Thermochimica Acta, 40 (1980) 63—71

MICROCALORIMETRIC INVESTIGATIONS OF PARTIALLY SYNCHRONOUS CULTURES OF BAKER'S YEAST

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Abstract

Synchronous cultures of Baker's yeast were obtained by use of a centrifugation and filtration technique. Synchrony was maintained over three cell cycles with a degree of at least 60%. The fraction of double cells, the cellular dry weight, the cellular volume and the cellular heat evolution oscillated with the periodicity of the cell cycle, whereas the total rate of heat production increased exponentially with time and linearly with the total dry mass. Addition of the antibiotic nystatin yielded an immediate decrease of heat production with a higher sensibility during the late G_2-phase than during the S-phase of the cell cycle.

INTRODUCTION

The energy metabolism of microorganisms has often been studied calorimetrically (review in: Beezer 1980). But due to the nonspecifity of calorimetric methods and mainly due to the heterogenous mixture of

cells in different phases of cell cycle within a microbial culture, usually the detected heat signal can hardly be associated with single events going on in the organisms. The use of cultures synchronized with respect to cell division gives the advantage to relate the heat effects to the temporarily organized biochemical events during the cell division cycle. Calorimetric measurements on synchronously dividing yeast cells were reported for Saccharomyces pombe prepared by a sedimentation-velocity selection method (Poole, Lloyd & Kemp 1973) and for Saccharomyces cerevisiae obtained in a continuous chemostat culture after nutritional treatment (Brettel 1977).

In the present investigations, the heat evolution of synchronously dividing cells of baker's yeast is compared with the cellular weight, volume and cell concentration exhibited during the cell cycle. The antibiotic nystatin was added at two different time points of the cell cycle in order to test its activity in different cell phases of growth. The influence of nystatin on the heat production in non-proliferating yeast cells was reported previously (Beezer et al. 1977, Schaarschmidt et al. 1979).

METHODS

Yeast cells were prepared from a commercially available baker's yeast by repeated centrifugation and resuspension of the supernatant in 0.15 M NaCl-solution and subsequent filtration over a membrane filter with a pore size of 8 µm. The cell layer remaining above the filter was then resuspended in 0.05 M KH_2PO_4-buffer. By means of the centrifugation technique the bigger cells as well as the budding cells and double cells were separated from the suspension whilst by filtering, the smallest cells and single buds were removed. With this double selection technique cell-collectives were obtained with a mean cell volume of $(48 \pm 10)\,\mu m^3$ against $(92 \pm 35)\,\mu m^3$ for the original cell collection as measured by a coulter counter (TELEFUNKEN-AEG, Germany, type: Partikelvolumenanalysator).

The selected cells were inoculated into growth medium prepared from yeast-extract (1%), peptone (1%) and glucose (2%). The culture vessel had a volume of 500 ml, was kept at 30°C and was provided by an aerating-stirring system. A small part of the suspension was continuously pumped through a flow microcalorimeter (LKB/Sweden, type 10700-1 with a working volume of 0.5 ml). Every 10 to 20 minutes small samples were taken from the culture vessel for determining the cell titer,

the cell volume or the cell dry weight. The degree of synchrony was assessed by an index (Nüsse 1973).

$$S = 1 - A_{ms}/A_{es}$$

where S has a maximum of 1.0 for a culture dividing perfect-synchronously with a stepwise growth curve, and a minimum of 0.0 in case of an exponentially growing culture. A_{es} is the area between the exponential and the ideal-synchronous curve during one cell cycle, A_{ms} is the area between the measured curve and the ideal-synchronous curve. A detailed description of the calorimetric method and the synchronizing procedure was given previously (Brettel 1974, Sayyadi 1978). The nystatin tests were performed by adding appropriate amounts of a concentrated nystatin-ethanol solution to the yeast culture (Schaarschmidt et al. 1979).

RESULTS

Synchronous growth

The baker's yeast Saccharomyces cerevisiae propagates mainly by budding. The emergence and the growing of a bud can be microscopically observed and is thus a convenient visual marker of the cell's state in the division cycle. Due to common designations for mammalian cells the yeast cell cycle is divided into the G_1-, S-, G_2- and M-phase (Nüsse 1973, Mitchinson et al.1975). The cycle starts with a single unbudded cell in the G_1-phase, which covers about one tenth of the whole cycle time t_c. During the following S-phase DNA-synthesis takes place in the course of which the new bud appears on the cell wall. In the G_2-phase, between 0.4 t_c and 0.7 t_c, the nuclear material doubles and the bud increases to normal size by filling with cytoplasma. Nuclear division, the mitosis, and migration of the nucleus into the new bud happen during the following M-phase finished by the cell wall synthesis. The last tenth of the cycle is passed by cell wall separation between mother cell and daughter.

Figure 1 shows the course of the growth for a culture started with a low titer of selected cells as described above. After a relatively long lag-phase, due to adaptation processes within the cells to the new medium conditions, the yeast count N increases in a stepwise fashion through three doublings in numbers. The cycle time was 1.85h, 1.65h and 1.55h respectively, the degree of synchrony were S_1=0.67, S_2 = 0.62, S_3 = 0.60. The fraction of undivided double cells DC (counted as such only if the bud was of the same size as the mother

cell) corresponded well with the stepwise cell concentration, since the maxima of DC occured each time shortly before the cell doubling. These maxima exhibited values of 90%, 75%, 50% and 45%, which show that there is a obviously scattering of synchrony. The mean cell volume V and mean cell dry weight W corresponded well with the cell counts, oscillating between 50 and 100 μm^3 or 40 and 70.10^{-12}g, respectively.

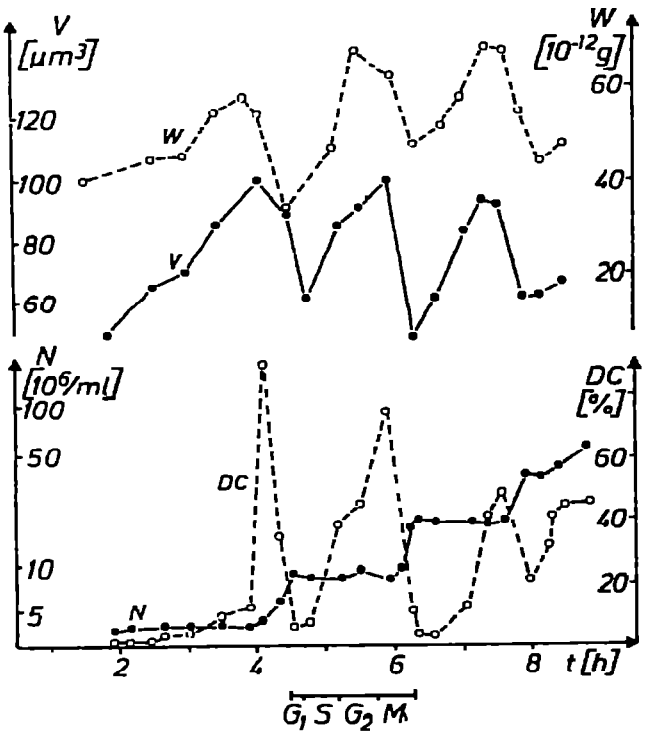

Fig.1: Growth of a partially synchronous yeast culture. N = number of yeast cells per ml of suspension, DC = fraction of double cells as percentage of total cell count, V = mean cellular volume, W = mean cellular dry weight. A diagram of the phases of a cell cycle is given below the time axis.

Heat evolution

The rate of heat production dQ/dt of the yeast culture increased shortly after inoculation at an exponential rate (Fig.2) with an overall doubling during each of the three cell cycles. Calculation of the specific heat production per cell dq/dt however, yielded an oscillating course (Fig.2) which corresponded in time exactly with those of the dry weight (Fig.1). dq/dt and W rose to maxima shortly before the cell volume V and the fraction of double cells DC did so (Fig.1), i.e. a short time before the buds separated from their mother cells.

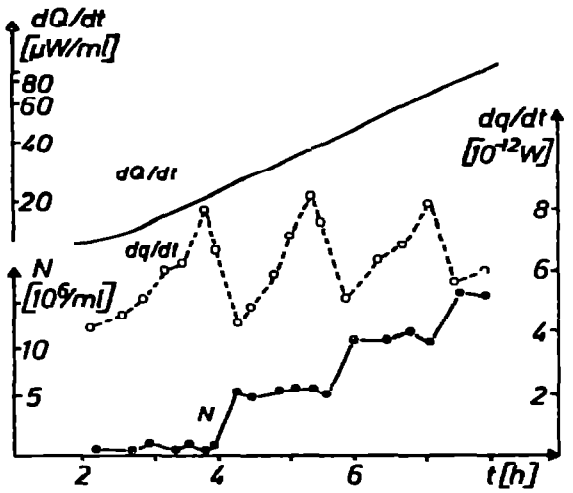

Fig.2: Heat evolution of a partially synchronous yeast culture. N = number of yeast cells per ml, dQ/dt = rate of heat production per ml of suspension (logarithmic scale!), dq/dt = 1/N.dQ/dt = rate of heat production per cell.

Nystatin addition

The early S-phase and the late G_2-phase were chosen for testing the influence of nystatin, because they mark two important events in the cell cycle, the beginning DNA-synthesis and the beginning nuclear division respectively which were each thought to give a distinct calorimetric response (Poole 1973). In both cases the addition of nystatin with a final concentration of 0.5, 1.0 and 2.0 µg/ml of yeast culture, caused within 15 minutes a pronounced decrease in the rate of heat evolution with a subsequent decay on a constant minimum value (Fig.3 and 4) which was dependent on the nystatin concentration. Cell growth did not proceed any longer, since the cell count N and the fraction of double cells DC remained constant throughout the next cycle. An analysis of the decaying part of the thermograms is given in the table. A steeper decrease of the heat production after nystatin addition was observed for G_2-cells than with S-cells. The decrease itself and the final minimum depended on the given concentration.

DISCUSSION

By the centrifugation-filtration technique used a synchronously dividing yeast culture could be maintained throughout three cell cycles as was demonstrated by the stepwise cell count and the coinci-

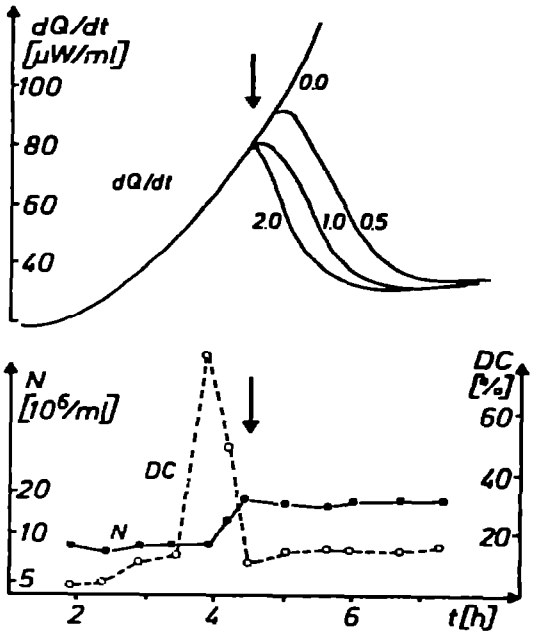

Fig.3: Influence of three concentrations of nystatin (0.5, 1.0 and 2.0 µg/ml) on the heat evolution during the S-phase of a partially synchronous yeast culture. Symbols as in Figs.1 and 2. The arrow indicates the time of nystatin addition.

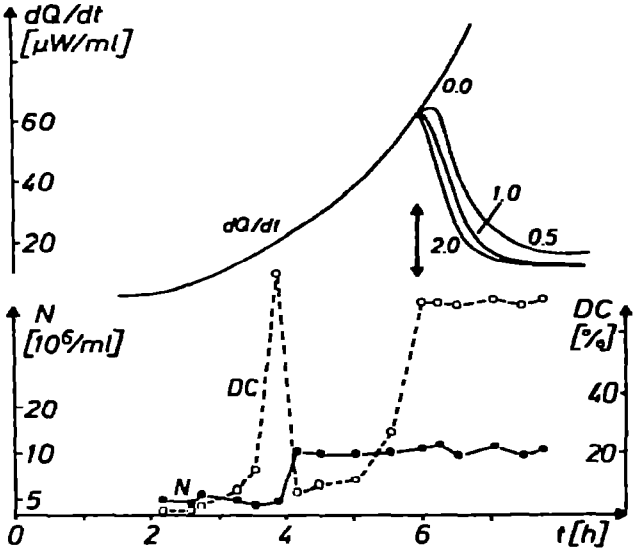

Fig.4: Influence of three concentrations of nystatin on heat production during the late G_2-phase of a partially synchronous yeast culture, symbols as in Fig.3.

ding fraction of double cells. The synchrony was not stable since the mean duration of a full cycle diminished from 1.85 h down to 1.55 h which is close to the mean generation time of 1.5 h for an exponentially growing culture of the same organism (Sayyadi 1978). Furthermore the degree of synchrony dropped continually as well as did the maxima of the fraction of double cells. The cessation of synchrony was not only due to age of the culture (as estimated by the number of cycles since inoculation) but also to the cell titer which reached a count of 5.10^7 organisms/ml after the third cycle. Cultures could be run synchronously only for one cell division before scattering into exponential growth, when they were started from beginning on with a high titer of 3.10^7 freshly prepared cells per ml.

Table:

Decrease of heat evolution of a partially synchronous culture of yeast cells after addition of nystatin in the S-phase and G_2-phase. The final concentrations of nystatin were 0.5, 1.0 and 2.0 µg/ml of suspension.

Nystatin concentration (µg/ml)	Rate of decrease of heat evolution (µW/h.ml)		Final minimum of heat evolution (µW/ml		Time of decrease (h)	
	G_2	S	G_2	S	G_2	S
0.5	29	20	18	35	1.6	2.2
1.0	45	26	13	33	1.1	1.6
2.0	49	30	12	31	1.0	1.5

The oscillations of the mean cellular volume V and dry weight W support the assumption of synchronous growth because their frequency equaled that of the cell counts and their maxima occured just before the cell separations. But it should be noted that the peaks for the cellular weight were shifted about 15 minutes earlier, indicating an uptake of water and formation of vacuoles during this time, which gave a rise of volume but not of dry weight as observed. In spite of the stepwise increase of cell count the heat evolution of the cultures grew exponentially as in an asynchronous culture (Brettel 1974). This is due to the fact that heat evolution in microorganisms is

mainly caused by catabolic processes (Forrest 1972), so that heat output is directly proportional to the biomass (dry weight) (Lamprecht et al. 1976).

Consequently the specific heat evolution of a single cell (dq/dt in Fig.2) corresponded to its dry weight, i.e. it oscillated in the same pattern with a maximum a few minutes before cell doubling as shown in Fig.1. A similar time shift was found for a synchronous culture of Saccharomyces cerevisiae run in a continuous chemostat (Brettel 1977). A correlation with the rate of oxygen consumption was reported. These results are in contrast to respiration oscillations without any correspondence to a non-oscillating heat evolution in a batch culture of Saccharomyces pombe (Poole et al. 1973) as was observed in the present investigations.

Nystatin concentrations $\geq$ 0.5 µg/ml given in S- or late G_2-phase acted at least bacteriostatically as drawn from constant cell counts. This was supported by the decrease of the heat production to well detectable minimum values between 1.4 and 2.0 10^{-12} W/cell which correspond fairly with a figure of $1.8.10^{-12}$ found for endogenously metabolizing cells (Schaarschmidt et al. 1979).

The decrease of the heat production did not obey a simple order kinetic, therefore the data of the table represent only an overall description. Nevertheless it is evident that the inhibition of the heat evolution with G_2-cells is stronger than with S-cells, both with respect to time and to nystatin concentration. Thus the addition of 0.5 µg/ml in the G_2-phase yielded the same inhibition as 2.0 µg/ml given in the S-phase. Since nystatin is known to act on cytoplasmatic membranes its higher influence on late G_2-cells may be due to membrane synthesis and changing properties of membrane happening during this time (Mitchinson 1971) and making the cell more sensitive to the drug.

Further work is necessary to analyze in more details the calorimetric response on drug influence, since it is evident that the heat signal contains more information as can be evaluated and interpretated at present.

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