ENERGY CONSERVATION IN ARUM SPADIX MITOCHONDRIA

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1. Introduction

Energy conservation reactions of the mitochondria from the spadix of *Arum maculatum* have been studied by Bonner and Bendall [1]. They found that, although there was no phosphorylation, the mitochondria were capable of reversed electron transport which was inhibited by uncoupling agents. Oxidation of succinate in the presence of cyanide, via the cyanide-resistant pathway present in these mitochondria [2], was apparently also able to sustain reversed electron transport on the cyanide-sensitive pathway.

According to the chemi-osmotic hypothesis of Mitchell [3], any mitochondria which are capable of an energy-coupled process should be capable of respiration-driven proton translocation reactions, although the finding of such reactions does not require that the chemiosmotic hypothesis be correct [4]. We have therefore studied the effect of valinomycin on these mitochondria, since in other mitochondria it is known that addition of valinomycin in the presence of potassium ions and a permanent anion leads to energy-dependent swelling and proton ejection [5,6].

2. Materials and methods

Aroid spadices were harvested from their natural habitat just before the spathes opened. The outer pigmented layer was removed by wiping with cotton wool before the tissue was homogenised. Mitochondria were prepared by the method of Ikuma and Bonner [7] except that the wash medium contained 5 mM Hepes adjusted to pH 7.2 with KOH and the mitochondria were finally suspended in a small volume of this wash medium.

The protein concentration of the mitochondrial preparation was determined by the Biuret method [8] after clarification with Triton X-100. Bovine serum albumin was used as a standard.

Reactions were carried out in a glass cell maintained at 25° and fitted with a Clark-type oxygen electrode and a Jena micro combined pH electrode (Electronic Instruments Ltd., Richmond, Surrey, England). Changes in optical extinction, taken as indication of mitochondrial swelling or contraction, were recorded by means of a light passing through the cell and a Wratten 29F filter (Eastman Kodak, Rochester, New York) onto a photocell. The photocell output was monitored with a log recorder, enabling extinction to be measured on a linear scale.

3. Results and discussion

Fig. 1A shows that addition of valinomycin to Arum spadix mitochondria oxidising succinate causes an increase in the rate of swelling, accompanied by an acidification of the external medium. When the medium becomes anaerobic, there is a partial reversal of swelling and an uptake of hydrogen ions by the mitochondria. In the absence of valinomycin there is some slow respiration-dependent swelling (fig. 1B) which ceases when the system becomes anaerobic. In this case, however, anaerobiosis causes a decrease in external pH. Addition of valinomycin after anaerobiosis has no effect.

Fig. 1C shows that addition of the uncoupling agent carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) both inhibits the slow respiration-driven valinomycin-independent swelling and prevents the valinomycin-induced swelling.

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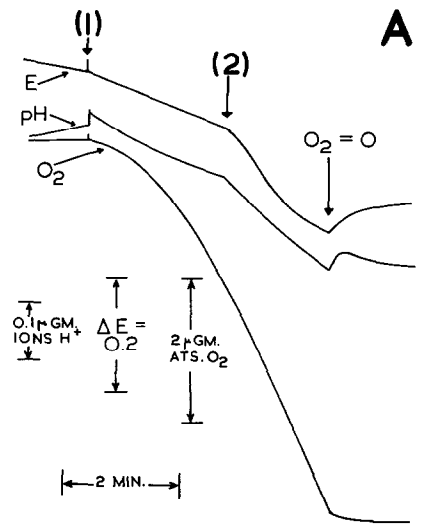


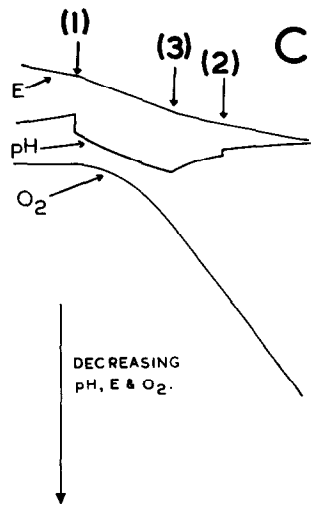
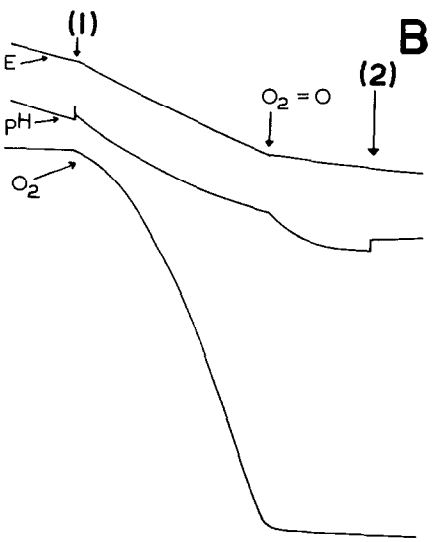


Fig. 1. Mitochondrial light scattering and pH changes induced by valinomycin. The total volume was 10 ml. The reaction mixture contained 0.3 M mannitol, 10.0 mM KCl, 5.0 mM potassium phosphate pH 7.4, 2.5 mM $MgCl_2$, 0.1 mM EGTA and 12 mg mitochondrial protein. Additions: (1) 50 mmoles sodium succinate, (2) 50 ng. valinomycin, (3) 5 nmoles FCCP. The temperature was 25°.



The cyanide-insensitive pathway is also apparently capable of sustaining energy-dependent valinomycin-induced swelling (fig. 2A, B). In this case, however, the rate of swelling is much lower than in the absence of cyanide. It is inhibited by FCCP (fig. 2B), but is not reversed on anaerobiosis (fig. 2A).

These results confirm the finding of Bonner and Bendall [1] that these mitochondria are capable of energy-coupled reactions, and also demonstrate that the mitochondria are capable of hydrogen ion pumping reactions. The presence of an energy-conserving site on the cyanide-insensitive bypass is, however, still open to question. The fact that succinate in the presence of 1 mM KCN will still sustain energy-dependent valinomycin-induced swelling would indicate that there is such a site but two possibilities cannot be ruled out. Firstly, there may still be a small turnover of cytochrome oxidase in the presence of this concentration of cyanide, although it is doubtful as to whether this would be fast enough to account for the rate of swelling observed. Secondly, the oxidation of malate by these mitochondria is very fast, and in ex-

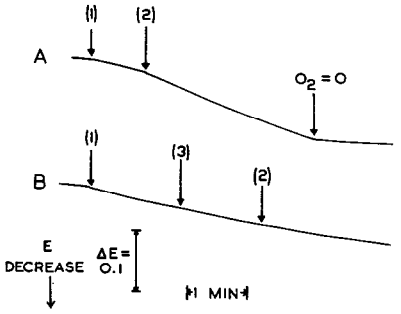


Fig. 2. Light scattering changes induced by valinomycin in the presence of cyanide. Assay medium and additions were as for fig. 1, but the medium also contained 1 mM KCN.

periments similar to those described above we have shown that this oxidation is also capable of sustaining valinomycin-induced swelling. Any malate produced during succinate oxidation would probably therefore be oxidised also, and might lead to energy conservation at site 1 rather than at a site in the cyanide insensitive bypass. This possibility cannot at the moment be tested since these mitochondria are resistant to the usual site 1 inhibitors such as rotenone and amytal.

Acknowledgements

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