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SCIENTIFIC CORRESPONDENCE

Heat Generation and Dissipation in Plants: Can the Alternative Oxidative Phosphorylation Pathway Serve a Thermoregulatory Role in Plant Tissues Other Than Specialized Organs?[1]

R. William Breidenbach*, Michael J. Saxton, Lee D. Hansen, and Richard S. Criddle

Thermalytics, Inc., 2545 Boatman Avenue, West Sacramento, California 95691 (R.W.B.); Institute of Theoretical Dynamics (M.J.S.), and Section of Molecular and Cellular Biology, Division of Biological Sciences (R.S.C.), University of California, Davis, California 95616; and Department of Chemistry and Biochemistry, Brigham Young University, Provo, Utah 84602 (L.D.H.)

A cyanide-insensitive alternative respiratory pathway mediated by a mitochondrial inner membrane oxidase is found in higher plants, a number of fungi and algae, and a few protozoa (Henry and Nyns, 1975; Siedow and Umbach, 1995). The amount of oxidase protein and its relative activity varies among species and among organs of the same plant (Day and Wiskich, 1995; Siedow and Umbach, 1995, and citations therein). The dynamic regulation of the pathway (Day and Wiskich, 1995; Siedow and Umbach, 1995; Wagner and Krab, 1995) supports the idea that the alternative oxidase pathway plays important roles in the metabolism of plants during development and adaptation to environmental conditions. These roles are poorly understood, except for the participation of the alternative oxidase in the exceedingly rapid oxidation of substrate by specialized fleshy plant tissues such as aroid spadices. In these tissues the heat from rapid oxidation regulates tissue temperature significantly above ambient as part of the reproductive process (Seymour et al., 1983; Meeuse, 1984; Seymour and Shultze-Motel, 1996; Seymour, 1997).

Three recent papers describing calorimetric studies (Ordentlich et al., 1991; Nevo et al., 1992; Moynihan et al., 1995) suggest that in some crop species the alternative oxidase also participates in limited thermoregulation to aid survival in cold conditions. Nevo et al. (1992) proposed that thermogenesis resulting from increased engagement of the alternative pathway may be a factor in genetic adaptation to low temperature in "nonovertly thermogenic" plants. When leaf tissues from wild progenitors of wheat (*Triticum dicoccoides*) and barley (*Hordeum spontaneum*) were exposed to low temperatures (5°C for 10 h), metabolic heat rates measured at 20°C increased markedly as a result of cold treatment. This response was cultivar specific, and for 10 barley accessions from cold regions the average rate increased by 41% over controls, whereas warmer-climate populations increased an average of 27%.

Moynihan et al. (1995) found that heat evolution in chilling-sensitive species increased by 47 to 98% after chilling. Smaller increases in response to cold were seen in chilling-resistant species. These authors concluded that the thermogenic effect of alternative pathway engagement could heat cold-sensitive tissues to temperatures significantly above ambient and protect the plants from chilling injury. They suggested that respiratory heat may have a pronounced local effect at the subcellular level, and that the temperature increase could be large enough to prevent the loss of fluidity of mitochondrial membranes, thereby causing faster growth rates and shorter life cycles that would be of adaptive value to populations in colder climates.

If these proposals are correct, they might explain the occurrence of the alternative pathway in temperate zone plants. Although we do not doubt the metabolic heat rate measurements or the observed responses, we disagree with the postulate that the alternative pathway is thermoregulatory, protecting plants from exposure to cold. The postulate of thermoregulation involves two assumptions: The first is that the reactions of the alternative oxidase pathway have much larger exothermic enthalpy changes than those of the Cyt oxidase pathway; the second is that the observed increases in rate after cold exposure are sufficient to raise tissue or organelle temperature significantly. As elaborated below, consideration of combined calorimetric and respirometric data invalidates the first premise and calculation of heat dissipation invalidates the second. Thermoregulation can-

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* Corresponding author; e-mail rwbreidenbach@ucdavis.edu; fax 1-916-752-4361.

not account for the widespread presence and engagement of the alternative pathway in plants exposed to low temperatures.

RELATION BETWEEN METABOLIC PATHWAYS, METABOLIC RATE, AND HEAT PRODUCTION

Calorimetric measurements of the rate of heat production by plants provide a useful indication of the rate of overall metabolic activity. The combination of calorimetry and respirometry provides further information regarding the efficiency of the metabolism and metabolic pathways involved in heat production. Three parameters are of interest: the heat rate (q), the ratio of the heat rate to the O_2 uptake rate (q/R_{O_2}), and the ratio of the heat rate to the CO_2 production rate (q/R_{CO_2}).

The rate of heat production is equal to the product of the reaction rate and the enthalpy change. Thus, there are only two ways to change the heat production rate: increasing the reaction rate or switching to a more exothermic enthalpy change, ΔH. Expressions relating q/R_{O_2} and q/R_{CO_2} to ΔH for respiratory reactions are as follows (Hansen et al., 1994):

$$q/R_{O_2} = \frac{-(1 - \gamma_P/4)\Delta H_{O_2} - (\varepsilon/1 - \varepsilon)\Delta H_B}{(1 - \gamma_P/4) + (\varepsilon/1 - \varepsilon)[(\gamma_B - \gamma_P)/4]} \quad (1)$$

$$q/R_{CO_2} = -(1 - \gamma_P/4)\Delta H_{O_2} - (\varepsilon/1 - \varepsilon)\Delta H_B \quad (2)$$

where γ_P is the average chemical oxidation state of the substrate for respiration, γ_B is the average chemical oxidation state of product biomass, ε is the substrate carbon conversion efficiency (the fraction of substrate carbon incorporated into structural biomass), and ΔH_B is the enthalpy of biomass synthesis as kilojoules per mole of carbon. ΔH_{O_2} has a value of -455 ± 15 kJ mol O_2^{-1} (Erickson, 1987).

Changing the ratio of Cyt oxidase to alternative oxidase has only a small effect on the ratios q/R_{CO_2} and q/R_{O_2}. For example, in young, growing plants typical values are $\gamma_P = 0 \pm \frac{1}{2}$, $\gamma_B = -\frac{1}{2} \pm \frac{1}{2}$, $\Delta H_B = 25$ kJ/mol, and $\varepsilon = 0.55 \pm 0.2$. Using the mean values, q/R_{CO_2} is calculated to be 424 kJ mol^{-1}. A complete shift from the Cyt pathway to the alternative pathway, where $\varepsilon = 0.18$, would change q/R_{CO_2} from 424 to 449 kJ mol^{-1}, or about 6%. A partial shift to the alternative pathway would give correspondingly smaller increases in the ratios of heat rates. Any temperature increase in plants resulting simply from shifting metabolism to the alternative pathway would be very small. Moynihan et al. (1995) measured up to a 98% increase in metabolic heat rate following low-temperature exposure of chilling-sensitive plants to 8°C. This increase in heat rate must come from an overall increase in rate of oxidative reactions, not simply from a switch to the alternative pathway.

The measurements of metabolic heat rates of barley and wheat tissues (Nevo et al., 1992) or other species (Moynihan et al., 1995) following cold exposure lacked parallel measurements of CO_2 and O_2 rates and showed only relatively small increases in heat rates compared with those observed during reproductive-related thermogenesis in aroids. Measurement of q and R_{CO_2} on spadix tissue of aroids such as *Sauromatum guttatum* and *Philodendron selloum* shows large respiratory rates that account for most of the large temperature increases in these specialized tissues (Seymour et al., 1983), but there is little if any difference in q/R_{CO_2} during thermogenesis compared with that during nonreproductive growth (Seymour et al., 1983; Lamprecht et al., 1991). This result is expected from the arguments above if γ_P and γ_B do not change. If the small increases in heat rates observed in wheat and barley (Nevo et al., 1992) and other species (Ordentlich et al., 1991; Moynihan et al., 1995) have a thermoregulatory role, it must therefore depend on slow dissipation of the heat and small but significant local temperature increases.

HEAT DISSIPATION

To estimate tissue temperature increases resulting from metabolic heat, we consider a simple model based on a long cylindrical stem of radius R_{stem}, insulated by a boundary layer of stagnant air of thickness δ. The boundary layer thickness around an object depends on the geometry of the object, decreases as the size of the object decreases, and decreases as the wind speed increases (Nobel, 1983). We assume that δ is 0.1 to 10 mm. Outside the boundary layer, the air is assumed to be well mixed and at a uniform temperature (Nobel, 1983). We assume that the stem tissue produces heat at a constant rate, and that heat loss across the boundary layer occurs by conduction alone. By neglecting heat losses by radiation, convection, and transpiration, this assumption provides an upper limit on the temperature increase. McNulty and Cummins (1987) have calculated a maximum respiration-caused temperature increase of 0.02 K for leaves of the arctic plant *Saxifrage cernua* based on a radiative cooling model.

This problem is described by the same equations as the heating of an insulated wire by an internal heat source. From the solution to the heat conduction equation for this case, the increase above the ambient temperature (ΔT K) is given by the equation (Carslaw and Jaeger, 1959):

$$\Delta T_v = (q_v R_{stem}^2/4K)[1 - r^2/R_{stem}^2 + (2K/K_A)\ln(1 + \delta/R_{stem})], \quad (3)$$

where q_v is the rate of heat production per unit volume, K is the thermal conductivity of the stem, K_A

is the thermal conductivity of air, R_{stem} is the stem radius, δ is the boundary layer thickness, r is the radial position in the stem, and ln is the natural logarithm.

Application of this model to calculate the temperature increase in cereal grasses using the results of Nevo et al. (1992) yields the values in Table I. The largest heat production measured in wheat and barley at 20°C was approximately 15 mW g^{-1} dry weight. Assuming the tissue is 90% water and has a density of 1g cm^{-3}, then q_v = 1.5 mW g^{-1} fresh weight, only 3% of that for *P. selloum* during thermogenesis (Seymour et al., 1983). With the further assumption of a 2-mm stem radius, we obtain the values in Table I as upper limits of the temperature increase. Furthermore, because metabolic heat rates decrease with temperature (Q_{10} is approximately 2), the heat rate at 5°C, where metabolic rate is postulated to have protective effects, would be only about one-fourth that at 20°C, and the heat rate differences between wheat and barley genotypes would only be about 6% of the differences at 24°C.

Metabolic heat cannot heat the entire stem significantly. Neither can it heat small regions such as mitochondria significantly because the surface-to-volume ratio (S/V) of mitochondria is far higher than the ratio for the stem. For a stem with a radius (R_{stem}) of 2 mm, $S/V = 2/R_{stem} = 1$ mm^{-1}. For a spherical mitochondrion with a r of 1 μm, $S/V = 3/r = 3000$ mm^{-1}. This excludes the possibility of any significant steady-state local heating.

Applying the heating model to the temperature increase in aroids is an instructive test of the validity of the model. Increases of 15 to 30 K above ambient temperature have been observed in the spadix of *P. selloum* during flowering (Seymour et al., 1983; Meeuse, 1984). The radius of the spadix is 1.0 to 1.75 cm in the region of the sterile male flowers, where most of the heat is produced. The maximum metabolic heat rate in the tissue is 40 to 70 mW g^{-1} fresh weight (Seymour et al., 1983), and for this calculation we assume a value for q_v of 50 mW g^{-1} fresh weight. The calculated temperature increases are shown in Table II. Calculated values are similar to the measured increases in spadix temperature. A large increase in temperature occurs because the metabolic heat rate is very high and the surface-to-volume ratio is low compared with barley. It is plausible that the spadix boundary layer is thick because the spadix is sheltered by the spathe, but the highest ΔTs shown in the table are not attained, because convective heat loss becomes important at a large ΔT (Nobel, 1983).

Table I. *Calculated temperature increases at the center and the surface of the stem for barley with q_v = 1.5 mW cm^{-3}*

The thermal conductivity of the plant is assumed equal to that of water, so K = 0.6 W m^{-1} K^{-1} and K_A = 0.0257 W m^{-1} K^{-1} (Nobel, 1983). R_{stem} is taken to be 2 mm.

δ	ΔT (Center)	ΔT (Surface)
mm	*K*	
0.1	0.004	0.003
1.0	0.025	0.024
10.0	0.106	0.105

Table II. *Calculated temperature increases at the center and the surface of the spadix for Philodendron selloum with q = 50 mW cm^{-3}*

The thermal conductivities are as in Table I.

R_{stem}	δ	ΔT (Center)	ΔT (Surface)
cm	*mm*	*K*	
1.0	0.1	3.0	1.0
1.0	1.0	11.3	9.3
1.0	10.0	69.4	67.3
1.5	0.1	6.1	1.5
1.5	1.0	18.8	14.1
1.5	10.0	116.5	111.9

Van der Straeten et al. (1995) used IR cameras to observe temperature increases in tobacco leaves after treatment with salicylic acid, and found an increase in surface temperature of 0.5 to 1.0 K. These results might be inferred to support the idea that the alternative oxidase pathway is thermogenic in these tissues in the field. The temperature increase in a leaf can be estimated by a similar model. The leaf is assumed to be an infinite slab of thickness 2L that loses heat to the atmosphere on both sides through boundary layers of thickness δ. The steady-state temperature increase in the leaf is then (Carslaw and Jaeger, 1959):

$$T = q_v[(L\delta/K_A) + (L^2 - x^2)/(2K)] \quad (4)$$

where x is the distance from the center of the leaf. If we use their maximum O_2 uptake of 1.5 nmol g^{-1} fresh weight s^{-1} and take $\Delta H_{O_2} = -455$ kJ mol^{-1}, we find that q_v = 0.68 mW g^{-1} fresh weight, around one-half of the value we used for barley stems. If the leaf thickness is 235 μm (Avery, 1933), we obtain a maximum temperature increase of 0.032 K for the largest boundary layer thickness used above, δ = 10 mm. Boundary layer thicknesses of 100 to 300 mm are required to give temperature increases of 0.32 to 0.95 K. The boundary layer thickness in the experimental chamber is not known, but Van der Straeten et al. (1995) state that special care was taken to provide maximum thermal insulation of the plants against air drafts. The observed temperature increase is likely to be a consequence of the experimental conditions, and is unlikely to occur in the field.

SUMMARY

A number of hypothetical physiological roles have been proposed for the cyanide-insensitive alternative

pathway in plants (Palmer, 1976; Laties, 1982; Meeuse, 1984; Purvis and Shewfelt, 1994; Wagner and Krab, 1995). The calorimetric observations of Raskin and co-workers (Ordentlich et al., 1991; Nevo et al., 1992; Moynihan et al., 1995) are significant contributions showing an interesting metabolic, chilling-induced response of the alternative pathway activity and differences in the low-temperature response among species adapted to different climates. Since different oxidative pathways do not have large differences in enthalpy, and observed heat rate increases are insufficient to cause significant temperature increases of physiological importance in nonthermogenic plants, other explanations must be developed for the relationship between the partitioning of electron flow and physiological conditions such as low temperature.

The induction and engagement of the alternative respiratory pathway is involved in metabolic stasis, maintaining proper balance between carbon flow, ATP-ADP ratio, and electron flow during fluctuating or extreme temperature conditions. The alternative oxidase is engaged when ATP requirements are adequately met, as discussed by Palmer (1976), Meeuse (1983), Lambers (1985), and Wagner and Krab (1995). The expression and kinetic activity of the alternative oxidase are regulated by concentrations of key metabolites (Day and Wiskich, 1995; Siedow and Umbach, 1995; Wagner and Krab, 1995; Day et al., 1996). Dynamic partitioning of electron flow between Cyt oxidase and the alternative oxidase depends on the kinetic behavior of the two oxidases and the substrate dehydrogenases (Day and Wiskich, 1995; Siedow and Umbach, 1995; Wagner and Krab, 1995; Day et al., 1996). Furthermore, Moynihan et al. (1995) found that *Episces cupreata* Hook, adapted to the tropics, has very little alternative oxidase activity compared with wheat (Nevo et al., 1992), adapted to a large range of temperate climates. This result is consistent with the general relation between the apparent alternative oxidase activity and the climate of origin of the species.

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