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Microcalorimetry studies on the antibacterial effect of crude monkshood polysaccharide*

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Abstract: In this paper, crude monkshood polysaccharide was isolated from Radix Aconiti Lateralis Preparata. The effects of crude monkshood polysaccharide on *Escherichia coli* and *Staphylococcus aureus* were investigated by microcalorimetry. The power-time curves of the bacterial growth at various concentrations (c) of crude monkshood polysaccharide were plotted with a TAM air isothermal microcalorimeter at 37 °C. The growth rate constant (μ), inhibitory ratio (I), peak-height (P_m), and peak-time (t_m) were calculated. From the data, the relationship between μ and c also was established. The growth rate constant μ decreased with the increasing concentrations of crude monkshood polysaccharide. Moreover, P_m reduced and t_m increased with increasing concentrations. The experimental results revealed that crude monkshood polysaccharide had inhibitory activity towards *S. aureus* and *E. coli*. Results obtained from our study strongly suggest that microcalorimetry is a fast, simple, and more sensitive technology that can be easily performed to study the effect of drugs on bacteria.

Key words: Crude monkshood polysaccharide, Microcalorimetry, *Escherichia coli*, *Staphylococcus aureus*, Inhibitory
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1 Introduction

Radix Aconiti Lateralis Preparata (also known as monkshood or aconite), is the daughter root of *Aconitum carmichaeli* Debx. It is a traditional Chinese medicinal (TCM) herb that belongs to the family of Ranunculaceae. It has been reported that Radix Aconiti Lateralis Preparata presents some pharmacological activities, including reviving yang for resuscitation, alleviating pain, anti-inflammatory activity, hypoglycemic effects, and so on (China Pharmacopoeia Committee, 2005). In recent research, more attention has been paid to the polysaccharides. Some activities of crude monkshood polysaccharide have been reported, including immunological activity (Li *et al*., 2008), anti-cancer activity (Ren *et al*., 2008), and hypoglycemic effect (Konno *et al*., 1985; Yu and Wu, 2009). Few studies concerning the effect of crude monkshood polysaccharide on bacteria have been reported. Thus, our aim is to investigate this further.

Microcalorimetry, as a quantitative, inexpensive, and versatile method for measuring heat production, has been successfully applied in biochemistry, biophysics, and environmental sciences in recent years (Wadsö, 1995; 1997; 2001). It allows for interaction in a heterogeneous medium, monitoring the process without disturbing the system, measuring the thermal effect of the system, and giving abundant thermodynamic and kinetic information (Critter *et al*., 2001; Yan *et al*., 2007). It is a good method to study the effect of drugs on bacteria. The heat output can be recorded in real time. Then the power-time curves of bacteria can be plotted. Using a mathematical growth model, a series of kinetic parameters can be calculated, such as the growth rate constant (μ), generation

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time (t), inhibitory ratio (I), minimal inhibitory concentration (MIC), peak-height (P_m), peak-time (t_m), and total heat (Q_{tot}). Microcalorimetry appears as a suitable technique to study the microbial activity (Li *et al*., 2001; 2002; Wadsö, 2002; Wu *et al*., 2005; Kong *et al*., 2008). The purpose of this study is to investigate the effect of crude monkshood polysaccharide on *Escherichia coli* and *Staphylococcus aureus* by microcalorimetry.

2 Materials and methods

2.1 Instrument

A 3114/3236 TAM air microcalorimeter (Thermometric AB, Sweden) was used to determine the metabolism of *E. coli* and *S. aureus*. The TAM air microcalorimeter is an eight-channel heat conduction calorimeter for heat flow measurements under isothermal conditions. Reactions could be carried out in the temperature range 5 to 90 °C. The temperature error was ±0.02 °C. The detection limit was 2 μW and baseline stability was 2×10^{-6} μW over a period of 24 h (Kong *et al*., 2008; Yang *et al*., 2008).

2.2 Materials

Crude monkshood polysaccharide was isolated from Radix Aconiti Lateralis Preparata through water extraction and alcohol precipitate, and sequentially purified through Sevage method and dialysis (Yang *et al*., 2007). It was a white powder, easily soluble in water.

Bacteria *E. coli* (CMCC (B) 44102) and *S. aureus* (CMCC (B) 26003) were used as the tested bacteria, provided by the Shandong Institute for Drug Control. They were routinely cultured in a Luria-Bertani (LB) culture medium, which contained 5 g/L NaCl, 5 g/L yeast extract, and 10 g/L peptone. Medium pH was adjusted to 7.0–7.2. The LB culture medium was sterilized by autoclaving at 121 °C for 20 min before the experiment.

2.3 Methods

The microcalorimetric measurement was made with the ampoule method. The LB culture medium, containing bacteria, was placed in 20 ml glass ampoules. Then different concentrations of crude monkshood polysaccharide were inoculated into each ampoule with a final volume of 10 ml. One ampoule without crude monkshood polysaccharide was used as the blank control. The ampoules were sealed and put into an eight-channel calorimeter block. The temperature was controlled at 37 °C. The power-time signals were recorded at an interval of 1 min until the recorder returned to the baseline (Burt, 2004; Yang *et al*., 2008).

3 Results and discussion

3.1 Thermokinetics

In the growth phase, theoretical model is in accordance with the following law (Burt, 2004):

$$\mathrm{d}N_t/\mathrm{d}t=\mu N_t-\beta N_t^2, \quad (1)$$

where N_t is the bacterial number at time t, μ is the growth rate constant, and β is the fungi static rate constant. The integral of Eq. (1) is given by

$$N_t=K/(1+\alpha e^{-\mu t}), \quad (2)$$

where K is the maximum density, and α is integral constant. If the power produced by every bacterium is P, then we can obtain $PN_t=KP/(1+\alpha e^{-\mu t})$. Making $P_t=PN_t$ and $P_m=PK$, where P_t is the power output at time t, and P_m the maximum power output, then Eq. (2) can changed to

$$P_t=P_m/(1+\alpha e^{-\mu t}). \quad (3)$$

Eq. (3) is the logistic equation. According to the data P_t and t obtained from the bacterial growth curves, the rate constant μ can be calculated.

The inhibitory ratio I is an excellent index to indicate the inhibition of crude monkshood polysaccharide on *E. coli* and *S. aureus*, and it can be defined as:

$$I=[(\mu_0-\mu_c)/\mu_0]\times100\%, \quad (4)$$

where μ_0 and μ_c are the growth rate constants of bacteria without and with crude monkshood polysaccharide, respectively.

The corresponding values of μ, t_m, P_m, and I are shown in Table 1.

Table 1 Parameters of bacteria growth at different concentrations of crude monkshood polysaccharide

Bacteria	c (mg/ml)	μ (min^{-1})	r	t_m (min)	P_m (mW)	I (%)
E. coli	0	0.3584	0.9998	80	1.2313	
	0.1	0.3387	0.9998	81	1.2226	5.50
	0.4	0.3000	0.9997	89	1.1938	16.29
	0.8	0.2770	0.9997	98	1.1678	22.71
	1.6	0.2516	0.9997	100	1.1410	29.80
	2.0	0.2341	0.9998	111	0.9403	34.68
S. aureus	0	0.2380	0.9998	120	0.4860	
	0.2	0.2068	0.9997	122	0.4757	13.11
	0.4	0.1734	0.9997	133	0.4438	27.14
	0.8	0.1380	0.9996	140	0.4226	42.02
	1.6	0.1256	0.9998	143	0.4006	47.22
	2.0	0.1150	0.9999	154	0.3900	51.68

c: concentration of monkshood polysaccharide; μ: growth rate constant; r: correlation coefficient; t_m: peak-time value; P_m: maximum heat output; I: inhibitory ratio

3.2 Power-time curves

The power-time curves of *E. coli* and *S. aureus* growths of black control were plotted in Fig. 1. We could see that the shapes of the curves were different. The focal points were power-time curves for the exponential growth, which were plotted in Fig. 2. It could be seen that the increase of the heat output became slower with increasing concentration of crude monkshood polysaccharide. High concentration of crude monkshood polysaccharide needed more time to reach the same heat output.

3.3 Relationship between μ and c

The values of the growth rate constant μ in Table 1 showed that crude monkshood polysaccharide had potent antibacterial activity against *E. coli* and *S. aureus*. The relationships between μ and c were demonstrated in Fig. 3. The μ of the bacterial growth declined with increasing of the c. That was mainly because some bacteria were killed, and some metabolized continuously at a lower heat production rate. This rate directly depended on the concentration of crude monkshood polysaccharide. The correlations between μ and c could be formulated according to following equations: for *E. coli*: $\mu=-0.0324c^3+0.1252c^2-0.1822c+0.3569$ (r=0.9993); for *S. aureus*: $\mu=-0.0325c^3+0.1433c^2-0.2189c+0.2406$ (r=0.9984).

3.4 Relationship between I and c

According to Eq. (4) and the relation between μ and c, the inhibitory ratio I was calculated. As shown in Table 1, the inhibition ratio increased with increasing concentration of crude monkshood polysaccharide. It demonstrated that crude monkshood polysaccharide had inhibitive effect on *E. coli* and *S. aureus*. At the same concentration, the inhibitory ratio I was larger on *S. aureus* than on *E. coli*. The phenomenon illustrated that crude monkshood polysaccharide had a greater inhibitive effect on *S. aureus* than on *E. coli*.

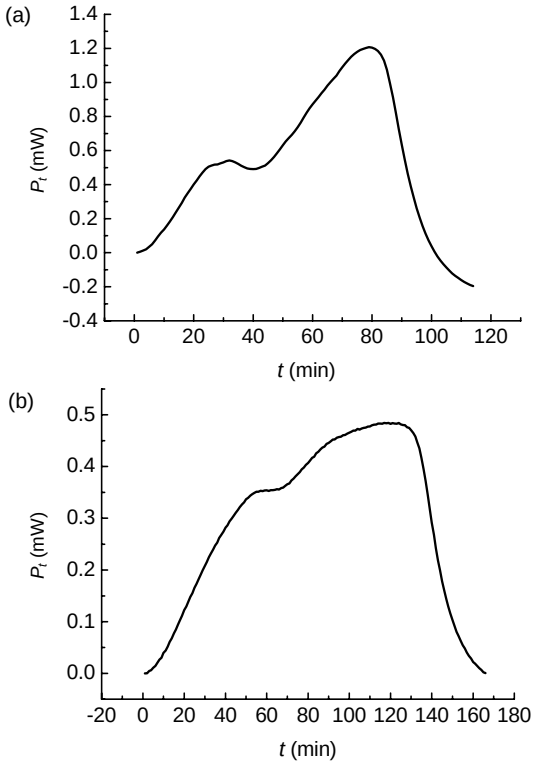

Fig. 1 Power-time curves of *E. coli* (a) and *S. aureus* (b)

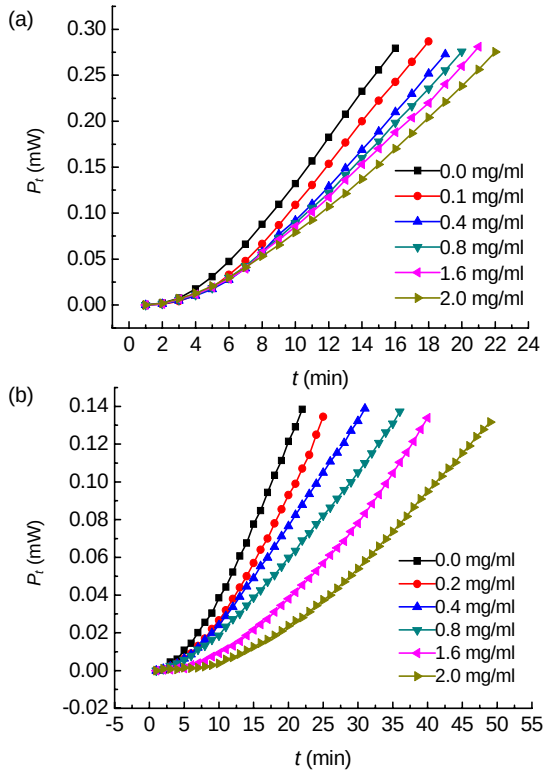

Fig. 2 Power-time curves for the exponential growths of *E. coli* (a) and *S. aureus* (b) affected by various concentrations of crude monkshood polysaccharide

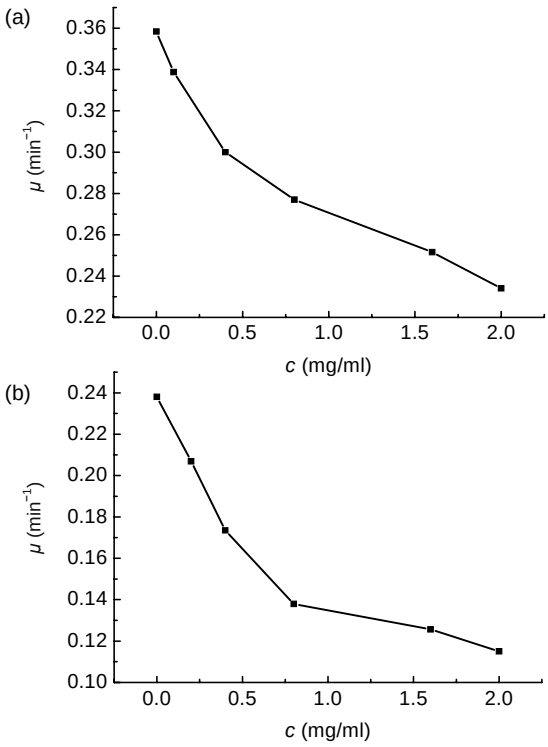

Fig. 3 Relationship between rate constant μ and concentration c for *E. coli* (a) and *S. aureus* (b)

3.5 Relationship between P_m and c

From Table 1, it could be seen that the max heat output P_m with crude monkshood polysaccharide was less than that of blank control, and a decrease in the value of P_m was observed when c was increasing. The main reason could be that the number of the survivors decreased with increasing concentration of crude monkshood polysaccharide. There was a similar effect on *E. coli* and *S. aureus*. The results indicated that crude monkshood polysaccharide had an inhibitory effect on the growths of *E. coli* and *S. aureus*.

3.6 Relationship between t_m and c

Experimental results for the t_m as a function of c are presented in Table 1. The peak-time t_m of crude monkshood polysaccharide was longer than that of blank control. With increasing concentration of crude monkshood polysaccharide, the t_m prolonged. This was mainly because, after the treatment with crude monkshood polysaccharide, there was a partial inhibition of the bacteria and the remaining survivors maintained growth and metabolized at a lower rate. There was a similar effect on *E. coli* and *S. aureus*. The results also indicated that crude monkshood polysaccharide had an inhibitory effect on the growths of *E. coli* and *S. aureus*.

4 Conclusions

As shown in this work, the inhibitory effect of crude monkshood polysaccharide on the growths of *E. coli* and *S. aureus* was found by microcalorimetry. The power-time curves of *E. coli* and *S. aureus* at different concentrations of crude monkshood polysaccharide were plotted. The parameters such as the growth rate constant (μ), inhibitory ratio (I), peak-height (P_m), and peak-time (t_m) were calculated. The relationships between μ and c were also established. The μ decreased with increasing concentration of crude monkshood polysaccharide. Crude monkshood polysaccharide showed stronger inhibitory effect on *S. aureus* than on *E. coli*. Microcalorimetry is a useful technique that can be applied to study microbial growth and estimate the efficiency of drugs.

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