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SIMULTANEOUS CALORIMETRIC AND RESPIROMETRIC MEASUREMENTS ON PLANT TISSUES *

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SUMMARY

Simultaneous measurements of the rates of heat production, CO_2 production, and O_2 utilization by living plant tissues provide a useful means of determining metabolic efficiencies and detecting and quantifying changes in the metabolic pathways active in the tissues. This paper describes a convenient, simple method for simultaneously measuring metabolic heat rate and the rate of CO_2 production directly in the calorimetric reaction vessel. The method requires only a slight modification of existing, commercially available, heat conduction calorimeters. Calorimetric measurement of the rate of heat production by reaction of CO_2 with a base is used to obtain CO_2 production rates. The detection limit for CO_2 production is 50 picomole/second, which is similar to the detection limits reported for commercially available respirometers of various types.

INTRODUCTION

Simultaneous measurements of the rates of heat production, CO_2 production, and O_2 utilization by living tissues provide a useful means of detecting and quantifying changes in the metabolic pathways active in the tissues (ref. 1-5). The ratio of heat rate to the rate of CO_2 production, or to the rate of O_2 utilization, have been called the calorespirometric ratio or the indirect calorimetric constant. The calorespirometric ratio has a value of approximately 460 kJ/mole for most animal tissues that have been studied with tightly coupled, aerobic, steady-state metabolism (ref. 6). The ratio is not a constant, however, as it differs in tissues or cell cultures with altered metabolic pathways, e.g. those with different primary energy sources or those capable of anaerobic metabolism. The experimentally determined ratio for any tissue depends on the stoichiometry and metabolic heat associated with each of the different metabolic pathways in the integrated metabolism. Measurements of R_C and R_O along with metabolic heat can, therefore, provide information on tissue metabolic pathways.

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Even without precise information on the detailed meaning of the calorespirometric ratio, measurements of changes in the ratio in response to metabolic or environmental effectors provide important information on changes in tissue activities. Consequently, the need exists for a general method for rapidly and easily measuring this ratio on a given organism, tissue, or cell culture. The purpose of this paper is to describe such a method, requiring only slight modification of existing, commercially available heat conduction calorimeters.

Most measurements of the rates of change of CO_2 and O_2 directly in calorimetric reaction vessels have employed microelectrodes. Such measurements are readily performed in circumstances where the electrode can be submerged in a uniform, stirred solution in the reaction vessel. These electrodes do not function well, however, if the solution cannot be stirred, if tissue samples are solid, or if insufficient liquid is present to cover the electrodes. Accordingly, electrodes do not work well in small microcalorimeter vessels. Other types of sensors for CO_2 and O_2 generate too much heat to be used within the reaction vessel or are too large to place within the reaction vessel and require gases to be flushed through the vessel and into the detector.

This paper reports the use of calorimetric measurements to determine both the metabolic heat rates and CO_2 production rates, by combining measurement of metabolic heat production with the heat resulting from reaction of metabolic CO_2 with a base in the calorimeter reaction vessel. Determination of the rate of CO_2 production by this method requires two measurements, one of the metabolic heat rate and one of the sum of the metabolic heat rate and the heat rate from reaction of the CO_2. The difference between the two measured values gives the rate of CO_2 production. Obviously, the higher the ΔH value for reaction of the CO_2 with the absorbent, the more sensitive the measurement will be. The CO_2 collecting reaction must be fast and well defined both thermodynamically and stoichiometrically. Given these requirements and considerations of convenience and availability, the following reactions were chosen for testing.

$$Ba(OH)_2(aq,sat'd) + CO_2(g) = BaCO_3(s) + H_2O(l) \qquad (1)$$

$$\Delta H = -113.2 \text{ kJ/mole}$$

$$2NaOH(aq,\ 0.4M) + CO_2(g) = Na_2CO_3(aq,\ 0.2M) + H_2O(l) \qquad (2)$$

$$\Delta H = -108.5 \text{ kJ/mole}$$

Significantly higher ΔH values could be obtained by reaction of the CO_2 with crystalline metal hydroxides, e.g. $Ba(OH)_2(s)$, ΔH = -165 kJ/mole and $Sr(OH)_2(s)$ ΔH = -153 kJ/mole, but these reactions proved too slow to be useful (numerical data from ref. 7).

At the ΔH values for reactions 1 and 2 and an uncertainty in the combined measurements of ±5 μW, the predicted detection limit for CO_2 production is 50 picomole/second (about 4 μL/hour) which is similar to the detection limits reported for commercially available respirometers of various types (ref. 8-11).

In the presence of a CO_2 absorber in a sealed ampule, uptake of O_2 results in a pressure decrease. The rate of decrease is proportional to the rate of O_2 use by the sample. In a one mL chamber, a 50 picomole/second (4 μL/hour) change in the O_2 content results in a pressure change of 0.12 Pa/second. Thus, a sealed calorimeter vessel can be used to simultaneously measure metabolic heat rate, CO_2 evolution rate, and O_2 use rate. This paper reports the method of CO_2 measurement. The method of O_2 measurement will be reported in a later paper.

EXPERIMENTAL

Calorimetric measurements were made with a Hart Scientific model 7707 DSC operated in both isothermal and scanning modes. This calorimeter has four, removable, cylindrical, 1 cm^3 Hastelloy ampules with screw caps. One of the ampules is used as a reference, so three simultaneous measurements of rates of heat production can be made. Two different types of ampules were constructed for measurement of the rate of CO_2 production. One type consisted of an ampule with a removable 60 μL container for either water or hydroxide solution. Early experiments in this series were done simply with glass vials which could be placed into or removed from the ampule. The glass vials were made from 3 or 4 mm glass tubing and were longer than the ampule diameter so that they could not tip over. Later experiments were done with specially constructed Hastelloy vials which were placed in a ring welded to the inside of the ampule. No significant improvement in performance was noted with the Hastelloy vials, but they were more convenient.

To perform an isothermal measurement, the metabolic heat rate was measured with water in the vial, then the ampule was opened and

50 μL of hydroxide solution was inserted. The ampule was resealed and replaced in the calorimeter, and the heat rate was again determined. The water blank measurement was normally then repeated on the same sample. Fig. 1 schematically illustrates how measurements are made and the appearance of the results. To perform a temperature scanning experiment for measuring both the metabolic heat rate and the CO_2 production rate as a function of temperature, one ampule was loaded with a weighed amount of sample and a vial of water, a second ampule was loaded with a closely similar amount of sample and a vial of hydroxide solution of equal volume. Temperature scans of metabolic heat rates were then performed as described by Hansen and Criddle (ref. 12). The CO_2 rate was determined throughout the temperature range as the difference between the two curves after correction for any differences in the amounts of sample. The presence of the liquid filled vials was determined not to measurably affect calorimeter performance in control experiments.

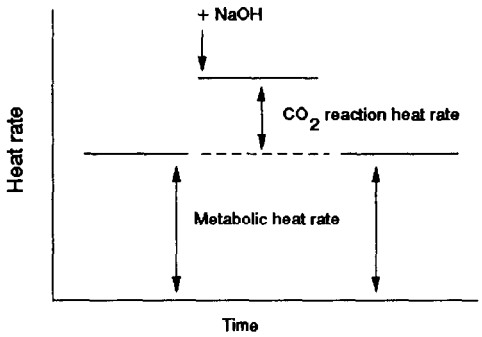

Fig. 1. Method for determination of CO_2 and heat production in calorimetery.

A second type of ampule for CO_2 analysis was constructed with two ampule lids connected by a minimum length of small diameter tubing. The tissue sample was placed in one ampule and the hydroxide solution directly in the other. With this system, the metabolic heat rate is measured directly in one ampule and the rate of CO_2 production in the other.

The absolute accuracy of this method for CO_2 production rate measurements was verified by measurements of heat produced while pumping CO_2 gas at constant rates into an ampule containing 0.4 ml of 0.4 M NaOH. The heat rate measured for carbonate formation agreed within 8% (low) of the value calculated from chemical thermodynamic properties.

It is convenient to express experimental results from CO_2 and metabolic rate measurements as a unitless ratio, R, of the heat rate produced by the CO_2 reaction, q_{CO2}, plus the metabolic heat rate, q_{met}, divided by q_{met}, equation 4.

$$R = (q_{met} + q_{CO2})/q_{met} = 1 + q_{CO2}/q_{met} \quad (4)$$

R is obtained directly from measured values (see Fig. 1). From equation 4, it is apparent that:

$$(R-1) = q_{CO2}/q_{met} = \frac{(-\Delta H)}{R_C} \quad (5)$$

Thus, the unitless value of (R-1) equals $-\Delta H$ for the CO_2 reaction with base, divided by the calorespirometric ratio. If we assume the commonly reported ratio of 460 kJ/mole for animal respiration and CO_2 reaction with 0.4 M NaOH, the resultant value of (R-1) is 0.24.

The value of (R-1) may be considered as an index of metabolic efficiency. The value will depend on the relation between growth and metabolic pathways and may, therefore, differ from plant to plant either within a species or among species. (R-1) can also vary for an individual plant in response to stress when alternate metabolic pathways are engaged or induced (see Fig. 4). Measurement of this value can provide important information about plant metabolic differences and their responses to stress. Determination of the functional relationship between growth, q_{met}, and R will yield important information about plant metabolic efficiencies.

RESULTS

Fig. 2 shows results obtained with corn (maize, Zea mays) meristem tissue at 25°C with 0.4 M NaOH as the CO_2 absorbent. The nonlinear data, shown during the first 20 min. of each segment of the experiment, is the thermal equilibration of the calorimeter following insertion or removal and reinsertion of the ampules. The approximately horizontal portions of the curves are used to determine q and R_C. These data show that a steady state rate of CO_2 uptake by the absorbent has been reached by the time the calorimeter has stabilized. A value of (R-1) = 0.28 ± 0.01, or a ratio of q/R_C = 361 ± 9 was calculated from 12 measurements, with the uncertainty given as the standard deviation.

Fig. 3 shows representative data collected on tomato leaves with $Ba(OH)_2$ absorbent, spruce meristem tissue with NaOH absorbent, and redwood meristem tissue with $Ba(OH)_2$ absorbent, all at 25°C. These data show problems encountered when dealing with less stable

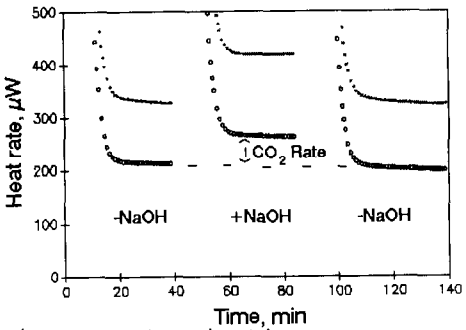

Fig. 2. Determination of (R-1) value for corn meristem.

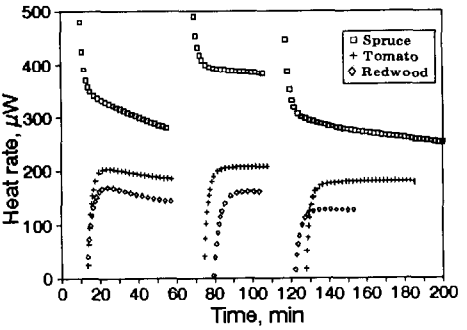

Fig. 3. Measurements of heat rates for plant tissues in the absence, presence, and again absence of CO_2 absorbent.

tissues. With these tissue samples during the first hour in the calorimeter q decreases nonlinearly. After the first hour, the tomato and redwood tissue metabolism becomes stable for several hours, but the spruce tissue continues to decrease. The long term decrease in the spruce metabolic heat rate may be due to a limited substrate supply (ref. 13). (R-1) ratios for several plants and conditions are shown in Table 1.

TABLE 1

Representative examples of efficiency index results on various species.

Species tissue	Conditions	R-1
Tomato, leaf	12°C < T < 27°C	0.26 ± 0.01
Corn, epicotyl	Grown at 25°C, measured at 25°C	0.28 ± 0.01
Corn, epicotyl	Grown at 2.5°, measured at 25°C	0.18
Coast redwood branch meristem tissue	Outdoors, mid-May, Davis, CA	0.18 ± 0.03
Yeast	Anaerobic	0.18
Yeast	Aerobic	0.31
Apple, leaf	Greenhouse, potted	0.29
Potato, tuber	Fresh slices	0.38 ± 0.06
Potato, tuber	8-hour slices	0.28 ± 0.04
Blue spruce branch meristem tissue	Outdoors, mid-May, Provo, UT	0.29
Mammals	Calculated from q/R_c=460 kJ/mole	0.24

Caution must be used in selection of growth-medium to ensure that no CO_2 absorption can occur. For example, some of our early

attempts to measure plant CO_2 emission rates had dilute Hoagland's solution included with the tissue. This solution traps a portion of the metabolic CO_2, presumably as $CaCO_3$, and interferes with assays of CO_2 by the methods described.

Data collected to date with two ampules linked by tubing show that a steady state of CO_2 transport between ampules is achieved within about 30 minutes. At present it is difficult to obtain reproducible, isothermal baselines with this experimental set-up because the tubing extends beyond the boundaries of the calorimetric measurement. Solving the problems encountered with this calorimetric measurement will allow study of unstable systems which change too rapidly with time to be done by the vial replacement method, study of thermal effects on metabolism, and studies with altered atmospheric conditions in the sample chambers.

Fig. 4 shows metabolic heat rate data and (R-1) data collected during temperature scanning of tomato leaflets. For this experiment, intact, oppositely paired leaflets from a branch of a tomato plant were placed in two different ampules, one with a vial of water, the other with a vial of NaOH solution. Scanning measurements were run from 25° up to 55°C, from 25° down to 3°C and up and down from 13° to 28°C. Both the upward and downward scans were at rates of about 4°C/hour. These data show changes in (R-1) with temperature. In the range from about 12°C to nearly 30°C, (R-1) is 0.26. Below 12°C where tomato sensitivity to cold is noted, (R-1) increases. Above 30°C the ratio also increases to a maximum of about 0.35, then declines rapidly towards zero as thermal inactivation of the tissues parallels a corresponding decrease in the metabolic heat rate.

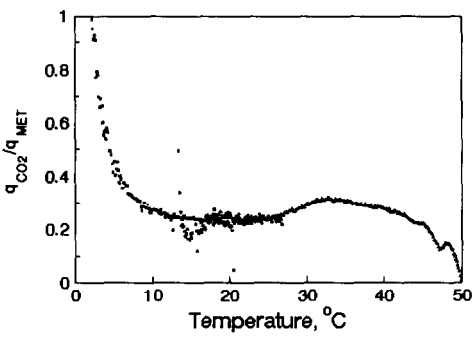

Fig. 4. Changes in metabolic activity and (R-1) for tomato leaflets with temperature.

DISCUSSION

The data collected in this study illustrate a simple method for measuring CO_2 production rates, and hence the calorespirometric ratio, by calorimetry alone. In favorable cases, this ratio can

be determined to within ±2.5% with the equipment described in this paper. Changes in this ratio can be determined as a function of temperature by scanning methods. A new parameter, (R-1), that is related to the inverse of the calorespirometric ratio, is proposed as a better way of presenting this ratio. (R-1) measures a fundamental property of metabolism as it is directly proportional to the efficiency at which the tissue can produce biomass from substrate at a specified set of conditions.

Values of R or (R-1) reported here depend upon the rate of CO_2 production and the conversion of heat rate values to micromoles of CO_2 produced per sec. was based on a value of 108.5 kJ/mole heat produced for CO_2 reaction with NaOH. Our measurements of this value in calibration studies were about 8% low. This makes conversion of (R-1) values to CO_2 production rates somewhat uncertain at this time. The values of (R-1) and changes in (R-1) are, however, highly reproducible and act as accurate indices of relative metabolic efficiencies of different plants and of changes in efficiency with changing plant conditions.

The data show that (R-1) differs with plant species and may also be different for individual plants of the same species. The values of (R-1) deviate both positively and negatively from the indirect calorimetric "constant" derived from animal tissues. Differences in (R-1) among different plants reflect differences in net metabolic pathways. Changes in (R-1) with temperature reflect differential activation of metabolic pathways in plants in response to environmental changes. Measurements of (R-1) should be a useful factor in selecting plants for rapid growth and stress resistance.

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