Study of Thermokinetic Properties of Sodium Selenite on *Bacillus thuringiensis* Cry B by Microcalorimetry

LI, Xi[a,b](李曦) LIU, Yi*,[a](刘义) ZHAO, Ru-Ming[c](赵儒铭) YU, Zi-Niu[d](喻子牛)
QU Song-Sheng[a](屈松生)

[a] *Colloege of Chemistry and Environment Science, Wuhan University, Wuhan, Hubei 430072, China*
[b] *School of Material Science and Engineering, Wuhan University of Technology, Wuhan, Hubei 430070, China*
[c] *College of Life Science, Wuhan University, Wuhan, Hubei 430072, China*
[d] *Agriculture Microbiology Laboratory, Huazhong Agriculture University, Wuhan, Hubei 430070, China*

By using an LKB2277 Bioactivity Monitor, the power-time curves of *Bacillus thuringiensis* Cry B at 28℃ effected by Na_2SeO_3 were determined. Some parameters, such as growth rate constants k, inhibitory ratio I, the maximum heat production rate P_{max}, heat output Q, were obtained. Considering both the growth rate constant k and heat output Q, we found that a low concentration of Na_2SeO_3 had a promoting action on the growth of *Bacillus thuringiensis* Cry B, but a high concentration of Na_2SeO_3 had an inhibitory action. The toxicity of a toxicant can also be expressed as half inhibitory concentration IC_{50} of toxicant, *i.e.*, 50% effective in this inhibition. The value of IC_{50} of Na_2SeO_3 on *Bacillus thuringiensis* Cry B is 117 μg/mL. This microcalorimetric bioassay for cellular toxicity is based on metabolic heat evolution from cultured cells. The assay is quantitative, inexpensive, and versatile.

Keywords *Bacillus thuringiensis* Cry B, Na_2SeO_3, microcalorimetry, metabolic action

Introduction

At present, metabolic event concerns with protecting environment, such as chemical effects on the environment and threats to human health. However, agriculture, industry and many other fields need chemicals. A practical resolution to these conflicting interests requires accurate toxicological information. Acute toxicity test is the most important. An acute toxicity study can establish the relationship between the dose of a toxicant and its effect on the tested organism. The toxicity of substances can be expressed as LC_{50}, IC_{50}, or EC_{50} values. The accurate measurement of the effects of potential toxic materials depends on the reproducibility of acute toxicity tests.[1]

Bioenergetic investigations, which should be the most important in the field of the assessment of harmful properties of substances in ecotoxicology, are closely related to the applicability of the calorimetry in biology because there is scarcely another method to analyze metabolic activities possessing such general validity as calorimetry.[1] In a living system, all the metabolic processes occurring within the cells produce heat. Continuous measurements of heat during microbial growth enable the assessment of the main metabolic process in a cellular culaical, therefore, microcalorimetry is a valuable tool for the control of a biological process. Power-time curves contain a lot of kinetic information. By analysis of the power-time curves, we have studied microbial metabolism and the effect of toxic agents on microbes.[2-4]

Selenium presents a nutrition conundrum through its

* E-mail: liuyi@chem.whu.edu.cn; Fax: (027) 87647617.
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dual status as an essential and highly toxic nutrient. From early days in this century, selenium has been known to cause toxicity in animals producing conditions, such as blind staggers and alkali disease.[5] In 1957, Schwardz and Foltz demonstrated trace elements of selenium protected against liver necrosis in vitamin E-deficient rats and established nutritional essentiality.[6,7] Selenium deficiency has been indicated as one of the causes of two human diseases found in China with particularly low soil selenium: the cardiomyopathy, Keshan disease, and the Kaschin-Beck disease, involving osteoarthropathy.[8,9] In the experimental models of insects, selenium deficiency resulted in impaired mitochondria substrate oxidation and lowered thiol level.[10] Selenium status has a very close relationship with a wide range of disorders, including heart disease, cancer, and acquired immunodeficiency syndrom(AIDS).[11,12] Since selenium is an essential and toxic nutrient, studying the effect of selenium on microbe can help elucidate the effect on the biological and environmental processes.

Bacillus thuringiensis is one of the biggest-production microbial insecticide instead of chemical insecticide. It is widely applied to control crop pests, forestry and horticulture pests, medical and storage pests, and get remarkable ecological benefit. Therefore the study of the *Bacillus thuringiensis* metabolic process is important for the research of action mode of *Bacillus thuringiensis*. In this work, the power-time curves of the metabolism of *Bacillus thuringiensis* Cry B and the effect of Na_2SeO_3 were investigated using an LKB-2277 Bioactivity Monitor.

Experimental

Instruments

An LKB-2277 Bioactivity Monitor, manufactured by LKB corporation of Sweden, was used to measure heat output of the metabolism of *Bacillus thuringiensis*. The microcalorimeter was thermostated at 28.00 ℃. The voltage signal was recorded by means of an LKB-2210 recorder (1000 mV range). The baseline stability was 0.2 μW/24 h. The details of the performance and structure of the instrument was described in Ref. 13.

Materials

Bacillus thuringiensis Cry B was provided by Agriculture Microbiology Laboratory, Huazhong Agriculture University, Wuhan 430070, China. The medium contains NaCl (5 g), peptone (10 g) and beef extract (5 g) per 1000 mL (pH = 7.0—7.2). It was sterilized in high-pressure steam at 120℃ for 30 min. Na_2SeO_3 (analytical grade) was supplied by the Second Chemical Reagent Factory of Shanghai.

Method

The metabolic power-time curves of *Bacillus thuringiensis* Cry B were recorded using flow-through method. Firstly, the flow cell was cleaned and sterilized. The procedure was: sterilized distilled water, 0.1 mol/L NaOH, 75% alcohol solution, 0.1 mol/L HCl and sterilized distilled water were pumped by an LKB-2132 microperplex peristaltic pump through the cell, each for 15 min at a flow rate of 50 mL/h.

Once the system had been cleaned and sterilized and the baseline had been stabilized, 50 mL of bacterial suspension and Na_2SeO_3 were pumped through the cycle-flow system with an LKB-2132 perplex peristaltic pump at a flow rate of 20 mL/h. The temperatures of the calorimeter system and the isothermal box were maitained at 28.00℃. in the meantime the LKB2210 recorder recorded the power-time curves of *Bacillus thuringiensis* Cry B growth continuously.

Results and discussion

Power-time curves

The metabolic power-time curve of *Bacillus thuringiensis* Cry B is shown in Fig. 1, and Fig. 2 shows the metabolic curves of *Bacillus thuringiensis* Cry B with different concentration of Na_2SeO_3.

From Figs. 1 and 2, it can be seen that the power-time curves contained two distinct phases: the first reflects the growth phase, and the second phase corresponds to the sporulation process.[14]

These curves show that a low concentration of Na_2SeO_3 has a promoting action on the growth of *Bacillus thuringiensis* Cry B, and a high concentration of Na_2SeO_3 has an inhibitory action. This result is in agreement with the previous report.[15]

Thermokinetics

These metabolic power-time curves indicate that the

log phase of the growth power-time curves obey the following equation:

$$\ln P = kt + \ln P_0$$

Using this equation, the growth rate constants k of all experiment curves were calculated and the generation times (G), which equal to $(\ln 2)/k$, were also obtained. Corresponding k and G are shown in Table 1.

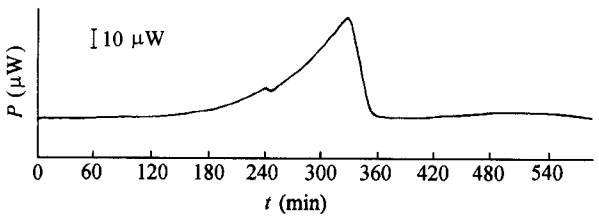

Fig. 1 Metabolic power-time curves of *Bacillus thuringiensis* Cry B at 28.00℃.

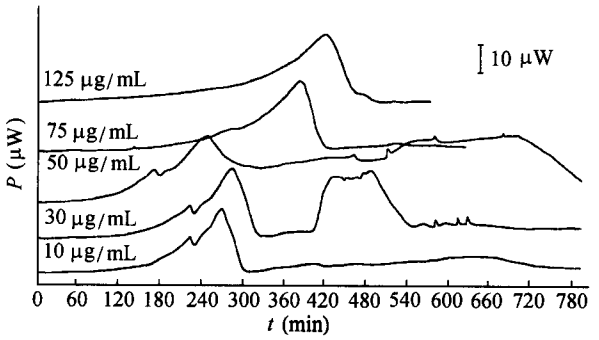

Fig. 2 Metabolic power-time curves of *Bacillus thuringiensis* Cry B with different concentration of Na_2SeO_3 at 28.00℃.

Inhibitory ratio I and the half inhibitory concentration IC_{50}

Inhibitory ratio I is defined as:

$$I = [(k_0 - k_\rho)/k_0] \times 100\%$$

where k_0 is the rate constant of the control, k_ρ is the rate constant of bacterial growth inhibited by inhibitor when concentration is ρ. The values of I are also shown in Table 1.

When inhibitory ratio I is 50%, the corresponding concentration of inhibitor is called the half inhibitory concentration IC_{50}. IC_{50} can be regarded as inhibiting concentration causing a 50% decrease of *Bacillus thuringiensis* Cry B growth rate constant in the log phase. From the data in Table 1, we can obtain the value of the half inhibitory concentration of Na_2SeO_3, (IC_{50}), which is equal to 117 μg/mL.

Table 1 Parameters of *Bacillus thuringiensis* Cry B growth in the solutions with different concentration of Na_2SeO_3 at 28℃

ρ (μg/mL)	k (min^{-1})	I (%)	Q_f (J)	Q_s (J)
0	0.02055	—	1.413	0.181
10	0.02124	-3.3	1.020	1.154
30	0.02247	-9.3	1.192	2.076
50	0.02842	-38.3	1.451	5.515
75	0.01533	25.4	1.686	0.066
125	0.00995	51.6	1.860	0.024

Heat output Q

The area under the curves is the heat output released by *Bacillus thuringiensis* Cry B during the metabolic progress. There are two peaks in the metabolic power-time curve of the bacterial, and this indicates that there are two stages of releasing heat. We calculated the releasing heat output of the two stages: the first heat output (Q_f) and the second output (Q_s), respectively. The values of the heat output are shown in Table 1.

Relationship between the growth rate constant k and the concentration of Na_2SeO_3 (ρ)

The data in Table 1 show that the growth rate constant k changes with an increase in the concentration of Na_2SeO_3, k increases with the concentration in the range of 0—50 μg/mL Na_2SeO_3 and decreases with the concentration in the range of 50—125 μg/mL Na_2SeO_3. Values of k are correlated to the concentration of Na_2SeO_3 (ρ) as

$$k = 0.01866 + 1.795 \times 10^{-4}\rho, \text{ and}$$
$$R = 0.9349 \ (\rho: 10—50\ \mu g/mL)$$

$$\ln k = 0.8138 - 1.133 \ln\rho, \text{ and}$$
$$R = 0.9860 \ (\rho: 50—125\ \mu g/mL)$$

Relationship between the concentration of Na_2SeO_3 (ρ) *and the first heat output* (Q_f)

Analysis of the values of the concentration of $Na_2SeO_3(\rho)$ and the first heat output (Q_f) shows that

the values of Q_f increase with the addition of Na_2SeO_3. Values of Q_f are corrected to the concentration of Na_2SeO_3 (ρ) as

$$Q_f = 1.008 + 7.483 \times 10^{-3}\rho, \text{ and}$$
$$R = 0.9669 \ (\rho: 10—125 \ \mu g/mL)$$

Discussion

Direct microcalorimetry of continuous cultures is a fast response technique to determine the toxic properties of chemicals and seems to be qualified for a feed forward control strategy within biological savage treatment. Calorimetry can enhance the accuracy of the determination of the physiological activity of the culture. The calorimetric determination of toxicity results in lower as well as higher values of standard data within the range of variances of toxicity determinations by different laboratories using one standard method.[16]

In the present study, analysis of the power-time curves of *Bacillus thuringiensis* Cry B affected by Na_2SeO_3 indicated that low concentration of Na_2SeO_3 (10—50 $\mu g/mL$) had promoting action on *Bacillus thuringiensis* Cry B, but high concentration of Na_2SeO_3 (50—125 $\mu g/mL$) inhibited action on *Bacillus thuringiensis* Cry B. The factors that determine the characteristics of a dose-response curve are the drug's mode of action in cells, its number of target sites, and its affinity for those target sites. Selenium is an active center of glutathione peroxidase (GSH-Px), which can catalyze and decompose lipid hydroperoxide or hydrogen peroxide.[17,18] At a low concentration, selenium can decompose reactive oxygen radical and hydroxyl radical, and therefore prevent the oxidative damage; but at a high concentration, selenium can catalyze the production of reactive oxygen radical resulting in the oxidative damage. In this study, the growth of *Bacillus thuringiensis* Cry B was inhibited by selenite excess probably through the catalysis of oxidation reactions of SH groups to S—S or S—Se—S bonds. During this process, more active free radicals may be produced that further damage the membrane structure and functions of cells.

Results from the microcalorimetric investigations of the metabolism of *Bacillus thuringiensis* Cry B effected by Na_2SeO_3 have shown that the calorimetry is a power tool for the monitoring and controlling the growth process of microbes. The very broad application range for non-specific methods like calorimetry can be attractive both in thermodynamic measurements and in analytical work. As pratically most processes are accompanied by heat effect, calorimetry is particularly well suited to the discovery of unexpected or unknown processes in samples of any aggregation state. By combining calorimetry and other specific methods, several different and important goals may be reached.

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