Microcalorimetric Studies on Thermochemical Characteristics of *Escherichia coli* NCTC 10418 Aerobic Growth Metabolism in Basic Media

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By using an LKB-2277 bioactivity monitor, cycle-flow mode, the thermogenic curve of metabolism of *Escherichia coli* NCTC 10418 growth at 37℃ in basic media was determined. The experimental results indicate that the relationship between cell concentration and power output can be characterized by the equations:

$$C = k'P + a$$

$$\mathrm{d}C/\mathrm{d}P_0 = KC^n, \qquad n = 1$$

where P is the power output (μW), C is cell concentration (mg/mL), P_0 is the power output produced by the metabolism of one unit of cell ($P_0 = P/0.6C$), the order of metabolism n is 1, and k', a and K are constants which depend on the culture condition and phylogenic state of the cells. These equations are different from those of the non-growth metabolism of resting cells and endogenous metabolism of cells. For different kinds of metabolism of cells, the order of metabolism, n, is different. For endogenous metabolism, $n = 0$, for growth metabolism, $n = 1$, and for non-growth metabolism of resting cells, $n = 2$. The equations are general thermochemical equations for the various different kinds of metabolism of cells.

Keywords Growth metabolism, thermochemical equations, *Escherichia coli*, microcalorimetry

Introduction

In general, all phenomena of physical, chemical, and biological processes are accompanied by heat effect, the precise magnitude of which may be of considerable practical and theoretical importance. Such measurements provide the fundamental data upon which studies of heat exchange in biological processes depend, and in pure research, they may give valuable information concerning the stability and structure of molecules, in predicting the course of reactions, and the conditions of chemical equilibrium, *etc*.

Studies on the metabolism of microorganisms are the central part of microbial physiological research. The various metabolic reactions, which occur within cells, all 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 is very complicated. In order to facilitate our research, we have studied the thermokinetics of the classic metabolic processes of bacteria, such as growth metabolism, endogenous metabolism and non-growth metabolism of resting cells, respectively.[1] In present work, an LKB-2277 bioactivity monitor was used to determine the thermogenic curves of the growth metabolism of *Escherichia coli*. Thermochemical equations of the various different kinds of metabolism in cells can be described by the common equations:

$$C = k'P + a$$

$$\mathrm{d}C/\mathrm{d}P_0 = KC^n$$

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Received May 23, 2000; accepted November 27, 2000.

Project supported by the National Natural Science Foundation of China (No. 29973030), and Natural Science Foundation of Hubei Province (No. 98J052).

For different kinds of metabolism of cells, the order of metabolism, n, is different. For growth metabolism, $n = 1$, for non-growth metabolism of resting cells, $n = 2$,[2] and for endogenous metabolism, $n = 0$.[3] The various different kinds of metabolic processes in cells can be described using a set of thermochemical equations obtained by microcalorimetric methods. All of these results are very significant for microbiology and thermochemistry.

Experimental

Instrument

An LKB-2277 bioactivity monitor was used to determine the growth metabolic process of *Escherichia coli*. The performance of this instrument and the details of its construction have been previously described.[4,5]

Materials

Standard *Escherichia coli* NCTC 10418 was provided by Wuhan General Hospital of Kwangchow Military District, Wuhan 430070.

Basic medium contained in every 1000 mL the following: glucose 5 g, NaCl 5 g, $NH_4H_2PO_4$ 1 g, K_2HPO_4 1 g, $MgSO_4 \cdot 7H_2O$ 0.2 g, pH = 7.3. They were sterilized in high pressure steam at 120℃ for 20 min.

General procedure

Microcalorimetric experiments were performed in cycle-flow mode (see the schematic diagram in Ref. 1). The *E. coli* NCTC 10418 was inoculated into basic medium. This bacterial sample was pumped into the flow-cell and the thermogenic curves were monitored by the cycle-flow method at 37℃.

In order to measure the cell concentration, three 10 mL bacterial samples were removed from the culture medium, centrifuged, and washed with distilled water to remove the residual medium, and then heated at 100℃ and weighed. The average dry weight of the cells (mg/mL) can then be calculated.

Results and discussion

Growth thermogenic curve

By using the cycle-flow method, we determined the thermogenic curve of *Escherichia* coli NCTC 10418 growth at 37℃. The experimental curve is shown in Fig. 1, and part AB is the logarithmic growth phase.

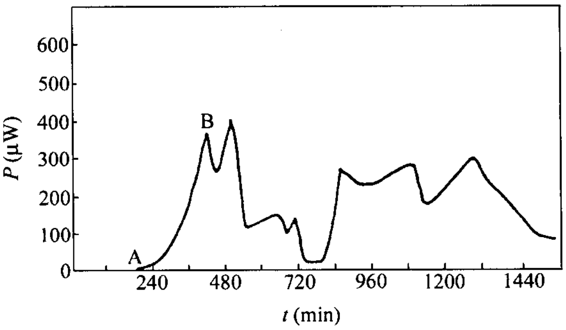

Fig. 1 Thermogenic curve *Escherichia coli* NCTC 10418 growth at 37℃.

Rate constants and thermokinetic equation for growth metabolism

In the log phase of growth, the cell is growing exponentially. If the cell number is n_0 at time 0, and n_t at time t, then

$$n_t = n_0 \exp(kt) \qquad (1)$$

where k is the growth rate constant. If the power output of each cell is w, then

$$n_t w = n_0 w \exp(kt) \qquad (2)$$

$P_{t=0} = n_0 w$ and $P_t = n_t w$, giving

$$P_t = P_{t=0} \exp(kt) \text{ or } \ln P_t = \ln P_{t=0} + kt \qquad (3)$$

The thermogenic curves of the log phase of growth correspond to Eq. (3). So, making use of the data P_t and t taken from the curves (shown as AB part) to fit a linear equation, one can obtain the growth rate constant k. The rate constant k of *Escherichia coli* growth was shown in Table 1.

From the data in Table 1, it is apparent that all of the correlation coefficients, r, are greater than 0.9950, indicating a good reproducibility and correlationship.

Eq. (3) can be rewritten as

$$dP/dt = kP = kP^n,\ n = 1 \qquad (4)$$

Eq. (4) is the thermokinetic equation of *Escherichia coli* growth metabolism, with the order of growth, $n = 1$.

Table 1 Rate constants for *Escherichia coli* NCTC 10418 growth at 37℃

Experiment no.	1	2	3	4	5	Mean value
k (min^{-1})	0.0229	0.0244	0.0236	0.0244	0.0242	0.0239 ± 0.0003
r	0.9972	0.9959	0.9989	0.9990	0.9958	0.9974

Relationship for heat output power and cell concentration

The $C \sim P$ data for the metabolism of the growth metabolism of *Escherichia coli* NCTC 10418 were obtained from the log phase of growth thermogenic curve in Fig. 1 (shown as AB part), and the corresponding average dry weight of the cells (mg/mL). The data are presented in Table 2.

The $C \sim P$ data given in Table 2 indicated that C and P are linearly proportional, as shown in Fig. 2.

Table 2 C ~ P data for *E. coli* NCTC 10418 growth metabolism

Experiment no.	1	2	3	4	5
C (mg/mL)	0.0635	0.0686	0.1024	0.1446	0.2410
P (μW)	5.4	12.5	50.1	105.1	252.6
P_0 (μW/mg)*	141.7	303.7	815.4	1211.4	1746.9
$\ln C$	−2.7567	−2.6795	−2.2789	−1.9338	−1.4230

* $P_0 = P/(0.6C)$ (flow cell volume $V = 0.6$ mL).

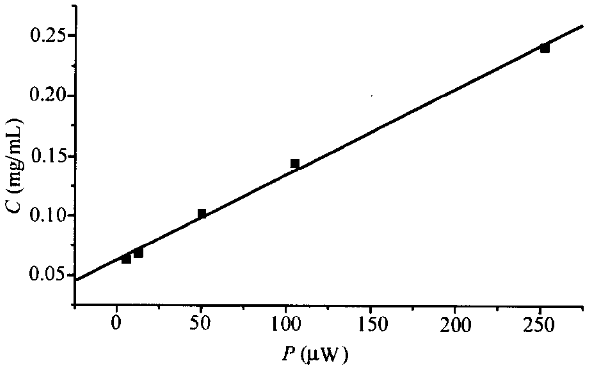

Fig. 2 $C \sim P$ relation for *E. coli* growth metabolism.

The corresponding linear equation is

$$C = 7.1685 \times 10^{-4}\ P + 0.06299,$$

with correlation coefficient $r = 0.99804$. That is to say the relation between C and P is

$$C = k'P + a$$

Relationship for the mean heat output power and cell concentration

From the data of P and C in Table 2, we can obtain the mean heat output P_0 of one unit of cells. Using the $\ln C$ and P_0 data values from Table 2 to fit a linear equation (see Fig. 3), another equation can be obtained as

$$\ln C = 8.3611 \times 10^{-4} P_0 - 2.9199,$$
$$\text{with } r = 0.99759.$$

The relation between $\ln C$ and P_0 is

$$\ln C = KP_0 + A,$$

it can be rewritten as:

$$dC/dP_0 = KC^1,$$

with the order of metabolism $n = 1$. So, the order of growth metabolism is one. It is one order metabolism.

Characteristics of different kinds of metabolism

The heat output of growth metabolism of *E. coli* has been determined. The experimental results indicated that the relationship between cell concentration and heat output could be characterized by equations

$$\begin{aligned} & C = k'P + a \\ \text{and} \quad & \mathrm{d}C/\mathrm{d}P_0 = KC^n, \quad n = 1. \end{aligned}$$

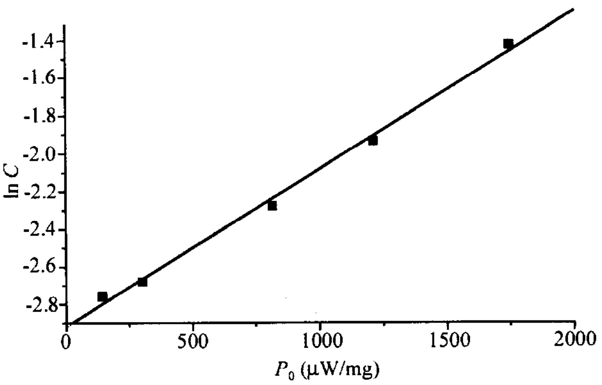

Fig. 3 $\ln C \sim P_0$ relation for *E. coli* growth metabolism.

As reported previously,[2,3] the non-growth metabolism of resting cells can be characterized by equations

$$\begin{aligned} & C = k'P + a \\ \text{and} \quad & \mathrm{d}C/\mathrm{d}P_0 = KC^2. \end{aligned}$$

And the endogenous metabolism of *E. coli* can be characterized by equations

$$\begin{aligned} & C = k'P + a \\ \text{and} \quad & \mathrm{d}C/\mathrm{d}P_0 = KC^0. \end{aligned}$$

So, we can obtain that three different kinds of metabolism of bacterial cells can be characterized by the general equations

$$\begin{aligned} & C = k'P + a \\ \text{and} \quad & \mathrm{d}C/\mathrm{d}P_0 = KC^n, \end{aligned}$$

where n is the order of metabolism. The metabolic order for endogenous metabolism is zero, the metabolic order for growth metabolism is one, and the metabolic order for non-growth metabolism of resting cells is two. All of the experimental results confirmed the applicability of the equations, with the correlation coefficient r being greater than 0.99, although cells were obtained from different conditions. In general, different cultural conditions and cells may only change the values of the constants k', a, K and A of the equations, and the metabolic order depends on the type of metabolism. Thus, these equations specifically characterize the metabolic process of cells and provide a functional relationship for the metabolism of cells. Combining these thermal equations with the thermokinetic equations in Ref. 1, it has provide a lot of significant information for the metabolism of cells. All of the results are the basic characteristics of the metabolism of cells.

Conclusions

The metabolism can be divided into three kinds, growth metabolism, endogenous metabolism and non-growth metabolism of resting cells. These three different kinds of metabolism can be characterized by the general equations

$$\begin{aligned} & C = k'P + a \\ \text{and} \quad & \mathrm{d}C/\mathrm{d}P_0 = KC^n. \end{aligned}$$

Different cultural conditions and cells only change the values of the constants k', a, K and A of the equations, and the metabolic order depends on the type of metabolism.

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(E200005111 JIANG, X.H.; LING, J.)