Journal of Thermal Analysis, Vol. 33 (1988) 1013–1018

PHARMACY, BIOLOGY, MEDICINE

OSCILLATORY HEAT PRODUCTION IN CELL FREE EXTRACT FROM YEAST: BATCH AND INFUSION EXPERIMENTS

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Microcalorimetric experiments on the periodic heat production in metabolizing cell free cytoplasmic extract from baker's yeast are described. The sugar substrates for glycolysis are supplied by two different techniques: (a) one-time addition of trehalose; (b) continuous infusion of glucose. For the first time the combination of calorimetry and substrate infusion technique is reported for oscillating biochemical reactions.

While oscillating biochemical reactions were originally assumed to be an interesting, but physiologically useless frill of nature more and more evidence arose in recent years that dynamic instabilities leading to self-oscillation are important regulatory effects in cells and organisms [1]. Acting against homeostasis they may produce temporal and spatial structures in cells, cell layers or even in layers of cell free extracts [2]. As these biochemical oscillations and especially those found during glycolysis are strongly connected with energy turnover [3] it was worthwile to monitore the temporal energy flow in cell extracts by means of modern microcalorimetry [4].

Cell extracts are advantageous because of good reproducibility, ease of experimental handling, and – most important – of the introduction of glycolytic substrates without cellular transport processes. To obtain glycolitic oscillations it is important to fit the energy input to the "oscillatory window", i.e. a well defined limited range of flow rates suitable for the maintenance of sustained oscillations [5]. This condition may be met in two manners: (a) by a continuous inflow of glucose at a special rate (infusion technique); (b) by offering a pool of the saccharides trehalose or glycogen as an energy source. In the latter case rate determining enzymes split the saccharides up in glucose which flows at a proper rate into the glycolytic pathway. While calorimetric experiments with trehalose are already elaborated [4, 6–8], for the first time combination of glucose infusion technique with microcalorimetry is reported here.

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Methods and materials

All experiments were performed with a modified isothermal batch calorimeter, type Triflux (Thermanalyse/Grenoble) with vessels of 2 ml and a sensitivity of 81.9 mV/W. Infusion was performed by a precision piston pump Precidor (Infors/Basel) at rates varying between 10 and 150 μl/h. A pulsation free pump is essential as small periodic pulses may be amplified in a solution tending to dynamic instabilities. Even more than with batch experiments an effective stirring is essential to ensure substrate homogeneity in the sample. It is obtained by a small penumatic pump which introduces neglectable thermal noise [6].

The cell free extracts from baker's yeast used throughout these experiments were prepared after an established recipe [9]. The protein content of the preparation was 47 mg/ml. An active medium in batch experiments consisted of: 0.430 ml extract, 0.045 ml 1M potassium phosphate, 0.015 ml 20 mM NAD and 0.015 ml 15 mM AMP plus varying amounts of 0.7 M trehalose. All additives were dissolved in 0.1 M potassium phosphate buffer. In infusion experiments trehalose was replaced by a continuous flow of 10% or 20% glucose in buffer, corresponding to fluxes of 5.6 mM/h to 120 mM/h.

Results and discussion

Figure 1 shows the result of a batch experiment with addition of 10.5 mM trehalose. The power-time-curve is clearly divided into three segments: an oscillatory phase at the beginning, a short period of nearly constant heat production after leaving the "oscillatory window" and the drop to the base line at the end. By integration of the area under the curve the total heat Q is determined. Experiments with increasing amounts of trehalose rendered an enthalpy change of $\Delta H = -139.7$ kJ pro mol glucose [10] in excellent agreement with data from the literature [11]. This simultaneous determination of heat production rate and total heat enables one to transform the p-t-curve (Fig.1A) into a quasikinetic graph (Fig.1B). For convenience, trehalose concentrations are given in glucose units.

Extract samples without addition of trehalose show a few oscillations and a reduced heat production due to endogenous sugar reservoirs. From p-t-curves one can calculate an energy pool of 14 kJ/ml extract or 102 μmole glucose/ml [10]. This pool is exhausted within 4 to 6 hours in experiments with complete cells in pure buffer or extracts without trehalose.

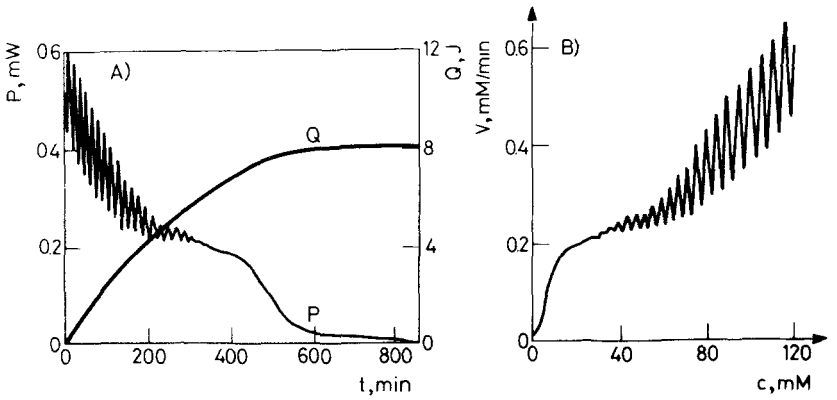

Fig. 1 Oscillatory and non-oscillatory phase during glycolysis of 5.57 μmol trehalose in a cell free cytoplasmic extract from baker's yeast.
a): Heat flow P and total heat production Q as function of time t;
b): Glycolytic flow V in glucose units as function of trehalose concentration c in glucose units.

The oscillations vanish at glucose concentrations of 64.4 mM and glycolytic fluxes of 12.6 mM/h independent of the initial sugar concentration [10]. These numbers indicate the lower boundary of the oscillatory domain. The data are confirmed by the observation that glucose infusion rates lower than 10 mM/h never lead to oscillations nor sustain them. Corresponding figures from the literature are 15.6 mM/h for 47 mg/ml protein [12]. On the other hand, an extract oscillating with trehalose can be shifted out of the window into a non-oscillating state by infusion of glucose at a rate which induces oscillations in "starved" extracts. By lowering the infusion rate the oscillations return after a few minutes and may be "killed" again by too high infusion rates. This shows how sensitively the extracts react on the sugar concentration near the boundaries of the oscillation domain.

Figure 2 depicts a train of glycolytic oscillations approximately 3 hours after the start of the infusion. The sequence of oscillations looks like a chain of pulses and is not as regular as that in trehalose experiments but the period length stays constant at 13.5 ± 0.6 min while the heat production per pulse shows a decreasing tendency as seen also in Fig. 1. The mean heat per pulse amounts to 32.1 mJ (± 7.5 mJ) and may be calculated to 0.23 μmoles glucose using the enthalpy change given above. The value corresponds to a modulated glucose flux of 1 μmol/h or 3% of the infusion rate. This percentage is much lower than for trehalose experiments (about 15%) which are in good agreement with literature data [13]. It may be due to unfavourable infusion and/or mixing conditions or to a shift of the glucose flux from glycolysis to gluconeogenesis.

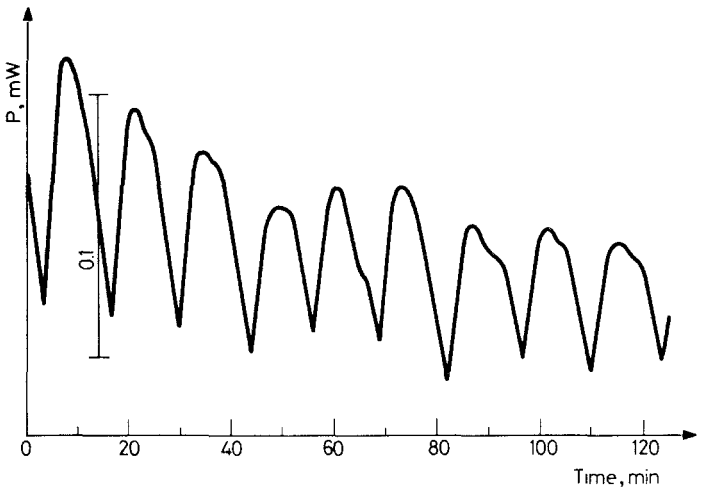

Fig. 2 Rate of heat production P as function of time t during infusion of a glucose solution at a rate of 59 mM/h, 3 h after the start of the experiment.

This question becomes even more acute in Fig. 3 where the periodic heat production is shown in dependence of stepwise changed infusion rates to a "starving" extract. A decreasing train of oscillations drops to a lower level when infusion is stopped and the extract consumes the last reservoir sugars. After starting glucose infusion again the low level is kept while the energy per pulse slightly increases. Even at a sixfold higher infusion rate the total rate of heat production stays constant. One may interprete this observation with gluconeogenetic activities of the extract [1, 14] or a refill of reservoirs. This opinion is strengthened by findings from another calorimetric experiment where fresh extracts from yeast cells with full energy depots showed an immediate upward jump of 0.335 mW (calculated to a flux of 8.6 mM/h) in heat production when the infusion rate was set from 33.6 to 42.6 mM/h. On return to the former level the rate of heat production decreased by 0.348 mW (9.0 mM/h) in excellent agreement with the glucose figures.

Infusion experiments may be run over many hours as long as the concentrations of essential substrates in the extract are not altered too strongly due to the dilution effect of infusion. To avoid such changes high glucose concentrations in the infusion medium are wanted which on the other hand demand for a particularly effective stirring at these small infusion volumes (a few μl/h). Until now such experiments were run up to 12 h.

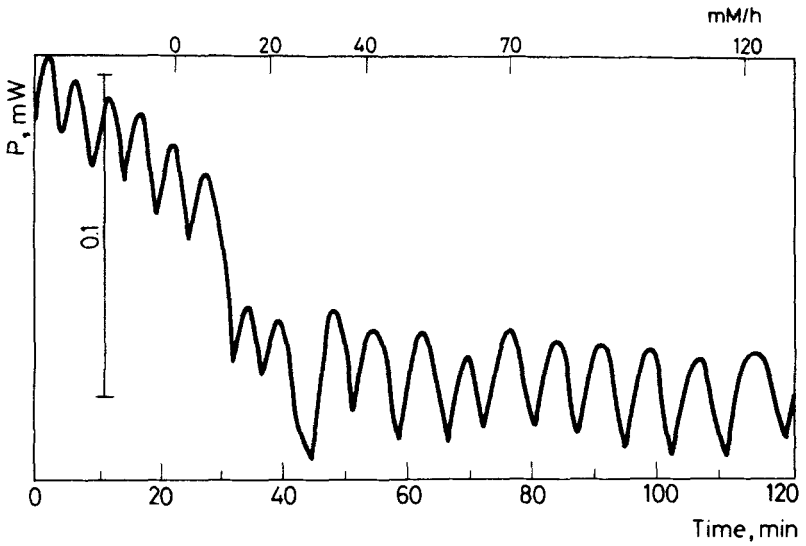

Fig. 3 Rate of heat production P as function of time t for various infusion rates of glucose. The numbers at the horizontal line indicate the infusion rate adjusted at that moment and lasting up to the next change.

References

1 A. Boiteux, B. Hess and E. E. Sel'kov, Current Topics in Cellular Regulation, 17 (1980) 171.
2 S. C. Müller, Th. Plesser and B. Hess, Naturwissenschaften, 71 (1984) 637.
3 H. A. Krebs and H. L. Kornberg, Energy Transformations in Living Matter, Springer-Verlag, Berlin 1957.
4 Th. Plesser, S. C. Müller, B. Hess, I. Lamprecht and B. Schaarschmidt, FEBS Letters, 189 (1985) 42.
5 B. Hess and A. Boiteux, Ann. Rev. Biochem., 40 (1971) 237.
6 B. Schaarschmidt, I. Lamprecht, Th. Plesser and S. C. Müller, Thermochim. Acta, 105 (1986) 205.
7 I. Lamprecht, B. Schaarschmidt and Th. Plesser, Thermochim. Acta, in press.
8 K. H. Müller and Th. Plesser, Thermochim. Acta, in press.
9 B. Hess and A. Boiteux, Hoppe-Seyler's Z. Physiol. Chem., 349 (1968) 1567.
10 Th. Plesser and I. Lamprecht, in: Springer Series in Synergetics, Springer-Verlag, Berlin-Heidelberg, in press.
11 E. A. Battley, Physiologia Plantarum, 13 (1960) 192.
12 B. Hess, A. Boiteux and J. Krüger, Adv. Enzyme Regul., 7 (1969) 149.
13 A. Betz, Physiologia Plantarum, 19 (1966) 1049.
14 C. J. Barwell and B. Hess, FEBS Letters, 19 (1971) 1.

Zusammenfassung – Mikrokalorimetrische Experimente über oszillierende Wärmeerzeugung in stoffwechselndem zellfreiem Hefeextrakt werden beschrieben. Die Zuckersubstrate für die Glykolyse werden nach zwei verschiedenen Methoden zugeführt: (1) einmalige Addition von Trehalose, (b) Kontinuierliche Infusion von Glukose. Erstmalig wird die Kombination von Kalorimetrie und Substratinfusion bei oszillierenden biochemischen Reaktionen beschrieben.

РЕЗЮМЕ — Описаны микрокалориметрические измерения теплоты, периодически образующейся в метаболитной клетке несвязанного цитоплазменного экстракта пекарных дрожжей. Сахарные субстраты для гликолиза вводились двумя различными методами; а) одноразовым прибавлением трегалозы и б) непрерывным прикапыванием глюкозы. Впервые для колебательных биохимических реакций сообщен комбинированный метод калориметрии и инфузионный метод прикапывания субстрата.