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Calorimetric study of the metabolic activity of *Escherichia coli* B infected by T4 phage in restricted medium

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Abstract

The effects of T4 phage on the metabolic rate of resting cells of *Escherichia coli* B in a restricted medium during the early phase of phage infection were measured calorimetrically. Heat output rate (P) and total quantity of heat (Q) of culture, heat output rate (P_0) and quantity of heat (Q_0) of single cell were determined. P and Q of *E. coli*–T4 phage system, or P_0 and Q_0 of infected cell, are all higher than that of control. The metabolic activity of infected cell is enhanced by 45–62%. P has a linear correlation with cell concentration (C), $P\,(\mu W) = 1.68 \times 10^{-6} C\,(\text{cfu/mL}) + 1.86$, for *E. coli* B cells, and for *E. coli* B–T4 phage system, $P\,(\mu W) = 2.24 \times 10^{-6} C\,(\text{cfu/mL}) + 3.60$. Q does not change significantly with the change of cell concentration. But Q_0 decreases with C increasing, indicating that Q_0 is restrained by cell density.

Keywords: Calorimetry; Phage–host interaction; Resting metabolism; Metabolic activity

1. Introduction

We previously investigated interaction between *Escherichia coli* B and its T4 bacteriophage by monitoring thermokinetic processes of phage infection to and amplification in *E. coli* in a complete medium by calorimetric methods [1]. It was found that metabolic rate of infected cells is higher than non-infected cells, indicating a higher metabolic activity of infected cells than that of non-infected cells. It remained unclear whether the higher metabolic rate of *E. coli* B is due to phage infection or other factors.

To investigate what contributes to the higher metabolic rate of infected cells, thermokinetics processes of early stage of phage infection were measured in a restricted medium. Due to the very low non-growth metabolism of resting cell in the restricted medium, this allows measurement of thermokinetics associated with phage–host cell interaction in the early stage of T4 phage infection and multiplication.

2. Materials and methods

2.1. E. coli B cells, T4 phages and growth medium

Strain *E. coli* B and bacteriophage T4 were obtained from China Center for Virus Collection (Wuhan Institute of Virology, Academia Sinica). Preparation of host cells and high titer T4 phage suspension was performed as described [1]. Briefly, host cells were prepared as follows: (1) Pick a single colony of *E. coli* B to liquid culture medium and cultivate at 37 °C for 8–10 h. (2) Centrifuge the cells at 5000 rpm for 10 min, wash the cells twice and re-suspend the cells in sterile 0.01 mol L^{-1} $MgSO_4$. (3) Determine the viable cells of *E. coli* B with spread-plate technique. The high titer T4 phage

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suspension was prepared as follows: (1) Pick a single plaque from these double layer plates, inoculate to the exponential phase culture of *E. coli* B and incubate for 6–8 h. (2) Mix above lysate with cells suspension, pour double plates and incubate for 6–8 h at 37 °C. (3) Immerse with 4 °C SM liquid (NaCl 5.8 g/L, $MgSO_4$ 2 g/L, 1 M pH 7.5 Tris·HCl 50 mL/L, 2% glutin 5 mL/L) for several hours. (4) Harvest and centrifuge the SM liquid, sterilize the supernatant by filtration through a 0.22 μm filter and assay the virus infectivity with double layer plate technique.

A restricted medium was used (MSG medium) for calorimatry containing NaCl 4 g/L, glucose 1 g/L, K_2HPO_4 0.4 g/L, KH_2PO_4 0.1 g/L, $MgSO_4·7H_2O$ 0.1 g/L, $CaCl_2$ 1 mg/L, $FeCl_3$ 10 μg/L, distilled water, pH 7.0.

2.2. Calorimetry setup and heat measurement assay

An LKB 2277 Bioactivity Monitor [2] stop-flow mode was employed to measure the thermogenic curves of phage–host interaction. After the equipment was thermostated at 37 °C, the sample cells of the calorimeter were cleaned and sterilized. When a stable baseline was established, *E. coli* B cell suspension and T4 phage solutions were mixed at various ratios and incubated for 5 min at 37 °C, the mixture was then inoculated into MSG medium and heat production in the culture measured at intervals of 60 s. Duplicates of experiments were carried out to verify the repeatability of the results.

2.3. Turbidity determination

Turbidity of *E. coli* culture with or without phage infection was determined by measuring absorbance at 600 nm at 30 min intervals [1].

3. Results

3.1. Effect of T4 phage infection on heat production of resting cells in restricted medium

Thermogenic curves of cultures of *E. coli* B–T4 phage incubated in the restricted medium MSG at various multiplicity of infection (MOI) are shown in Fig. 1. Four phases, ascending phase (A phase), stationary phase (S phase), degressive phase (D phase) and resting phase (R phase), can be observed on each curve. In phase A, the heat production of each culture increases to the highest value very quickly, usually in 5–6 min after recording. The heat output rate at phase S shows a plateau. In phase D, nutriments inside and outside the cells are exhausted and the metabolism decreases quickly. In phase R, the heat output rate decreases slowly and the low level indicates the cells are at resting state.

The maximum heat output rate (P_{max}), the mean heat output rate (P_{med}) and the total heat output (Q) during S phase are given in Table 1 for each MOI shown in Fig. 1. The heat output rate of a single cell (P_0) during S phase, including highest and mean value (SCP_{max} and SCP_{med}) were calculated based on P_{max}, P_{med} and cell concentration (Table 1 and Fig. 2). At low MOIs (0.007 and 0.07), P_{max}, P_{med}, SCP_{max} and SCP_{med} are very similar to that of the control, see Fig. 1, curves 3, 4 and 5. With rising of MOI, the heat output rate increases significantly. Infected cells of *E. coli* B exhibit a significantly higher metabolic activity than non-infected cells. At an MOI of 7, P_{max} was similar to that of MOI 0.7, but the heat output rate during S phase decreases quickly (Fig. 1), and P_{med} and SCP_{med} are smaller than that of control test (Table 1), indicating "lysis from without" [3].

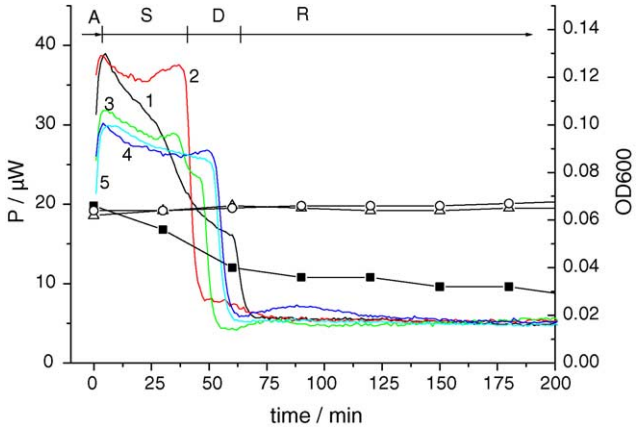

Fig. 1. Heat output rate curves and turbidity change of *E. coli* B–T4 phage system at various MOIs. Cell concentration: 1.69×10^7 cfu/mL. The MOI values of curve 1 to curve 5 were 7, 0.7, 0.07, 0.007 and 0 (control). A, ascending phase; S, stationary phase; D, degressive phase; R, resting phase. The line and symbol curves, change of OD_{600}: squares, MOI = 7, corresponding to curve 1; triangles, MOI = 0.7, corresponding to curve 2; circles, MOI = 0 (control), corresponding to curve 5.

3.2. Heat production of E. coli B–T4 phage living system at various cell concentrations

With the MOI fixed at 0.7 for maximal change in P_{max} and P_{med}, heat output rate of the cultures at various cell concentrations with or without T4 phage infection was determined

Table 1
Thermal data of *E. coli* B–T4 phage system at different MOIs in restricted medium[a]

	MOI				
	7	0.7	0.07	0.007	Control
P_{max} (μW)	38.98	38.70	31.81	30.20	29.91
P_{med} (μW)	26.32	36.74	29.58	27.17	27.54
Q (J)	0.083	0.081	0.069	0.075	0.075
Q_0 ($\times 10^{-9}$ J)	8.19	7.99	6.80	7.40	7.40
SCP_{med} (pW)	2.60	3.62	2.92	2.68	2.72

[a] MOI, multiplicity of infection; P_{max}, maximum heat output rate of cultures; P_{med}, median heat output rate of cultures during S phase; Q, total heat output of culture in S phase; Q_0, total heat output of single cell in S phase. Cell concentration, 1.69×10^7 cfu/mL; SCP_{med}, median of P_0 during S phase.

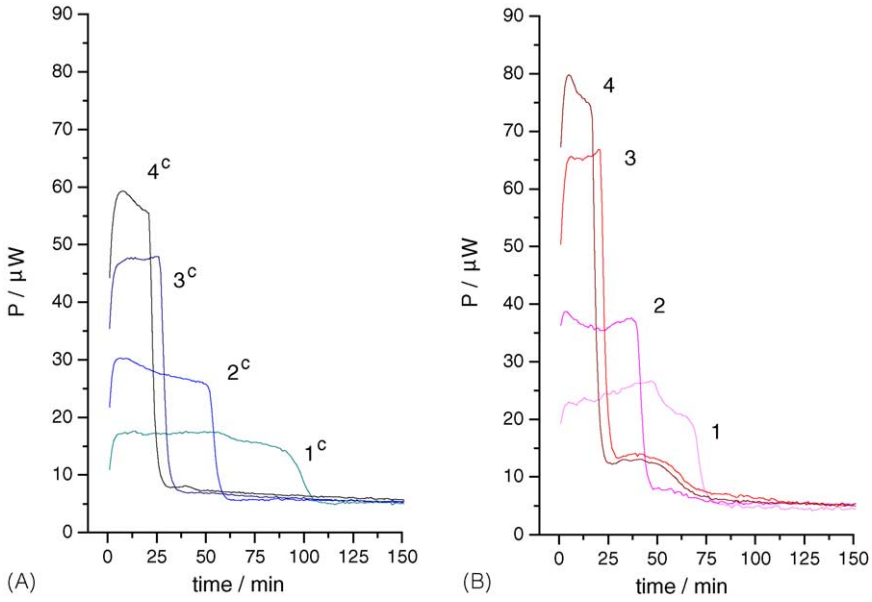

Fig. 2. Heat output rate curves of *E. coli* B–T4 phage system at various cell concentrations with same MOI value of 0.7. A, *E. coli* B (control). B, *E. coli* B–T4 phage system. Cell concentrations ($\times 10^7$ cfu/mL) of curves 1 and 1^c to 4 and 4^c are: 0.845, 1.69, 2.53 and 3.38, respectively.

(Fig. 2). Heat output rate and cell concentrations are given in Table 2. According to Xie's results [4,5], the relation between cell concentration and heat output rate can be characterized by the equations

$$P = kC + a \tag{1}$$

or

$$\frac{dC}{dP_0} = KC^N \tag{2}$$

where P is the heat output rate of the culture, C the cell concentration, P_0 the heat output rate produced by a single cell ($P_0 = P/C$). When $N = 2$, the equations are for resting metabolism (washed cells added to a restricted medium with a little external energy source but can't grow); $N = 0$, are for endogenous metabolism (washed cells added to a buffer solution without external energy source) [6,7]. A linear correlation was also observed here between P and C for both infected and non-infected cells in MSG medium. In MSG medium, the metabolism of cells should be resting metabolism. According to the data of P_{med}, with non-infected cells C–P correlation can be presented as

$$P\,(\mu W) = 1.68 \times 10^{-6} C\,(\text{cfu/mL}) + 1.86$$
$$(\text{correlation coefficient } r = 0.99) \tag{3}$$

and with infected cells their correlation can be presented as

$$P\,(\mu W) = 2.24 \times 10^{-6} C\,(\text{cfu/mL}) + 3.60 \quad (r = 0.99) \tag{4}$$

The slope and intercept of the lines with the infected cells in comparison to non-infected cells indicated that T4 phage infection enhances the metabolic rate of *E. coli* host cells.

Calculating P_0, including SCP_{max} and SCP_{med}, it shows that P_0 of non-infected cell is always less than infected cell at the same cell concentration. Considering that 70% of *E. coli* cells at the utmost are infected at MOI of 0.7, the theoretical value of P_0 for infected cell was calculated (Table 2) according to the formula, $SCP^t = (P_0$ of *E. coli* B–T4 phage

Table 2
Thermal data for *E. coli* B–T4 phage system at various cell concentrations[a]

	C ($\times 10^7$ cfu mL^{-1})							
	0.845		1.69		2.53		3.38	
	Curve 1^c	Curve 1	Curve 2^c	Curve 2	Curve 3^c	Curve 3	Curve 4^c	Curve 4
P_{max} (μW)	17.64	26.64	30.31	38.70	47.91	66.86	59.30	79.79
P_{med} (μW)	16.66	23.87	27.99	36.74	47.47	65.58	57.59	77.29
Q (J)	0.070	0.081	0.075	0.081	0.073	0.084	0.071	0.080
Q_0 ($\times 10^{-9}$ J)	13.81	15.98	7.40	7.99	4.80	5.52	3.50	3.94
SCP_{med} (pW)	3.29	4.71	2.76	3.62	3.12	4.31	2.84	3.81
SCP^t_{med} (pW)	–	5.32	–	3.99	–	4.82	–	4.23

[a] C, cell concentration; cfu, colony formation unit; P_{max}, maximum heat output rate of cultures; P_{med}, median heat output rate of cultures during S phase; Q, total heat output of culture in S phase; Q_0, total heat output of single cell in S phase. SCP_{med}, median of P_0 during S phase; SCP^t_{max}, maximum of theoretical P_0 of infected cell; SCP^t_{med}, median of theoretical P_0 of infected cell. MOI for tests, 0.7.

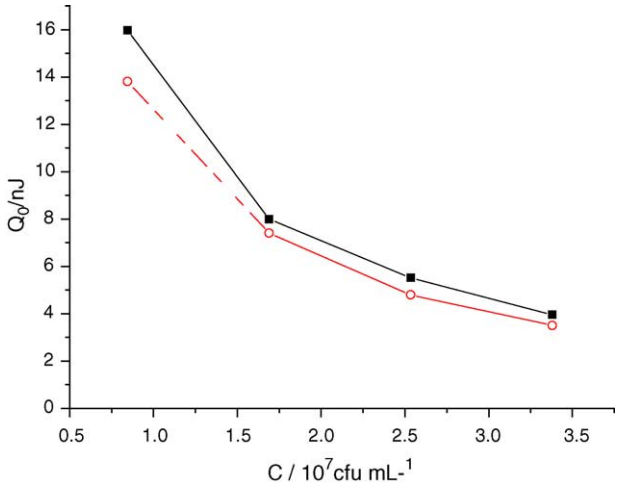

Fig. 3. C–Q_0 relation for resting metabolism of *E coli* B. Squares, infected cell. Circles, non-infected cell.

$-0.3 \times P_0$ of control)/0.7. The actual value of P_0 of infected cell is lower than SCP^t calculated with above formula, maybe because two or more phage particles can infect one host cell, so the percentage of infected cell is less than 70%. The heat output rate of infected cells increases by 45–62% in comparison to non-infected *E. coli* B cells.

3.3. Turbidity change of E. coli B and E. coli B–T4 phage system incubating in restricted medium

For *E. coli* B culture, the turbidity does not change during incubation, indicating that the number of cells does not change with incubation time (Fig. 1, curve with circles). For cultures of *E. coli* B–T4 system, the OD_{600} value does not change significantly (Fig. 1, curve with triangles) at low MOI ($\leq$0.7), indicating cell lysis cycle has not been completed. At an MOI of 7, the turbidity drops during S phase (Fig. 1, curve with squares), which indicates a decrease in number of intact cells. The rapid decline in stationary phase on the thermogenic curve 1 is a display of phenomenon of "lysis from without".

3.4. Total quantity of heat during A to D phase

The area under each curve from A to D is the quantity of heat (Q) released. At low MOI, no significant difference in total heat between *E. coli* B culture and *E. coli* B–T4 system was observed. Q does not change significantly with the cell concentration, despite the increase in the rate of heat production with the rising of cell concentration. More heat was produced at MOI of 0.7 in *E. coli* B–T4 system culture than in *E. coli* culture. The heat produced by a single infected cell is higher than non-infected cell for various cell concentrations. Q_0 decreases with the increasing of C indicating that Q_0 is restricted by cell density (Fig. 3).

4. Conclusion

It is a feasible strategy combining resting culture method with calorimetric technique to study the metabolic activity of infected cell. In the restricted medium, host cells are forced to use endogenous material and restricted carbon source outside cells more rapidly for metabolic activity, and concomitantly produce more quantity of heat. The enhancement of metabolic activity of host cell by infection of T4 phage is significant for phage development, which accommodates to the faster progeny production rate and larger burst size.

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