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# RESPIRATORY CONTROL AND OXIDATIVE PHOSPHORYLATION IN *ARUM MACULATUM* MITOCHONDRIA

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## SUMMARY

Oxidative phosphorylation can be directly demonstrated in *Arum maculatum* mitochondria by polarographic measurement of $O_2$ uptake. During the youngest stages of inflorescence development, the efficiency is only about 30% of the accepted normal value. As the inflorescence develops, the efficiency of oxidative phosphorylation decreases still further owing to increased participation of an alternative non-phosphorylating electron transport pathway, endogenous uncoupling and contribution of an active ATPase.

## INTRODUCTION

James and Beevers[1] have demonstrated that respiration in the spadix of *A. maculatum* is a very active process showing complete resistance to cyanide or carbon monoxide. At some stage in the development of the inflorescence, the respiratory rate is so high[1—3] that all the energy derived from electron transport is released as heat, resulting in a warming up of the tissue. Heat production was traced to the uncoupling of oxidative phosphorylation and until recently the belief was that respiration in *A. maculatum* is endogenously uncoupled.

In contrast to some other inflorescences of *Araceae (Sauromatum guttatum, Symplocarpus foetidus)*, in which energy conservation has been demonstrated[4—6], mitochondria from *A. maculatum* have not lent themselves to such a demonstration. Failure to demonstrate oxidative phosphorylation[7] or very poor P/O ratios with α-ketoglutarate[8] were reported. However, from the observation of energy-dependent processes such as reversed electron transport[7] or valinomycin-induced swelling[9], it could be inferred that some capacity for

Abbreviation: TMPD, tetramethylparaphenylene diamine.

energy conservation was still retained by these mitochondria. Direct demonstration of oxidative phosphorylation in *A. maculatum* mitochondria has been provided only recently[10–12].

In the course of a study on the alternative electron transport pathway which is operative in *A. maculatum* mitochondria, as in all cyanide-insensitive mitochondria[13], oxidative phosphorylation was also observed. This paper is a contribution to the understanding of energy conservation and electron transport in *A. maculatum* mitochondria.

## MATERIALS AND METHODS

*Arum maculatum* L. inflorescences were collected at different stages of their development[1,3] from wooded, shaded spots near Paris from mid-April to mid-May. Only the upper sterile part of the spadix was used.

Mitochondria were prepared at 2—4° as follows: 50 to 200 g of tissue were gently disintegrated in a medium containing: 300 m*M* mannitol, 10 m*M* EDTA and 3 g/l bovine serum albumin. Neither cysteine nor phosphate buffer was present, but large amounts of polyvinylpyrrolidone (3 g/l) were added to the medium. The pH was initially adjusted to 7.70 and maintained at a value of 7.30 during grinding. The volume of grinding medium was about 10 times the weight of tissue used. According to usual preparative procedures[14,15], the following steps were performed: squeezing through cheese cloth, low speed centrifugation (10 min x 1000 *g*), high speed centrifugation of the supernatant (15 min x 10 000 *g*), resuspension of the mitochondrial pellet in half the volume of medium used for grinding and further low speed (10 min x 500 *g*) and high speed (10 min x 10 000 *g*) centrifugations. The final mitochondrial suspension was made in 2 to 5 ml of grinding medium adjusted to pH 7.30.

Oxygen uptake and oxidative phosphorylation were assayed at 25° by a conventional polarographic technique[16,17], in a medium containing 300 m*M* mannitol, 10 m*M* KCl, 5 m*M* $MgCl_2$, 1 g/l bovine serum albumin, 100 m*M* phosphate buffer, pH 7.20. Mitochondrial proteins were estimated by mineralisation and nesslerization[15].

## RESULTS

### *Oxidative activities of* A. maculatum *tissue and mitochondria*

Respiratory activity in the sterile part of the spadix is highly dependent on the stage of development of the inflorescence[1–3]. The rate of respiration steadily increases from stage $\alpha$ to stage $\delta$, when the spathe opens (Fig. 1A). When the anthers open, a very brief respiratory crisis takes place, characterized by heat production and extremely high rates of $O_2$ uptake (average: 40 000 $\mu$l/h/g fresh weight[3]). After the crisis, the rate decreases as the spadix wilts.

The same evolution is found in the mitochondrial oxidative activities[2,18,19]. With various substrates (Fig. 1 B), the maximal activity is encountered at stage $\delta$. In particular, NADH oxidation reaches extremely high values. Whatever the

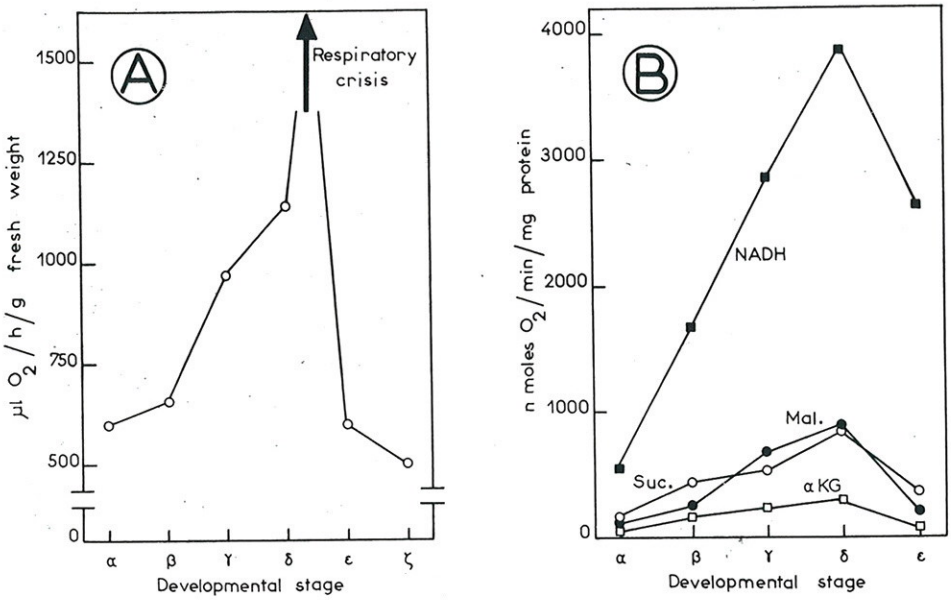


**Fig. 1. Oxidative activities in *A. maculatum* tissue and mitochondria in relation to the stage of development of the inflorescence. (A), $O_2$ uptake by the sterile part of the spadix (taken from ref. 3). (B), oxidation of succinate (10 m*M*), malate (30 m*M*), α-ketoglutarate (10 m*M*) and NADH (1 m*M*) by mitochondria. Developmental stages are named after ref. 1 and 3.**

substrate or the stage of development, these rates of oxidation are significantly higher than those previously reported for such mitochondria[2,8,18,20]. We ascribe the high levels of activity to the preparative technique which includes the use of very large volumes of grinding medium, the omission of cysteine and the presence of high levels of bovine serum albumin and polyvinylpyrrolidone as efficient trapping agents of fatty acids or polyphenols endowed with uncoupling properties[14,21].

*Oxidative phosphorylation*

Fig. 2 presents typical oxygen electrode tracings which show that additions of limiting amounts of ADP to mitochondria oxidizing various substrates, induce a transition from state 3 rate to state 4 rate of oxidation[15,16], with the possibility of measuring ADP/O ratios and respiratory control values.

Mitochondria isolated from spadices up to stage γ but, at variance with a recent report[12], not from stage δ or later stages, showed respiratory control by ADP. It must be pointed out, however, that not all mitochondrial preparations displayed such a behaviour. The number of preparations showing respiratory control decreased as stage δ was approached. Similarly, respiratory control was easily obtained with α-ketoglutarate as substrate, but was very difficult to observe with succinate except for the youngest stage of development. Substrates linked to NAD-dehydrogenases showed an intermediate behaviour.

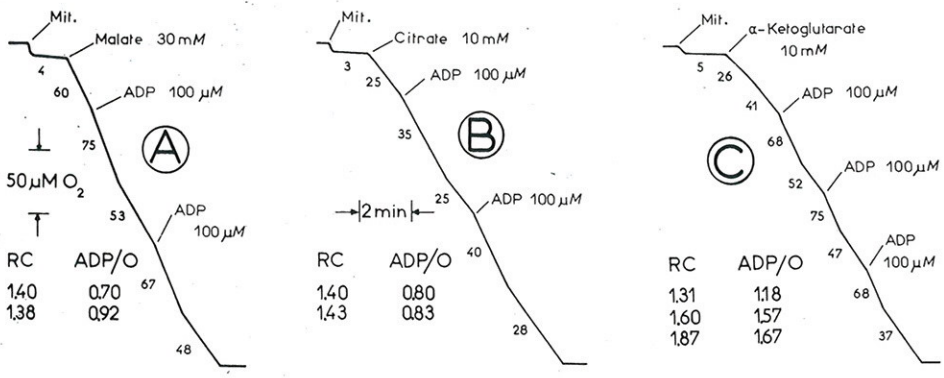


Fig. 2. Oxygen electrode tracings showing oxidation of various substrates by *A. maculatum* mitochondria. (A), oxidation of malate by stage α mitochondria (0.57 mg protein/ml). (B), oxidation of citrate by stage α mitochondria (0.74 mg protein/ml). (C), oxidation of α-ketoglutarate by stage β mitochondria (0.46 mg protein/ml); 2 m*M* malonate and 50 μ*M* thiamine pyrophosphate were present in the medium. Numbers refer to $O_2$ uptake in nmoles/min/ml of medium.

The above observations are summarized in Table I. For most substrates the efficiency of oxidative phosphorylation decreases from stage α to stage γ. This is particularly clear with malate and glutamate. In stage α, the efficiency for most substrates is about one third of the accepted maximal values. With ascorbate-TMPD, a substrate whose oxidation involves one site of phosphorylation only (site III), oxidative phosphorylation is fairly constant and approximately equal to one half of the maximal efficiency. For α-ketoglutarate, whose oxidation is associated with one substrate-level phosphorylation step, the efficiency is very low, if one considers that substrate-level phosphorylation cannot be uncoupled. No respiratory control was observed with NADH, which is oxidized at the highest rate (Fig. 1B).

## DISCUSSION

The above results show that oxidative phosphorylation in *A. maculatum* mitochondria can be directly demonstrated using the polarographic technique of oxygen uptake measurement. Very recently, Wedding *et al.*[12], using a similar technique, reported ADP/O ratios from 0.5 to 1.0 with succinate or malate, but gave no values for other substrates, including NADH, for which they observed respiratory control by ADP. So far, the best values for P/O ratios in *A. maculatum* mitochondria, obtained by measurement of $O_2$ uptake in a Warburg apparatus and chemical determination of ATP synthesis, were those of Hackett and Simon[8] (P/O: 0.4 with α-ketoglutarate) and those of Passam and Palmer[11] (P/O: 0.28 with succinate and 0.43 with malate). To our knowledge, the values of Table I are the highest reported. It should be pointed out that the demonstration of oxidative phosphorylation by polarographic techniques requires mitochondria of good quality. Oxidative phosphorylation

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TABLE I

OXIDATIVE PHOSPHORYLATION IN *A. MACULATUM* MITOCHONDRIA

The number of mitochondrial preparations ($n$) in which respiratory control (RC) was observed is indicated within brackets (out of 30 preparations: 13α, 9β, 8γ). All preparations were tested for every substrate. Due to the many factors preventing its demonstration: ATPase, uncoupling, alternative pathway, preparative procedure (see text), respiratory control is thought to be significant and to represent the best performance of the *A. maculatum* mitochondria, even if it is observed in a few instances. No respiratory control could be demonstrated with preparations from stage δ and later stages; RC: ratio of state 3 rate of oxidation/state 4 rate of oxidation[16,17].

| Substrate | Stage of development of the inflorescence | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Stage α | | | Stage β | | | Stage γ | | |
| | RC | ($n$) | ADP/O | RC | ($n$) | ADP/O | RC | ($n$) | ADP/O |
| Succinate (10 m*M*) | 1.17 | (2) | 0.65 | — | — | — | — | — | — |
| Malate (30 m*M*) | 1.39 | (6) | 0.85 | 1.18 | (3) | 0.66 | 1.35 | (1) | 0.24 |
| α-Ketoglutarate (10 m*M*) | 1.60 | (11) | 1.15 | 1.40 | (4) | 1.17 | 1.18 | (4) | 0.68 |
| Glutamate (10 m*M*) | 1.46 | (3) | 1.20 | 1.15 | (2) | 1.05 | 1.43 | (3) | 0.78 |
| Citrate (10 m*M*) | 1.36 | (2) | 0.82 | — | — | — | 1.50 | (1) | 1.17 |
| Isocitrate (10 m*M*) | 1.53 | (1) | 1.20 | 1.15 | (1) | 1.35 | 1.23 | (1) | 0.52 |
| Ascorbate (20 m*M*)-TMPD (2 m*M*) | 1.37 | (1) | 0.42 | 1.17 | (1) | 0.52 | 1.30 | (1) | 0.42 |

probably still takes place, but with a low efficiency, when respiratory control is no longer observable. Direct determination of ATP synthesis should prove a more sensitive technique, particularly for mitochondria from later stages of development.

By measuring the influence of various inhibitors on the efficiency of oxidative phosphorylation in these mitochondria, Passam and Palmer[11] were able to demonstrate that, in the presence of KCN or antimycin, electrons are diverted to an alternative electron transport pathway. In the absence of inhibitors, this pathway may also function in competition with the normal respiratory chain. It would also diverge from the normal respiratory chain before the site of antimycin inhibition[5,22–25] and would not be phosphorylative[5,11,24,26], although it has been reported that one site of phosphorylation could be associated with it[10].

Although no inhibitor was used in the present study, the above results point to the same general conclusion. The ADP/O ratios observed with a number of substrates clearly show that oxidative phosphorylation takes place at all sites of energy conservation in the normal respiratory chain. However, the low efficiency of the process which, in the best conditions, is roughly one third of the normal efficiency, can be interpreted as a diversion of the electrons to the alternative non-phosphorylation pathway.

As the inflorescence develops, the contribution of the alternative pathway to electron transport increases. This is exemplified by the general decrease in the ADP/O ratios as the spadix grows older and, also, by the behaviour of succinate oxidation. For this substrate, in marked contrast to all other substrates, no oxidative phosphorylation can be demonstrated after stage $\alpha$, at least by the polarographic technique. Since electrons originating from succinate enter the respiratory chain after site I of phosphorylation and, moreover, if they are diverted before site II, no phosphorylation should occur if the alternative pathway contributes to some extent to electron transport. This indeed happens between stages $\alpha$ and $\beta$ with succinate only, but not with an NAD-linked substrate like malate.

From the ADP/O ratios measured with ascorbate-TMPD, it can be assumed that, because of some endogenous uncoupling, the efficiency of oxidative phosphorylation in *A. maculatum* mitochondria is probably not higher than 50%. If one associates 3 sites of phosphorylation with the normal pathway and only 1 site (site I) with the alternative pathway (for an NAD-linked substrate), the contribution of both pathways to electron transport can be easily estimated from the data of Table I. For the oxidation of succinate, the alternative pathway would contribute 35% at stage $\alpha$ and 100% at stage $\beta$. For malate, the figures would be 65% at stage $\alpha$ and 84% at stage $\beta$. Some discrepancy then exists between the two sets of values. Moreover, one cannot compute the values for malate at stage $\gamma$ because, according to our hypothesis, the value of ADP/O could not be lower than 0.50, even if the alternative pathway were contributing 100% to electron transport. This also has to be related to the very low ADP/O ratio found for the oxidation of $\alpha$-ketoglutarate at the

same stage. For this substrate, the ADP/O ratio should not be lower than 1.50. Therefore it appears that, in order to account for the very low efficiency of oxidative phosphorylation, one has to postulate the participation of an active ATPase, which specifically lowers the levels of ATP synthesized.

Whatever the exact situation, which is certainly not as clear as in the skunk cabbage[5], a similar cyanide-insensitive plant material, these results show that in *A. maculatum* mitochondria: (*a*) oxidative phosphorylation takes place at all three sites of energy conservation in the normal respiratory chain, (*b*) endogenous uncoupling and an alternative non-phosphorylative pathway contribute to lower the efficiency of this process, (*c*) an endogenous ATPase seems to be required to account for the extremely low values of ADP/O measured as the spadix approaches the respiratory crisis.

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