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High- and low-temperature limits to growth of tomato cells

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Abstract. The temperature dependence of the metabolic rates of cultured tomato cells (*Lycopersicon esculentum* Mill.) has been studied by differential scanning calorimetry as a continuous function over the range from near 0 to above 45°C. Metabolic rates increase exponentially with temperature over the permissive range for growth (approx. 10–30°C). Outside this range irreversible loss of metabolic activity occurs. The rate of activity loss is time and temperature dependent, increasing as the exposure temperature diverges from the permissive range and increasing with time at any nonpermissive temperature. Metabolic heat rates obtained while scanning down from intermediate (25°C) to low temperature (0°C) yielded Arrhenius plots with pronounced downward curvature below about 12°C. The increase in apparent activation energy below 12°C is a function of the scan rate, showing its time dependency. This time dependency caused by inactivation confounds many estimates of apparent activation energy. Scanning up to high temperature shows that activity loss at high temperature is also time and temperature dependent. No first-order phase transitions associated with the changes in metabolism were detected at either low or high temperatures. Studies with lamellar lipid preparations added to cells show that temperature-induced transitions of lipids at levels equivalent to 4% of the lipid content of the cells were detectable. Cells with altered lipid composition showed altered temperature dependence of inactivation. High pressures (in the range from 10 to 14 MPa) shift the high temperature threshold and the rate of metabolic activity loss, supporting a postulate that higher-order transitions may be associated with inactivation of metabolism. Higher-order transitions of lipids or first-order transitions encompassing only a small fraction of total lipid remain among several viable postulates to explain temperature-dependent loss in activity. Alternative postulates are discussed.

Key words: Calorimetry – Chilling – Heat-stress injury – *Lycopersicon*

Introduction

Chilling-sensitive plants exposed to temperatures below 10–12°C exhibit precipitously decreased growth, respiration, and photosynthesis rates. A variety of cell and tissue functions are impaired. Prolonged exposure to chilling leads to acute dysfunction and death of the cells. Although it has been seriously questioned (Bagnall and Wolfe 1978; Low et al. 1984; Minorsky 1985; Martin 1986; Terzaghi et al. 1989), the "thermotropic membrane lipid phase transition" hypothesis (Lyons and Raison 1970; Raison et al. 1971; Lyons et al. 1979; Raison and Orr 1990) has been widely used to explain the alterations in metabolic activity induced in many poikilothermic organisms at low nonfreezing temperatures.

Plants are also injured at high temperatures. The temperature at which injury begins to occur is a characteristic of the species or ecotype. Inactivation of metabolism accompanies high-temperature injury. Injury at high temperature has been ascribed to lipid phase transitions that decrease membrane stability (Pike et al. 1979; Raison et al. 1980; Berry and Raison 1981; Fork et al. 1985).

A phase transition is a change in molecular order accompanied by changes in the macroscopic physical properties of the system. In an ordinary phase transition, e.g. ice melting, the first derivative of the Gibbs free energy with respect to temperature (i.e. entropy) is discontinuous and the enthalpy change must be nonzero. In higher-order transitions, the first derivative is continuous and the enthalpy change must be zero, but some higher-order derivative must be discontinuous. Phase transitions are

Abbreviations: BASF 13-338 = [4-chloro-5(dimethylamino)-2-phenyl-3(2H)-pyridazinone]; DEPC = Dielaidoyl phosphatidylcholine

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thus described as first, second, third, etc. order according to the order of the derivative of the Gibbs free energy that is discontinuous (Guggenheim 1950, pp. 276–288; Landsberg 1961, p. 316).

Several methods, have been used to seek evidence for phase transitions occurring at onset temperatures of physiological effects (see Raison and Orr 1986a for a comparison of methods). The results of these studies are inconsistent. In some cases the temperature at which changes in lipid structure were detected corresponded to the temperature at which physiological effects were observed. Others either failed to detect any transition or detected transitions that occurred at temperatures other than those inducing injury. Thus, attempts to verify the lipid-phase-transition hypothesis have not been conclusive. Raison has recently discussed his interpretation of the disparities in these studies (Raison and Orr 1990).

A number of cell components have been used to test for correlation between temperatures at which lipid properties change and temperatures at which the physiology or biochemistry of plants change at both high and low temperatures. Most studies used extracted lipids, usually either polar lipids or polar lipid classes derived mostly from chloroplast thylakoids (Murata 1983; Raison and Wright 1983; Roughan 1985; Raison and Orr 1986a). Other studies used mitochondrial membrane lipids (Raison et al. 1977; Dalziel and Breidenbach 1982; Raison and Orr 1986b). A few studies have used isolated subcellular organelles or membranes (Lyons and Raison 1970; Raison et al. 1971; Wade et al. 1974; Chapman et al. 1979; Low et al. 1984), but results of membrane and organelle studies have not been as readily interpreted as studies of isolated lipids.

Prior calorimetric studies (Dalziel and Breidenbach 1982; Raison and Wright 1983; Low et al. 1984; Raison and Orr 1986a,b; Raison et al. 1987; Orr and Raison 1987, 1990; Raison and Brown 1987) have focused on a search for phase transitions in isolated lipids or membranes (McMurchie 1979) that could then be correlated with in-vivo physiological changes. Recently we have studied intact tissues using isothermal and scanning calorimetric methods to characterize the time courses and reversibility of low and high temperature inactivation of metabolism (Rank et al. 1991). These methods measure metabolic heat arising primarily from oxidative reactions of respiration occurring in mitochondria. The kinetics of inactivation observed in isothermal temperature-cycling experiments (Rank et al. 1991) demonstrated that loss of metabolic activity at both low and high temperatures is a function of both the temperature and time of exposure. The temperature course of inactivation implied that higher-order phase transitions may be associated with temperature-induced metabolic inactivation.

Temperature-scanning calorimetric methods allow further examination of metabolic activity as a continuous function of temperature allowing observation of simultaneous changes in lipid or protein structures detectable as phase transitions (Hansen and Criddle 1990). To our knowledge there has been no previous in-vivo investigation of chilling and heat injury in plants by measurement of the temperature dependence of inactivation of metabolism under conditions where the enthalpy change accompanying transitions could be detected.

While continuously scanning temperature, phase transitions or lipid phase separations may appear as peaks superimposed on the metabolic heat rate versus temperature profiles. Peaks will be observable if the transitions have a nonzero enthalpy change, occur over a narrow temperature range, and involve a sufficient fraction of the tissue mass. First-order transitions such as liquid-gel phase transitions have relatively large enthalpy changes and sharp transitions in vitro and would be expected to be observed in plant cells if an appreciable fraction of the cell mass is involved. No peaks will be observable in the thermograms when structural changes occur over a broad temperature range or have near-zero enthalpy change. First-order transitions or phase separations occurring in complex mixtures of lipids and proteins are often very broad (Blume 1991) and consequently difficult to detect during temperature scans. Experimentally higher-order transitions have near-zero enthalpy changes. Thus, the absence of observable thermal events, while scanning temperature, can not be interpreted as an absence of structural changes.

During scanning experiments, thermally induced structural transitions can be distinguished from changes in metabolic activity by the nature of the signals produced. An exothermic effect in a downward temperature scan, and an endothermic effect in an upward scan indicates a structural transition and not simply an effect on metabolic heat rate. It does not follow, however, that all such transitions are reversible, particularly in vivo. Thus, a complete test of this condition in vivo is usually impractical.

If structural transitions do occur, they may or may not be causative or even associated with changes in metabolic activity. However, if structural transitions indeed trigger metabolic changes, these changes must be initiated by and coincide with some feature of the transition.

In the present study of cultured tomato cells, we have employed continuous temperature-scanning methods to determine whether significant changes in the apparent activation energy for metabolic heat rate occurring over a narrow temperature range are accompanied by thermal events indicative of phase transitions or other structural changes in components of cultured tomato cells. Further, we examined the effect of high pressure on the temperature and time course of high-temperature inactivation. While no direct evidence for a transition was observed by scanning alone, the response of the cells to the application of high pressures during temperature-scanning of metabolic rates at high temperature supports the conclusion that some structural change is involved, since pressure increased the temperature necessary to induce inactivation. These data and previously published data on the kinetics of inactivation (Rank et al. 1991) suggest that higher-order structural changes, perhaps in the membranes, are associated with both high- and low-temperature inactivation.

Materials and methods

Tomato cells. Tomato (*Lycopersicon esculentum* Mill.) cells (cell line #45, Thomas and Pratt 1981) were grown as suspension cultures or on agar media as described previously (Criddle et al. 1988). Cells (30–300 mg fresh weight) were removed from agar plates with a sterile spatula or from liquid media with a sterile pipette and deposited on a glass-fiber filter. The cell samples were placed in a sterilized calorimeter ampule, and wetted with up to 50 µl of liquid growth media. After each experiment the cells were examined for microbial contamination with a microscope. BASF 13–338 [4-chloro-5(dimethylamino)-2-phenyl-3(2H)-pyridazinone], an inhibitor of fatty-acid desaturation, was added to the cells by irrigating the agar plates they grew on with 0.5 ml of a solution of the compound (25 mg·ml^{-1} BASF 13–338, 10% acetone, 90% nutrient media) one week after subculturing. Cells were grown for one week in the presence of the inhibitor before measuring their metabolic rates and fatty-acid composition. Control cells were irrigated with 0.5 ml of 10% acetone containing media lacking BASF 13–338.

Calorimetry. A model-7707 differential-heat-conduction scanning calorimeter (Hart Scientific, Pleasant Grove, Utah, USA) was used for isothermal studies, as described by Criddle et al. (1988) or in the scanning mode as described by Hansen and Criddle (1990). In the scanning mode, data points were recorded at 1-min intervals. Because of the large numbers of points in some scanning experiments, the figures in this paper show only every third point or are shown as smooth curves. Temperature-scanning calorimetry at high pressure used special sample ampules connected to a compressed-gas cylinder by glass capillary chromatography tubing (50 µm i.d., Altech Inc., Deerfield, Ill., USA). In most experiments the ampules were flooded with O_2 from a standard laboratory bottle, sealed and then pressurized up to bottle pressure with N_2 or Ar. The partial pressure of O_2 at the start of each experiment was thereby established at about one atm irrespective of the total pressure employed. The high-pressure modification of the differential scanning calorimeter is described in detail in Criddle et al. (1993).

Lamellar lipid preparations. Dielaidoyl phosphatidylcholine (DEPC) was obtained from Avanti Polar Lipids (Birmingham, Ala., USA) as a chloroform solution. The chloroform was evaporated with a N_2 stream and a 1-h lyophylization. Aqueous buffer (0.1 M NaCl, 0.01 M 3-(N-morpholino)propanesulfonic acid (Mops), 0.1 mM EDT, adjusted to pH 7.6 with NaOH) was added to give a final lipid concentration of 5 mg·ml^{-1} and briefly sonicated to give a multilamellar vesicle suspension. Multilamellar preparations were then either mixed directly with the cells or were placed in a sealed glass capillary tube that was inserted into the ampule along with the cells for the scanning studies.

Fatty-acid compositions of control and BASF 13–338 cells were determined by GC/MS on lipids extracted by the method of Bligh and Dyer (1959).

Results

The isothermal heat rate for metabolism of tomato cells was determined at temperatures from 5 to 40°C. Three major regions are apparent in Arrhenius-type plots of these data (Fig. 1). The central region, from about 12 to 34°C, has a relatively constant slope. This slope indicates a temperature coefficient of metabolic activity (µ) of 3.3 to 3.5 kK (µ = E/R, where E is the apparent activation energy and R is the gas constant, (Johnson et al. 1974)). Below this region, the curve has a steeper slope (increased µ). At higher temperatures, µ begins to decrease, eventually becoming negative. Activity reaches a maximum where µ changes sign.

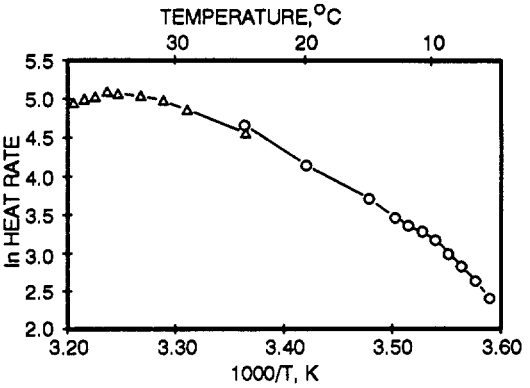

Fig. 1. Arrhenius plot of isothermal measurements of the metabolic heat rates of tomato cells at various temperatures from 0 to 40°C. *Open circles* represent measurements from 25 down to 4°C on one sample of cells. The *triangles* represent measurements on a second sample from 25 to 40°C. Masses of the samples were close, but not identical. The duration of the exposure of the cells to any given isothermal temperature while making a measurement was 45–60 min

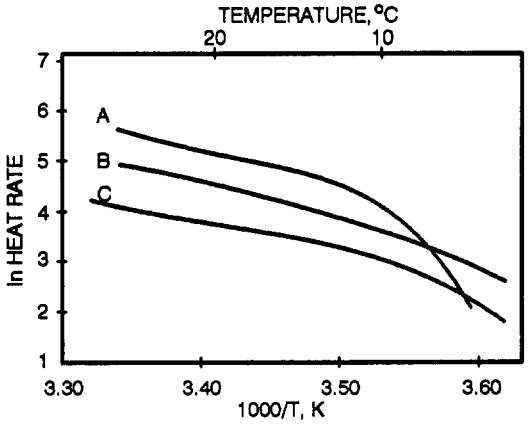

Fig. 2. Data from differential-scanning-calorimeter scans from 25 to 3°C on three different samples of tomato cells presented as Arrhenius plots. Scans were made at three nominal rates, $A = -2$°C/h, $B = -10$°C/h, $C = -5$°C/h. The actual scan rate changes slightly as a function of temperature. The scan rate change is small, experimentally known, and accounted for in the heat-rate calculations. Metabolic heat rates were recorded at 90-s intervals

Figure 2 shows metabolic heat rates of tomato cells as a continuous function of decreasing temperature from 25°C down to 3°C at three different scan rates. The scan rate determines the results obtained with continuous in-vivo temperature scanning calorimetry. In the limits, only phase transitions would be seen in a very fast scan while only metabolic heat would be seen in a very slow scan. Scan rate must therefore be optimized between these limits if both metabolic heat and phase transitions are to be observed simultaneously. Also, the rate of inactivation of cells has a complex dependence on the time and temperature above or below their stability range (Criddle et al. 1988; Rank et al. 1991). Thus, the scan rate will influence the degree of inactivation of cells at any given temperature during the scan. Table 1 illustrates the effect of scan rate on the fraction of metabolic activity

Table 1. Recovery of metabolic activity following scanning down to 4° C and rapid readjustment of temperature to 25° C. Initial metabolic heat rate is established at 25° C, isothermally for 2000 s. After scanning down at the indicated rate the samples are rapidly restored to 25° C (approximately 100° C h^{-1})

Scan rate (nominal), °C·h^{-1}	Actual range of scan rates °C·h^{-1}	% recovery of activity at 25° C
−8	−9.5 to −7.1	94
−6	−7.3 to −5.5	85
−5	−5.9 to −3.6	84
−4	−5.2 to −3.2	58
−1	−2.2 to −0.5	52

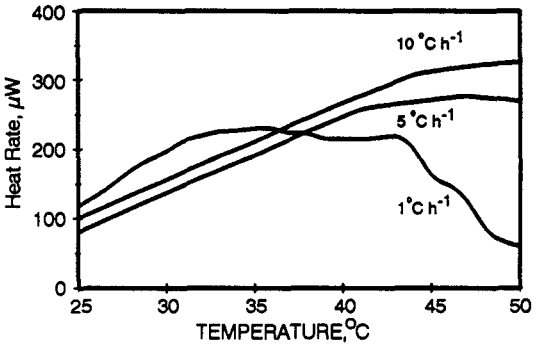

Fig. 3. Data from differential-scanning-calorimeter scans from 25 to 50°C. Scans were made at three nominal rates as explained in Fig. 2

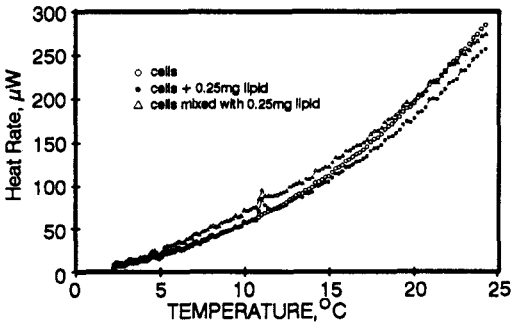

Fig. 4. Data from downward scanning experiments with tomato cells (250 mg FW). Two of the tomato cell samples had 0.25 mg of DEPC added prior to the scan. The DEPC was either mixed directly with the cells or placed in the calorimeter ampule in a sealed capillary. The samples were scanned at −6°C·h^{-1}

lost during scans over the same temperature range. The observed change in value of μ with temperature will also depend on scan rates. At the most rapid scan rate shown in Fig. 2, a small deviation from linearity in the Arrhenius plot begins at about 10°C. As the scan rate decreases, deviation from linearity becomes more pronounced and the onset is evident at higher temperature. For the slowest scan rate deviation begins near 12°C.

During upward scans to a high temperature the metabolic heat rate slows, and at some temperature deviates downward from the rate predicted by a linear Arrhenius extrapolation (Fig. 3). The temperature at which deviation from the Arrhenius relation occurs decreases as the scan rate decreases. At a scan rate of 10°C·h^{-1}, no significant change in the slope of the net activity rate occurs until the temperature reaches 43°C and the slope remains positive to at least 50°C. At 5°C·h^{-1}, the net rate begins to decrease near 41°C and the slope becomes negative near 46°C. These temperatures are above the recognized threshold for heat stress and heat shock for tomato. At a very slow scan rate (1°C·h^{-1}), deviation from the exponential increase with temperature becomes evident as low as 28°C. The net rate of increase becomes negative at a temperature slightly above 35°C. An abrupt decline in activity is seen near 43°C in this scan. Between 28°C and 43°C the curve is complex, suggesting that a number of metabolic changes are occurring. The overall form of the response at 1°C/h is similar to that observed in isothermal experiments reported earlier (Fig. 1 and Rank et al. 1991). No residual metabolic activity was detected upon returning the cells to 25°C following any of the scans of Figure 3. Cells exposed to temperatures greater than 45°C for as little as 10 min were totally and irreversibly inactivated.

Neither the scan-down nor the scan-up data in regions of low- or high-temperature inactivation show reproducible indications of peaks, characteristic of a first-order lipid transition. Careful examination of scan-down data at low temperature (Fig. 2) shows only a smooth change in slope between 12 and 10°C. Scan-up data from 3 to 25°C also show only a gradual rate of change in regions where inactivation of metabolism is evident in down-scans (data not shown). Similar conclusions can be drawn from scan-up data in the high-temperature region (Fig. 3).

To test our ability to see lipid phase transitions and establish a detection limit for observation of transitions, a pure lipid sample was added to the sample ampule. Figure 4 shows scanning data on metabolic heat rates for three samples of tomato cells (each 250 mg wet weight). A suspension of laminar vesicles amounting to 0.25 mg of dielaidoyl phosphatidylcholine (DEPC) was mixed with the cells in one of the samples. An identical aliquot enclosed in a glass capillary was inserted in the second sample. Thermally induced transitions, 2°C in width, near 11°C are clearly visible in the samples with added lipids. Transitions are observable with as little as 0.1 mg of added lipid (data not shown). Whether DEPC was dispersed with tomato cells or added in a glass capillary made no difference in the peak area or the transition temperature. Each tomato cell sample contained about 2.5 mg of naturally occurring lipid. Accordingly, the scanning studies measuring metabolic heat rates are capable of revealing transitions in the added lipids at levels lower than 4% of the amount of total cellular lipids. A peak to peak noise of about 5 μW limits the ability to detect transitions involving smaller amounts of lipids. The minimum detection limit expressed as a percentage of lipid undergoing phase transformation is given approximately by Eq. 1:

$$\text{Detection limit (\% of lipid)} > 6800\ \Delta T/bw \qquad (1)$$

Table 2. Fatty-acid compositions of tomato cells grown in the absence and presence of BASF 13-338 fatty-acid-desaturase inhibitor

Lipid	Mol % of total fatty acids	
	−BASF 13-338	+BASF 13-338
C16:0	25.2	24.5
C16:1	1.2	2.2
C18:0	0.7	1.2
C18:1	1.4	4.3
C18:2	29.0	44.8
C18:3	34.0	17.8
Other	8.5	1.5

where ΔT is the temperature range over which the transition occurs, b is the scan rate in $°C \cdot h^{-1}$, and w is the wet weight of the sample (Hansen and Criddle 1990).

The enthalpy change for transition of pure DEPC or DEPC mixed with tomato cells was determined to be $27\ J \cdot mg^{-1}$, a value in good agreement with reports by others (Small 1986). Thus, the presence of metabolizing cells does not perceptibly alter the ability to accurately measure the heats of transitions of lipids.

Data presented thus far show the absence of a first-order transition in a measurable fraction of the lipid of tomato cells at either high or low temperature. However, evidence of lipid involvement in the inactivation at high and low temperature was obtained using cells with altered lipid. Cells grown in the presence of BASF 13–338 to inhibit desaturation have altered lipid composition (Table 2). As expected BASF 13–338 treated cells have lower levels of C18:3 and higher C18:2 fatty acids. Overall unsaturation of lipids is markedly decreased. Isothermal studies (Fig. 5) indicate that BASF 13–338-treated cells undergo a chilling-induced decrease in activity at a temperature higher (13–15°C) than that observed for untreated cells (9–10°C).

Metabolic heat rates of cells treated with BASF 13–338 and untreated cells were compared at a scan rate of $-2°C \cdot h^{-1}$ (Fig. 6). In this scan the BASF 13–338 treated cells show a more rapid loss of activity in the low temperature region than untreated cells. These differences cannot be seen at faster scan rates ($-6.5°C \cdot h^{-1}$) and the curves are parallel (data not shown). When cells are taken to 4°C, held for 20 h, returned to 25°C, and the metabolic heat rate determined isothermally, BASF 13–338-treated cells lose activity more rapidly than untreated cells (Fig. 7). This difference in the stability of metabolic activity is consistent with the apparent shift in the temperature curves between treated and untreated cells (Figs. 5, 6). When at 4°C, the treated cells are 9–11°C below the inactivation temperature while the untreated are only 5–6°C below their inactivation temperature. In high-temperature-scanning studies, no difference was seen in the scans of BASF 13–338-treated cells compared to untreated cells and there was no noticeable difference in the time course of activity loss at 38.7°C.

Figure 8 shows the results from an experiment in which three tomato cell samples were scanned simultaneously over the temperature range from 25 to 60°C at a scan rate of $6°C \cdot h^{-1}$. Two of the samples, differing only in the mass of cells, were examined at high pressure (10 MPa) and one at ambient pressure. Differing sample sizes were used to demonstrate that the effects observed were not due to differences in the depletion of O_2, the accumulation of CO_2 or the accumulation of other volatile metabolic products. The metabolic activity response to temperature change for the sample at ambient pressure is essentially the same as that shown in Fig. 3. The activity profile for the samples at high pressure is the same as that for the ambient-pressure sample up to 36°C, but under high pressure, activity continues to increase with temper-

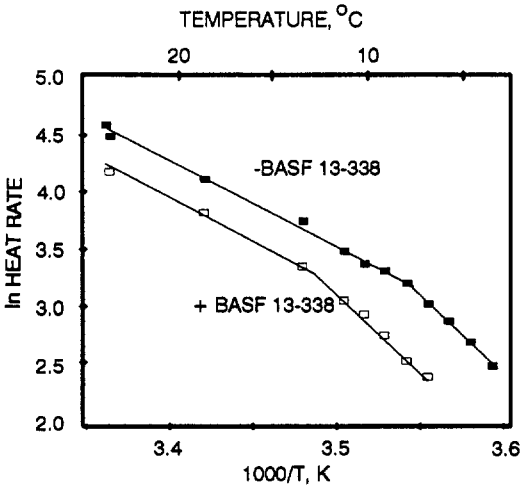

Fig. 5. Isothermal metabolic heat rates of tomato cells grown in the presence or absence of BASF 13–338 presented in Arrhenius form. The cells treated with BASF 13–338 were grown for one week on media containing the inhibitor added by irrigating with 0.5 ml of a solution of the compound in 10% acetone in nutrient media. Control cells were irrigated with the same volume of acetone containing media lacking BASF 13–338

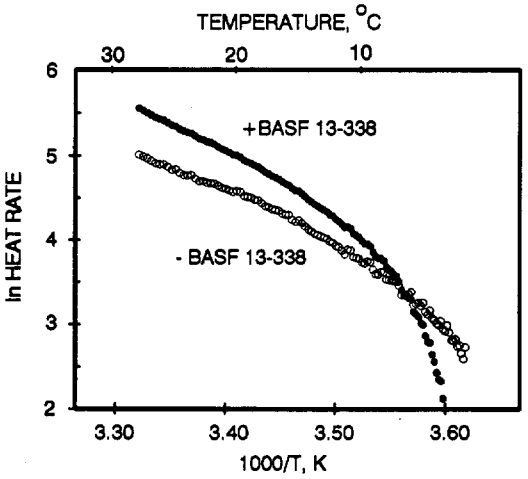

Fig. 6. Tomato cells treated with BASF 13–338 compared to untreated cells by scanning from 25°C to 3°C at $-2°C \cdot h^{-1}$. Data in Arrhenius form

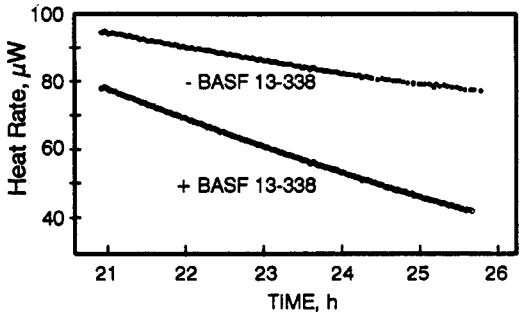

Fig. 7. Loss of metabolic activity by tomato cells treated with BASF 13–338 compared to that of controls when the cells are exposed to a temperature of 4°C for 20 h and then returned to 25°C for the times indicated on the abscissa

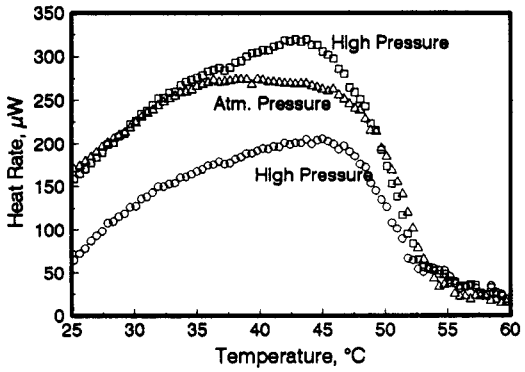

Fig. 8. Scanning-calorimetric determination of tomato metabolism at ambient and elevated pressure. In one ampule, a 220-mg-FW sample of cells was scanned while at 10 MPa (□). In a second ampule 115 mg FW of cells was scanned while at this same high pressure (○). The third ampule (△) contained 220 mg of cells at ambient pressure (0.1 MPa). The nominal scan rate was 6°C·h^{-1} (reprinted with permission of Thermochim. Acta, Elsevier Scientific Publishers, Amsterdam)

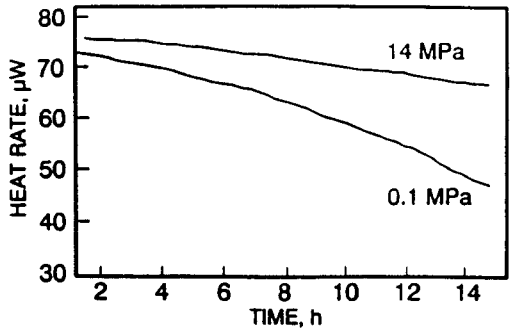

Fig. 9. Inactivation of tomato cells at 38.7°C at atmospheric (0.1 MPa) and at high (14 MPa) pressure. Approximately 100 mg of cells were rapidly heated by scanning up to the specified temperature at the maximum rate (99°C·h^{-1}) after pressurizing the ampules. Heat rates were then recorded isothermally for the duration of the treatment

ature up to at least 43°C. Above 45°C the abrupt inactivation of metabolism is again noted.

The reproducibility of the pressure effects was demonstrated with scans at several pressures between ambient and 14 MPa. The magnitude of the effect is proportional to pressure up to about 10 MPa, above which it becomes approximately constant. The observed effect does not appear to be caused by solubility of the pressurizing gas in the cells because no appreciable differences in cell responses resulted from using N_2 or Ar as the inert gas. The only observable difference between samples flooded with O_2 and those under air is that the experiment can continue for longer times without becoming O_2-limited.

The observed increase in temperature stability of metabolic activity at high pressure could result from pressure effects on rates of inactivation or from pressure effects on equilibria. At least a portion of the pressure effect on activity results from alteration of rates (Fig. 9). These data were obtained after rapidly raising the temperature to 38.7°C and measuring isothermal heat rates as a function of time. Inactivation of the sample at high pressure was slower than inactivation of the sample at atmospheric pressure.

Discussion

There are two fundamentally different models that can be used to explain the precipitous onset of injury to plants at either high or low temperature. The first of these models postulates the occurrence of a physical change in the state of some cellular component over a narrow temperature range. The thermotropic membrane-lipid phase transition hypothesis, which postulates a liquid crystalline to gel transition of bulk membrane lipids on lowering the temperature is only one of the possible models of this type (Raison et al. 1971; Lyons et al. 1979; Raison et al. 1980; Quinn et al. 1989; Raison and Orr 1990). Such a liquid crystalline to gel transition would be expected to have a large enthalpy change and be readily observable by scanning calorimetry, see Fig. 4.

Changes in protein conformation or aggregation within the cell as well as phase separations of lipids or of lipids and proteins also fit into the physical change category. Such changes of protein conformation, separation of gel lipid phases and extrusion or inclusions of proteins by the lipid membrane have often been vaguely linked with bulk lipid phase transition models. Many such changes would probably be accompanied by small enthalpy changes and hence may not be observable by scanning calorimetry.

The second type of model postulates a change in the kinetics of the rate-limiting step in the metabolic process not necessarily related to a change in physical state (Johnson et al. 1974). The altered kinetics could for example result from differences in temperature dependence of reaction rates in the complex of metabolic pathways, from changes in the amounts or nature of regulatory molecules or from differences in availability of key metabolites at different temperatures.

The results obtained in this study tend to support the first model but do not rule out the second. Measurements of metabolic heat rates by differential scanning calorimetry described here have shown that the deviation of metabolic rate from an exponential increase with temperature have the following characteristics. (i) Changes in

metabolic activity do not occur at a precise temperature, but instead occur over a span of a few degrees. Such metabolic changes are indicated by large changes in the temperature dependence of the metabolic rate (Figs. 2, 3, 6, 8). Prior experiments to answer this question depended at best on a relatively small number of imprecise points fitted statistically. The data given here are made up of thousands of data points taken at 30-s intervals as the temperature is changed at rates as slow as $2°C \cdot h^{-1}$. If a sharp "break" occurred, it would certainly be seen. (ii) The rate of activity loss is time and temperature dependent, increasing as the exposure temperature diverges increasingly from the permissive range for metabolism and increasing as the time at inactivating temperatures increases (Figs. 2, 3). At a fixed temperature, activity loss is approximately first order in time (Rank et al. 1991). While a time-temperature interaction for temperature stress has been recognized for a long time previously, it has been difficult to precisely quantify. (iii) Alterations of metabolic activity are not accompanied by detectable thermal events indicative of a first-order transition in cell components. (iv) At low temperature, activity loss is rapidly and largely reversible for at least a few hours of exposure of the cells to temperatures just below the temperature range where inactivation is initiated (Table 1). (v) Activity loss at high temperature is slow and partly reversible at temperatures just above the temperature where inactivation is initiated, but rapid and irreversible when temperature limits are greatly exceeded (Figs. 3, 8). (vi) At high temperature, high pressure raises the temperature of the onset of inactivation, indicating that a positive change in volume of some cellular component is required in the inactivation process (Fig. 8).

First-order transitions must invariably have large enthalpy changes and occur over a very narrow temperature range. No significant thermal event indicating a structural change was measured accompanying high- or low-temperature inactivation. Either no major physical changes accompany inactivation or a constituent comprising only a small fraction of the mass of the cells is involved in a phase transition, or a higher-order transition is involved.

The effects of lipid alteration using BASF 13–338 reported here as well as the studies of others where lipid composition has been altered by growth temperature (Raison et al. 1980; Raison et al. 1982a,b; Orr and Raison 1987), by catalytic hydrogenation (Vigh et al. 1985a,b) or by genetic manipulation (Murata et al. 1992; Hugly and Sommerville 1992) indicate that, whatever the mechanism, lipid composition is importantly involved in physiological dysfunction induced at both low and high temperature. A number of studies have provided evidence that saturated lipid components present in only small amounts may be important in determining susceptibility to chilling injury affecting development of the photosynthetic apparatus and/or photosynthetic activity (see Murata and Nishida 1990 for a recent review). These changes can be detected by scanning calorimetry of polar-lipid extracts and in fractions containing the classes of chloroplast lipid with high proportions of saturated fatty acids, i.e. phosphatidylglycerol and sulfoquinovosyl diacylglycerol. Few data are available on mitochondrial membranes or lipids. Earlier reports of scanning-calorimeter studies of total mitochondrial lipids (Dalziel and Breidenbach 1982) or membrane fractions (McMurchie 1979) from tomato showed broad transitions beginning at temperatures above those where metabolic rates are affected. On the other hand, extracted polar lipids from mitochondria gave transitions with onsets correlated with chilling sensitivity (Raison and Orr 1986b). From the enthalpy it was estimated that 3% of the lipid underwent a transition.

Murata and coworkers (Murata et al. 1992) provided strong support for the causal involvement of chloroplast membrane lipids in the chilling responses evoked in the photosynthetic apparatus. Transformed tobacco plants with genes encoding for the glycerol-3-phosphate acyltransferases were cloned from chilling-sensitive squash or chilling-insensitive *Arabidopsis*. The chilling responses manifested in the photosynthetic apparatus of the transformants were characteristic of the species that served as the source of the acyl-transferase gene. Moreover the fatty-acid composition of phosphatidylglycerol of the transformed tobacco plants was altered to closely resemble that of the gene donor. Cis-unsaturated fatty-acid content in the sn-1 position of phosphatidylglycerol was lowered in tobacco plants transformed with the squash gene and increased in tobacco plants transformed with the *Arabidopsis* gene.

The temperature dependence of inactivation seen here and in previous work (Rank et al. 1991) is consistent with a higher-order transition as the cause of inactivation. Higher-order transitions must invariably have near-zero enthalpy changes and may occur over several degrees. Phase separation is a special case of the latter phenomena. Others have proposed that higher-order transitions in the membrane trigger temperature inactivation (Lyons et al. 1979; Raison and Orr 1986a, 1990; Quinn et al. 1989). Our results are consistent with this proposition.

The scanning-calorimetric studies at atmospheric pressure rule out a first-order transition involving a major fraction of the cell mass as a cause of inactivation (Fig. 4); nevertheless, studies at high pressure provide direct evidence for structural change associated with high-temperature inactivation of tomato cells (Figs. 8, 9). The pressure dependence of the high-temperature threshold shows that a transition having a positive change of volume is tightly linked to loss of metabolic activity. The volume change may be associated with either an equilibrium or kinetic process. The effect of pressure is described by the isotherm in Eq. 2

$$(\partial \ln K / \partial p)_T = \Delta V / RT \qquad \text{(Eq. 2)}$$

where ΔV is the difference in partial molal volumes of reactants and products, T is the absolute temperature, p is the pressure and K is either an equilibrium constant or a rate constant. The slope of a plot of $(\partial \ln K / \partial p)$ vs. $1/T$ is $\Delta V/R$. Therefore, if an event is moved to a higher temperature by increased pressure, ΔV must be positive and nonzero. Lipid melting and protein denaturation have positive ΔV values, and thus the data are consistent with

postulating a higher-order transition in a major cell component as the proximate cause of thermal inactivation. However, neither are the data inconsistent with inactivation being caused by alteration of the rate(s) of reaction(s) associated with a volume increase in forming a transition-state complex. An increased volume of the transition state is observed in most reactions. The only means available for distinguishing between the two possibilities is to determine activity as a function of time, temperature and pressure. The activity, time, temperature surface at atmospheric pressure was reported previously by us (Rank et al. 1991). The additional evidence reported here that the inactivation process has a positive ΔV makes it possible to proceed with experiments identifying the chemical nature of the events leading to high-temperature injury. The same evidence is more difficult to obtain at low temperatures because of the slow reaction rates but potentially allow similar information on events associated with chilling.

Taken together these results rule out a first-order transition of a lipid fraction encompassing more than 4% of the lipid accompanying high- or low-temperature inactivation, and provide evidence to support a postulated structural transition such as a higher-order transition or small changes in lipid and/or protein conformations as the proximate cause of chilling and heat injury in plants.

This manuscript is dedicated to the memory of John Raison, a good friend and an inspiring plant physiologist. The authors thank Anne Bahr for assistance with the data analysis. The authors thank Dr. J.B. St John for the gift of BASF 13–338.

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