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Microcalorimetric Study on the Toxic Effect of Pb^{2+} to *Tetrahymena*

Liu Peng · Zhang Weiying · Li Xi · Chen Xiaojuan · Zhang Chaocan

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Abstract The toxic effect of Pb^{2+} has been studied in eukaryotic cells by using *Tetrahymena* as a target. The maximum power (P_m) and the growth rate constant (k) were determined, which showed that values of P_m and k were linked to the concentration (C) of Pb^{2+}. The addition of Pb^{2+} caused a decrease of the maximum heat production and growth rate constant, indicating that *Tetrahymena* growth was inhibited in the presence of Pb^{2+}, and Pb^{2+} took part in the metabolism of cells. From micrographs, morphological changes of *Tetrahymena* were observed with addition of Pb^{2+}, indicating that the toxic effect of Pb^{2+} derived from destroying the membrane of surface of *Tetrahymena*. According to the thermogenic curves and photos of *Tetrahymena* under different conditions, it is clear that metabolic mechanism of *Halobacterium halobium* R1 growth has been changed with the addition of Pb^{2+}.

Keywords Pb^{2+} · Toxic effect · *Tetrahymena* · Inhibitor · Membrane

Introduction

Tetrahymena, as a kind of ciliated protozoa, belongs to a free-living, freshwater genus that is highly successful ecologically. Its ultrastructure, cell physiology, development, biochemistry, genetics, and molecular biology have been extensively investigated [1–3]. The richness of *Tetrahymena*'s biology makes it a unicellular animal model organism "for all seasons". As a eukaryotic monocellular animal, *Tetrahymena* is often used as a biological indicator in biological and environmental study [4]. Consequently, it has been

L. Peng (✉) · Z. Weiying · L. Xi · Z. Chaocan
Department of Applied Chemistry, School of Sciences, Wuhan University of Technology, Wuhan 430070, People's Republic of China
e-mail: liup567@163.com

C. Xiaojuan
Institute of Hydrobiology, Chinese Academy of Science, Beijing, People's Republic of China

widely used as a "test animal" to evaluate, among others, the effects of toxic substances, nutrients, antibiotics and anticancer medicaments.

Lead is naturally present in the environment and probably has been a source of pollution for centuries. In more recent time, lead from a number of other sources has become a hazard. Besides leaded gasoline, still in use in some countries, has resulted in a drastic increase of lead particles in air. Soil and water pollution from industry and discarded batteries are still worldwide environmental problems [5, 6].

As to the complex living system, microcalorimetry can record easily its specific thermogenetic curves, which offers not only thermal data but also kinetic data. The thermal characteristic may be helpful for studying the metabolism differences. In this study, the toxic effect of Pb^{2+} on the eukaryotic organism was investigated using *Tetrahymena* as the target.

Materials and Methods

Materials

Cells and Reagents

Tetrahymena thermophila BF_1 was provided by East China normal university. The culture medium containing the Tryptone 15 g (Oxoid), yeast extract 5 g (Oxoid), and glucose 1 g per 1,000 mL. It was sterilized in high pressure steam at 121°C for 20 min. Analytical grade reagent lead nitrate was purchased from China Medicine (Group) Shanghai Chemical Reagent Corporation.

Instruments and Methods

Microcalorimetric Measurement

TAM Air (Thermometric AB, Sweden), which is an eight-channel heat conduction microcalorimeter for measurements in the milliwatt range, was designed to monitor continuously heat released or absorbed in a series of processes, such as metabolism of cells. The performance and the details of this instrument have been described previously [7].

Initially, *Tetrahymena* was inoculated in the prepared culture medium, which was put into the ampoule at once. Then, Pb^{2+} solution of different concentrations (0, 0.1, 0.2, 0.3, 0.5, 1.0 μg/mL) were added into the culture medium, respectively. The metabolic thermogenic curves of *Tetrahymena* were recorded using ampoule method (one datum per min). One sealed ampoule contained a reference solution such as the cultural medium, the other ampoule contained the sample. The sample normally occupied position A in the monitor and reference occupied B. The temperature of all calorimetric experiments was 28°C.

Calculation of the Growth Rate Constant of Halobacterium Halobium

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

$$n_t = n_0 \exp(kt) \tag{1}$$

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

$$n_t w = n_0 w \exp(kt) \tag{2}$$

$$P_0 = n_0\ w \text{ and } P_t = n_t\ w$$

$$P_t = P_0 \exp(kt) or \ln P_t = \ln P_o + kt \tag{3}$$

The growth thermogenic curves of the log phase correspond to Eq. 3. At the same time, in accordance with the data, $\ln P_t$ and t, taken from the curves to fit a linear equation, we can obtain the growth rate constant (k). Thermokinetics equations were shown in Table 1.

Microscopic Observation

Four groups of *Tetrahymena* were observed by Axioplan 2 imaging and Axiophot 2 universal microscope (Carl Zeiss) after addition of Pb^{2+} solution of 0, 0.3, 0.5, 1.0 μg/mL, respectively.

Results

Growth Thermogenic Curve of Tetrahymena

The curve in Fig. 1 is typical for the growth metabolism of *Tetrahymena*, from which we can see that the metabolic process can be divided into four parts: lag phase, log phase, stationary phase, and decline phase.

The curves in Fig. 2 have shown the effects of Pb^{2+} on *Tetrahymena* growth metabolism. Clearly, the heat output of *Tetrahymena* growth decreased as Pb^{2+} was added in the medium.

Thermokinetic Equation

According to the method in Peng et al. [8], the following thermokinetic equation (Eq. 1) can be applied to processing growth parameters.

$$\ln\ P_t = \ln\ P_0 + k\ t$$

Table 1 Kinetic Data of *Tetrahymena* Growth

Pb concentration/μg/mL	Kinetic equation	k/min^{-1}	P_m/mW
0	$\ln P_t = -4.33 + 2.28 \times 10^{-3} t$l	2.28×10^{-3}	0.3542
0.1	$\ln P_t = -3.81 + 1.83 \times 10^{-3} t$	1.83×10^{-3}	0.28336
0.2	$\ln P_t = -2.98 + 1.57 \times 10^{-3} t$	1.57×10^{-3}	0.22669
0.3	$\ln P_t = -2.68 + 1.60 \times 10^{-3} t$	1.60×10^{-3}	0.21252
0.5	$\ln P_t = -3.31 + 1.29 \times 10^{-3} t$	1.29×10^{-3}	0.14168
1.0	——	0	0

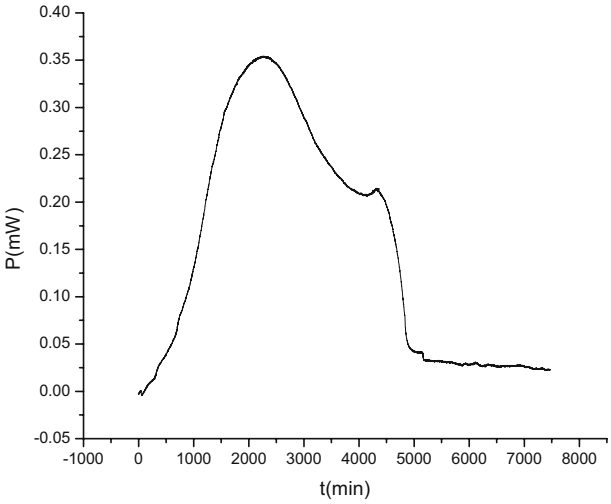

Fig. 1 Metabolic thermogenic curve of *Tetrahymena*

where P_t and P_0 are the power output at time t and 0, k is the growth rate constant. Thermokinetics equation was shown in Table 1 of *Tetrahymena* growth.

Relationship Between Pm and Concentration

The toxic effect of Pb^{2+} was concentration-dependent. With the addition of Pb^{2+}, The maximum power (P_m) and the growth rate constant (k) were increased. There was a linear relation among them, which was shown in Fig. 3 and the following equations.

$$k = 0.0216 - 0.0021C \tag{4}$$

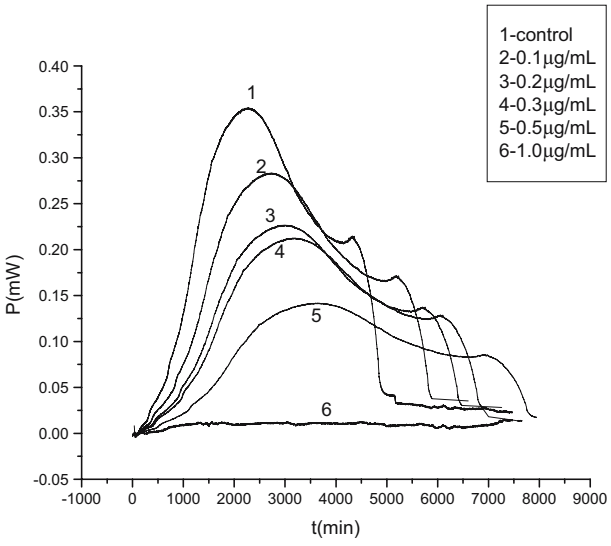

Fig. 2 Metabolic thermogenic curves of *Tetrahymena* growth affected by Pb^{2+}

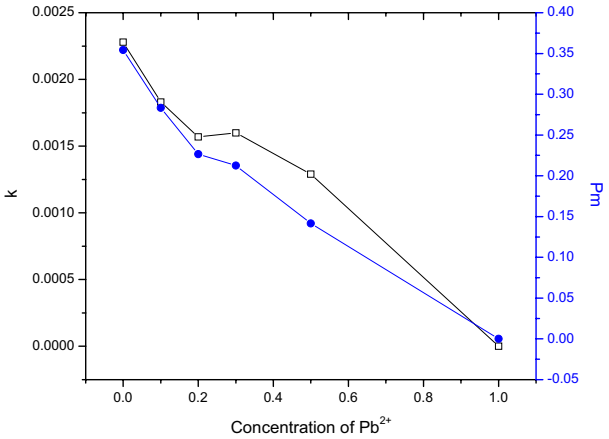

Fig. 3 Relationship between P_m and Pb^{2+} concentration

$$P_m = 0.320 - 0.333C \tag{5}$$

Inhibitory Ratio and Half-Inhibitory Concentration

The inhibitory ratio can be defined as [8]:

$$I = [(k_0 - k_c)/k_0] \times 100\% \tag{6}$$

where k_0 is the rate constant of the control, and k_c is the rate constant for *Tetrahymena* growth in the presence of an inhibitor with a concentration of C. Their relationship is shown in Fig. 4

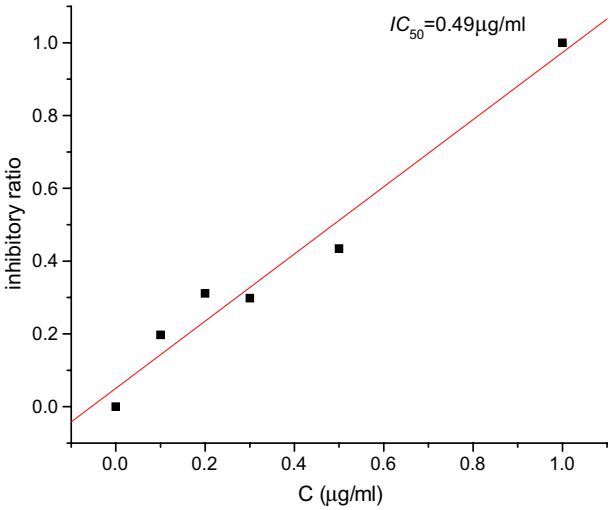

Fig. 4 Relationship between inhibitory ratio and the concentration of Pb^{2+}

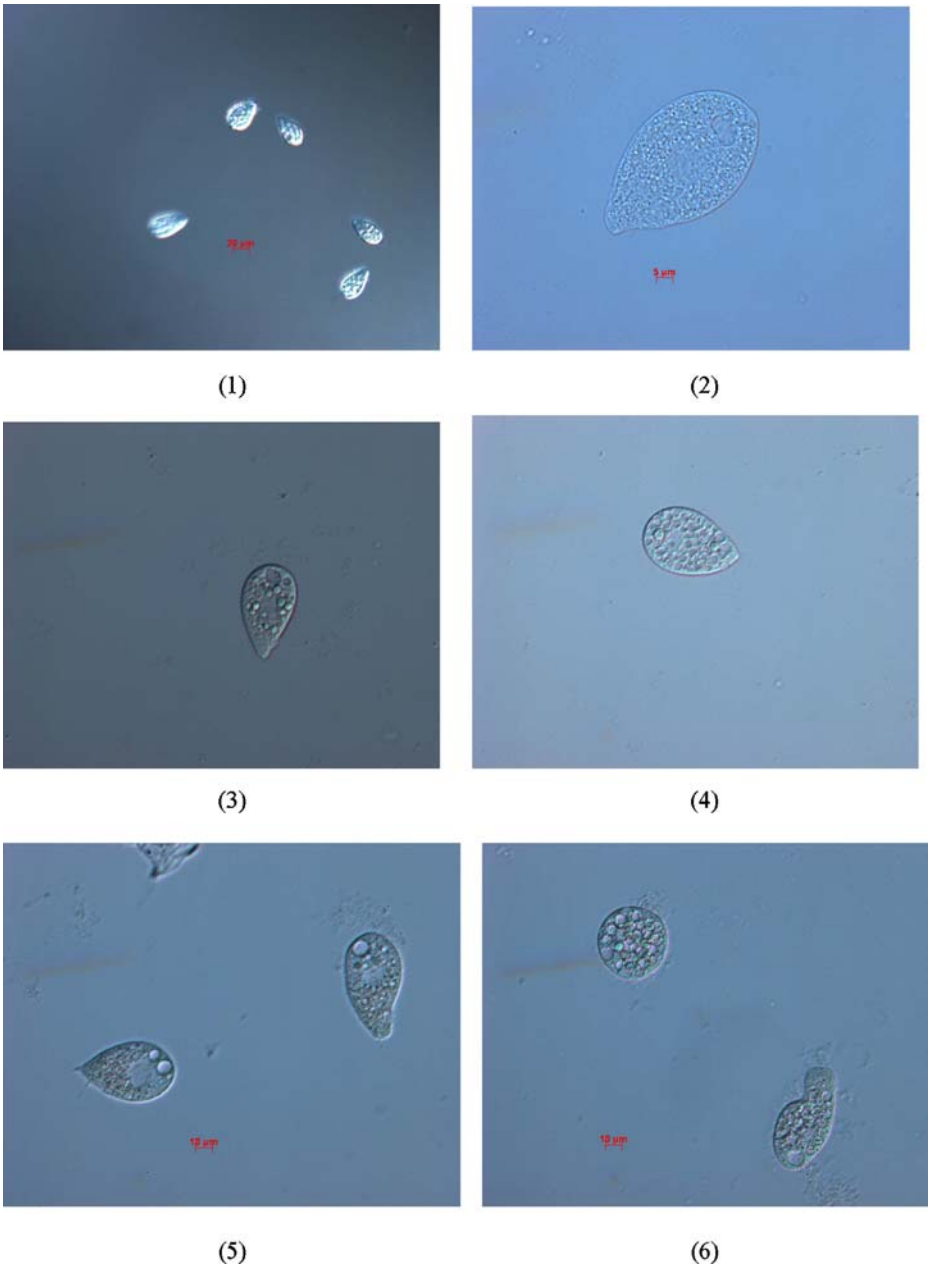

Fig. 5 Micrographs of *Tetrahymena* with addition of Pb^{2+} *1*and *2*—Control; *3* and *4*—0.3 μg/mL; *5* and *6*—0.5 μg/mL; *7* and *8*—1.0 μg/mL

When the inhibitory ratio (*I*) is 50%, the corresponding half-inhibitory concentration of the inhibitor can be represented as IC_{50}. IC_{50} can be regarded as the inhibiting concentration causing a 50% decrease of the growth rate constant. From Fig. 3 and Table 1, we can obtain directly that IC_{50} is about 0.49 μg/mL.

Fig. 5 (Continued)

Morphological Change

Microscopic method (Axioplan 2 imaging and Axiophot 2 universal microscope, Carl Zeiss, Hong Kong) was used to monitor the change of *Tetrahymena* under the presence of La^{3+}. Figure 5 displayed the morphological changes of *Tetrahymena* in the progress of growth with addition of different concentrations of Pb^{2+}.

Discussions

Comparing Fig. 1 with Fig. 2, we can see that the growth curves differ from each other when different amounts of Pb^{2+} was added. Clearly, Pb^{2+} has taken part in the metabolism of *Tetrahymena* growth. From the growth curves under different conditions, to some degree, we can throw light on metabolic mechanism of microorganism.

To analyze the results, the maximum power (P_m) and the growth rate constant (k) were determined, which show that values of P_m and k are linked to the concentration of Pb^{2+}. With the addition of Pb^{2+}, both the maximum heat production and growth rate constant decreased. Meanwhile, the prolonged lag phase indicated that *Tetrahymena* lose the ability to adapt themselves to environment. According to values of those growth parameters, it can be seen that Pb^{2+} has an inhibitory effect on *Tetrahymena.* With the increasing concentration of inhibitor, the inhibitive effect becomes more and more obvious. If the concentration increases to 1.0 μg/mL, growth of *Tetrahymena* was inhibited completely, and IC_{50} is about 0.49 μg/mL.

Being a eukaryotic monocellular animal, *Tetrahymena* often was used as a biological indicator in biological and environmental study. Inhibitive effect of toxic metal ions, such as Mn^{2+}, La^{3+}, on *Tetrahymena*, has been reported [9, 10]. Compared with these results, the value IC_{50} of Pb^{2+} was much lower than other metal ions, suggesting that Pb^{2+} has more formidable toxic effects and is of more threat to human health when released into the environment.

Through the imaging microscope, we took the photos of *Tetrahymena* when different concentrations of Pb^{2+} was added afterwards. According to Fig. 5 (1) and (2), the shapes of *Tetrahymena* were normal without Pb^{2+}, and the individuals swam freely. When Pb^{2+} was

added, their shape had a little change and the swimming rate slowed down, which was shown in Fig. 5 (3) and (4). Furthermore, when the concentration of Pb^{2+} was more than 0.5 μg/mL, they could not swim any more, and their membrane had been affected. Furthermore, it could be observed that the membrane of *Tetrahymena* had been affected because void space appeared on the surface of *Tetrahymena.* The degree of closeness of membrane decreased, and its surface was hollowed-out. Permeability of membrane of *Tetrahymena* has been changed. As a result, absorption of nutrients and discharge of waste will be affected. The growth of *Tetrahymena* was undoubtedly affected in the presence of Pb^{2+}. When the concentration of Pb^{2+} increased to 1.0 μg/mL, the morphology experienced an obvious change, and some of them broke into pieces. The morphological changes, with the addition of Pb^{2+}, indicate that the toxic effect of Pb^{2+} on *Tetrahymena* was derived from destroying the membrane of the surface.

To conclude, The microcalorimetric method requires only an observable difference between the power production in the treated and controlled incubations. Unlike many other procedures, transparent solution is not required. Colored or turbid solution, even suspension, can be put into the calorimeter. That approximates more closely the in vivo state than many other techniques do. Comparing with the thermocurves of *Tetrahymena* growth, microcalorimetry is a more simple, more sensitive, and more economic technique in the study among eukaryotic cells. Certainly, it is safe too. The appropriate thermokinetic parameters could quantitatively study the distances among *Tetrahymena* and overcome the difficulties of operation of eukaryotic cells.

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