrifugation at 4500g and finally resuspended in mannitol–tris to give a mitochondrial fraction. All operations were carried out at 4°. Samples of the mitochondrial fraction were examined under the electron microscope by negative contrast, which indicated that it consisted almost entirely of intact mitochondria.

* Present address: Physiologisch-Chemisches Institut, Justus Liebig Universität, Giessen, Germany.

The results are shown in Table 1. The cytochrome content of the mitochondria was determined by room-temperature difference spectra with succinate or dithionite as reducing agent. These indicated the presence of mammalian-type cytochromes *a*, *b* and *c*. Carbon monoxide-treated dithionite-reduced minus oxidized difference spectra indicated the presence of cytochrome *a*₃. The concentrations of these carriers were calculated on the assumption that the molar extinction coefficients are similar to those of other mitochondrial systems (van Gelder & Muijsers, 1967; Chance & Williams, 1956). Quinone concentrations were determined by the semi-micro method of Pumphrey & Redfearn (1960). Oxidase activities were determined at 23° by using a Clark electrode. The reaction mixture contained 0.25 M-mannitol–5 mM-tris-HCl buffer, pH 6.7, 1.0–2.0 mg. of mitochondrial protein and 9 μmoles of substrate in a final volume of 2.2 ml. The mitochondria oxidized succinate, NADH and ascorbate + 2,6-dichlorophenol-indophenol. There was only low malate oxidase activity, and this was stimulated by

Table 1. Cytochrome and quinone concentrations and oxidase system activities from the mitochondrial fraction of the myxamoebae of the cellular slime mould compared with those from rat liver (Chance & Hess, 1962) and *Hartmanella* (Lloyd & Griffiths, 1968)

See the text for details of assays.

Mitochondrial component	Content (nmoles/mg. of protein) or activity (nmoles of O_2 /min/mg. of protein)		
	Rat liver	<i>H. castellanii</i>	<i>D. discoideum</i>
Cytochromes <i>a</i> + <i>a</i> ₃	0.42	0.10	0.16
Cytochrome <i>c</i>	0.34	0.26	0.25
			0.32*
Cytochrome <i>b</i>	0.18	0.08	0.17
			0.27*
Ubiquinone	1.8	—	2.7
Succinate oxidase	45	28	25
NADH oxidase	0	22	50

* Dithionite-reduced.

NAD⁺. The mitochondria showed no respiratory control.

Studies with inhibitors showed that both the succinate oxidase and the NADH oxidase systems are completely inhibited by 0.1 mM-cyanide and 0.3 μ mole of antimycin A/mg. of protein. Rotenone (15 μ moles/mg. of protein) and sodium Amytal (8 μ moles/mg. of protein) inhibited the NADH oxidase system by 55% and 22% respectively. The succinate oxidase system was only slightly inhibited by sodium Amytal.

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