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Estimation and Analysis of Cucumber (*Cucumis sativus* L.) Leaf Cellular Heat Sensitivity[1]

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Triphenyl tetrazolium chloride (TTC) reduction by cucumber (*Cucumis sativus* L. cv Poinsett 76 and cv Ashley) leaf discs was used as a viability assay to examine the effect of temperature pretreatment on the tissue response to acute hyperthermia. Semilogarithmic plots of TTC reduction as a function of incubation time at different temperatures from 40 to 60°C resembled the heat survival curves of animal cells. Heat inactivation rates were obtained and subjected to "quasi" Arrhenius analyses by analytical methods derived from the animal studies. The Arrhenius plots of TTC reduction rates for cv Ashley leaf discs preincubated at 25 or 37°C and for cv Poinsett 76 preincubated at 37°C were linear with the same activation energy (E_a) of about 80 kcal mol^{-1}. The Arrhenius plot of cv Poinsett 76 preincubated at 25°C was nonlinear with an E_a of about 80 kcal mol^{-1} at temperatures below 46°C and an E_a of about 27.5 kcal mol^{-1} at temperatures above 47°C. The significance of these differences is discussed in terms of the role of protein denaturation in the thermal sensitivity of cucumber disc reduction of TTC and the applicability of these methods to the analysis of plant cellular heat sensitivity.

The responses of plants to hypo- and hyperthermia have been the subject of numerous investigations (Berry and Bjorkman, 1980; Levitt, 1980). Many of these studies have involved the search for new heat-tolerant germplasm of agriculturally important plants (Chen et al., 1982). Even though the physiological, biochemical, and biophysical responses of plants to temperature extremes have been extensively investigated, much of this research has been concerned with those processes that may be related to plant damage and resistance to reduced temperatures (Levitt, 1980; Graham and Patterson, 1982).

Until recently, physiological and biochemical studies of plant responses to elevated temperatures have emphasized the effects of temperature on plant photosynthetic reactions (Berry and Bjorkman, 1980). The emphasis has shifted recently to the molecular biology of plant hyperthermia (Key et al., 1981; Kimpel and Key, 1985). The involvement of heat-shock proteins in plant thermotolerance has been demonstrated (Kimpel and Key, 1985; Krishnan et al., 1990), even though the specific function of many of these proteins is not known. Nonetheless, plants can undergo induced thermotolerance in a manner similar to that found in animal cells and tissues (Krishnan et al., 1990).

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Researchers of the hyperthermic responses of animal and microbial systems have often applied the methods of radiobiology to analyze the cellular responses of cells and tissues to elevated temperatures (Bauer and Henle, 1979; Henle, 1987; Alpen, 1990). They have used cell survival curves obtained at different incubation temperatures to estimate certain "quasi" thermodynamic properties of the cells or tissues (Westra and Dewey, 1971; Alpen, 1990). Such studies have permitted the identification of potential cellular lesions that influence the cell's ability to withstand hyperthermia (Henle, 1987). Using these methods, Henle (1987) demonstrated that drugs and other chemicals, thermal history, developmental stage, ions, and previous exposure to ionizing radiation can modify a cell's ability to respond to acute hyperthermia.

In this report, approaches developed for the analysis of animal and microbial hyperthermia (Westra and Dewey, 1971; Bauer and Henle, 1979) have been applied to the analysis of the temperature dependence of TTC reduction by leaf discs of two cucumber (*Cucumis sativus* L.) cultivars. The TTC viability assay has been used previously to study the basic thermal responses of different plants (Steponkus, 1971; Towhill and Mazur, 1974; Chen et al., 1982) and to demonstrate the role of heat-shock proteins in plant thermotolerance (Krishnan et al., 1990). Because UV-B radiation and hyperthermia cotolerance has been observed in both animal and microbial cells (Dewey and Holahan, 1987; Koval, 1991), the cucumber cultivars were selected based on their differing responses to UV radiation (Krizek, 1978; Kramer et al., 1991) and their use in previous studies of thermotolerance (Mahan et al., 1990; LaFuente et al., 1991).

MATERIALS AND METHODS

Plant Growth Conditions

Seed of UV-sensitive cv Poinsett 76 and UV-resistant cv Ashley cucumber (*Cucumis sativus* L.) were germinated and grown in Jiffy Mix[2] (Ball Jiffy, Chicago, IL) at 25°C day and 17°C night temperatures and 65% RH in an EGC plant

Abbreviations: D_o, time at 50°C that results in a 37% decrease in TTC-reducing activity; D_q, lag time before the onset of first-order inactivation kinetics; TTC, triphenyl tetrazolium chloride.

[2] Mention of trademark or proprietary products does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

growth chamber. Light was supplied by cool-white fluorescent lamps (300 μmol m^{-2} s^{-1}) in a 16-h photoperiod.

Temperature Treatment Procedure

Leaf discs (5 mm) were cut with a No. 3 cork borer from both cotyledons (7 d old) and first leaves (14 d old), avoiding major veins. The discs were floated adaxial side down on 10 mM potassium phosphate buffer (pH 7.4) in Petri dishes at either 25 or 37°C for 6 h under cool-white fluorescent lamps (300 μmol m^{-2} s^{-1}) before being assayed for thermal sensitivity and/or cellular viability. The cutting of the leaf discs and transfer to the temperature pretreatments were staggered to ensure that the assays for thermal sensitivity of TTC reduction began 6 h after the onset of the temperature treatment. In all experiments we used leaf discs cut at the 8th h of the 16-h photoperiod.

Individual discs were transferred to covered 12- × 75-mm culture tubes containing 1 mL of 10 mM potassium phosphate buffer (pH 7.4) that had been preincubated in a water bath set at the desired temperature (±0.2°C). Sufficient discs were transferred to permit triplicate viability determinations for each time period. After the desired treatment time, the discs were rapidly removed and immediately assayed for viability as described below.

Viability Assay

The cellular viability of the leaf discs was determined by the TTC reduction assay described by Chen et al. (1982). The three discs for each time/temperature treatment were transferred into a 50 mL-Erlenmeyer flask containing 15 to 20 mL of 0.8% (w/v) TTC in degassed 50 mM potassium phosphate buffer (pH 7.4). The leaf discs were vacuum infiltrated with the TTC solution by four cycles of vacuum on/off of about 15 s each. Infiltration was considered to be complete when the leaf discs became uniformly colored and sank to the bottom of the flask. Each disc was then transferred to 1 mL of fresh TTC solution in a 24-well microtiter plate. The plates were wrapped in foil and incubated at 25°C in the dark for 18 to 20 h. The rest of the procedure was the same as that described by Chen et al. (1982) except that 1 mL of 95% ethanol was used to extract and redissolve the reduced tetrazolium salts.

Data Presentation and Analysis

The As at 485 nm of the leaf extracts were divided by the average leaf disc weight (3.8 mg) to give A/mg fresh weight. These values for the acute hyperthermia treatments were then converted to percentages relative to the TTC reduction values of control discs from the 25 and 37°C pretreatments. The log of the average percentage with SE was then plotted as a function of incubation time to give the heat inactivation curves.

The rates for heat inactivation (D_o) were derived from standard regression analyses of the linear portions of the heat survival curves, assuming pseudo-first-order kinetics. Only data sets that gave a linear correlation coefficient of 0.975 or better were used. D_o represents the time (min) required for an inactivation of TTC reduction ability of 37% relative to some arbitrary value in the linear portion of the inactivation curve (Johnson et al., 1974; Alpen, 1990). Because analyses of this type have not been applied to the thermal sensitivity of a plant viability assay, the methods for the calculation of the various inactivation curve parameters are depicted in Figure 1. The Arrhenius analyses of the D_o values were done according to the procedures described in detail elsewhere (Bauer and Henle, 1979), using the equation

$$\log(k) = \log(D_o^{-1}) = -(E_a/[2.3RT])(T^{-1}) + \log(A) \quad (1)$$

where k was the rate constant or reciprocal of D_o; E_a, the Arrhenius activation energy; A, Eyring constant; T, temperature (°K); R, the universal gas constant (1.99 cal $°K^{-1}$ mol^{-1}). The values of D_q or, quasithreshold dose, were also obtained by regression analysis and used as a measure of the heat inactivation curve "shoulders" or the lag time before the onset of first-order kinetics (Henle, 1987; Alpen, 1990). Although all numerical values given in the text and used for subsequent analyses were obtained by linear regression analyses, the lines were plotted according to the best visual fit to the data.

RESULTS

TTC Reduction Assay

Initially, 6-h pretreatments at 42°C were tested to evaluate the effect of pretreatment temperature on the thermal inactivation of TTC reduction. These pretreatment conditions had been previously shown to induce heat-shock protein synthesis and increased resistance to chilling in cucumber (cv Poinsett 76) (LaFuente et al., 1991). However, the 42°C pretreatment reduced the control TTC reduction values by 85 and 65% for cvs Poinsett 76 and Ashley, respectively (data not shown).

The heat-killing time and heat-killing temperatures were

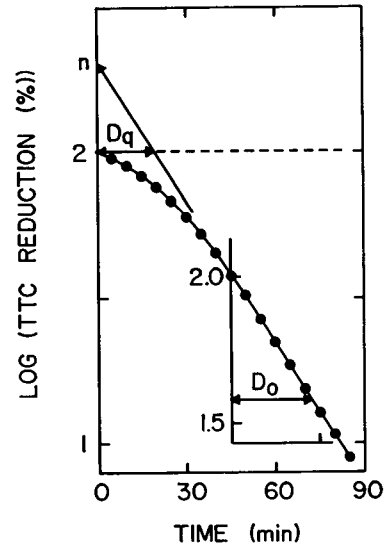

Figure 1. General characteristics of the heat inactivation curves for TTC reduction by cucumber leaf discs, demonstrating the source of the various parameters described in the text.

determined for leaf discs of the two cucumber cultivars pretreated at either 25 or 37°C for 6 h. The heat-killing times at 50°C for cv Poinsett 76 (26.5 min) and cv Ashley (30.3 min) preincubated at 25°C were longer than those obtained after 6 h at 37°C (23.5 min for Poinsett 76, 26.0 min for Ashley). The heat-killing temperatures for leaf discs of cv Poinsett 76 and cv Ashley preincubated for 6 h at 25°C were 46.4 and 49.5°C, respectively. After 6 h at 37°C, the heat-killing temperature for leaf discs of cv Poinsett 76 increased to 50.4°C, whereas that of cv Ashley was essentially unchanged at 49.7°C. Even though there was little effect on cv Ashley, the heat-killing temperatures indicated increased thermotolerance of cv Poinsett 76 after the 37°C preincubation. By both methods, it was apparent that cv Ashley was inherently more tolerant of hyperthermia than cv Poinsett 76.

Effect of Hyperthermia on the Inactivation Rate of TTC Reduction

Because the heat-killing times presented above were not consistent with the expected increase in thermotolerance after pretreatment at 37°C, more detailed analyses were performed to determine the kinetics of heat inactivation by subjecting the leaf discs to temperatures varying between 40 and 60°C for different time intervals. As shown in Figure 2, plotting the logarithm of TTC reduction (percentage of control) as a function of time at different temperatures resulted in a series of curvilinear plots. These plots are similar to the heat survival curves obtained with various animal cells and tissues (Henle, 1987). The linear portion of these curves indicated pseudo-first-order kinetics and permitted use of the slopes to quantify the inactivation rates (D_o) (Alpen, 1990). It should be noted that, to permit inspection of the data obtained at short time intervals (<15 min), the TTC reduction values obtained at times >120 min were not included in the figures. However, for treatment temperatures below 50°C, the incubations were extended to 360 min at 30-min intervals to permit the accurate calculation of the inactivation rates.

The results presented in Figure 2 indicated significant differences in the thermal inactivation rates for TTC reduction by the two cucumber cultivars preincubated at 25°C. An analysis of the slopes of the linear portions of the curves showed that pretreatment at 37°C resulted in a similar temperature dependence of the inactivation rates for cv Poinsett 76 and cv Ashley (Fig. 3). The significance of the differences in the shoulder region (D_q) will be considered below. It should be noted that the 37°C pretreatment resulted initially in increased rates of TTC reduction by leaf discs from cv Ashley relative to the control at temperatures below 47.5°C (Fig. 3B), suggesting that the temperature pretreatment directly modified the TTC-reducing capability of cv Ashley. A similar phenomenon was not observed for cv Poinsett 76 preincubated at 37°C (Fig. 3A).

Thermodynamic Analysis of the Inactivation Rates for TTC Reduction

Arrhenius plots were prepared for use as a convenient representative method to compare the effect of the temperature pretreatments on the temperature dependence of the rate of thermal inactivation of TTC reduction. As shown in Figure 4A, plotting the reciprocal of the inactivation rates as a function of temperature (°K^{-1}) for cv Ashley pretreated at 25°C resulted in a straight line that gave an apparent E_a of 81.2 kcal mol^{-1}. Although the data began to deviate from linearity at temperatures above 55°C, there was insufficient data to accurately calculate the change in E_a. The Arrhenius plot for cv Poinsett 76 preincubated at 25°C was nonlinear with E_a values of 78.2 and 27.5 kcal mol^{-1} below and above the "break" at 46.5°C, respectively.

Pretreatment of the cucumber leaf discs at 37°C resulted in inactivation rates that produced linear Arrhenius plots (Fig. 4B). The E_a for cv Poinsett of 78.8 kcal mol^{-1} was essentially the same as those for cv Ashley pretreated at 37°C (79.4 kcal mol^{-1}) and the 25°C pretreated cv Ashley and cv Poinsett 76 (<46.5°C) (Fig. 4A).

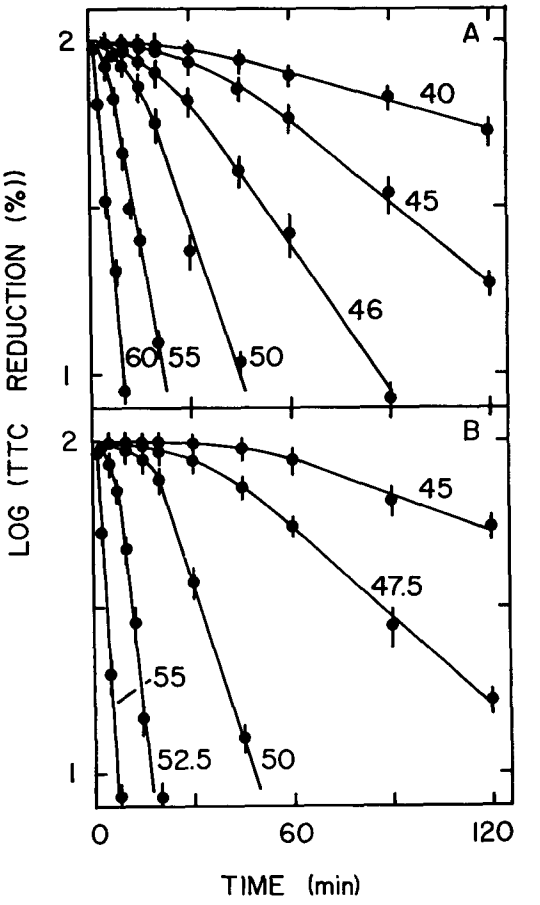

Figure 2. Effect of temperature on the time-dependent inactivation of TTC reduction by cucumber leaf discs pretreated at 25°C. Discs (7 mm) were cut from the leaves of 14-d-old cucumber cv Poinsett 76 (A) or cv Ashley (B) and pretreated at 25°C for 6 h. The ability of the leaf discs to reduce TTC was determined after treatment for the desired time at the temperature (°C) given next to the curve. The data are the results of triplicate determinations and are presented as the means and SES of percentages relative to control values (A, 0.224 ± 0.012 A/mg fresh weight; B, 0.198 ± 0.010 A/mg fresh weight) obtained immediately after the temperature pretreatment.

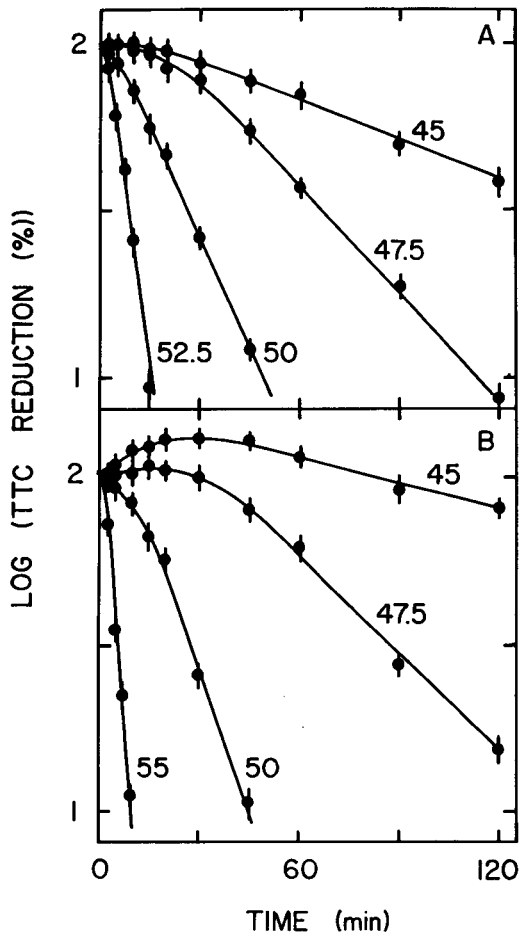

Figure 3. Effect of temperature on the time-dependent inactivation of TTC reduction by cucumber leaf discs pretreated at 37°C. Discs (7 mm) were cut from the leaves of 14-d-old cucumber cv Poinsett 76 (A) or cv Ashley (B) and pretreated at 37°C for 6 h. The ability of the leaf discs to reduce TTC was determined after treatment for the desired time at the temperature (°C) given next to the curve. The data are the results of triplicate determinations and are presented as the means and SES of percentages relative to control values (A, 0.202 ± 0.015 *A*/mg fresh weight; B, 0.183 ± 0.009 *A*/mg fresh weight) obtained immediately after the temperature pretreatment.

To permit comparisons between the results presented in Figure 3 and those reported for animal cells, tissues, and isolated proteins (Joly, 1965a; Westra and Dewey, 1971; Bauer and Henle, 1979), the various values used in the thermodynamic analyses of simple kinetic systems were computed. As will be discussed in more detail later, this type of analysis is not strictly valid, if for no other reason than the obvious problems of reversibility and conceptual definition of a mole of leaf discs. Nonetheless, this procedure has been previously utilized to infer possible mechanisms for cellular heat sensitivity (Rosenberg et al., 1971; Bauer and Henle, 1979).

With the exception of temperatures above 46.5°C for cv Poinsett 76 and above about 55°C for cv Ashley pretreated at 25°C, the inactivation enthalpies for the temperature dependence of cv Ashley and cv Poinsett 76 TTC reduction were essentially the same, ranging from 77.5 to 80.6 kcal mol^{-1} (Table I). Similarly, other than at temperatures above 46.5°C for cv Poinsett 76 pretreated at 25°C, the inactivation entropies were similar for both pretreatments and cultivars (177.2–187.3 cal mol^{-1} $°K^{-1}$). The Gibbs free energy for inactivation was essentially constant, ranging from 20.1 to 21.6 kcal mol^{-1} (Table I).

Effect of Temperature Pretreatment on the TTC Reduction Curve Shoulders

As noted above, the pretreatment temperature significantly influenced the shoulders or lag time (D_q) before the onset of first-order kinetics in plots of the time dependence of TTC reduction at different temperatures (Figs. 2 and 3). D_q may partially result from the time for the leaf discs to reach the treatment temperature (Henle, 1987). However, it seems unlikely that D_q values that exceed 1 min can be explained in terms of tissue temperature equilibration, considering the results of preliminary experiments in which leaf discs with an imbedded needle thermocouple (data not shown) were used.

The D_q values declined with increasing treatment temperature with the magnitude of the change being greater for cv Ashley, particularly after the 37°C pretreatment (Fig. 5). For cv Ashley pretreated at either 25 or 37°C, the 50% maximal response in D_q occurred at about 47.5°C. The cv Poinsett 76 had a similar response except that the 50% maximal response occurred at 49°C.

DISCUSSION

In general, all eukaryotic cells probably have some degree of induced thermotolerance (Henle, 1987). Results of studies of animal cells are often expressed in terms of clonogenic cell survival (Henle, 1987). Because the survival of individual cells cannot be readily measured in intact tissues (Schlesinger, 1990), other criteria to assess thermally induced tissue damage, resistance, and/or repair are necessary. In animal studies, several physiological endpoints believed to be important in cellular responses to hyperthermia (e.g. membrane structure and composition, DNA polymerase) were subsequently found to be unaltered in thermotolerant cells (Henle, 1987).

The TTC reduction assay has been extensively used as a suitable physiological endpoint in plant temperature studies of both intact tissues and cultured plant cells (Steponkus, 1971; Towhill and Mazur, 1974; Chen et al., 1982). Modification of TTC reduction by temperature is considered to be indicative of the cellular and tissue response to temperature and, therefore, should be affected by induced thermotolerance. The correlations between TTC reduction, vital staining, and conductivity measurements support this conclusion (Chen et al., 1982). The reduction of TTC measures enzyme activity, primarily that associated with mitochondria (Steponkus, 1971), and it is possible that specific thermally induced lesions could occur in the plant cells that the TTC reduction assay would not measure. Nonetheless, if we consider the importance of the biochemical reactions measured by the TTC reduction assay in plant metabolism, the inactivation of

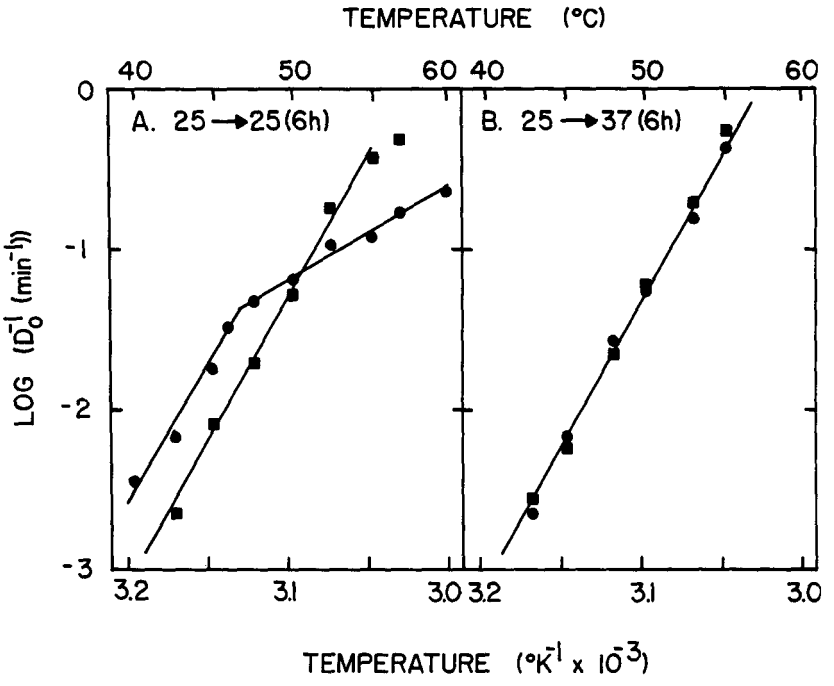

Figure 4. Arrhenius plots of the inactivation rates for TTC reduction by cucumber leaf discs pretreated at 25°C (A) or 37°C (B) for 6 h. The D_o values were obtained by linear regression analysis of the data presented in Figures 2 and 3, as well as data obtained at other temperatures and were graphed as $\log(D_o^{-1})$ as a function of the reciprocal of the absolute temperature for cv Poinsett 76 (●) and cv Ashley (■).

TTC reduction by elevated temperatures remains a convenient means to evaluate damage at the cellular level.

Using the method recommended by Chen et al. (1982) to measure plant tissue damage by elevated temperatures, we found that cucumber cv Ashley is inherently more resistant than cv Poinsett 76 to hyperthermia. Even though the measurement of heat-killing times and temperatures are useful in screening plants for differences in their thermal responses, little information is derived about potential mechanisms for variations in the plant's responses to temperature. For example, after the 37°C pretreatment, both cv Ashley and cv Poinsett 76 had very similar heat-killing temperatures. However, as shown in Figure 3, the shapes of the thermal inactivation curves are different, suggesting that the two cucumber cultivars have different responses to elevated temperatures.

The shapes of the curves presented in Figures 2 and 3 are very similar to those obtained with animal cells and tissues, supporting the contention that the overall responses of eukaryotic cells to temperature extremes may be similar. This also supports the application of the methods developed to quantitate the thermal responses of animal cells and tissues to the study of plant high-temperature stress. As noted previously, Arrhenius plots have been used to evaluate the effects of temperature on animal heat survival curves. Even though the use of Arrhenius plots to analyze the thermodynamic properties of complex, irreversible systems is conceptually invalid, they do provide the means to compare the results obtained with the cucumber cultivars with those acquired with animal cells and tissues.

The nonlinear Arrhenius plot for the inactivation rates of TTC reduction for cv Poinsett 76 pretreated at 25°C (Fig. 4A) is remarkably similar to those obtained with nonthermotolerant animal cells (Bauer and Henle, 1979). The nonlinear Arrhenius plots for the animal systems generally showed a critical or break temperature at about 43°C (Lepock and Kruuv, 1980). In the animal studies, the variation of the inactivation rates above and below this critical temperature have been interpreted as indicating either two different mech-

Table I. *Thermodynamic parameters calculated from the Arrhenius plots*

The inactivation enthalpies (ΔH, kcal mol^{-1}), entropies (ΔS, cal mol^{-1} °K^{-1}), and Gibbs free energies (ΔG, kcal mol^{-1}) were computed by standard linear regression analyses of the Arrhenius plots presented in Figure 4 after determination of the inflexion or break point by inspection. The linear correlation coefficients for the regression lines exceeded 0.95. ΔH and ΔS are shown ±SE.

Cultivar	Temperature Range	ΔH	ΔS	ΔG
Poinsett 76[a]	40 → 45°C	77.5 ± 5.8	177.2 ± 12.3	21.6[c]
Poinsett 76[a]	47.5 → 60°C	26.8 ± 2.1	20.81 ± 1.7	20.1[d]
Ashley[a]	40 → 60°C	80.6 ± 6.2	187.3 ± 14.0	20.3[d]
Poinsett 76[b]	40 → 60°C	78.8 ± 5.1	180.2 ± 9.4	20.6[d]
Ashley[b]	40 → 60°C	79.4 ± 4.4	180.12 ± 10.1	21.2[d]

[a] Preincubated at 25°C for 6 h. [b] Preincubated at 37°C for 6 h. [c] Determined at 315.5° K (42.5°C). [d] Determined at 323°K (50°C).

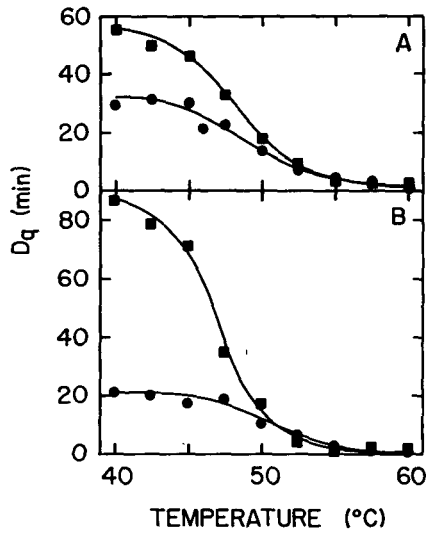

Figure 5. Plots of D_q or lag time before the onset of first-order inactivation rates of TTC reduction. The linear regression analyses used to obtain the D_o values used in Figure 4 were also utilized to determine the time delay (D_q) before the onset of the linear portions of the curves shown in Figures 2 and 3, as well as curves obtained at other temperatures. The D_q values are plotted as a function of the treatment temperature for cucumber cv Poinsett 76 (●) and cv Ashley (■) pretreated at either 25°C (A) or 37°C (B).

anisms for cell damage or the development of thermotolerance in the cells at temperatures below the break temperature (Lepock and Kruuv, 1980). It seems unlikely that the nonlinear Arrhenius plot for cv Poinsett 76 resulted from the induction of thermotolerance by temperatures below 46.5°C, considering the loss of TTC reduction ability after transfer of cv Poinsett 76 from 25 to 42°C for 6 h.

Denaturation reactions with small inactivation enthalpies (35–50 kcal mol^{-1}) may involve the disruption of a few strong cooperative bonds, whereas large inactivation enthalpies (>50 kcal mol^{-1}) usually indicate the rupture of a large number of weak, noncooperative bonds (Joly, 1965b). Therefore, the computed thermodynamic values (Table I) suggest that the change in inactivation rates above 46.5°C for the cv Poinsett 76 may result from a localized lesion that directly reduces the activity of mitochondrial dehydrogenases.

With the exception of temperatures above 46.5°C for cv Poinsett 76 pretreated at 25°C, the inactivation enthalpies for cv Ashley and cv Poinsett 76 were essentially the same (Table I) and within the 35 to 198 kcal mol^{-1} range reported by Joly (1965a) for the denaturation of a variety of proteins. The values for inactivation entropy (177.2–187.3 cal mol^{-1} $°K^{-1}$) were also similar and consistent with protein denaturation (Johnson et al., 1974). Although the compiled values for enthalpy and entropy for protein denaturation can vary widely (Joly, 1965a; Johnson et al., 1974), the Gibbs free energy for inactivation is more specific for the class of macromolecule that is denatured (Bauer and Henle, 1979) and occurs within the narrow range of 22 ± 5 kcal mol^{-1} for proteins (Johnson et al., 1974). If one considers the Gibbs free energy values presented in Table I, the temperature-dependent inactivation of TTC reduction in the two cucumber cultivars appears to be related to protein denaturation. These results indicate that, with the possible exception of the 25°C pretreated cv Poinsett 76 at temperatures above 46.5°C, the temperature dependence of the inactivation rates of TTC reduction for both cucumber cultivars are essentially the same and may result from protein denaturation.

Rosenberg et al. (1971) presented evidence that certain thermodynamic parameters in protein thermal denaturation can be correlated with cellular death rates and are related by a simple linear relationship. They concluded that all proteins denature at elevated temperatures according to the compensation law,

$$S = a\Delta H + \Delta b \quad (2)$$

where ΔS and ΔH are the activation entropy and activation enthalpy, respectively. For proteins, the constants in Equation 2 are -64.9 cal mol^{-1} $°K^{-1}$ for b and 3.04×10^{-3} $°K^{-1}$ for a. This relation indicates that variations in enthalpy are partially compensated by variations in entropy. With Equation 2 and the enthalpy values from Table I, we calculated the expected entropies to be within the range of 170.7 to 180.6 kcal mol^{-1} or within the error of the entropies obtained directly from the Arrhenius plots (Table I). However, application of Equation 2 to the data obtained with 25°C pretreated cv Poinsett 76 at temperatures above 46.5°C produced a predicted entropy of 16.6 kcal mol^{-1}, which does not correspond well with the actual value of 20.8 ± 1.7 cal mol^{-1} $°K^{-1}$. Therefore, with one possible exception, the results presented in Table I are consistent with the proposition that the reduction in the ability of cucumber leaf discs to reduce TTC at elevated temperatures is the result of protein denaturation. However, the constraints described above for the use of Arrhenius plots preclude any definitive identification of the type of cellular lesion that results in the observed temperature-induced changes in the ability of cucumber to reduce TTC.

The obvious differences in D_q (Figs. 2, 3, and 5) between the two cucumber cultivars may have significance in explaining the variations in the temperature sensitivity of TTC reduction and the inherent differences in the responses of these cultivars to elevated temperatures. Even though the computed thermodynamic parameters (Table I) suggest that cv Ashley did not have any increased thermotolerance after the 6-h 37°C pretreatment, the TTC reduction values and shape of the initial portion of the inactivation curves were different from those of cv Ashley preincubated at 25°C (Figs. 2B, 3B, and 5). In fact, TTC-reducing capability was enhanced below 47.5°C after the 37°C pretreatment. Regardless of pretreatment temperature, the D_q values for cv Ashley were significantly greater than those of cv Poinsett 76 until the heat treatment temperature exceeded about 52.5°C (Fig. 5). This further supports the conclusion that cucumber cv Ashley is inherently more thermotolerant than cv Poinsett 76. Although the significance of the variations in D_q is difficult to interpret, animal studies have suggested that D_q values corrected for any lag time in temperature equilibration may be related to the ability of the cells to repair nonlethal thermal damage (Henle, 1987; Alpen, 1990).

Although pretreatment of the cucumber leaf discs at 37°C

modified their response to subsequent heat treatments, it is apparent from the slope of the Arrhenius plots (Fig. 4) that it did not appreciably alter the D_o values relative to discs preincubated at 25°C. Nonetheless, the changes in D_q for cv Ashley (Fig. 5) and linearization of the Arrhenius plot for cv Poinsett 76 (Fig. 4B) suggest increased thermotolerance of these cucumber cultivars after the 37°C pretreatment. The mechanism for this increased thermotolerance may be different for the two cultivars. Thermodynamically, cv Ashley, unlike cv Poinsett 76, was unchanged by the 37°C pretreatment. This suggests that cv Ashley was already thermotolerant relative to cv Poinsett 76. The 37°C treatment apparently modified the thermal sensitivity of cv Ashley by increasing its ability to repair temperature-induced damage ($>D_q$). Conversely, the decreased thermosensitivity of cv Poinsett 76 may be related to reduced temperature-induced damage.

Lafuente et al. (1991) demonstrated that a 6-h incubation at 37°C induced the synthesis of heat-shock proteins in discs cut from cv Poinsett 76 cotyledons. Heat-shock proteins have been shown to be intimately related to the induction of thermotolerance in both plant (Krishnan et al., 1990) and animal cells (Schlesinger, 1990). However, Lafuente et al. (1991) also observed the synthesis of heat-shock proteins after 6 h at 42°C, a temperature that caused significant damage to both cucumber cultivars, as measured by TTC reduction. This suggests that TTC reduction is more sensitive to heating than heat-shock protein synthesis, and the synthesis of heat-shock proteins does not necessarily contribute to the thermotolerance of certain metabolic processes.

The results presented in this report demonstrate the applicability of more detailed analyses of temperature-dependent changes in TTC reduction rates in the evaluation of plant responses to hyperthermia. Although the thermodynamic analyses are necessarily speculative, similar studies with animal and microbial materials have permitted the identification of potential cellular lesions and, more important, potential methods to alleviate heat-induced cellular damage. Experiments of the type presented here may be useful in identifying appropriate crops and possible counteractive measures to permit continued, efficient crop production, considering the possibility that the climate in the agriculturally productive regions of the world may become warmer.

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