Plant Physiol. (1970) 45, 386–389

Oxidative Activity of Mitochondria Isolated from Plant Tissues Sensitive and Resistant to Chilling Injury[1]

Received for publication August 13, 1969

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ABSTRACT

Arrhenius plots of the respiration rates of mitochondria isolated from chilling sensitive plant tissues (tomato and cucumber fruit, and sweet potato roots) showed a linear decrease from 25 C to about 9 to 12 C (with Q_{10} values of 1.3 to 1.6), at which point there was a marked deviation with an increased slope as temperatures were reduced to 1.5 C (Q_{10} of 2.2 to 6.3). The log of the respiration rate of mitochondria from chilling resistant tissues (cauliflower buds, potato tubers, and beet roots) showed a linear decrease over the entire temperature range from 25 to 1.5 C with Q_{10} values of 1.7 to 1.8. Phosphorylative efficiency of mitochondria from all the tissues, as measured by ADP:O and respiratory control ratios, was not influenced by temperatures from 25 to 1.5 C. These results indicate that an immediate response of sensitive plant tissues to temperatures in the chilling range (0 to 10 C) is to depress mitochondrial respiration to an extent greater than that predicted from Q_{10} values measured above 10 C. The results are also consistent with the hypothesis that a phase change occurs in the mitochondrial membrane as the result of a physical effect of temperature on some membrane component such as membrane lipids.

Mitochondria isolated from chilling resistant tissues show the capacity to swell to greater extent than the mitochondria from chilling sensitive tissues (14), indicating that the membranes of mitochondria from chilling sensitive tissues are less flexible than those from chilling resistant tissues. Furthermore, this difference in flexibility was related to the relative proportion of saturated and unsaturated fatty acids of the membrane lipids. A study of the temperature at which mixtures of the predominant saturated and unsaturated fatty acids, found in the membranes of plant mitochondria, solidify showed that a small increase in the proportion of unsaturated fatty acids causes a large decrease in the solidification temperature (13). Thus differences in the lipid composition of membranes would appear to determine the physical state of membranes at a given temperature. While the implications of this difference in physical properties may be profound, no definitive link between mitochondrial behavior and the metabolic events associated with chilling injury has been established.

Previous reports (12, 16) have shown that oxidative and phosphorylative activities (assayed at 25 C) of mitochondria, isolated from chilling sensitive sweet potato roots, were impaired as a result of chilling treatment. However, many factors, such as the polyphenol content of the parent tissue (4, 11), can markedly influence both oxidative rates and apparent phosphorylative ability of isolated plant mitochondria. Thus, where plant material is given a differential pretreatment, it is most difficult to ascertain whether the observed differences in activity represent a direct effect of the chilling treatment on the mitochondria or an indirect effect due to the release or activation of some components which might be operational during isolation. It has also been noted that the method of tissue disruption (8), the amount of mitochondrial protein in the assay mixture (20), and the time involved between tissue disruption and assay (18) influence the activity of isolated mitochondria but which may be of little significance *in vivo*.

The experiments reported here were carried out with mitochondria from plant tissue not subjected to chilling treatment, with the aim of evaluating the influence of temperature on oxidation and phosphorylation of these isolated mitochondria. The results show that in mitochondria from chilling sensitive tissues there is a marked depression in the respiratory rates below the critical temperature for chilling injury (10 C) which is not observed with mitochondria from resistant tissues. It was also established that temperatures between 1.5 and 25 C do not influence the phosphorylative efficiency of mitochondria from either sensitive or resistant plant tissues.

MATERIALS AND METHODS

Mitochondrial preparations were made from the following chilling sensitive plant materials: mature green tomato fruit (*Lycopersicon esculentum* Mill. cv. Grosse Lisse); sweet potato roots (*Ipomoea batatas* L. cv. White Maltese); and cucumber fruit (*Cucumus sativus* L.). The chilling resistant plant material included the following: potato tubers (*Solanum tuberosum* L. cv. Sebago); cauliflower buds (*Brassica oleraceae* L. var. botrytis); and beet roots (*Beta vulgaris* L.).

The mitochondria were prepared and respiratory activity was determined by the methods described in the preceding paper (20). Since it was shown (20) that the respiration of isolated mitochondria was greatly reduced and the mitochondria lost respiratory control when the concentration of mitochondrial protein in the reaction mixture was less than some critical amount (0.25–0.75 mg of protein/4.2 ml of reaction mixture for mitochondria from the various tissues), concentrations above these amounts were used at each of the temperatures. In all of the mitochondrial preparations studied it was noted that there was some inhibition of the initial state 3 respiration rate, probably because of endogenous oxaloacetate (30). Therefore, both the state 3 and state 4 respiration rates were calculated by averaging the rates obtained with three or more cycles of ADP addition after the first two additions. Respiration rates at various temperatures from at least three separate preparations of mitochondria from differ-

[1] This work was supported in part by a Commonwealth Scientific and Industrial Research Organization, Australia, study grant awarded to J. M. Lyons while on sabbatical leave from the Department of Vegetable Crops, University of California, Riverside, California 92502.

ent samples of the various plant tissues were adjusted by a common factor to give a value of 100 nmoles of O_2 per min per mg of protein at 25 C and are presented as an Arrhenius plot ($\log_{10}$ respiration rate against the reciprocal of the absolute temperature). Reaction temperatures for each separate preparation were selected to sample the full temperature range (*i.e.*, 1.5–25 C) in addition to a reaction at 25 C. Reactions of subsequent preparations of the same tissue were determined at a different set of four temperatures, again including 25 C. Because of the differential decrease in activity with storage time (20), reactions at several temperatures were started simultaneously. The slow reactions at lower temperatures required long time periods for completion, but several reactions at high temperature (15–25 C) were completed within a relatively short period after isolation. The deviation of some state 3 rates of the Arrhenius plots, from the line of approximate best fit, probably represents small differential variations in the temperature response of the individual preparations.

RESULTS

The respiration data, presented as Arrhenius plots in Figure 1, clearly demonstrate a differential response to temperature of mitochondria isolated from chilling sensitive plant tissues as compared to chilling resistant tissues.

Each of the chilling resistant tissues examined showed an approximately linear slope for state 3 succinate oxidation rates, with Q_{10} values of 1.7 to 1.8, over a temperature range from 25 to 1.5 C. In contrast, the plots for mitochondria from the chilling sensitive tissues showed similar linear slopes (Q_{10} of 1.4–1.6) from 25 C to only 9 or 12 C. At lower temperatures the slope of the plot for tomato fruit mitochondria increased sharply; the Q_{10} value increased to 6.1. Data obtained with sweet potato mitochondria followed a similar pattern with a change in slope between 9 and 12 C, and a Q_{10} value of 6.3 for the lower temperatures. The response of cucumber mitochondria followed a somewhat different pattern in that a sudden decrease in respiration occurred at 12 C followed by an increase in the slope of the plot; the Q_{10} value in the lower temperature range was 2.2. While the Q_{10} value of 2.2 for mitochondrial respiration from cucumber fruit below the critical temperature is lower than the Q_{10} values determined for tomato (6.1) and sweet potato (6.3), the initial drop in respiration at this temperature is much greater. If the slope from 10 to 25 C is projected to 2 C, the drop in activity reflects a depression in respiration at 8 C, for example, of nearly 60% for cucumber mitochondria, and 13 and 20% for sweet potato and tomato mitochondria, respectively. At 2 C, this depression in respiration is between 61 and 63% for each of the three sensitive tissues.

While temperatures below 9 and 12 C markedly reduced the rates of succinate respiration of mitochondria from chilling sensitive plant tissues, there was no concomitant reduction in phosphorylative efficiency, as demonstrated by both the ADP:O and respiratory control (RC) ratios (Table I). Mitochondria from both the chilling sensitive and resistant tissues demonstrated good respiratory control and phosphorylative ability over the entire temperature range studied. The RC ratios for mitochondria from both chilling sensitive and resistant tissues, with succinate as substrate, were within the range of 2.2 to 3.4 (Table I). Although not approaching infinity, as reported by Ku *et al.* (8) for mitochondria from tomato fruit and Romani *et al.*

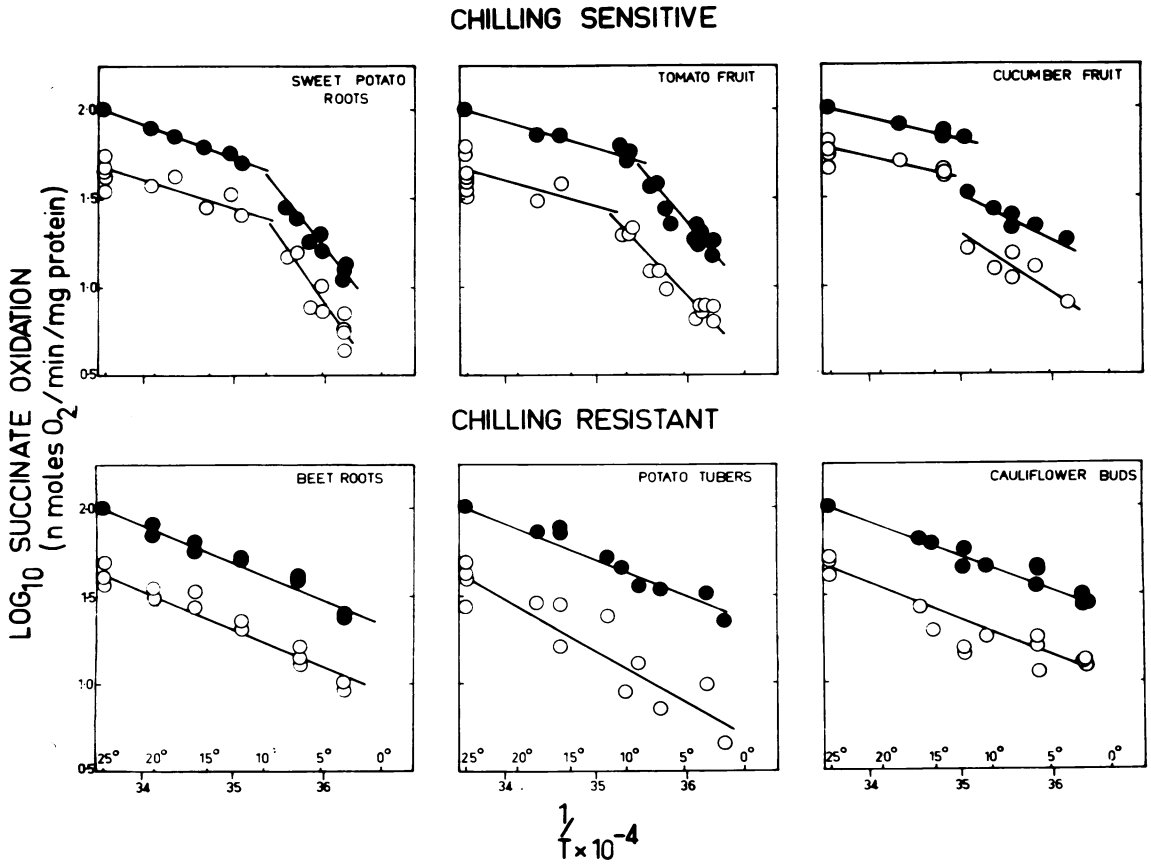

FIG. 1. Arrhenius plots of succinate oxidation by plant mitochondria. Each plot showing state 3 (●) and state 4 (○) respiration represents data from three or more mitochondrial preparations. The log values were adjusted by a factor to a common value at 25 C in order to compensate for differences in the rates (nanomoles of O_2 per min per mg of protein) between the different preparations.

(22) for mitochondria from pears, the RC ratios shown in Table I fell within the range usually observed for succinate oxidation by plant mitochondria (1, 3, 5, 23, 26, 27, 30) and by rat liver mitochondria (6). Similarly, the ADP:O ratios, although exhibiting some variability, show no consistent pattern which can be related to temperature effects and approach the theoretical value of 2 for succinate oxidation. Discontinuities in the Arrhenius plot for succinate oxidation and for the ADP-ATP and P_i-ATP exchange reactions catalyzed by rat liver mitochondria have been reported (7). Each of the reactions measured show a change in the temperature coefficient at approximately 17 C with a 2-fold increase in the Q_{10} value below the critical temperature. We have measured the rate of succinate oxidation by rat liver mitochondria and found a discontinuity in the Arrhenius plot between 23 and 25 C for both state 3 and state 4 respiration.

DISCUSSION

The results clearly demonstrate that the respiration of mitochondria from chilling sensitive plant tissue, in contrast to the mitochondria from resistant tissue, is significantly reduced at temperatures below 10 C, indicating that the immediate response of sensitive tissue to chilling is a depression of respiratory activity. This depression in activity observed in each of the sensitive tissues below a critical temperature is consistent with the hypothesis that reduced temperatures affect some physical property of the mitochondrial membranes, *e.g.*, membrane lipids (13, 14, 21). At the same time, the data show that phosphorylative efficiency of mitochondria from sensitive as well as resistant tissues is not affected directly by low temperatures although the rate of phosphorylation would be reduced concomitant with reduction in oxidative activity.

Table I. *Respiratory Control and Oxidative Phosphorylation for Succinate Oxidation by Plant Mitochondria*

Data for respiratory control (RC) ratios and phosphorylation (ADP:O ratios) are averages ± the standard error of two to five cycles from three to five experiments for each of the tissues shown in Figure 1. Procedures are described in "Materials and Methods."

Temp	Respiratory Control		ADP:O	
	Resistant	Sensitive	Resistant	Sensitive
C				
0–4.9	3.4 ± 1.1	2.4 ± 0.3	1.35 ± 0.12	1.44 ± 0.10
5–9.9	2.8 ± 0.3	2.2 ± 0.3	1.40 ± 0.07	1.67 ± 0.15
10–25	2.5 ± 0.3	2.3 ± 0.2	1.76 ± 0.13	1.47 ± 0.18

The relationship between respiratory activity and chilling injury has been investigated as a postharvest problem with intact tissues (*cf.* References 2, 9, 19, 28), tissue slices (10, 17, 24), excised roots (29), and mitochondria (12, 16). In relating these studies on respiratory behavior and chilling injury, it is essential to distinguish between the primary response to chilling temperatures, and subsequent responses associated with altered metabolism. For example, if the data of Eaks and Morris (2) for chilling sensitive cucumber fruit are presented as an Arrhenius plot of the respiration rates at 24 hr and 5 and 10 days (Fig. 2) after being placed at the test temperatures, the differences between the initial effect of temperature on respiration and those changes in respiration which occur after injury are readily apparent. At 24 hr (the earliest measurement reported), the respiration rates diminish with a Q_{10} value of 1.7 from 30 to 13 C. A change occurs between 10 and 13 C. Below 10 C there is an increase in the rate of reduction of respiration as the temperature is decreased further. The Q_{10} value rose to 2.9. Eaks and Morris (2) reported visible injury between 24 hr and 5 days in the fruit held at 0 and 5 C, and this injury was reflected by the increased respiration rates at these temperatures. By 10 days in storage, injury and increased respiration had occurred at all temperatures at or below 10 C, causing the slope of the Arrhenius plot in this temperature region to be elevated to the slope exhibited at the higher temperatures (Fig. 2). An Arrhenius plot of the data for intact potato tubers (19) shows a linear slope over the entire temperature range from 25 to 0.5 C (Fig. 2). While the rate of respiration gradually decreases with time in storage (as does the respiration of cucumbers at nonchilling temperatures, Fig. 2), there is no deviation in the linearity of the slope. With the use of respiration data obtained from intact tissues as well as the mitochondrial data (Fig. 1), it is evident that a very early response to temperatures below the critical point for injury is a depression in respiratory activity. This depression in respiration is not observed with mitochondria (Fig. 1) or plant tissues (Fig. 2) resistant to chilling injury.

Impairment of mitochondrial phosphorylation is not an immediate result of chilling (Table I), but probably follows after the tissues have been injured for some time period. Schichi and Uritani (24) demonstrated that sweet potato disks did not increase respiration in response to dinitrophenol after the roots

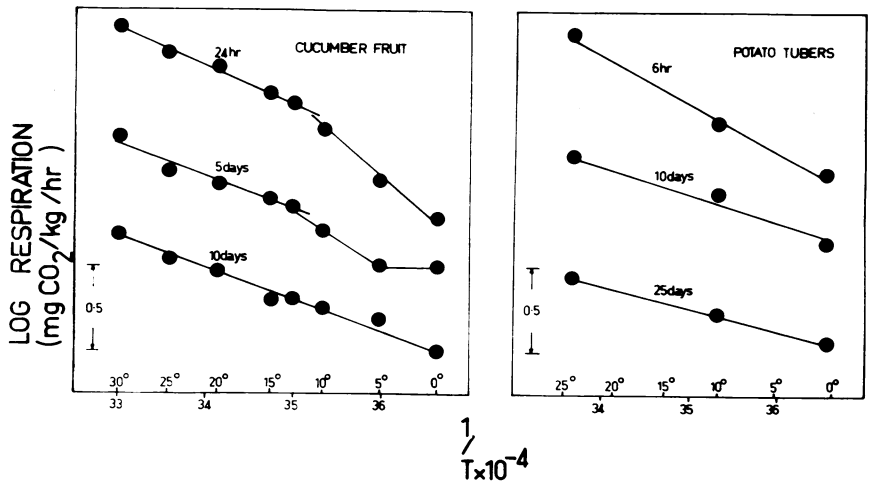

FIG. 2. Arrhenius plots of respiration data for intact cucumber fruit and potato tubers. Data for cucumber fruit calculated from Eaks and Morris (2) and for potato tubers from Platenius (18). The different slopes represent different time periods (as indicated) after tissues were placed in the temperature treatments.

had been stored for 8 to 10 days at 0 C. In addition, Lewis and Workman (10) found that chilling tomato tissues for 12 days at 0 C decreased their phosphorylative capacity, as demonstrated by their inability to incorporate ^{32}P at 20 or 0 C. On the other hand, Wheaton (29) found that dinitrophenol stimulated the respiration of chilling sensitive corn roots after 3 hr at 5 C to the same extent as insensitive wheat roots. In this example, the corn roots presumably had not been injured sufficiently in the 3-hr period to alter coupled respiration, whereas with the studies on sweet potato (24) and tomato disks (10) injury had occurred. Wheaton (29) discarded the idea that altered respiration was a primary step in chilling injury because he did not observe a difference in respiration between excised roots from chilling sensitive and resistant seedlings over 4½ days at 5 C. However, this conclusion is not valid since he did not consider in what manner the respiration at 5 C varied with respect to respiration at temperatures above those critical for chilling.

While the descriptive aspects of the influence of low temperature on mitochondrial oxidation from chilling sensitive plant tissues are apparent from the data in Figure 1, the mechanism of this response is not known. However, previous studies (13, 14, 21) have pointed to the role of fatty acids in the mitochondrial membranes in determining their physical (and thus physiological) characteristics at different temperatures. Evidence was presented in the work of Luzzati and Husson (15) that a lipoprotein complex (such as a mitochondrial membrane) is on the borderline of a reversible phase transition from a liquid-crystalline structure to a coagel, and they suggested that, when one of the controlling parameters (such as temperature) is altered, the hydrocarbon chains crystallize and modify some physiological function. Since the mole percentage of unsaturated fatty acids in the mitochondrial membranes of chilling resistant tissues is greater than in those from chilling sensitive tissues (14), and this concentration is very close to the point in the phase diagram of mixtures of fatty acids where only a 5% increase in the amount of unsaturated fatty acid lowers the temperature at which solidification occurs by nearly 15 C (13), it appears likely that the respiration data in Figures 1 and 2 reflect the consequence of such a phase change.

The less flexible nature of the mitochondrial membranes from sensitive tissues, below the critical temperature for chilling injury, could alter the oxidative rate, for example, by reducing permeability to oxidizable substrates. This depressed rate of mitochondrial oxidation could lead to an accumulation of metabolic intermediates produced by extramitochondrial systems not adversely influenced by chilling temperatures and thus induce injury. Indeed, evidence that these events occur can be found in studies by Murata and Ku (15) with tissue slices from banana fruit where it was shown that ethanol and acetaldehyde accumulate as a result of temperature treatments inducing chilling injury. In addition to accumulation of metabolic intermediates leading to cellular injury, reduction in the rate of ATP production could also induce injury. Stewart and Guinn (25) found that ATP levels decreased within 13 hr of chilling 2-week-old cotton seedlings at 5 C and suggest that below a certain level of available energy the tissue is unable to maintain the metabolic integrity of the cytoplasm necessary for survival. Either of these events, *i.e.*, accumulation of metabolites or reduced ATP supply, resulting from depressed mitochondrial respiration at chilling temperatures could ultimately lead to injury.

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