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Communication

Thermal Dependence of the Apparent K_m of Glutathione Reductases from Three Plant Species

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ABSTRACT

The thermal dependencies of the apparent K_m of the glutathione reductases from spinach (*Spinacia oleracea* L.) corn (*Zea mays* L.), and cucumber (*Cucumis sativus* L.) were determined. The apparent K_m of the enzymes were found to vary up to 9-fold between 12.5 and 45°C. Values of the apparent K_m in excess of 200% of the observed minimum are suggested to be detrimental to the normal function of the enzyme. We propose the term "thermal kinetic window" to describe to the range of temperatures over which the apparent K_m of the glutathione reductase is within 200% of its minimum and suggest that it may be a useful indicator of the limits of thermal stress for a given species. The thermal kinetic windows determined in this study are: <16°C for spinach, 23 to 32°C for corn, and 35 to 41°C for cucumber.

The effects of thermal stress on plants are substantial and have been extensively investigated and reviewed (6, 14). Thermal stress has been defined as a temperature-induced aberration in the metabolism of the plant that, under certain conditions, is expressed as a reduction in growth, yield, or value of the plant (7). Temperature-induced aberrations in metabolism are often related to changes in the activity of enzymes and thus the thermal dependence of enzyme function may prove to be a useful indicator of the onset of thermal stress.

GR[1] is thought to play an important role in the protection of the plant from both high and low temperature stresses by preventing the oxidation of enzymes and membranes (5, 8, 13, 16). In the light of this role, a temperature-induced decline in GR activity could adversely affect the metabolism of the plant and thereby increase its susceptibility to thermal stresses. The effect of temperature on reaction rate and molecular integrity of GR has been addressed by Burke and Hatfield (2) who predicted changes in maximal velocity associated with changes in plant temperature. However, maximal velocity is not generally indicative of the *in vivo* rates of enzyme reactions and thus may not be predictive of the effects of temperature on metabolism (15).

The *in vivo* rate of an enzyme reaction is dependent upon interactions between the enzyme and specific ligands. The value of the apparent K_m has been used as an indicator of enzyme:ligand interactions. These interactions are often temperature dependent and it has been suggested that the thermal dependence of the K_m of various plant enzymes may play a role in the thermal dependence of whole plant responses (6, 15).

The objective of this study was to determine if the differential sensitivity of some plant species to thermal stresses could be related to differences in the thermal dependence of the K_m of their GRs. We have chosen spinach, corn, and cucumber as representatives of cool, moderate, and warm environment species, respectively. The thermal dependence of apparent K_m of the GRs from these species for NADPH was determined over a 12.5 to 45°C thermal gradient.

MATERIALS AND METHODS

Plant Material

Cucumber (*Cucumis sativus L.* cv Ashley) and corn (*Zea mays*) seeds were planted in vermiculite and watered twice weekly with deionized water. The plants were grown for two weeks under fluorescent lights at 28°C.

Enzyme Sources

Glutathione reductase (EC 1.6.4.2) from spinach (*Spinacia oleracea L.*) leaves (100–200 units/mg protein) was purchased from Sigma Chemical Co.[2] The GR from cucumbers and corn was purified from the cotyledons and leaves of 14 d old seedlings by the procedure of Kalt-Torres *et al.* (10). The purity of the enzymes from both sources was verified by SDS-PAGE using the method of Laemmli (12).

Enzyme Assays

Assays were carried out with a Gilford Response spectrophotometer that was equipped with a thermostated rapid sampler. Enzyme activity was monitored by the decrease in A at 340 nm. After a 15 s interval for thermal equilibration, the progress of the reaction was monitored for 15 s at a rate of 100 observations/min. The assay temperature was maintained by a thermostated micro flow cell (volume = 700 μL)

[1] Abbreviations: GR, glutathione reductase; TKW, thermal kinetic window.

[2] Mention of a trademark or proprietary product 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.

that was controlled by the spectrophotometer. The effect of temperature on the reaction was monitored from 12.5 to 45°C (±0.1°C). The pH of the reaction mixture varied <0.03 units over a temperature gradient from 10 to 50°C. Absorbance and time data were analyzed by linear and quadratic regressions to determine the initial velocity. The assay mixtures contained the following in a 1 mL volume: 100 mM Hepes:NaOH (pH 8), 500 μM oxidized glutathione (GSSG), 0.5 to 100 μM NADPH, and purified GR (0.02 units). A unit of activity is that amount of enzyme that will catalyze the reduction of 1 μmol of GSSG per min at 25°C. The reaction was initiated with the addition of the enzyme (10 μL).

Apparent Michaelis Constants

The determination of the K_m for NADPH was accomplished by assays of the enzyme at a fixed, saturating concentration of GSSG (>30 times the K_m at 25°C). Initial velocity was determined at several concentrations of NADPH ranging from 0.5 to 10 K_m. The concentration of NADPH in the assays was calculated from the absorbance at 340 nm. A minimum of five assays were conducted for each concentration of NADPH at each assay temperature. The K_m values were determined by direct linear plots as described by Cornish-Bowden (4).

RESULTS AND DISCUSSION

Figure 1 shows the thermal dependence of the K_m of GR for NADPH from spinach, corn, and cucumber. These results establish that the K_m of the GRs from these three species is temperature dependent and the pattern of this dependency is determined by the species in question. With respect to our objective of relating the differences in the thermal dependence of these species to the thermal dependence of the apparent K_m of their GRs, the GR from spinach, a cool season plant

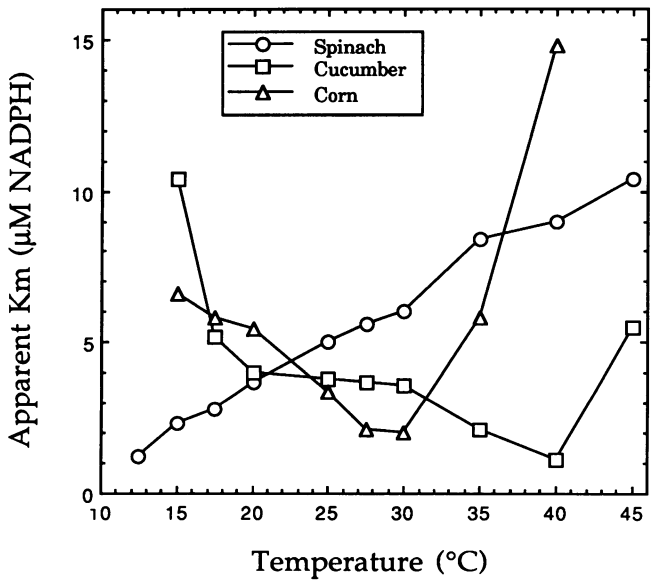

Figure 1. Apparent K_m for NADPH as a function of temperature for glutathione reductases from spinach, corn, and cucumber.

has its lowest K_m value at the lower end of the 10 to 45°C thermal gradient. Similarly the K_m of the GR from corn, the moderate temperature species, was lowest at 30°C and higher at temperatures above or below that value. Finally, the apparent K_m for the warm season species, cucumber, was lowest in the 35 to 40°C range, and increased as the temperature either increased or decreased from this range.

Temperature-induced changes in the value of the K_m similar to those seen in this study have been suggested to be causal factors in the thermal dependence of metabolism in a variety of plant species. Patterson and Graham (15) have stated that, for plants, the effect of temperature on the value of the K_m may be of 'paramount importance' in the overall response of the plant to thermal stress. The thermal dependence of the K_m of phospho*enol*pyruvate carboxylase has been suggested to account, at least in part, for the declines in enzyme activity in the dark (17). Patterson and Graham (15) correlated differences in the thermal dependence of the K_m of phospho*enol*-pyruvate carboxylase in tomato and peas with the thermal dependence of the growth in these species. On an ecological level, the thermal dependence of the K_m of malate dehydrogenase has been proposed to be a factor in the geographic distribution of some plant ecotypes (18, 19).

The variation in the value of the K_m of GR that we have observed suggests that the protective activity of GR may be limited to species-specific thermal ranges. Several investigators have suggested that temperature induced variation in the apparent K_m may be a determining factor in the thermal range which an organism can tolerate. Patterson and Graham (15) suggest that, in the case of the enzyme phospho*enol*pyruvate carboxylase, evolutionary pressures have resulted in a constant and low K_m within the range of temperatures over which growth is optimal. Hochachka and Somero (9) have stated that, for a variety of animal enzymes, the value of the K_m is conserved around the habitat temperature and that changes in this value tend to occur at the thermal limits of the habitat.

In light of the reported correlations between the thermal dependence of the K_m and the optimal thermal range for a variety of plant and animal species, we believe that the thermal dependence of the K_m of GR might be a useful indicator of the optimal range of temperatures for plants. To define such an optimal thermal range on the basis of the thermal dependence of the apparent K_m we must have a means of assessing the effect of such variation on the enzymic function. Teeri (19) has suggested that changes of less than twofold in the value of the K_m will not significantly impair enzyme function. Thus, for comparative purposes, we have defined the range of temperatures over which the K_m is within 200% of the observed minimum as conducive to optimal enzyme function. We propose the term TKW for this thermal range of ostensibly optimal K_m values. Figure 2 shows the value of the K_m as a percentage of the minimum observed (minimum = 100%). The TKWs for the three species thus defined are: <16°C for spinach, 23 to 32°C for corn, and 35 to 41°C for cucumber. While we believe this '200% of minimum' criterion sufficient for discrimination between species, it must nonetheless be viewed as speculative. We propose that a TKW for GR may be a useful indicator of the range of temperatures over which

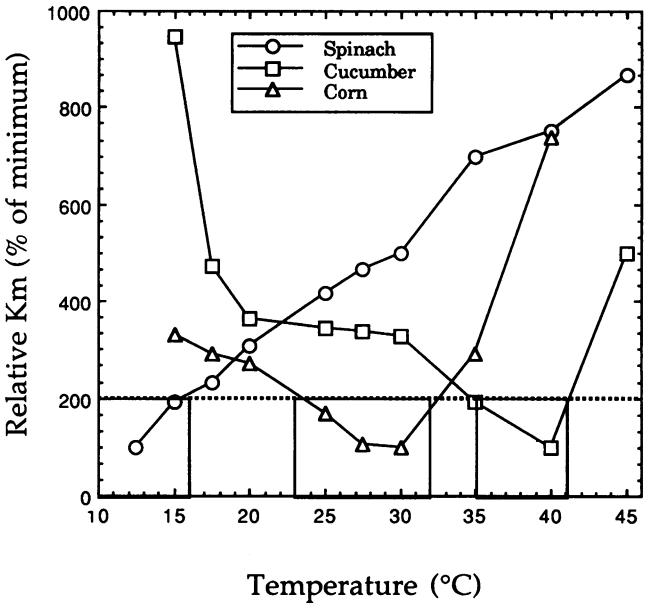

Figure 2. Relative apparent K_m as a function of temperature for the glutathione reductases from spinach, corn, and cucumber. Percent of minimum was determined from the minimum K_m observed in each species. The dashed line indicates a twofold change in the apparent K_m. The boxed regions represent the thermal kinetic windows for these species.

a transition from normal metabolism to abnormal metabolism (*i.e.* thermal stress) begins.

Kidambi *et al.* (11) have recently investigated the thermal dependence of the apparent K_m for GRs from several cultivars of alfalfa and sainfoin. They identified intraspecific variations and described a TKW for each cultivar. Additional support for the utility of such TKWs comes from the work of Burke *et al.* (3) who determined TKWs for glyoxylate reductase in both cotton and wheat. In field experiments they found that the cumulative time that canopy temperature was within the TKW was positively correlated with the rate of biomass accumulation for both species. In a study of the thermal behavior of cotton, Upchurch and Mahan (20) found that under a variety of environmental conditions cotton leaf temperatures were maintained at a normative temperature of 27 + 2°C, well within the TKW reported for cotton. Recent work by Burke (1) has shown that the thermal dependence of PSII fluoresence is in agreement with TKWs previously determined for a variety of plant species.

CONCLUSIONS

The observed thermal dependencies for the K_m of the GRs from spinach, corn, and cucumber suggest that their *in vivo* function may be thermally limited. Such thermal limitations may play a role in the ability of the plant to withstand both low and high temperature stresses. For each of the three species we have defined a TKW within which we propose the function of the GR is not thermally limited. We believe that an ability to predict the range of optimal temperatures for a given species with a TKW based upon the thermal dependence of the apparent K_m of a purified GR might be a useful tool in studies of thermal stress in plants.

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