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Effects of Temperature on Electron Transport in *Arum maculatum* Mitochondria[1]

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ABSTRACT

The effects of temperature upon the respiratory pathways of *Arum maculatum* mitochondria have been studied. The alternate oxidase sustained a greater proportion of the total respiration at low temperatures than at higher temperatures. Arrhenius plots of respiratory activities show two discontinuities, one at 14°C and one at 21°C. The lower temperature discontinuity was associated with electron transport from succinate dehydrogenase to the alternative oxidase, enzymes that face the inner side of the membrane while the higher temperature discontinuity was associated with electron transport from the external NADH dehydrogenase to cytochrome *c* oxidase, which face the outer side of the membrane. Both discontinuities resulted in a decrease in the activation energy for electron transport on one side of the membrane. Arrhenius plots of transmembrane electron transport showed discontinuities at both 14° and 21°C but the upper discontinuity resulted in an increase in the activation energy. Activation energies determined for the respiratory activities show that above 21°C the exogenous NADH-cytochrome pathway and the succinate-alternative oxidase pathway were lower than those for the NADH-alternative pathway or the succinate cytochrome pathway.

The effect of temperature on membrane-bound enzymes has been studied in some detail. The temperature-dependent activations (E_a[3]) calculated from Arrhenius plots are considered to characterize candidates which are rate-limiting in a reaction sequence (2). Studies of the effects of temperature on the respiratory activities of plant mitochondria have primarily involved investigation of chilling-sensitive plants (8, 9). The changes observed in the thermodynamic and kinetic properties have been attributed to changes in the molecular ordering of lipids within the membrane rather than to temperature-dependent changes of the respiratory enzymes (9).

The mitochondria of *Arum maculatum* spadices are capable of substantial rates of cyanide-insensitive respiration. While the function of the cyanide-insensitive alternative oxidase in many species remains conjecture, in the *A. maculatum* spadix it is almost certainly thermogenic in function. The alternative oxidase activity of these mitochondria allows the spadix to maintain a temperature estimated to be up to 20°C above the ambient temperature (5). The *A. maculatum* flower emerges in early spring when the ambient temperature may be as low as 10°C. The heat generated by the alternative oxidase is thought to help volatilize amines which attract pollinating insects to the flower. However, the heat generated may also effect the mitochondrial function itself.

A. maculatum mitochondria contain not only large quantities of alternative oxidase but also substantial amounts of the externally facing NADH dehydrogenase. However, *A. maculatum* mitochondria do not normally exhibit the apparent association between the exogenous NADH dehydrogenase and the Cyt pathway which has been observed in some other plant mitochondria (4) and the dehydrogenase appears to have total access to the alternative pathway. This access is not seen in mitochondria isolated from other species such as winter wheat (*Triticum aestivum*) (7), sweet potato (*Ipomoea batatus*) (13), or cassava (*Manihot esculatenta*) (4). This difference might be related to the disproportionately high amounts of the enzymes of the alternate pathway in *A. maculatum* mitochondria.

In this paper, we investigate the effect of temperature upon the proportion of cyanide-sensitive and cyanide-insensitive respiration in thermogenic *A. maculatum* mitochondria. The association between the external NADH dehydrogenase and succinate dehydrogenase with the two oxidases in *A. maculatum* mitochondria was investigated with respect to temperature.

MATERIALS AND METHODS

Arum maculatum inflorescences were collected from the wild. Spadices were picked in the thermogenic γ-stage (5) and stored at 6°C for not more than 3 d. The sterile portion of the spadix was removed just prior to the preparation of the mitochondria. Mitochondria were isolated by the method of Cammack and Palmer (1). The concentration of mitochondria in the assays was approximately 0.1 mg protein/ml.

O_2 consumption was measured in a water-jacketed O_2 electrode (Rank Brothers, Cambridge) in medium A (3). The electrode was calibrated with air-saturated water at each temperature using values previously reported (14). The temperature of the assay medium was monitored with a digital thermometer (Data Scientific Ltd., Princes Riseborough, United Kingdom HP17 9BH).

NADH and carbonylcyanide-trifluoromethoxy phenylhydrazone were purchased from Boehringer (London) Ltd., Lewes, East Sussex, United Kingdom. SHAM and rotenone were purchased from Sigma Chemical Co. Ltd., Poole, Dorset, United Kingdom.

RESULTS

Effect of Temperature on the Relative Activity of the Two Oxidases. Figure 1 depicts the change in cyanide-sensitivity of *A. maculatum* mitochondria with temperature. The cyanide-insensitive respiration, through the alternative oxidase, sustained a greater percentage of the total respiration at 12°C than it did at 25°C. This phenomenon appeared to be more marked for

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[3] Abbreviations: E_a, activation energy; SHAM, salicylhydroxamic acid.

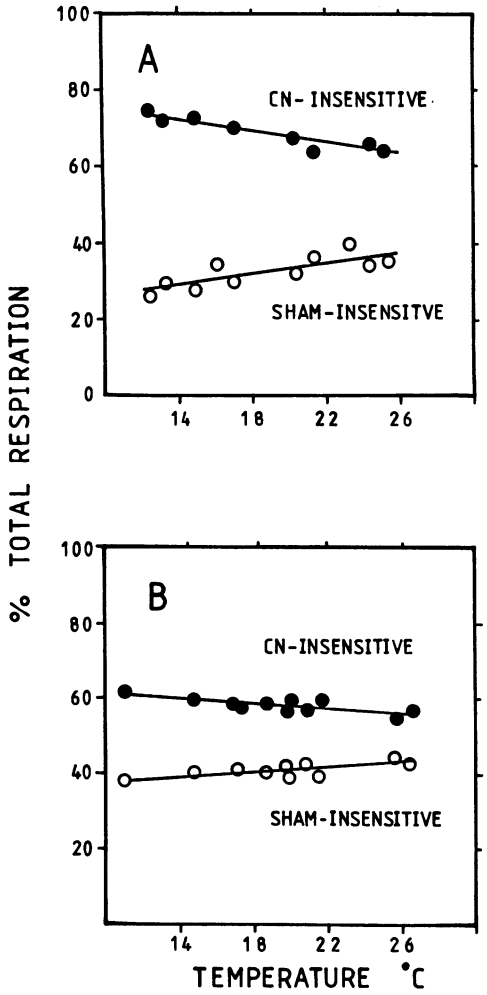

FIG. 1. Change in cyanide sensitivity with temperature in *A. maculatum* mitochondria. O_2 consumption was measured as in "Materials and Methods." Cyanide (CN)-insensitive rates were determined in the presence of 1 mM KCN, SHAM-insensitive rates were determined in the presence of 1 mM SHAM. Rates were expressed as nmol O_2/min·mg protein. At all temperatures, 1 mM KCN together with 1 mM SHAM achieved greater than 98% inhibition of O_2 consumption. Exogenous NADH oxidation (A) was measured using 1 mM NADH in the presence of 40 μM Rotenone. Succinate oxidation (B) was measured after incubation of the mitochondria in the cuvette for 2 min with 200 μM ATP, and the reaction was started by adding 10 mM succinate.

exogenous NADH oxidation than for succinate oxidation. A decrease in temperature from 25° to 12°C resulted in an increase of 11% in the contribution of the alternative pathway to the total respiration (Fig. 1). This diversion of electrons from the cyanide-sensitive pathway to the alternative pathway varied in different preparations between 11 and 30% for exogenous NADH oxidation. These results suggest that at low temperatures a greater proportion of the total respiration is through the thermogenic alternative pathway. As thermogenic respiration proceeds, the temperature of the spadix will rise and proportionately more of the total respiration will proceed through the cyanide-sensitive pathway.

Effect of Temperature on the Relative Activities of NADH and Succinate Oxidation. In *A. maculatum*, both oxidase activities increased with temperature. However, there was a marked difference in the increase of succinate oxidase activities in comparison with the exogenous NADH oxidase activities. Table I shows the increase in electron transport between 11° and 21°C.

The oxidation of succinate by both oxidases was stimulated approximately 6-fold between 11° and 21°C. The oxidation of exogenous NADH however, showed a discrepancy between the two oxidases over a similar temperature range. Exogenous NADH oxidation by the alternative pathway was stimulated 5.5-fold by increasing the temperature from 11° to 21°C, while exogenous NADH oxidation by the Cyt pathway was only stimulated 3.5-fold over this temperature range.

Table I. *Effect of Increase in Temperature on Electron Transport in Arum maculatum Mitochondria*

Rates of oxidation are for coupled mitochondria. O_2 consumption was measured in Rank O_2 electrode as described in Figure 2.

Activity	O_2 Consumed		
	11°C	21°C	Increase
	nmol min^{-1} mg^{-1} protein		*-fold*
Succinate-alternative oxidase	39	270	6.9
Succinate-Cyt oxidase	34	207	6.1
NADH (exogenous)-alternative oxidase	68	372	5.5
NADH (exogenous)-Cyt oxidase	43	159	3.7

This suggests that the two pathways have different temperature-dependent rate-limiting steps. For NADH oxidation by the Cyt pathway, the rate-limiting step is not the external NADH dehydrogenase, because the rate of NADH oxidation through the alternative oxidase was much higher; nor does it appear to be due to a limiting capacity of the Cyt system as reflected by the higher rate of cyanide-sensitive succinate oxidase activity. The difference in the temperature dependence of the rates of cyanide-sensitive and cyanide-insensitive NADH oxidation would therefore appear to be due to the involvement of a component between the dehydrogenase and the oxidase. The most likely explanation is therefore a difference in the involvement of ubiquinone in the two pathways.

Figure 2 depicts the temperature dependence of NADH and succinate oxidation via the Cyt pathway. In this figure, the data are presented as Arrhenius plots (log rate *versus* reciprocal temperature) and the various sections have been fitted to straight lines. Although there is some scatter in the points, very similar results were obtained in three separate experiments. The slopes of the lines have been converted to E_a values, although it should be noted that probably none of these values corresponds to the activation of a simple one-step reaction.

The uncoupled oxidation of exogenous NADH by this pathway showed a slight discontinuity at approximately 20°C resulting in the lowering of the E_a. This discontinuity was not observed in coupled *A. maculatum* mitochondria. Succinate oxidation, however, appeared to have two discontinuities, one at 16°C and one around 21°C.

Figure 3 depicts the Arrhenius plots for NADH and succinate oxidation via the alternative pathway. Succinate oxidation by this pathway showed a sharp discontinuity at around 14°C. Exogenous NADH oxidation, however, showed a similar pattern to succinate-Cyt oxidase activity. There was a decrease in the E_a around 13°C. Above 20°C, the E_a of exogenous NADH-alternative oxidase activity increased 2.5-fold coincident with an upward break in the Arrhenius plot. It is clear that the upward deflection is independent of the participation of any one of the dehydrogenases or oxidases. The upward deflection appears to be associated with the transport of electrons across the membrane, presumably through ubiquinone. Table II summarizes the calculated E_a for the activities studied within the temperature ranges between the discontinuities of the Arrhenius plots.

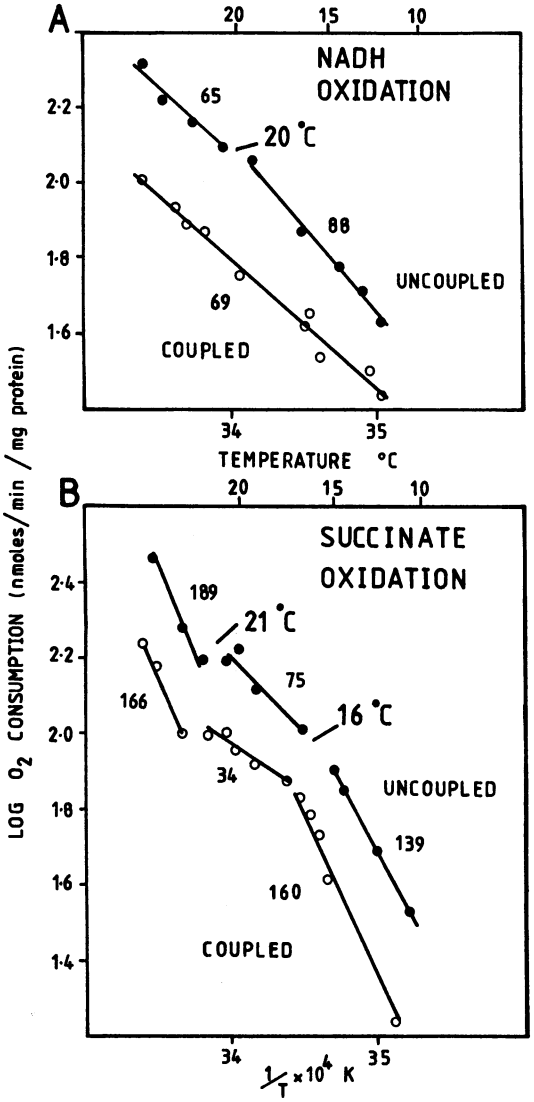

FIG. 2. Arrhenius plot of cyanide-sensitive exogenous NADH and succinate oxidation in *A. maculatum* mitochondria. Activities were measured as for Figure 1, in the presence of 1 mM KCN. Values on the lines represent the E_a in kJ mol^{-1}. Mitochondria were uncoupled using 0.2 μM carbonylcyanide-trifluouromethoxy phenylhydrazone.

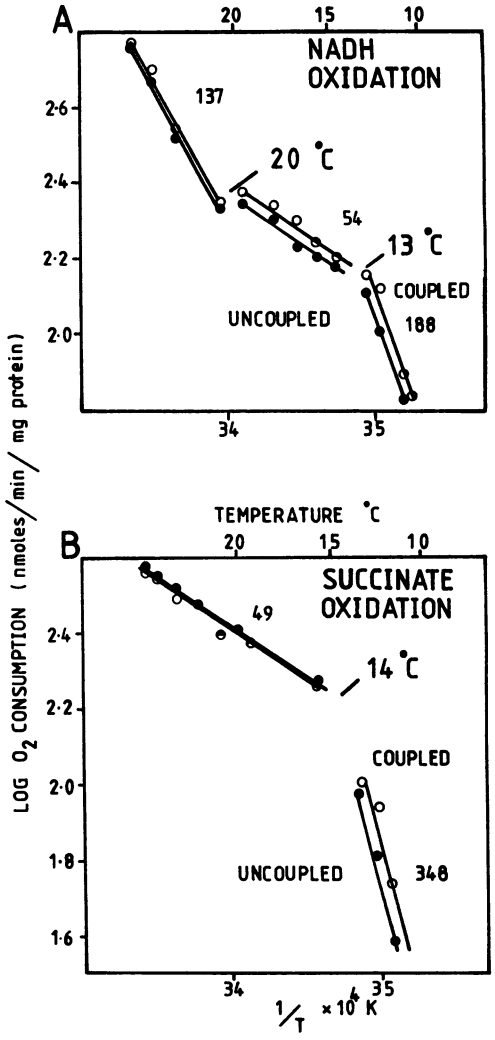

FIG. 3. Arrhenius plot of cyanide-insensitive exogenous NADH and succinate oxidation in *A. maculatum* mitochondria. Activities were measured as for Figure 1, in the presence of 1 mM SHAM. Mitochondria were uncoupled using 0.2 μM carbonylcyanide-trifluoromethoxy phenylhydrazone.

DISCUSSION

The results presented here suggest that at lower temperatures the thermogenic alternative pathway is proportionately more active than it is at higher temperatures. As the temperature of the spadix rises, either by thermogenesis or increase of the ambient temperature, respiration by the thermogenic alternative oxidase would give way to the energy-conserving cyanide-sensitive pathway.

The preponderance of alternative oxidase activity at low temperatures has been noted before. Yoshida and Tagawa (15) have noted that chilling-sensitive *Cornus* callus mitochondria showed a diversion of flux from the Cyt pathway to the alternative pathway at temperatures below the break in the Arrhenius plot of respiration.

It has been noted that in mitochondria containing both cyanide-sensitive and cyanide-insensitive respiratory pathways that the external NADH dehydrogenase and the Cyt pathway appear to be closely associated functionally (4, 7, 13). A similar functional association has been postulated between the succinate dehydrogenase and the alternative pathway (6, 10). In agreement with this, the results presented here would suggest that the activation energies for the exogenous NADH-Cyt oxidase and succinate-alternative oxidase pathways are significantly lower than those of the NADH-alternative pathway and the succinate-Cyt oxidase pathways. Interpretation of the physical basis for the breaks in the Arrhenius plots is difficult. Listed below are three features of the electron transport chain that might give rise to

Table II. *Calculated E_a for the Activities Studied within the Temperature Ranges between the Discontinuities of the Arrhenius Plots*

Activity	E_a Calculated from Arrhenius Plots of Coupled O_2 Consumption		
	11–15°C	15–21°C	21–30°C
	kJ mol⁻¹		
NADH (exogenous)-alternate oxidase	188	54	137
NADH (exogenous)-Cyt oxidase	65	65	69
Succinate-alternate oxidase	348	49	49
Succinate-Cyt oxidase	160	34	166

such discontinuities.

The Mobility of the Lipid Phase. Solid-liquid phase transitions are unlikely as the lipids are expected to be fluid over the temperature range studied, but more subtle changes of lipid organization or lipid-protein interactions can take place.

The Enzymes Themselves. Possible effects include reversible denaturation or conformational changes in proteins and cooperative changes in the interaction of the enzymes with the bilayer (11). Moreover, in a complex series of electron transfer steps, one step might be rate-limiting at one temperature and another step at a higher temperature above the break.

Diffusion of Ubiquinone and Its Interactions with the Electron Carrier Proteins.

Studies of the temperature dependence of respiratory rates cannot by themselves distinguish between these possibilities. Some further information would be provided by specific probes of components of the system. Such an approach was made by Wright and Raison (unpublished data, cited in [8]) who found that the membrane fluidity, as measured by spin labels, of Jerusalem artichoke tuber mitochondria increases during the winter. During this period of increased fluidity, they observed an increase in the E_a of succinate oxidase activity. This result suggests a relationship between the E_a and the fluidity of the bulk membrane lipids. Our observation in *A. maculatum* that the E_a of cyanide-sensitive succinate oxidase activity increased at temperatures above the break in the Arrhenius plot, may indicate a similar mechanism.

In the present case, electron transport through the succinate-alternative oxidase pathway, which involves proteins facing the inner surface of the membrane, showed only one, downward break in the Arrhenius plot around 14°C (Fig. 3B). Electron transport through the external NADH dehydrogenase-Cyt *c* oxidase pathway, which involves proteins facing the outer surface of the membrane displayed a slight downward break in the plot around 20°C (Fig. 2A). Transmembrane electron transport through the NADH-alternative oxidase pathway, or, in the opposite direction, through the succinate-Cyt oxidase pathway, displayed the more complex pattern with an upward break at 20°C.

The downward breaks in the Arrhenius plots associated with the succinate-alternative oxidase pathway and with the NADH-Cyt pathway are consistent with an increase in the membrane fluidity, if diffusion of proteins or ubiquinone were rate-limiting. However, the discontinuities around 20°C in the NADH-alternative oxidase and succinate-Cyt oxidase activities are in the opposite direction; the E_a for succinate-Cyt oxidase activity is increased 4.8-fold above 21°C. The upward deflection of Arrhenius plots has been observed in other membrane processes such as transmembrane sugar transport in *Escherichia coli* (12). In this case, it is possible that the upward deflection is associated with the transmembrane movement of reducing equivalents by ubiquinone.

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