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*Short Communication*

# Occurrence of a Temperature-induced Phase Transition in Mitochondria Isolated from Apple Fruit

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W. B. McGLASSON AND J. K. RAISON
*Plant Physiology Unit, Commonwealth Scientific and Industrial Research Organization, Division of Food Research, and School of Biological Sciences, Macquarie University, North Ryde 2113, Sydney, Australia*

## ABSTRACT

**Mitochondria were isolated from fruit of six cultivars of apples differing in susceptibility to the physiological disorder, low temperature breakdown. The state 3 rate of succinate-dependent oxygen uptake and the motion of a spin label were measured at from 0 to 25 C. Arrhenius plots of the data showed that the apparent energy of activation of both respiration and motion of the spin label increased abruptly at low temperatures indicative of a temperature-induced phase change in the membrane lipids. The changes were detected with mitochondria from all of the cultivars, but the temperature at which the changes occurred did not correlate with the susceptibility of the cultivars to low temperature breakdown.**

The Arrhenius activation energy of respiratory enzymes of mitochondria from chilling-sensitive plant tissues exhibit a marked increase when the temperature is reduced below about 10 C (4, 10). This increase in Ea[1] is considered to be a consequence of a temperature-induced phase change in the lipid components of mitochondrial membranes (11) and is not shown with mitochondria from chilling-resistant plant tissues where no lipid phase transitions are detected (4, 11). Thus plants sensitive to chilling exhibit characteristic changes in both the physical properties of membrane lipids and the kinetics of enzymes associated with these membranes. This structure-function relationship of the mitochondria has practical implications, since the temperature at which these changes are observed approximates the lower limit for normal physiological function of the particular tissue.

Different cultivars of apple fruit vary considerably in susceptibility to the physiological disorder known as "low temperature breakdown" 1, 3). This breakdown is observed when the fruits are held below 3 to 4 C, and in terms of time-temperature relationships is similar to injury incurred at 8 to 12 C by plant tissues of tropical or subtropical origin (2). In the latter tissues, chilling injury has been related to the phase changes in the mitochondrial membranes (4, 10). An investigation was therefore undertaken to determine if the membranes of apple mitochondria undergo temperature-induced phase changes and whether there are variations among cultivars in the temperatures at which these phase changes occur that could be related to susceptibility of the fruit to low temperature breakdown. The results show that a phase change occurs in the membrane lipid components of mitochondria from apples and that there is an increase in the Ea of respiratory enzymes at temperatures below the phase transition. However, the results show that both these changes were exhibited by all of the cultivars examined and do not correlate with the differing susceptibility of the fruit to low temperature breakdown.

[1] Abbreviation: Ea: Arrhenius activation energy.

## MATERIALS AND METHODS

Mitochondria were isolated from apples by methods similar to those described by Raison and Lyons (9). The rate of succinate-dependent oxygen uptake was measured polarographically over a temperature range of 0 to 25 C (5) in the medium described by Raison and Lyons (9). Supplementary measurements were made of the effect of a detergent (Teric X8, a nonionic detergent consisting of a alkylphenyl-ethylene oxide condensate, supplied by Shell Chemical Co., Sydney) and sodium deoxycholate, on the rate of oxygen uptake over the same temperature range. Treatment of mitochondria with phospholipase A consisted of incubating 0.5 ml of mitochondria (5 mg of protein) with 3 mg of phospholipase A (phosphatide acyl-hydrolase EC 3.1.1.4 from *Crotalus adamanteus,* Koch-Light Laboratories Ltd.) at 20 C for 15 min. Phase changes in the lipid components of mitochondrial membranes were inferred from changes in the effect of temperature change on the motion of the spin-labeled analogue of methyl stearate (M12NS) [3-oxazolidenyloxy, 2-(10-carbmethoxyl dicyl)-2-hexyl-4,4-dimethyl] by electron spin resonance spectroscopy. Preparations of isolated mitochondria were infused with spin label, and the spectra were recorded with a Varian E4 spectrometer. The instrument was fitted with a temperature control unit, designed and built by Mr. H. F. Symmons of the CSIRO Division of Physics, which maintained the temperature of the sample at ±0.1 C. The motion of the spin label ($\tau_0$) was calculated as described by Raison *et al.* (11) and for comparative purposes represents the time for the spin label to tumble through a significant arc, *e.g.* 40°.

## RESULTS AND DISCUSSION

The effect of temperature on the state 3 rate of respiration of mitochondria from Jonathan apples (ex Victoria) is shown in Figure 1 as a plot of the logarithm of respiration rate against the reciprocal of the absolute temperature (Arrhenius plot).

There was an abrupt increase in Ea from 9.8 kcal/mole to 22.3 kcal/mole as the temperature was reduced from 4.8 C to 2.5 C. Corresponding with the change in Ea of respiratory activity the motion of the spin label, M12NS, with mitochondria from the same Jonathan apples showed an abrupt change within the same temperature range (4.4 C to 3 C). The effect of temperature on the motion of the spin label is presented in Figure 2 as an Arrhenius plot. Changes in the slope of such

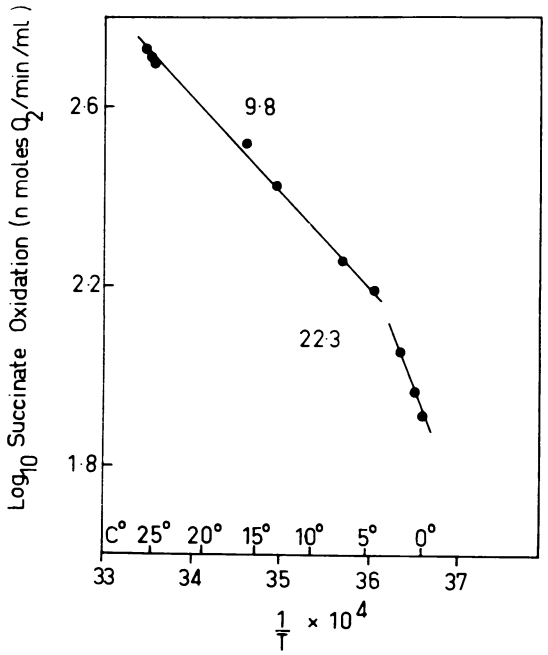


FIG. 1. Arrhenius plot of succinate oxidation (state 3) by mitochondria from Jonathan apples ex Scoresby, Victoria. The Arrhenius energies of activation (Ea, kcals/mole) are shown above and below the transition temperature range.

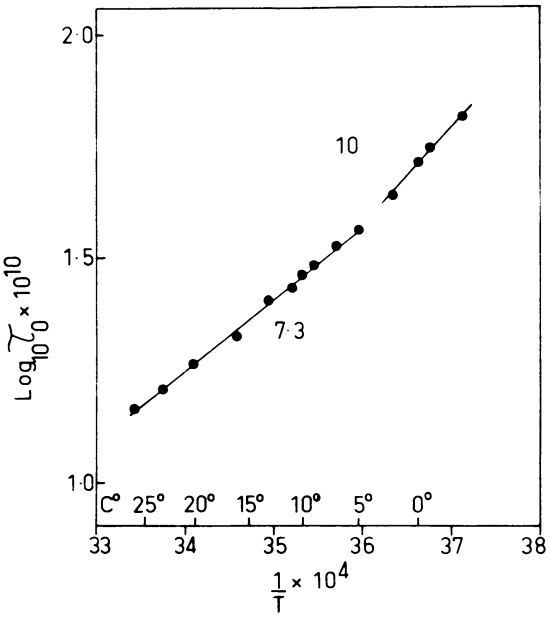


FIG. 2. Arrhenius plot of the molecular motion of spin label (M12NS) incorporated into a hydrophobic region of the membranes of mitochondria from Jonathan apples ex Scoresby, Victoria. The values of the apparent Ea (kcals/mole) are shown above and below the transition temperature range.

Table I. *Temperature of the Phase Transition Determined by Electron Spin Resonance Measurements and Ea*

Temperature was calculated from the rates of succinate oxidation above and below the transition temperature for mitochondria from six cultivars.

| Cultivar[1] | Temperature of Phase Change[2] | Ea above Transition Temperature Range | Ea below Transition Temperature Range |
|---|---|---|---|
| | *C* | *kcal /mole* | |
| Jonathan, Bathurst, N.S.W. | 3.6 | 10 | 20 |
| Jonathan, Scoresby, Vic. | 4.4 | 10 | 22 |
| Jonathan, Lenswood, S.A. | 9.5 | 7 | 21 |
| Coxs Orange Pippin, Tas. | 9.1 | 9 | 30 |
| Red Delicious (Richared), Orange, N.S.W. | 4.5 | 12 | 21 |
| Granny Smith, Bathurst, N.S.W. | 7.0 | 9 | 29 |
| Golden Delicious Orange, N.S.W. | 7.0 | 11 | 18 |
| Democrat, Bathurst, N.S.W. | 4.7 | 9 | 23 |

[1] Jonathan and Coxs Orange Pippin are considered susceptible to low temperature breakdown (1); Red Delicious, Granny Smith, Golden Delicious, and Democrat have low susceptibility (1, 3).

[2] The temperature of the phase change was estimated graphically from plots of the log of $\tau 0$ against the reciprocal of the absolute temperature and is within the limits ±1.5 C.

plots are indicative of a phase transition within the lipid components of the membrane (11). Table I lists the temperature at which the change in spin label motion was observed for mitochondria from the various cultivars, together with the Ea for the succinate oxidase system of the mitochondria above and below the transition temperature. Because of the time involved in measuring the rate of oxidation at low temperatures and the decrease in activity of isolated mitochondria with time of storage (9) the temperature at which the change in Ea occurred could not be determined, in all cases, as accurately as the temperature of the phase change by spin labeling. However, as shown in Figures 1 and 2 and previously (11) with chilling-sensitive plants, the temperature of the phase change and the temperature at which membrane-associated enzymes exhibit a change in Ea are within the same range. Consequently, only the temperature of the phase change is presented. This was derived from the intersection of the two straight lines of the Arrhenius plot of spin-label motion.

The addition of detergents such as Teric X8 and deoxycholate to reaction mixtures (0.05–0.5% [w/v]) did not affect the Ea of the succinate oxidase system above or below the temperature of the transition. In previous studies (10) with mitochondria from chilling-sensitive plants and warm blooded animals, these detergents at concentrations of 0.05% (w/v) abolished the discontinuity observed in Arrhenius plots of succinate oxidase activity; the Ea in the presence of detergent was usually intermediate to the values observed above and below the transition temperature with untreated mitochondria (10). Abolition of the change in Ea of succinate oxidase in chilling-sensitive plants by treatment with detergent is considered to involve solubilizing the lipid components of the membrane (10). The inability of the detergents to alter the Ea of succinate oxidase with apple mitochondria suggests the lipid components of these mitochondria are different from those of the chilling-sensitive plants studied previously.

Treatment of apple mitochondria with phospholipase A abolished both the change in Ea of succinate oxidation and

the phase change observed by spin labeling, thus demonstrating the involvement of lipid components as shown with chilling-sensitive plants. This treatment did not affect the rate of succinate oxidation measured at 25 C.

The temperature of the phase change in the different cultivars (Table I) varied considerably but was usually lower than the temperature at which similar changes were observed with mitochondria from chilling-sensitive plants (4). Furthermore the variations in the temperature of the phase change did not conform to a pattern which could be related to the known susceptibility of the different cultivars to low temperature breakdown. To explain this lack of correlation, it would be necessary to understand the physiological events which lead to the visual manifestation of breakdown. There is little information available regarding the biochemical aspects of low temperature breakdown in apples or chilling injury in plants of tropical origin. Raison (8) has proposed that as a consequence of the phase change in the membrane lipids and the related change in Ea of respiratory enzymes imbalances in metabolism occur which lead to the disorganization of metabolic processes and the visual symptoms of injury or breakdown. Thus while the primary event is thought to be the temperature-induced phase change in the membrane lipids the secondary events involving changes in metabolism are probably the actual cause of the visible injury (8). The accumulation of fermentation products such as ethanol and acetaldehyde has been noted in chilling-sensitive plants stored at chilling temperatures (6) indicative of a disproportionate decrease in respiratory activity compared to glycolysis below the temperature of the phase change. Overholser *et al.* (7) proposed in 1923 that low temperature breakdown in apples is caused by an accumulation of a toxic volatile compound. More recent studies (12) have shown that acetic acid accumulates in Jonathan apples early in storage at 0 C but later falls to low levels before the appearance of visible symptoms of low temperature breakdown. Such observations are consistent with a temperature-induced imbalance in metabolism in apples susceptible to low temperature breakdown. The observation of a phase change in the mitochondrial membranes of all of the cultivars of apple examined would suggest that all cultivars undergo some degree of imbalance in metabolism when stored below the temperature of the phase change. The differences in susceptibility to low temperature breakdown might therefore be a reflection of the ability of some cultivars to compensate for the imbalance in metabolism and thus prevent the visual manifestation of injury.

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