# Periodic heat production by oscillating glycolysis in a cytoplasmic medium extracted from yeast

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The rate of heat production in a periodically glycolysing cell-free cytoplasmic medium extracted from yeast *Saccharomyces cerevisiae* is measured with a batch calorimeter. The rate exhibits periodic variations of approx. 10% of the average heat production rate of about 54 mJ/ml per minute. From this rate and the enthalpy change fro glycolysis a glucose degradation rate of 0.43 mM/ming is calculated. The value fits into the 'oscillatory window' determined by a glucose injection technique.

*Glycolysis* *Oscillation* *Microcalorimetry* *Heat production* *Enthalpy*

## 1. INTRODUCTION

The production of heat and its regulation accompanied by metabolic turnover is an inherent property of living systems. Therefore calorimetry is an adequate tool for the analysis of dynamic processes in biology and biochemistry. Calorimetric measurements have clarified the relation between growth and heat production of biological systems. On the other hand, they have been helpful for the elucidation of elementary reaction and binding mechanisms in chemical as well as biochemical problems [1]. Much less is known about heat production by distinct metabolic pathways and their enzyme catalysed reaction steps. An obvious candidate for such an investigation is the highly exothermic anaerobic degradation of sugar by the sequence of glycolytic reactions. Examples of calorimetric research work on glycolysis in intact cells can be found in the literature [1–3]. Glycolysis in a cell-free cytoplasm may be considered as a better defined model system since other metabolic pathways are excluded and substrates as well as inhibitors specific for different reaction steps can be supplied in a precisely controlled manner [4].

In this paper a study of heat production during oscillating glycolysis is presented to determine the heat balance of this particular dynamic state of the glucose metabolism. In addition, the study was motivated by the pattern formation process in thin layers of cytoplasm from yeast first reported by Boiteux and Hess [5]. These patterns, observed at NADH-specific wavelengths, arise and disappear twice within one NADH cycle during those time intervals in which the NADH concentration changes at its maximum rate on the ascending or descending part of the cycle, respectively. The pattern is connected to convective flow driven by gradients in surface tension [6]. Since the surface tension depends on temperature, and heat may be produced when the 2 different parts of the glycolytic pathway are activated, one can speculate that the production of heat in a periodically metabolizing extract has half the period of the photometrically monitored NADH oscillations.

The experiments show for the first time oscillations of the heat production in a cell-free glycolytic system. The period of the oscillations is the same in calorimetric and photometric measurements.

Dedicated to Professor Carl Martius on the occasion of his 80th birthday



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## 2. MATERIALS AND METHODS

The cytoplasmic medium used for the experiments was extracted from yeast cells (*Saccharomyces Carlsbergensis*) grown under aerobic conditions according to a published procedure [7]. The protein content of 47 mg/ml corresponds to roughly one third of the concentration in the cytoplasm of intact cells. The procedure of sample preparation was the same for photometric and calorimetric experiments. For calorimetry the sample volumes are increased by a factor of 6 compared with the following volumes used for photometry: 300 $\mu$l extract; 40 $\mu$l of 0.7 M trehalose dissolved in 0.1 M potassium phosphate buffer; 10 $\mu$l of 20 mM NAD; 30 $\mu$l of 1 M potassium phosphate. The samples were thoroughly mixed before use. This recipe leads to oscillatory fermentation activity in the extract. All chemicals were of analytical grade.

Spectrophotometric measurements were done with a 2 mm quartz cuvette in a Zeiss PM4 spectrophotometer. Calorimetric measurements were performed with a microcalorimeter E. Calvet (SETARAM, Lyon) with 2 pairs of vessels allowing for 2 simultaneous experiments. In each pair one vessel served as the reaction chamber, the other as a reference. Their sensitivity amounts to 63.1 and 60.3 $\mu$V/mW, respectively. The vessels with a volume of 15 ml could be equipped with stirrers. For a technical description of the instrument see [8,9].

Preparing the experiment, samples of approx. 2 ml were filled into the reaction chambers. The reference chambers received the same volume of water. The top of each calorimeter vessel carried a small cup of 1 ml with a removable bottom. For titration experiments the titrand was placed into these cups and released, at an appropriate point in time, into the sample by pushing down the bottom. The temperature of the calorimeter was kept at 27.5°C. No signal corrections were made for the time constant of the calorimeter which is less than 2 min for a sample volume of about 2 ml.

## 3. RESULTS

Fig.1 shows the periodic rate of heat production $\dot{q}$ by a sample of 2.3 ml yeast extract without stirring. The difference between maximum and minimum rate during one period is 0.2 mW (0.087 mW/ml). Thus the periodic variation modulates the slowly decreasing overall rate $\dot{q} \approx$ 2 mW (0.87 mW/ml) by approx. 10%. The period of the oscillations is 12 min. This value is close to the period of 13 min which was measured with a 380 $\mu$l sample in a photometer at a NADH specific wavelength (fig.2).

The wave form of the oscillations in fig.1 has some characteristic features as for example the sharp rise after the minimum and its buckling ascend to the maximum. The descend from the maximum shows a smoother profile. The shape differs significantly from that of the curve in fig.2

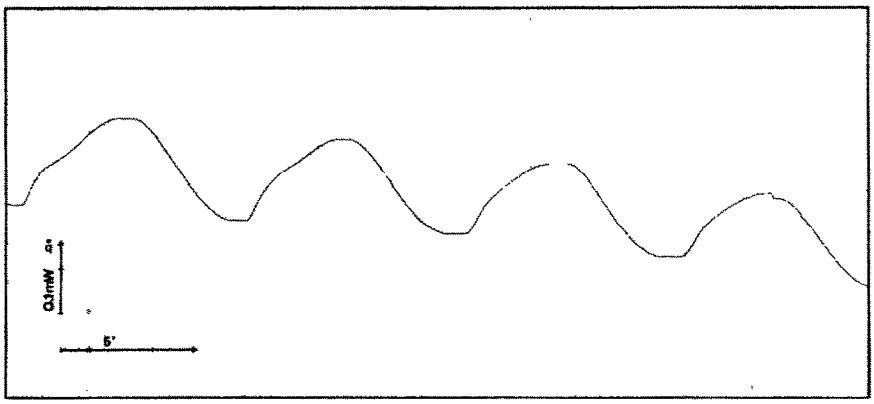


Fig.1. Oscillations of $\dot{q}$, the rate of heat produced by a 2.3 ml sample of cell-free extract of the yeast *S. Carlsbergensis* fed with trehalose. The difference between minimum and maximum $\dot{q}$ amounts to 2 mW (0.087 mW/ml). The period is 12 min.

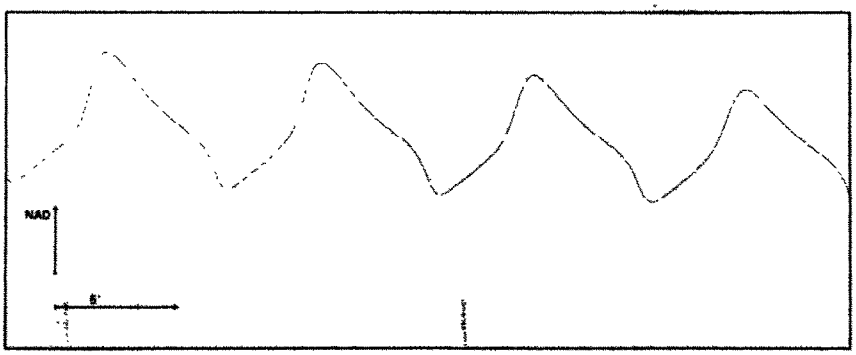


Fig.2. Oscillations of NADH concentration monitored photometrically at 340 nm in a sample of cell-free yeast extract from the same preparation as in fig.1 and at the same temperature within ± 0.5°C. The period is 13 min.

representing the time course of the concentration of NAD or NADH, respectively. Running the calorimeter with permanent stirring of the samples did not alter the results.

## 4. DISCUSSION

A consideration of the enthalpy change of the overall reaction [3,11]

Trehalose + $H_2O$ ⟶ 2 glucose

$\Delta H \approx 0$

Glucose ⟶ 2 $CO_2$ + 2 ethanol

$\Delta H = -126$ kJ/M

allows the calculation of the glucose turnover, which results in a rate of 0.43 mM/ml. This value fits into the 'oscillatory window' ranging from 0.3 to 2 mM/min as determined by a glucose injection technique [4,10].

The splitting of fructose 1,6-bisphosphate by the enzyme aldolase and the reduction of acetaldehyde by the enzyme alcohol dehydrogenase, the last step in the fermentation process, should be considered as the major sources for the varying rate of heat production. The aldolase reaction is endothermic with $\Delta H = +42$ kJ/mol glucose [2]. The production of ethanol from acetaldehyde is accompanied by a release of heat in the range of $\Delta H = -47$ kJ/mol [12] or $\Delta H = -94$ kJ/mol glucose.

Since preliminary titration experiments with AMP and ADP do not show right away the pronounced phase jumps as in spectrophotometric experiments [10], the phase correlation of heat production and NADH concentration has not yet been established. Further experiments are in progress which, by simultaneous measurements of NADH concentration and heat production in one sample cuvette, will allow for a better identification of the most significant heat producing and heat consuming steps in oscillating glycolysis.

## REFERENCES

[1] Lamprecht, I. and Zotin, A.I. (1978) Thermodynamics of Biological Processes, Walter de Gruyter, Berlin.
[2] Minakami, S. and De Verdier, C.-H. (1976) Eur. J. Biochem. 65, 451–460.
[3] Hoogerheide, J.C. (1975) Rad. and Environm. Biophys. 11, 295–307.
[4] Hess, B. and Boiteux, A. (1968) in: Regulatory Functions of Biological Membranes (Jarnefelt, J. ed.) pp.148–162, Elsevier, Amsterdam, New York.
[5] Boiteux, A. and Hess, B. (1980) Ber. Bunsenges. Phys. Chem. 84, 392–397.
[6] Müller, S.C., Plesser, T. and Hess, B. (1985) in: Temporal Order (Rensing, L. and Jaeger, N.I. eds) pp.194–196, Springer Series in Synergetics Vol.29, Springer, Berlin.
[7] Hess, B. and Boiteux, A. (1968) Hoppe-Seyler's Z. Physiol. Chem. 349, 1567–1574.
[8] Calvet, E. and Prat, H. (1956) Microcalorimetrie - Applications Physico-Chemiques et Biologiques, Masson et Cie, Paris.
[9] Hemminger, W. and Hoehne, G. (1984) Calorimetry, Verlag Chemie, Weinheim.
[10] Hess, B., Boiteux, A. and Krüger, J. (1969) in: Advances in Enzyme Regulation, vol.7, pp.149–167, Pergamon, Oxford.
[11] Wilhoit, R.C. (1969) in: Biochemical Microcalorimetry (Brown, H.D. ed.) pp.33–81, 305–316, Academic Press, New York.
[12] Burton, K. (1974) Biochem. J. 143, 365–368.