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A THERMOKINETIC STUDY OF BACTERIAL METABOLISM

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ABSTRACT

Thermograms of growth metabolism, endogenous metabolism and non-growth metabolism of resting cells of *E. coli* were obtained using an LKB-2277 Bioactivity Monitor. From these thermograms it can be established that the thermokinetic equation for the growth metabolism of *E. coli* is $dp/dt = k_1 p$, with order of metabolism $n = 1$, $k_1 = 0.03993\ \text{min}^{-1}$ (k_1, multiplication rate constant; p, power output; t, time). The corresponding thermokinetic equation for the non-growth metabolism of resting cells is $dp/dt = 0$, $k_0 = 0$. The equation for endogenous metabolism is dependent on the previous equation, but it can be divided into two parts: at an early phase, $dp/dt = 0$, $k_0 = 0$; at a later phase, $dp/dt = k_{-1} p$, $n = 1$, $k_{-1} = -0.01769\ \text{min}^{-1}$. Thus, thermokinetic equations for the various different kinds of metabolism in cells (in this case, bacteria) can be obtained by microcalorimetric methods.

INTRODUCTION

The various metabolic events which occur within cells are all reactions which produce heat. Thus, by monitoring the heat effects with sufficiently sensitive calorimeters, we can study the metabolic processes of living cells. Generally, the metabolism of cells (e.g. bacteria) is very complicated. For convenience, we studied the classic metabolic processes of bacteria: growth metabolism, endogenous metabolism, and non-growth metabolism of resting cells. An LKB-2277 Bioactivity Monitor was used to determine the thermogram curves for these three kinds of metabolic process (Figs. 1, 3, 4). Clearly, these thermogram curves have different patterns. From the results obtained from the thermogenesis curves (see Tables 1, 2, 3, 4) the following conclusions can be drawn. The thermokinetic equation for non-growth metabolism of resting cells is $dp/dt = 0$, $k_0 = 0$. The corresponding thermokinetic equation for growth metabolism is $dp/dt = k_1 p$, $k_1 = 0.03993\ \text{min}^{-1}$. The order of metabolism $n = 1$ (k_1, multiplication rate constant; p, power output; t, time). The equation for endogenous metabolism is more com-

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plicated, but can be divided into two parts: at an early phase, $dp/dt = 0$ ($k_0 = 0$); at a later phase, $dp/dt = k_{-1}p$ ($k_{-1} = -0.01769\ min^{-1}$), $n = 1$. Thus, the various different kinds of metabolism in cells (in this case, bacteria) can be described using a set of thermokinetic equations obtained by microcalorimetric methods.

EQUIPMENT

Instrument

An LKB-2277 Bioactivity Monitor was used to obtain the metabolic thermograms of the bacteria. The performance of this instrument and the details of its construction have been described previously [1,2].

Materials

Escherichia coli (ACCT). Peptone medium (A), containing in every 1000 ml the following: NaCl, 5 g; K_2HPO_4, 2 g; *p*-aminobenzoic acid, 0.02 g; peptone, 5 g; sodium citrate, 12 g; $MgSO_4$, 5 g; NaOH (2N), 2.5 ml; tryptone, 5 g; beef extract, 3 g; and tryptose, 5 g. Glucose medium (B), for the non-growth cultures: containing 400 μg glucose per ml buffer solution. Buffer (pH = 7), containing in every 1000 ml the following: 0.2 M KH_2PO_4, 66 ml; 0.2 M K_2HPO_4, 144.5 ml; NaCl, 0.5 g; $MgSO_4$, 0.12 g; $CaCl_2$ and $FeCl_2$, microaddition.

All the above materials were provided by the Army Hospital of the Kwangchow Military District.

EXPERIMENTAL AND RESULTS

Growth metabolism

Growth metabolic thermograms of *E. coli* were obtained by the same experimental method as has been described previously [1]. The *E. coli* strain

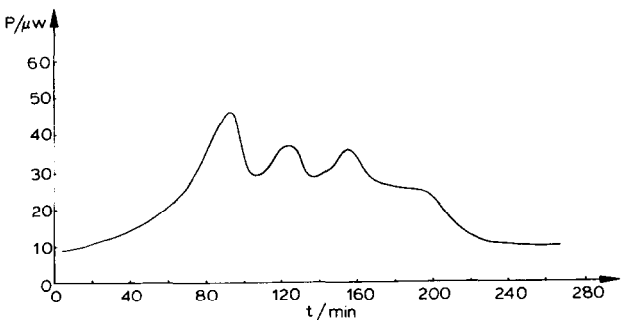

Fig. 1. Thermogram of growth metabolism of *E. coli* at 37 °C.

TABLE 1

Data corresponding to the growth metabolism of *E. coli* [a]

t (min)	p_t (μW)	ln p_t [b]
12	1.86	0.64
22	2.30	0.83
32	4.19	1.43
42	5.76	1.75
52	9.32	2.23
62	12.90	2.56
72	19.89	2.99
82	29.32	3.38
87	34.05	3.53

[a] $k_1 = 0.03993\ \text{min}^{-1}$, $r = 0.9981$.
[b] $\ln p_t = 0.09472 + 0.03993\ t$.

was inoculated into medium A at a concentration of about 10^5 cells ml^{-1}. The bacterial sample was then pumped into the flow cell. When the flow cell (volume 0.6 ml) was full, the pump was stopped and the monitor allowed to continue recording the thermogram for the bacterial growth (culture temperature 37 °C).

The thermogram curve is shown in Fig. 1; the corresponding p (μW) vs. t (min) data (log phase AB) are given in Table 1. From these data, it is clear that ln p vs. t is satisfied for a linear equation; more particularly we can say that the p vs. t is satisfied for the thermokinetic equation $dp/dt = k_1 p$, with order of growth metabolism $n = 1$, $k_1 = 0.03993\ \text{min}^{-1}$.

Non-growth metabolism of resting cells

Resting cells were prepared as follows. The *E. coli* strain was inoculated into medium A. When the cells were going into the log phase, a bacterial sample (10 ml) was removed, and separated using a centrifuge (10^4 r.p.m.). The residual medium was then washed away with buffer solution. Finally, these bacterial cells were suspended in 25 ml buffer solution containing 80 μg ml^{-1} glucose. This bacterial sample was pumped into the flow cell, and the thermogenesis curves were monitored, according to the cycle-flow method. A schematic representation of the experimental apparatus is shown in Fig. 2. When the pen of the chart recorder returns to the baseline, this indicates that the cells are going into the resting state [3]. This bacterial sample can then be used for the determination of non-growth metabolism.

The thermograms were obtained as follows. Medium B (5 ml) was added to the above sample of resting cells. As non-growth metabolism progressed, thermograms were obtained by the cycle-flow method. The resulting metabolic thermogenesis curve is shown in Fig. 3; the corresponding p vs. t data are given in Table 2.

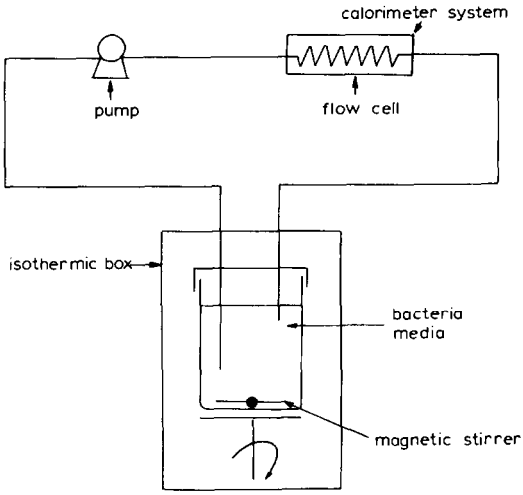

Fig. 2. Schematic diagram for culture in cycle-flow cell.

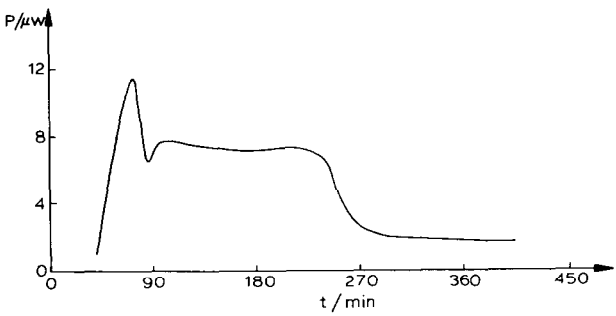

Fig. 3. Thermogram of non-growth metabolism of resting cell at 37 °C.

TABLE 2

Data corresponding the the non-growth metabolism of resting cells

t (min)	p_t (μW)	$\Delta p = p_0 - p$ (μW) [a]
90	5.5	−0.27
100	6.3	0.53
120	6.0	0.23
130	5.75	−0.02
140	5.75	−0.02
160	5.60	−0.17
180	5.70	−0.07
200	5.75	−0.02
220	5.60	−0.17

[a] $p_0 = 5.77 \pm 0.24$ μW.

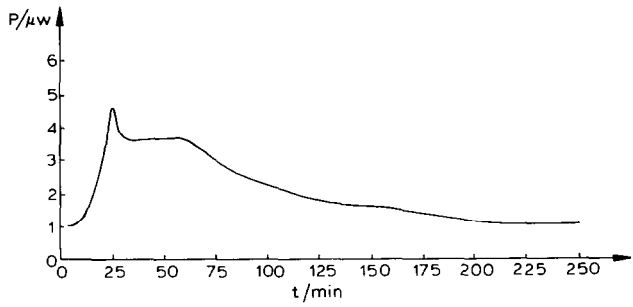

Fig. 4. Thermogram of endogenous metabolism at 37 °C.

These results clearly indicate that the metabolic power output is steady. These p vs. t data satisfy the thermokinetic equation $dp/dt = 0$, or $p = p_0 = 5.77 \pm 0.24$ μW. The multiplication rate constant $k_0 = 0$, which indicates a steady metabolic process.

Endogenous metabolism

The *E. coli* were inoculated into medium A, and cultured at 37 °C. When the cells were going into the log phase, a bacterial sample (10 ml) was removed, and separated using a centrifuge (10^4 r.p.m. for 5 min). The residual medium was washed away with buffer solution. The cells were then suspended in 10 ml buffer solution, and this sample (3×10^8 cells ml^{-1}) was immediately pumped into the flow cell, where a metabolic thermogram was obtained by the stop-flow method. As the buffer solution does not contain any nutrient, the bacteria are forced to undergo endogenous metabolism of nutrients inside the cells. The thermogram obtained is shown in Fig. 4; the corresponding p vs. t data are given in Tables 3 and 4.

The endogenous metabolic thermogenesis curve can be divided into two parts: (1) an early phase AB (30–60 min); and (2) a later phase BC (60–190 min) (Fig. 4). From every corresponding partial p vs. t data using the test

TABLE 3

Data corresponding to endogenous metabolism (early phase)

t (min)	p_t (μW)	$\Delta p = p_0 - p$ (μW) [a]
30	2.60	−0.05
35	2.60	−0.05
40	2.60	−0.05
45	2.65	0.00
50	2.70	0.05
55	2.70	0.05
60	2.70	0.05

[a] $p_0 = 2.65 \pm 0.05$ μW.

TABLE 4

Data corresponding to endogenous metabolism (later phase) [a]

t (min)	p_t (μW)	ln p_t [b]
60	2.70	0.9933
70	2.25	0.8109
80	1.75	0.5596
90	1.50	0.4055
100	1.25	0.2231
110	1.00	0.000
120	0.81	−0.2107
130	0.75	−0.2876
140	0.60	−0.5108
150	0.55	−0.5978
170	0.40	−0.9163
190	0.25	−1.3862

[a] $k_{-1} = -0.01769\ \text{min}^{-1}$, $r = 0.9973$.
[b] $\ln p_t = 2.0021 - 0.01769\ t$.

method as the reaction kinetic we can see their metabolism thermokinetic equations are respectively as follows

(1) $(\mathrm{d}p/\mathrm{d}t)_{AB} = 0$ or $p = p_0 = 2.65 \pm 0.05\ \mu\text{W}, \quad k_0 = 0$

(2) $(\mathrm{d}p/\mathrm{d}t)_{BC} = k_{-1}p$ or $\ln p_t = 2.0021 - 0.01769\ t, \quad r = 0.9973, \quad n = 1,$
$k_{-1} = -0.01769\ \text{min}^{-1}$

whereas the thermokinetic equations can be expressed as follows

early phase: $(\mathrm{d}p/\mathrm{d}t)_{AB} = 0$

later phase: $(\mathrm{d}p/\mathrm{d}t)_{BC} = k_{-1}p$

CONCLUSIONS

The thermogenesis curves obtained for the various kinds of metabolism studied have characteristic patterns. The thermokinetic equations corresponding to these metabolic processes provide some insight into the physical chemistry of cellular metabolic processes.

For example, when the resting cells are undergoing non-growth metabolism, the number of cells does not increase, and the multiplication rate constant $k_0 = 0$. The cell is therefore functioning as an active catalyst, serving only to transform glucose (as a substrate) to metabolic products. The metabolic power output is steady, indicated in the thermogenesis curve as a plateau. The corresponding thermokinetic equation is $\mathrm{d}p/\mathrm{d}t = 0$, or $p = p_0$.

When the cells are undergoing growth metabolism (log phase), the number of cells and the culture time correspond to the exponential law $n_t = n_0\ \mathrm{e}^{kt}$

[4], so the power output of metabolism and time have the same relation $p_t = p_0 \, e^{kt}$. The corresponding thermokinetic equation is $dp/dt = k_1 p$ (first order equation).

The results of the experiments support these thermokinetic equations for metabolism.

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