Thermochimica Acta, 216 (1993) 147–155
Elsevier Science Publishers B.V., Amsterdam

Reaction rates as a function of pressure, temperature and concentration by heat-conduction differential scanning calorimetry

Richard S. Criddle [a,*] R. William Breidenbach [b], Anthony J. Fontana [a] and Lee D. Hansen [c]

[a] *Department of Biochemistry and Biophysics, University of California, Davis, CA 95616 (USA)*
[b] *Department of Agronomy and Range Science, University of California, Davis, CA 95616 (USA)*
[c] *Chemistry Department, Brigham Young University, Provo, UT 84602 (USA)*

(Received 26 June 1992)

Abstract

New equipment and procedures to examine reaction and metabolic rates as functions of temperature and pressure by scanning and isothermal calorimetry are described. Three applications are presented to illustrate the capabilities of the methods. The first demonstrates the effects of pressure and temperature on the metabolic rates of cultured plant cells. Pressures in the range from 7 to 16 MPa shifted significantly the high-temperature inactivation of metabolic activities in tomato cells to higher temperatures. The second demonstrates the use of differential scanning calorimetry to investigate rapidly reaction kinetics and mechanisms. The chemical degradation rates of pineapple juice concentrate as a function of oxygen concentration and temperature were determined. The third demonstrates the use of the instrument in the isothermal mode to determine the effects of total pressure and oxygen partial pressure p_{O_2} on the metabolic rate in an ectothermic animal tissue. The metabolic rates of fresh-water mussel gill tissues were increased by both increased total pressure and increased p_{O_2}.

INTRODUCTION

Differential temperature-scanning calorimetric (DSC) analyses are used routinely to measure the thermodynamics of structural changes and reaction rates as a function of temperature (see, for example, ref. 1). Hansen and Criddle [2] used heat conduction DSC techniques simultaneously to monitor metabolic rate and to look for physical events

* Corresponding author.

associated with abrupt alteration of metabolic activities. No first-order phase transitions were found, but data consistent with a higher order phase transition were obtained. The data were also consistent with other explanations, not including a phase transition, however. Because pressure is an additional variable helpful in interpreting the nature of thermally induced structural changes, we have developed new equipment and techniques for DSC at high pressure. As emphasized by Hochachka et al. [3], temperature increase affects all chemical reactions in the same way, whereas pressure can activate, retard, or have no effect at all on reaction rate. Thus, pressure effects on rates can aid interpretations of structure-related factors influencing the temperature dependence of rates.

In previous studies of biological tissues, the temperatures of some structural transitions in lipid bilayers and chloroplasts were altered by increased pressures, indicating the importance of volume changes associated with these transitions [4–8]. In spite of these indications that pressure–temperature interactions may be significant in metabolism, pressure has not previously been included as a second intensive variable in calorimetric studies examining temperature effects on metabolic activities.

Studies examining the effects of symmetrically applied high pressures on growth rates and metabolic activities by non-calorimetric means have been reported for many tissues. In general, pressure effects on metabolic and growth rates are small [9]. However, in some cases distinct changes have been noted in respiratory rates [10], photosynthesis [4, 11], and structures [12–14]. Conclusions defining whether tissue metabolism and growth are pressure dependent are severely limited in these studies because they apply only to tissues exposed to a limited set of test conditions. If the rate–pressure–temperature surface including points and lines of critical structural transitions is obtained, the effects of pressure can be better defined.

In this communication, we describe equipment and methods that extend temperature–scanning methods to allow simultaneous measurement of the effects of increased pressure and of changed atmospheric concentrations on reaction rates. The methods are particularly well suited for metabolic rate studies and for measurements of slow chemical reactions such as oxidations of food products where variation of both p_{O_2} and temperature markedly influence rates.

The major barrier to temperature-scanning calorimetric measurement of metabolic heat rates of living tissues has been the difficulty of accurately separating the relatively small metabolic heat production rates of cells from the much larger heats required to change the sample and ampule temperatures [2]. This problem becomes even more acute with addition of any thermally conductive connections between the surroundings and the sample ampule. Thus, connecting metallic or thick-wall non-metallic high-pressure lines can result in sufficient uncontrolled and irreproducible

heat exchange with the surroundings during temperature scanning runs that analysis for metabolic heat rates becomes unfeasible. This difficulty was overcome by use of thin-wall, glass, microcapillary tubing to connect the calorimeter ampules to the high-pressure source. The low thermal conductivity and mass of the capillary tubing results in a minimal and reproducible thermal connection with the surroundings. With this equipment, pressures up to 15 MPa are achieved using compressed gases, allowing simple control of the pressure and composition of gases in the sample atmospheres.

As one example demonstrating the usefulness of measuring heat rates as pressure is varied, we studied the effects of pressure on the high-temperature inactivation of tomato cells as indicated by the metabolic heat rate. Metabolic heat rate measurements were conducted at temperatures from 25 to 60°C and at pressures from ambient to 12 MPa. The results show that a reaction with a positive volume change is associated with the high-temperature inactivation of tomato cells.

We have also used the pressurization system of our modified DSC to introduce different p_{O_2} while examining oxidative reactions of pineapple juice concentrate for O_2–temperature–pressure dependence. A single scanning experiment yields extensive information defining reaction stoichiometries and kinetics. In an additional demonstration of equipment applications, isothermal calorimetry was used to show an increased metabolic rate of mussel gill tissue as p_{O_2} and total pressure were increased.

MATERIALS AND METHODS

All measurements were conducted in a modified Hart Model 7707 heat-conduction differential scanning calorimeter. For high pressure and altered atmosphere runs, special thick walled, high-pressure calorimeter ampules were constructed from Hastelloy C according to the design obtained from Hart Scientific, except that the exit tube was a 0.5 cm long × 1.6 mm o.d., 1 mm i.d. stainless steel tube, silver soldered into the ampule lid (Fig. 1). An approximately 2 ft section of glass capillary tubing (25 μm i.d., 150 μm o.d.) was inserted through the ampule lid tubing and sealed in place with polyamide–epoxide resin. The other end of the capillary column was connected to a high pressure line, pressure gauge, bleeder valve, and gas cylinder (Fig. 1).

The general protocols for tomato cell sample preparation and for isothermal measurements have been described in ref. 15. The scanning measurement procedures have been previously described [2, 16]. These procedures were modified for the tomato metabolic rate analyses employed here in that scanning was run in two cycles: A. Measure heat rate isothermally at 25°C, scan up to 60°C at 6°C h^{-1}, measure isothermal heat

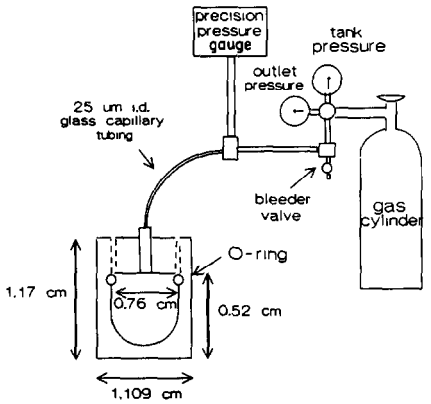

Fig. 1. Schematic of high pressure ampules and pressurization system.

rate at 60°C, scan down to 25°C at 99°C h^{-1}. This procedure, besides measuring metabolic rate changes during temperature increase, causes thermal inactivation of the cells at the high temperature. B. A second cycle following the identical protocol of isothermal and scanning measurements of part A was then run on the same samples to obtain data on killed cells. The heat rate differences between live and killed cells were interpreted as the heat of metabolism of the tomato cells. The isothermal measurements at 60°C and subsequently at 25°C provided tests of cell inactivation by the high temperature.

The tomato cells used in this study were Tom-45, obtained from D. Pratt [17]. Cells were cultured on agar medium and added directly to the calorimeter ampules under near-sterile conditions. The ampule head space was filled with O_2 prior to sealing the lid in place, to provide ample O_2 for aerobic cell metabolism during calorimetric examination. The ampules were pressurized with N_2 gas from a compressed gas cylinder. The pressurized ampules therefore contained O_2 at ambient pressure (approximately 82 kPa at the elevation of these studies). Cells in non-pressurized ampules were also filled with O_2 before sealing the ampules for thermal analysis.

Pineapple juice concentrate preparations were evaporatively concentrated (61° brix) commercial pineapple juice from Dole Packaged Food Co., stored frozen until use. In the calorimeter, thawed concentrate was covered with either N_2 or O_2 at ambient pressure or O_2 at 0.5 MPa pressure, and the heat rate was measured as a function of temperature. The scan rate for these studies was 6°C h^{-1}.

In isothermal studies of pressure effects on metabolic rates, approximately 1 cm^2 sections were cut from inner gill tissue of the fresh water mussel, *Elepio compleanatus* (purchased from Connecticut Valley Supply Co. Southampton, MA). Tissue sections were rinsed with chlorine-free tap

water then placed on a water-saturated filter disk in the calorimeter ampule for heat rate measurements.

RESULTS

The curves in Fig. 2 show the metabolic rates of three tomato cell samples scanned simultaneously over the temperature range from 25°C to 60°C. Two of the samples were the same mass, while the third was about half the mass of the other two. The two identical samples were scanned at high pressure and atmospheric pressure. The smaller sample was scanned at high pressure. The atmospheric pressure curve is the same as that reported earlier for tomato cells run in the standard low-pressure ampules of this calorimeter [18]. At atmospheric pressure, the temperature-dependent increase in activity begins to slow at about 36°C. Activity then becomes essentially constant between 38°C and 42°C, where it rapidly decreases to near zero. The pattern at high pressure is essentially identical up to 36°C, except that activity continues to increase with temperature up to 42°C where the abrupt inactivation of metabolism is noted.

Elevated pressure altered the temperature–metabolic–activity profile of tomato cells in a fashion consistent with pressure opposing some effects of

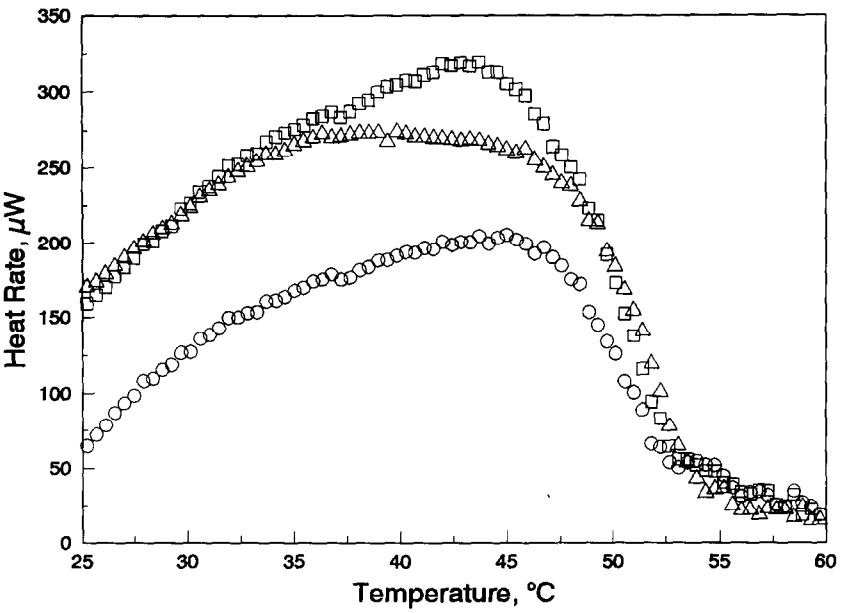

Fig. 2. Scanning calorimetric analysis of metabolic heat rates of tomato cells at 11.8 MPa (○) and (□), and at 0.1 MPa (△). Samples (□) and (△) contained equal amounts of cells; sample (○) contained about one-half as many cells.

high-temperature inactivation of metabolism (Fig. 2). Higher temperatures were required to cause inactivation at higher pressures. This indicates that a structural change with an increased volume of some cell component(s), or an increased volume of a transition state complex in a rate-limiting step, must accompany the high-temperature inactivation of metabolism.

The two high-pressure scans of Fig. 2, differing only in cell mass and corresponding total metabolic rate, plus the low pressure control were all run simultaneously and demonstrate the reproducibility of the pressure effects. They also show that the abrupt decrease of activity at high temperature is not simply due to a depletion in oxygen or a build-up of metabolites in the closed ampules. This follows because samples with more cells deplete O_2 from the atmosphere at a higher rate than the smaller sample, yet showed inactivation of metabolism at the same time and temperature as smaller samples.

Figure 3 shows the effects of increasing partial pressures of O_2 on the kinetics of the oxidative reactions in samples of pineapple juice concentrate. These three experiments were run simultaneously in the modified Hart calorimeter using N_2, O_2 and pressurized O_2 with the samples. Clear temperature and O_2 dependencies are indicated. An Arrhenius plot of the heat rate curve at 0.5 MPa O_2 is presented in the inset, with a linear regression fit of the data to indicate a probable change in reaction mechanism around 55°C. This is in agreement with data from other means

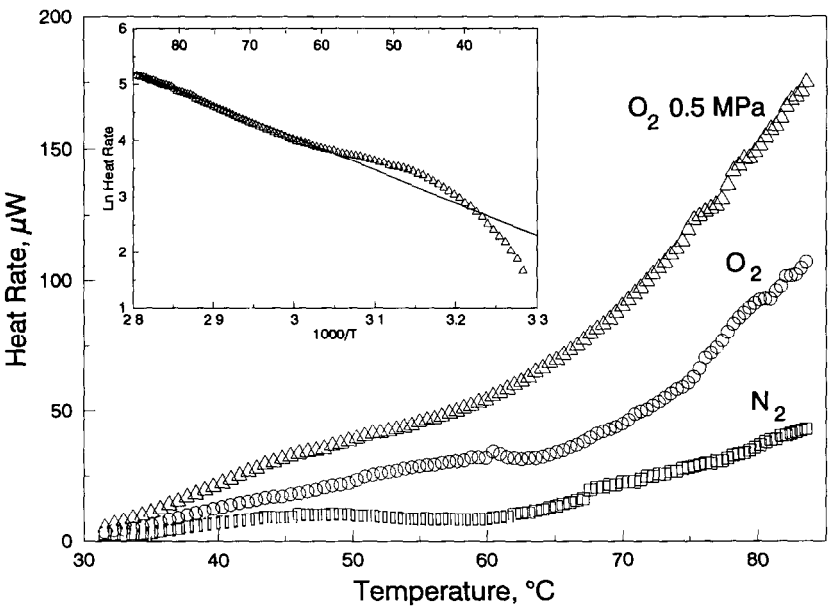

Fig. 3. Heat rates for reactions of pineapple juice concentrate in N_2, 0.1 MPa O_2 and 0.5 MPa O_2. The temperature scan rate was 6° h^{-1}. The inset shows an Arrhenius plot for the 0.5 MPa O_2 sample with a linear regression fit to the data.

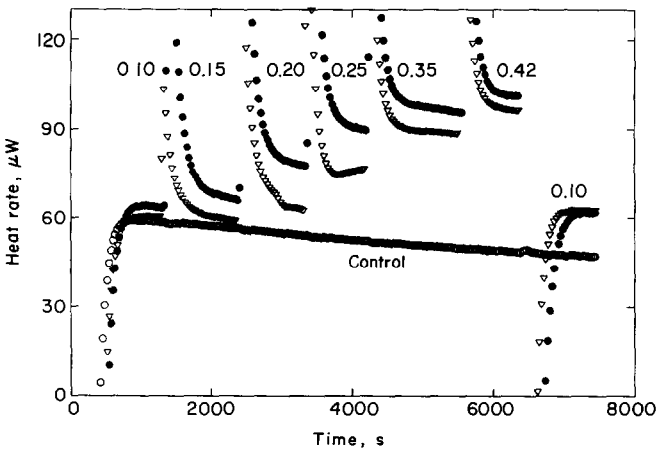

Fig. 4. Metabolic heat rates of mussel gill tissue samples as total pressure is increased with N_2 while maintaining p_{O_2} at 0.02 MPa (▽) and 0.1 MPa (●). The numbers are total pressure in MPa at each step of the experiment. The curve labeled "control" gives the time course of the metabolic rate for an unpressurized gill tissue sample from the same organism.

of analysis. These studies show an approximately half-order dependence on p_{O_2} and an apparent activation energy of approximately 50 kJ mol^{-1} above 55°C.

Figure 4 shows increasing heat rates of mussel gill tissues at 30°C in an experiment that held p_{O_2} constant at two different levels, while the total pressure was increased stepwise with N_2. Similar results were obtained using He for pressurization. The heat rates of the mussel tissues are a function of both total pressure and p_{O_2}. Effects of pressure can result from a shift in equilibrium due to some volume-related change in tissue lipids or proteins or to kinetic effects relating to changed solubility of gases. At the pressure levels employed in these experiments, significant changes in the equilibrium positions of the reactions of lipid or protein seem unlikely.

DISCUSSION

Determination of the activity–pressure–temperature surface provides a direct method for detecting structural changes that may accompany metabolic activity changes. Many structural changes in macromolecules or lipid membranes are known to be accompanied by changes in volume. In such cases, the temperatures of thermally induced metabolic changes will be altered by application of elevated pressures. Other workers have demonstrated experimentally a decrease in the ordered structure of simple lipid and protein complexes with increasing temperatures accompanied by an increase in volume, i.e. an increased volume upon denaturation [8]. Consequently, if metabolic activity changes are linked to structural changes in cells involving membrane or protein structures, it is expected that higher

temperatures will be required for structural alteration when samples are examined at higher pressures. This was the result with tomato cells.

While it is difficult to predict precisely either the magnitude or the sign of a thermally induced volume change in systems as complex as cells and membranes, it is clear that a changed thermal stability as a direct conseqence of a change in pressure provides unambiguous evidence for a volume change associated with the thermal event. Measuring the metabolic heat rate while temperature scanning in biological systems at high pressure promises to be a useful tool for studying mechanisms of thermal inactivation.

The data provided by a single scan on pineapple concentrate at different O_2 pressures form the basis for an extensive analysis of reaction rates, stoichiometries and mechanisms. The ability to pressurize the reaction vessel with a desired level of gaseous reactant and simply to examine reaction rates as a continuous function of temperature will greatly accelerate reaction analyses.

Measurement of pressure effects on cell metabolism at fixed temperatures and gas compositions, as illustrated in the mussel gill tests, are not unique to this study. The equipment described does, however, enhance previous capabilities and allow rapid, easily performed, and precise measurements of heat rates at various pressures. Because variable pressure calorimetry is non-invasive, it can be combined with other experimental manipulations and analytical methods to gain insights in biochemistry and physiology.

ACKNOWLEDGMENTS

We thank E. Wilhelmsen and Dole Packaged Foods for supplies of pineapple juice concentrate, J. Crawford and D. Paige for help with construction of the high pressure modifications to the Hart Calorimeter, and Marcus Cheney for conducting mussel tissue experiments. We acknowledge research support for these studies from Pioneer Hybrid Seed Co., the California Rice Research Counsel, and USDA CGRO 90-37280-5592.

REFERENCES

1 A.E. Beezer, Biological Microcalorimetry, Academic Press, New York, 1980.
2 L.D. Hansen and R.S. Criddle, Thermochim Acta, 160 (1990) 173–192.
3 P.W. Hochachka, T.W. Moon and T. Mustaga, The Effects of Pressure on Organisms, Academic Press, New York, 1972, pp. 175–195.
4 U. Schreiber and W. Vidaver, Photobiol., 18 (1973) 205.
5 U. Schreiber and W. Vidaver, Z. Naturforsch. Teil. C, Biochem. Biophys. Biol. Virol., 28 (1973) 704.
6 D.B. Mountcastle, R.L. Biltonen and M.J. Halsey, Proc. Natl. Acad. Sci. USA, 75 (1978) 4906.

7 N.D. Russell and P.J. Collings, J. Chem. Phys., 77 (1982) 5766.
8 J.T. Mason and T.J. O'Leary, Biophys. J., 58 (1990) 277.
9 J. Levitt, Responses of Plants to Environmental Stresses, Vol. II, Water, Radiation, Salt, and Other Stresses, Academic Press, New York, 1980, pp. 491–497.
10 D.H. Pope and L.R. Berger, Can. J. Bot., 52 (1974) 2375.
11 B.E. Janes and G.W. Gee, Physiol. Plant Pathol., 28 (1973) 201.
12 K. Suzuki and Y. Taniguchi, Symp. Soc. Exp. Biol., 26 (1972) 103.
13 M. Shameel, Pak. J. Bot., 7 (1975) 169.
14 G. Schmid, H.D. Luedemann and R. Jaenicke, Biophys. Chem., 3 (1975) 90.
15 R.S. Criddle, R.W. Breidenbach, E.A. Lewis, D.J. Eatough and L.D. Hansen, Plant Cell Environ., 11 (1988) 695.
16 R.S. Criddle and L.D. Hansen, J. Plant Physiol., 138 (1991) 376.
17 B.R. Thomas and D. Pratt, Theor. Appl. Genet. , 69 (1981) 215.
18 D.R. Rank, R.W. Breidenbach, A.J. Fontana, L.D. Hansen and R.S. Criddle, Planta, 185 (1991) 576.