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Microcalorimetric study on the aerobic growth of *Escherichia coli* ☆

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Abstract

By using an LKB-2277 Bioactivity Monitor, complete thermograms of aerobic growth at 37°C have been obtained for *Escherichia coli*. The amount of glucose consumed S_c has been measured by the glucose oxidase method, and its relationship with the quantities of heat evolved Q_t has been studied. Different linear relationships exist at different phases of Q_t and S_c, indicating that there are different metabolic controlling processes in the growth of *E. coli*. The turning point of the different metabolic controlling processes can be determined from the thermograms. Furthermore, through studies on the relationship between the dry weight of cells produced $\bar{W}$, and S_c together with the relationship between $\bar{W}$ and time t, the conclusion can be drawn that the different metabolic controlling processes reflected in the relationship between Q_t and S_c are just the change of the synthesising power of the cells.

Keywords: Aerobic growth mechanism; *Escherichia*; Microcalorimetry; Metabolic thermogram

1. Introduction

Studies on the metabolism of microorganisms are the central part of microbial physiological research. Traditional research used to concentrate on substrate metabolism and energy metabolism was only studied indirectly. Now with the creation and improvement of very precise calorimeters, studies on energy metabolism

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directly from energy variations have become possible. Because the samples can be kept intact while being studied calorimetrically, synchronous or follow-up analysis can be carried out. If studies on energy metabolism and substrate metabolism can be done simultaneously, metabolism research will certainly improve. Because the basic metabolic processes of all living things are similar, and microorganisms are rather simple living systems which breed quickly and can easily be treated and studied, research on the metabolism of microorganisms is not only significant in itself, but is also an additional tool for the study of living beings.

Some microcalorimetric studies on *Escherichia coli* have recently been reported. For example, we have obtained the growth rate constant, activation energy, activation entropy and activation free energy for the anerobic metabolism of *E. coli* [1]; the thermochemical equations [2] and the thermokinetic equation [3] for the various different kinds of metabolism in cells can be obtained by microcalorimetric methods. In this work, the complete thermograms of the aerobic growth metabolism of *E. coli* were obtained using an LKB-2277 Bioactivity Monitor in cycle–flow mode in simple glucose medium at 37°C. The relationship between the quantity of heat evolved Q_t and the amount of glucose consumed S_c in glucose metabolism was studied. We find that different linear relationships exist at different phases of Q_t and S_c. This result indicates that different controlling processes exist in the growth metabolism of glucose. Furthermore, by determining the variations of the dry weight of the cells produced $\bar{W}$ with $\bar{S}_c$, the amount of glucose consumed together with the variation of $\bar{W}$ with time t in the growth metabolism of *E. coli*, it can be concluded that the different metabolic controlling processes reflected in the relationship between Q_t and S_c is just the change of synthesising power of the cells. The changeover between the different metabolic pathways is determined at point B in the thermograms.

2. Instruments, materials and methods

2.1. Materials

Standard *Escherichia coli* NCTC 10418 were provided by Wuhan General Hospital of Guangzhou Military District.

The culture medium contained per 1000 ml, glucose 5 g, NaCl 5 g, $(NH_4)H_2PO_4$ 1 g, K_2HPO_4 1 g, $MgSO_4 \cdot 7H_2O$ 0.2 g [4]; its pH was 7.3 and it was sterilised in high pressure steam at 118°C for 20 min.

Glucose oxidase (EC 1.1.3.4), was produced by Sigma Corporation of America; its activity was 23 800 u g^{-1}. Horseradish peroxidase (EC 1.11.1.7) was produced by the Shanghai Biochemistry Institute of China; its activity was 250 000 u g^{-1}. O-Dianisidine was produced by the Fluka Corporation of Switzerland.

2.2. Methods

Microcalorimetric experiments were performed in cycle–flow mode (see the schematic diagram in Ref. [3]). The culture bottle was specially made so as to

culture thermostatically and to determine thermograms and take samples for analysis simultaneously. The bath temperature was 37°C. The amount of glucose consumed was estimated by the glucose oxidase method [5]. The method is based on the specific oxidation of glucose by glucose oxidase and the determination of the resulting hydrogen peroxide with peroxidase in the presence of O-dianisidine as chromogenic oxygen acceptor. The absorbance A was measured by spectrophotometry of 420 nm, and the concentration of glucose could be obtained by comparing A with the standard curve for glucose.

2.3. Instruments

The microcalorimetric experiments were performed on an LKB Bioactivity Monitor manufactured by LKB Corporation of Sweden. Glucose analysis was carried out with the WA759 type spectrophotometer manufactured by the Shanghai Optical Instrument Factory.

3. Experimental results

3.1. Thermograms

Complete thermograms for the aerobic growth of *E. coli* were obtained.

Fig. 1 is a thermogram of the aerobic growth of *E. coli* in cycle–flow mode with the glucose medium as the sole carbon source at 37°C. The *E. coli* strain was inoculated into the medium at a concentration of about 10^7 cells ml^{-1}. Part AC of the thermogram corresponds to the metabolism of glucose, with good duplication obtained in the experiments. The average time for glucose consumption is about 9 h, commencing from the starting point A in the thermograms.

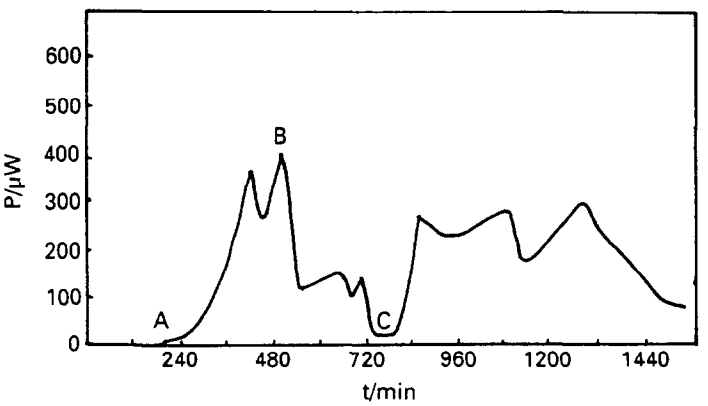

Fig. 1. Complete thermogram of aerobic growth metabolism of *E. coli* in the simple glucose medium at 37°C.

Table 1
Data of quantities of heat evolved and the amount of glucose consumed by the aerobic growth of *E. coli* in simple glucose medium [a]

Expt. 1							
t/min	120	180	240	300	360	540	
S_c/μmol	1.066	1.965	4.130	6.461	8.393	11.390	
Q_t/J	0.533	1.467	2.590	3.198	3.653	4.410	
Expt. 2							
t/min	145	205	265	325	385	525	
S_c/μmol	0.990	2.165	3.565	5.678	8.434	11.390	
Q_t/J	0.450	1.391	2.304	3.465	3.920	4.856	
Expt.3							
t/min	175	235	295	355	415	535	
S_c/μmol	0.999	2.165	4.230	5.062	8.819	10.724	
Q_t/J	0.447	1.428	2.442	3.360	3.779	4.679	
Expt. 4							
t/min	150	210	285	345	460	505	
S_c/μmol	1.166	2.415	4.796	6.944	10.375	11.390	
Q_t/J	0.455	1.346	2.807	3.652	4.490	4.739	
Expt. 5							
t/min	140	200	260	320	380	440	600
S_c/μmol	0.966	1.565	3.146	5.229	7.177	10.058	12.189
Q_t2/J	0.413	1.253	2.181	3.427	3.887	4.272	5.251

[a] t is the time counted from the peak starting point A in the thermograms; S_c and Q_t refer to 0.6 ml sample; the flow cell volume is 0.6 ml.

3.2. Relationship between heat evolved and glucose consumed

The quantities of heat evolved Q_t are treated as functions of the amount of glucose consumed S_c.

The amount of glucose consumed S_c was measured at various times during glucose metabolism, and the corresponding quantity of heat evolved Q_t was calculated by simultaneous graphical integration of the thermogram. The results are listed in Table 1. The Q_t versus S_c plot corresponding to experiment 1 is shown in Fig. 2. We can see that a different linear relationship exists at different phases: part I passes through the origin while part II does not. Two linear equations were obtained separately by the linear least squares regression method. All the results for the five experiments are listed in Table 2. Two linear equations were worked out according to the average values of all the experiments

Part I

$$Q_t = 0.6251 S_c$$

Part II

$$Q_t = 0.2609 S_c + 1.7477 J$$

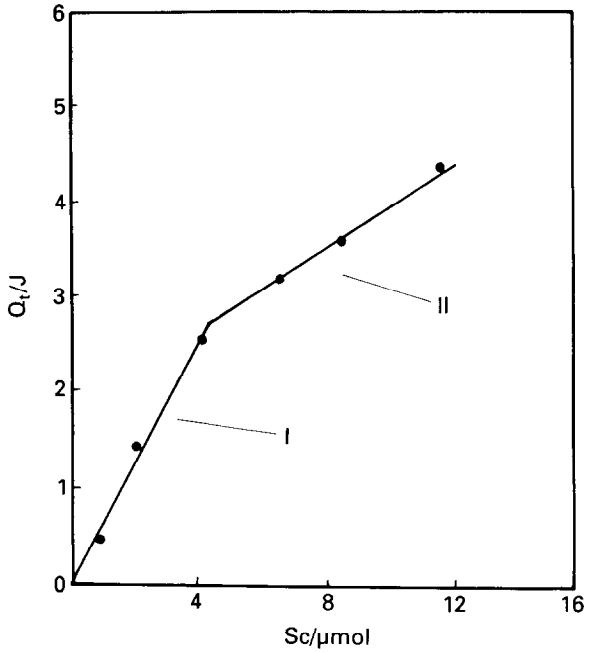

Fig. 2. Q_t vs. S_c plot for the growth of *E. coli*.

Table 2
Linear equation of Q_t vs. S_c obtained by linear least squares regression method [a]

Expt. no.	Part I		Part II	
	Equation	r	Equation	r
1	$Q_t = 0.6422S_c$	0.9911	$Q_t = 0.2469S_c + 1.5876$	0.9994
2	$Q_t = 0.6192S_c$	0.9965	$Q_t = 0.2446S_c + 2.0018$	0.9845
3	$Q_t = 0.5942S_c$	0.9936	$Q_t = 0.2833S_c + 1.5224$	0.9882
4	$Q_t = 0.6004S_c$	0.9965	$Q_t = 0.2855S_c + 1.5312$	0.9935
5	$Q_t = 0.6693S_c$	0.9931	$Q_t = 0.2440S_c + 2.0954$	0.9678

[a] Unit of coefficient J mol^{-1}.

where the unit of the coefficients 0.6251 and 0.2609 is J μmol^{-1}. The Q_t versus S_c plot drawn according to these two equations and the distributions of experimental points are shown in Fig. 3.

3.3. Relationship between dry weight of cells produced and glucose consumed

The relationship between the dry weight of cells produced $\bar{W}$ and the amount of glucose consumed $\bar{S}_c$ is that the former is treated as a function of the latter. The

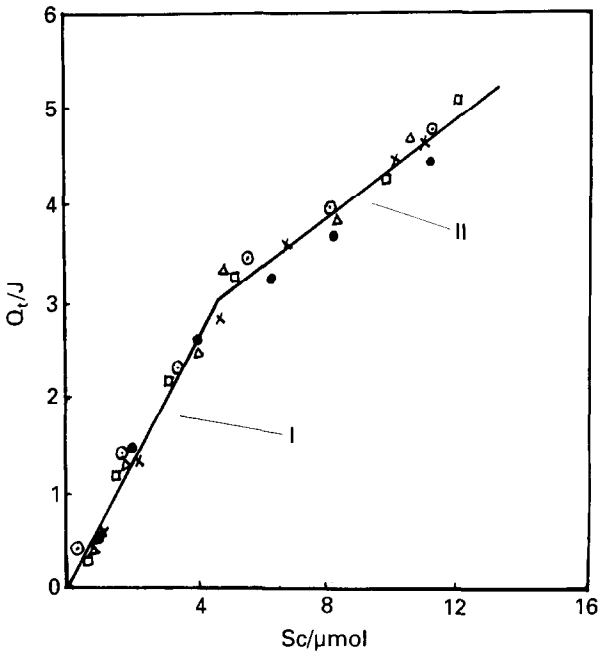

Fig. 3. Q_t vs. S_c plot and the distributions of each experimental points.

Table 3
Data of dry weight of cells vs. the amount of glucose consumed in the growth of *E. coli* [a]

t/min	40	100	140	260	320	380	440	600
$\bar{S}_c$/(μmol ml^{-1})	0.389	0.999	1.610	5.273	8.714	11.962	16.763	20.315
$\bar{W}$/(μg ml^{-1})	70.9	100.3	140.4	334.8	418.0	466.0	519.0	581.0

[a] $\bar{S}_c$ and $\bar{W}$ refer to 1 ml sample in all cases.

data determined in the experiments are shown in Table 3 and the $\bar{W}$ versus $\bar{S}_c$ plot drawn according to these data is shown in Fig. 4. We can see that there are different linear relationships at different phases. Two linear equations obtained by the least squares regression method are

$$W = 54.19S_c + 49.56 \text{ μg ml}^{-1} \quad r = 0.9997$$

for part I and

$$W = 13.56S_c + 300.75 \text{ μg ml}^{-1} \quad r = 0.9955$$

for part II. The unit of the coefficients 54.19 and 13.56 is μg μmol^{-1}. To facilitate discussion the W versus t plot is shown in Fig. 5.

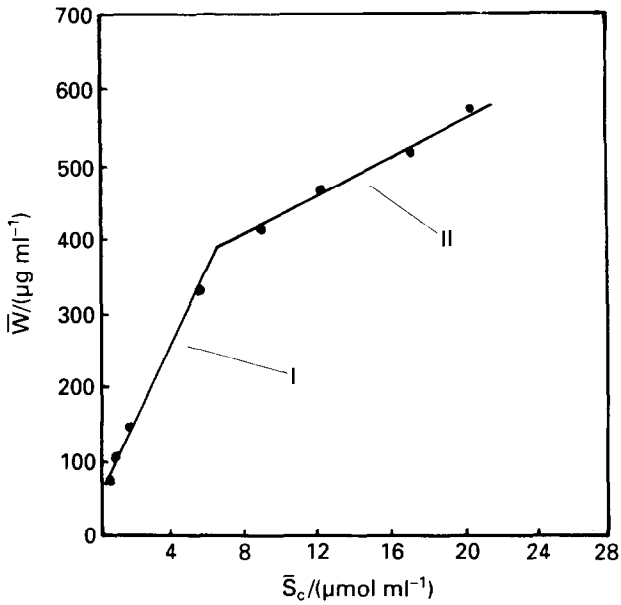

Fig. 4. Dry weight of cells produced $\bar{W}$ vs. glucose consumed $\bar{S}_c$ plot.

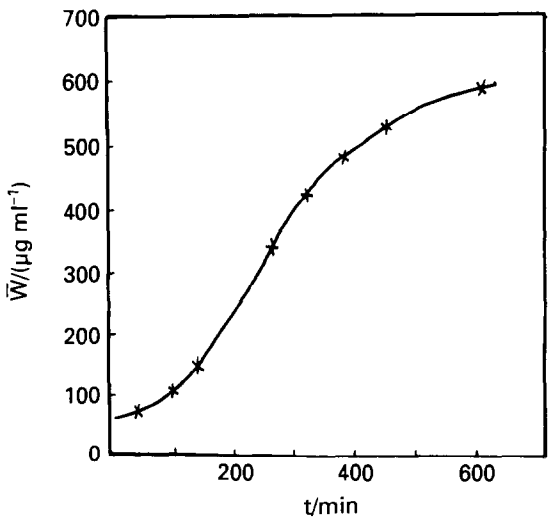

Fig. 5. Dry weight of cells produced $\bar{W}$ vs. time plot.

4. Conclusions

(1) On the Q_t versus S_c plot two sections can be noted, while the quantities of heat evolved are the same. Because the calorimetric study on metabolic processes of

living things is based on the principle that the heat effects change as the metabolic processes and activity vary, the two sections must obviously reflect two different metabolic processes.

(2) From the two linear equations obtained by applying a linear least squares regression treatment to the data of Q_t and S_c, it is found by solving the equations that the point at which they intersect is $S_c = 4.799$ μmol. Fortunately, in experiment 4 we found that the amount of glucose consumed by *E. coli* metabolising at point B ($t = 285$ min) in the thermogram, and $S_c = 4.796$ μmol; the two values are almost identical. This shows that point B is probably the turning point of the two different metabolic processes reflected in Q_t as a function of S_c.

(3) In our experiments glucose is not only the sole carbon source, but also the only energy source. The fact that the straight line in part I passes through the origin shows this distinguishing feature. However the quantities of heat evolved per micromole of glucose consumed in part I are greater than that in part II. The reason for the differences is probably that in part I all the glucose which has entered the cells takes part in the metabolism with no storage, but in part II storage occurs.

(4) The $\bar{W}$ versus $\bar{S}_c$ plot also consists of two straight lines. This indicates that there is a change of cell synthesising power of *E. coli* in the aerobic growth metabolism with glucose as the sole carbon source. We can obtain the point of intersection of these two straight lines by solving the equations. We found that the value of the point of intersection $\bar{W}$ is about 384.5 μg ml^{-1}, and from the $\bar{W}$ versus t plot (see Fig. 5), we can find that $\bar{W}$ is about 380.0 μg ml^{-1} corresponding to the point B (the time is about 290 min) in the metabolism thermogram. This result indicates that point B in the metabolic thermogram is the turning point of cell synthesising power. Hence, the conclusion can be drawn that the different metabolic controlling processes reflected in the variations between Q_t and S_c is just the change of synthesising power of the cells.

(5) The concentration of glucose [S_c] can be calculated from the equations of Q_t as a function of S_c. This will be significant in the research on bacterial growth kinetics measured by microcalorimetry.

Acknowledgement

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