A Microcalorimetric Method for Studying the Biological Effects of Mg^{2+} Ion on Recombinant *Escherichia coli*

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Power-time curves of growing recombinant *Escherichia coli* B1 cell suspensions, treated with different concentrations of Mg^{2+}, were recorded by microcalorimeter. The extent of the stimulatory effect of Mg^{2+} on the growth of recombinant *E. coli* B1 was compared by reference to the changes in the values of the growth rate coefficient of bacteria (k), the time of reaching the maximum effect in the log phase (t_D), the time of maintaining the maximum effect in the stationary period (t_S), and the maximum thermal power during the entire bacterial growth (P_m) at different Mg^{2+} doses and the optimal Mg^{2+} dose was calculated. The experimental results revealed that when Mg^{2+} concentration reached $\gamma = 2.2$ mg mL^{-1}, the stimulatory effect is the greatest. With more Mg^{2+} ($\gamma >$ 2.2 mg mL^{-1}) added, the promotive effect would decrease drastically.

Key words:
Mg^{2+}, microcalorimetry, recombinant *Escherichia coli* B1, metabolism, thermokinetic equation

Introduction

Magnesium ion (Mg^{2+}) is one of the most important mineral salts for the growth of microbes as the activator of many enzymes, so its doses will affect enzyme-catalysis and the turnover efficiency of the enzyme. Therefore, the cell growth, the energy mechanisms and the product of metabolism of microbes will be affected further. The recombinant *E. coli* B1 constructed in our lab[1] can produce a useful collagen, human-like collagen. In order to investigate the effect of Mg^{2+} ion on the growth and metabolism of recombinant *E. coli* B1 deeply and provide enough useful information, we want to find out the relationship between Mg^{2+} doses and the energy mechanisms of recombinant *E. coli* B1 growth.

Microcalorimetry can be used to measure the heat associated with chemical or biochemical reactions.[2,3] It has proven to be a useful tool for examining many types of cellular activities in a wide range of organisms.[4–6] In many circumstances, microcalorimetry can reveal facets of a process that would not be detected by other methods of biochemical analysis.[7] Application of this technique to the study of metabolic processes has also yielded important information, not only thermal data but also kinetic data, and shown the advantages of non-invasive methods for studying metabolism.[8–11] There should be a correlation between energy mechanisms and the growth states of biological systems.[12]

Until now, there have been no reports on the effects of Mg^{2+} concentrations on the growth of recombinant *E. coli* B1 by means of microcalorimetry, so it is of interest to study it. In this paper, the effect of different Mg^{2+} doses on the growth of recombinant *E. coli* B1 was investigated by reference to the changes in the values of k, t_S, t_D and P_m. Based on the data, Mg^{2+} ion has a promotive action on its growth, which can be described greatly by the generalized logistic equation, and the optimal Mg^{2+} concentration was calculated, which is $\gamma =$ 2.09 mg mL^{-1}. The experimental results revealed that Mg^{2+} concentrations increasing from $\gamma = 0$ to 2.2 mg mL^{-1}, had a promoting effect on the growth of cells; when the addition of Mg^{2+} was more than γ = 2.2 mg mL^{-1}, the promotive effect would decrease drastically.

Materials and methods

Chemicals

All chemicals used for this study were A.R. grade and solutions were prepared with double distilled water.

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Bacterial strains

Recombinant *E. coli* B1 used in this study was constructed in our lab and described previously.[1]

Material

Luria-Bertani (LB) medium contained (g L^{-1}): Glucose (10), Yeast extract (5), NaCl (10), tryptone (10), pH 6.5.

Growth medium contained (g L^{-1}): Glucose (30), Yeast extract (50), K_2HPO_4 (8.7), NaH_2PO_4 (4.2), $(NH_4)_2SO_4$ (5.5), EDTA (1.1), trace element (1), pH 6.5. The trace element solution was the same as described in the previous report.[13]

The recombinant strains were cultured in growth medium. The media were sterilized in high-pressure steam at 121 °C and 0.1 MPa for 30 min.

Preparation of the sample

In this type of experiment, the solution of Mg^{2+} was prepared with sterilized distilled water and prepared fresh every time. In the beginning of experiment, stock culture (–70 °C) was activated at 32 °C with LB broth in an orbital shaker (200 rpm) for 10–12 hours to get enough inoculums, which were inoculated into the growth media at a number concentration of $1.18 \cdot 10^7$ cells mL^{-1}. The cell number concentration was examined with a hemocytometer. Finally, the fresh Mg^{2+} solution was added into the cell suspension. The cell growth was measured turbidometrically in a spectrophotometer at λ_{600} nm.

Instruments

The calorimetric measurements of the bacterial growth were performed with a high sensitivity differential scanning calorimeter Micro DSC III (Setaram, France) based on the Calvet principle. The detection limit was ± 0.2 to 2 μW and the baseline stability over 3 days was better than ± 2 μW. The measurements were carried out in stainless "closed, batch" vessels with a volume of V = 1000 μL. The sample and reference vessels were sterilized. The sample ampoule was filled with 300 μL of cell suspension and the reference ampoule was filled with 300 μL of double distilled water. Power-time curves in our experiments were obtained at 34 °C.

Results and discussion

Fig. 1 shows the power–time curves (log phase AB and stationary phase CD) of the growth of recombinant *E. coli* B1 in the presence of Mg^{2+} of different concentrations at 34 °C.

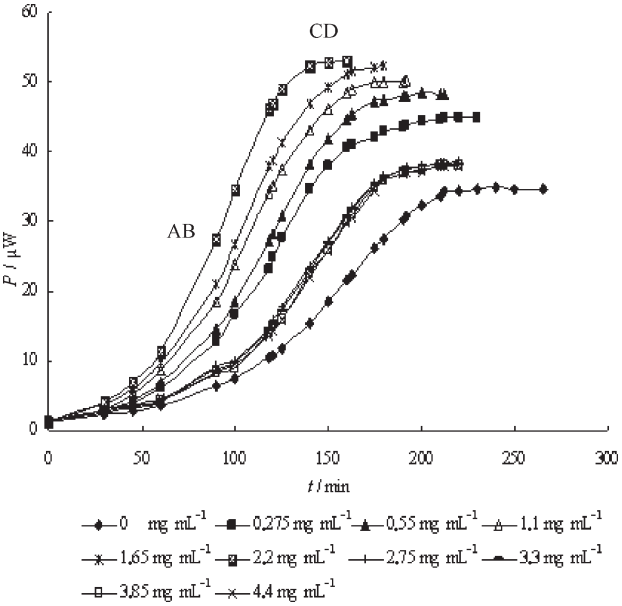

Fig. 1 – *The power–time curves (log phase AB and stationary phase CD) of E. coli B1 in the presence of Mg^{2+} of different concentrations at 34 °C. Inoculation size of E. coli B1 cells was $1.18 \cdot 10^7$ CFU mL^{-1}.*

Determination of thermo-kinetic equation of metabolism and calculation of the growth rate coefficient

It is well known that microorganism growth is often limited and influenced by external conditions. Considering the limitations, the growth process of microorganisms is inhibited and the heat output curve is often incomplete, non-characteristic "S" curves. So, modification and extension were made to the logistic equation in order to explain these curves better. We propose that in the log phase of growth, theoretical model is in accordance with the following generalized logistic equation[14] written as:

$$\frac{dN_t}{dt} = \frac{k N_t (N_m - N_t)}{N_m + v N_t} \qquad (1)$$

where $k > 0$, $N_m > 0$, $v \geq -1$, $N_t \geq 0$. N_t is the number of bacteria at time t, N_m the maximum number of bacteria in the growing period, k the growth rate coefficient of bacteria, t the time, and v a quantity that shows the extent bacteria used the environmental resources (including nutrients).

Under the assumption that the heat production rate P_t is proportional to the number of bacteria N_t,[14]

$$P_0 = w N_0, \quad P_t = w N_t, \quad P_m = w N_m \qquad (2)$$

where w is the thermal power produced by one cell, P_t the thermal power at time t, P_0 the thermal power at time 0, and P_m the maximum thermal power during the entire bacterial growth.

Inserting eq. (2) into eq. (1), we have

$$\frac{dP_t}{dt} = \frac{k\,P_t(P_m - P_t)}{P_m + v\,P_t} \qquad (3)$$

On integrating eq. (3) with respect to t, we can prove that

$$\ln\frac{P_t}{(P_m - P_t)^{v+1}} = \ln\frac{P_0}{(P_m - P_0)^{v+1}} + k(t - t_0) \qquad (4)$$

Using the experimental data of P_t and t obtained from the power-time curves, the growth rate coefficient k and the quantity v can be calculated from regression analysis with eq. (4), and it indicated that the thermal power-time curves of the log phase of growth were matched with eq. (4) greatly, only fitting with a correlation greater than 0.99 was considered. The corresponding results are listed in Table 1 and it is apparent that 0.0180 $min^{-1} \le k \le$ 0.0381 min^{-1} when Mg^{2+} ion varied in the concentration ranges of 0–4.4 mg mL^{-1}, showing during the growth of recombinant *E. coli* B1 in the presence of Mg^{2+}, the heat given out is greater than that of recombinant *E. coli* B1 without Mg^{2+}.

In the stationary phase of growth, Fig. 1 indicated clearly that the metabolic power output was steady, meaning a steady metabolic process took place. The kinetic interpretation of the modulatory effect was carried out by comparing the time of reaching the maximum effect in the log phase (t_D) and the time of maintaining the maximum effect in the stationary period (t_S) at different Mg^{2+}doses for cells. Fig. 2 shows the relations between Mg^{2+} doses and t_D, t_S and P_m. With the concentrations of Mg^{2+} increasing from 0 to 2.2 mg mL^{-1}, the values of t_D and t_S were decreased to a great extent and P_m was enhanced greatly, so it had a promoting effect in this period. When Mg^{2+} concentration reached 2.2 mg mL^{-1}, t_D, t_S and P_m all attained the extremum. With more Mg^{2+} ion (γ > 2.2 mg mL^{-1}) added, the promotive effect would decrease drastically. This probably resulted from the fact that Mg^{2+} ion may deposit when its concentration is higher than an extremum, and so depress its utilization further. But the true regulative mechanism itself is unclear and further study is necessary. During the microorganism growth in the presence of Mg^{2+}, the maximum thermal power (P_m) given out is more than that without Mg^{2+}.

Table 1 – *Equations for power-time curves and mean values of k, v, and R during the growth of E. coli B1 at different Mg^{2+} doses at 34 °C. Cell concentration: 1.18 · 10^7 CFU mL^{-1}. k, v, and R were obtained after fitting the mean P_t and t to Logistic Equation. Values show the mean for 3–5 experiments.*

γ mg mL^{-1}	Equations of power-time curves	k min^{-1}	v	R
0	$\ln\frac{P_t}{(34.5-P_t)^{0.1212}} = 0.0180\,t - 0.1621$	0.0180	–0.8788	0.9982
0.275	$\ln\frac{P_t}{(44.9-P_t)^{0.3454}} = 0.0267\,t - 1.0415$	0.0267	–0.6546	0.9940
0.55	$\ln\frac{P_t}{(48.3-P_t)^{0.3784}} = 0.0283\,t - 1.1945$	0.0283	–0.6216	0.9951
1.1	$\ln\frac{P_t}{(50.2-P_t)^{0.4943}} = 0.0322\,t - 1.6604$	0.0322	–0.5057	0.9939
1.65	$\ln\frac{P_t}{(52.3-P_t)^{0.5472}} = 0.0352\,t - 1.8891$	0.0352	–0.4528	0.9987
2.2	$\ln\frac{P_t}{(53.1-P_t)^{0.5026}} = 0.0381\,t - 1.7216$	0.0381	–0.4974	0.9960
2.75	$\ln\frac{P_t}{(38.8-P_t)^{0.3277}} = 0.0238\,t - 0.9242$	0.0238	–0.6726	0.9970
3.3	$\ln\frac{P_t}{(38.3-P_t)^{0.2964}} = 0.0239\,t - 0.8079$	0.0239	–0.7036	0.9990
3.85	$\ln\frac{P_t}{(38.0-P_t)^{0.2976}} = 0.0238\,t - 0.8098$	0.0238	–0.7024	0.9995
4.4	$\ln\frac{P_t}{(37.9-P_t)^{0.2631}} = 0.0237\,t - 0.6848$	0.0237	–0.7369	0.9960

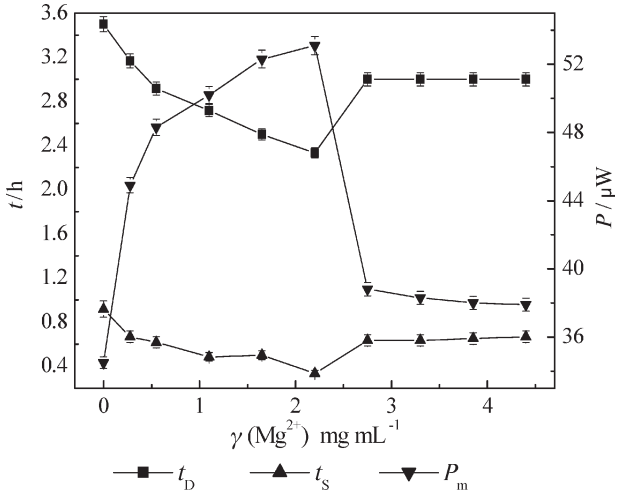

Fig. 2 – *Time of reaching maximum effect (t_D), maintaining maximum effect (t_S) and the maximum thermal output (P_m) at different Mg^{2+}doses*

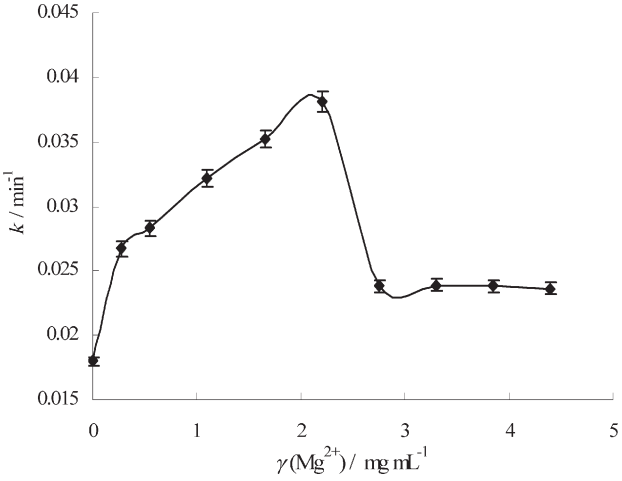

Fig. 3 – *Plot of γ vs k. Symbols show the mean ± standard error for 3–5 experiments.*

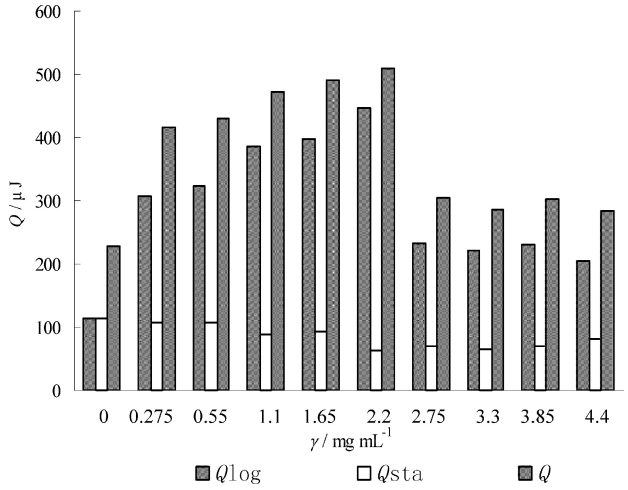

Fig. 4 – *Comparison of total heat of different phases for E. coli B1 in the presence of different Mg^{2+}doses. Cell concentration: 1.18 · 10⁷ CFU mL⁻¹.*

After calculation we noted the experimental data (P_t and t) in this period satisfied eq. (4) highly. So, the generalized logistic equation can greatly describe the power-time curves (log phase and stationary phase) of the growth of recombinant *E. coli* B1 in the presence of $Mg^{2+}$of different concentrations.

Relationship between *k* and concentration of Mg^{2+} γ, and determination of the optimal Mg^{2+} dose

As shown in Fig. 3, the relationship between k and γ (Mg^{2+}) is not linear. With the concentrations of Mg^{2+} increasing, the value of k first increased and then decreased. When the range of concentration of Mg^{2+} is γ = 0.275 to 2.75 mg mL^{-1}, analyses of the growth rate coefficient (k) and the corresponding Mg^{2+} mass concentration (γ), the k versus γ was established:

$$k = -0.0045\gamma^4 + 0.021\gamma^3 - 0.0326\gamma^2 + 0.0256\gamma + 0.0215$$

and R = 0.9919 (0.275–2.75 mg mL^{-1}) (5)

$$\gamma_{opt} = 2.09, \qquad k_{opt} = 0.0384 \text{ min}^{-1}$$

Calculation of the heat

In order to further show the results in a more quantitative way, we calculated the heat (Q_{log}) evolved in the log phases, the heat (Q_{sta}) evolved in the stationary phases and the total thermal effects (Q) from power-time curves of recombinant *E. coli* B1 in the presence of $Mg^{2+}$of different concentrations. These results are shown in Fig. 4.

From Fig. 4, it is clear that the heat (Q_{log}) evolved in the log phases and the total thermal effects (Q) increases while the heat (Q_{sta}) evolved in the stationary phase decreases as the concentrations of Mg^{2+} increase from 0 to 2.2 mg mL^{-1}, which are the same trends as the rate coefficient. For the region of γ = 2.2 to 2.75 mg mL^{-1}, the values of Q_{log} and Q decreased drastically with the increasing mass concentrations of Mg^{2+}, so the growth of cells were inhibited. When the mass concentration lied within γ = 2.75 to 4.4 mg mL^{-1}, all thermal effects (Q_{log}, Q_{sta} and Q) were independent of Mg^{2+} concentrations. In the whole ranges of γ = 0–4.4 mg mL^{-1}, because the total thermal effects (Q) in the presence of Mg^{2+} are much greater than those without Mg^{2+}, it had a promotive effect on the growth of recombinant *E. coli*.

Changes of recombinant *E. coli* B1, and determination of generation time

Culture turbidity was monitored by taking absorbance readings at λ = 600 nm. Fig. 5 shows

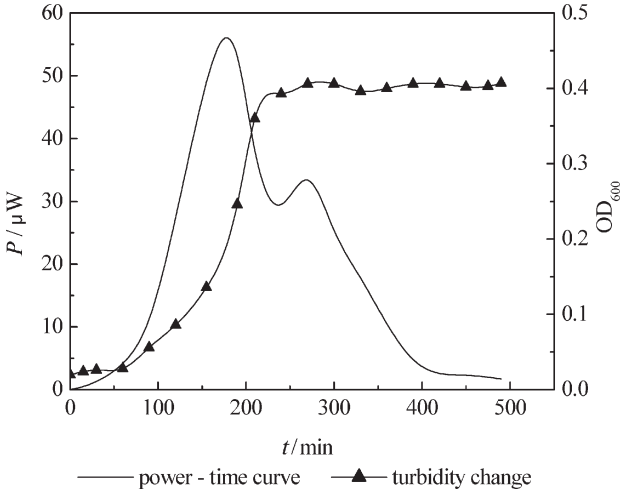

Fig. 5 – *Power-time curves and the corresponding turbidity changes for E. coli B1 in the CFU mL^{-1}*

the typical whole growth of recombinant *E. coli* B1 and the corresponding turbidity-time curves in the presence of Mg^{2+} of γ = 2.2 mg mL^{-1} at θ = 34 °C. It indicates that the results obtained calorimetrically coincide with those of the routine microbiology method and provide more details about the complex bioprocesses. From both, *P-t* and turbidity-time curves, the heat production rate per single cell can be calculated. It rises from a very small figure of $0.11 \cdot 10^{-12}$ W cell^{-1} to $0.77 \cdot 10^{-12}$ W cell^{-1}. The maximum OD_{600} and t_G, which are (ln 2)/k, in the presence of Mg^{2+} of different mass concentrations were also obtained and shown in Table 2. The generation time (t_G) of bacteria is a classic parameter of microbiology. It characterizes the multiplication rate of bacteria. The result indicated that in the range of γ = 0–4.4 mg mL^{-1}, the generation time (t_G) of bacteria first decreased and then prolonged with higher Mg^{2+} mass concentrations added.

Conclusion

During the growth of recombinant *E. coli* B1 in the presence of Mg^{2+}, the heat given out is greater than that without Mg^{2+}. So, Mg^{2+} ion has a promotive effect on its growth, which can be described greatly by the generalized logistic equation. With the concentrations of Mg^{2+} increasing, the stimulative effect first enhanced gradually and then decreased drastically. When Mg^{2+} concentration reached γ = 2.2 mg mL^{-1}, the effect attained the extremum. But the true regulative mechanism itself is unclear and further study is necessary. The relationship between k and γ (Mg^{2+}) is not linear and we calculated the optimal Mg^{2+} dose, which is 2.09 mg mL^{-1}, during the growth of recombinant *E. coli* B1.

Table 2 – *Values of k, the generation time (t_G) and turbidity (OD_{600}) for the growth of E. coli B1 in the presence of Mg^{2+} of different mass concentrations at 34 °C. Inoculation size of E. coli B1 cells was $1.18 \cdot 10^7$ CFU mL^{-1}. Values show the mean ± standard error for 3–5 experiments.*

γ mg mL^{-1}	k min^{-1}	t_G min	OD_{600}
0	0.0180 ± 0.0003	38.51 ± 0.65	0.244 ± 0.001
0.275	0.0267 ± 0.0005	25.96 ± 0.50	0.382 ± 0.001
0.55	0.0283 ± 0.0002	24.49 ± 0.18	0.390 ± 0.001
1.1	0.0322 ± 0.0006	21.53 ± 0.41	0.395 ± 0.002
1.65	0.0352 ± 0.0003	19.69 ± 0.17	0.403 ± 0.001
2.2	0.0381 ± 0.0005	18.19 ± 0.24	0.407 ± 0.001
2.75	0.0238 ± 0.0005	29.12 ± 0.63	0.296 ± 0.001
3.3	0.0239 ± 0.0007	29.00 ± 0.88	0.296 ± 0.002
3.85	0.0238 ± 0.0004	29.12 ± 0.50	0.295 ± 0.001
4.4	0.0237 ± 0.0003	29.25 ± 0.37	0.293 ± 0.001

In conclusion, our experiments show that microcalorimetic investigations on the stimulatory effects of Mg^{2+} on microorganisms are possible and promising. We believe that microcalorimetry is a useful, accurate system for studying the detailed mechanisms of microorganisms, which provides important information for microbiological research. All this information is significant for the synthesis of human-like collagen.

ACKNOWLEDGEMENTS

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List of symbols

k – growth rate coefficient, min^{-1}
N – number of bacteria
P – power, μW
Q – heat, μJ
R – determination coefficient
t – time, min
V – volume, μL
w – thermal power by one cell, μW
γ – mass concentration, g L^{-1}
θ – temperature, °C
λ – wave length, nm
v – extent bacteria

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