Journal of Thermal Analysis and Calorimetry, Vol. 93 (2008) 2, 417–421

MICROCALORIMETRIC STUDIES ON THE ANTIMICROBIAL ACTIONS OF DIFFERENT CEPHALOSPORINS

L. N. Yang[1,2], *F. Xu*[1,*], *L. X. Sun*[1], *Z. B. Zhao*[3] *and C. G. Song*[4]

[1]Materials and Thermochemistry Laboratory, Dalian Institute of Chemical Physics, Chinese Academy of Sciences 457 Zhongshan Road, Dalian 116023, P.R. China
[2]Graduate School of the Chinese Academy of Sciences, Beijing 100049, P.R. China
[3]Division of Biotechnology, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, 457 Zhongshan Road Dalian 116023, P.R. China
[4]Dalian Institute of Drug Detection, Dalian 116021, P.R. China

Microcalorimetry was applied to study the effect of cephalosporins (cefazolin sodium and cefonicid sodium) on the *E. coli* growth. The microbial activity was recorded as power–time curves through an ampoule method with a TAM Air Isothermal Microcalorimeter at 37°C. The parameters such as the growth rate constant (k), inhibitory ratio (I), the maximum power output (P_m) and the time corresponding to the maximum power output (t_m) were calculated. The change tendencies of k, with the increasing of concentration (C) of the two cephalosporins, are similar which show that cefazolin sodium and cefonicid sodium have the same inhibitory mechanism. The experimental results reveal that cefonicid sodium has a stronger antibacterial activity towards *E. coli* than that of cefazolin sodium and this was coincide with the clinical manifestations.

Keywords: *cefazolin sodium, cefonicid sodium, cephalosporins, E. coli DH5α, inhibition, microcalorimetry*

Introduction

The cephalosporins are the semi-synthetic antibiotics which were originally derived from the fungus cephalosporium acremonium about sixty years ago [1]. As a result of modification of the active nucleus 7-aminocephalosporanic acid side-chains, there are four generations of cephalosporins [2, 3]. Each newer generation of cephalosporins has significantly greater gram-negative antimicrobial properties than the preceding generation, in most cases with more extended spectrum activity. The mechanism of bactericidal action is cephalosporins can disrupt the synthesis of the peptidoglycan layer of bacterial cell walls.

Cefazolin sodium is the first-generation cephalosporin antibiotic which can eliminate infections that are caused by bacterial including lung, skin, bone, joint, stomach, blood, heart valve and urinary tract infections; it is also useful in preventing infection during surgery and patients mildly allergic to penicillin [4, 5]. As one of the second-generation cephalosporins, cefonicid sodium is effective against a wide range of gram-positive and gram-negative bacteria, and it is useful for anaerobic infections, gonorrhea and diabetic foot infections [6, 7]. The two antibiotics have been recognized in the United States Pharmacopeia. Since each individual antibiotic acts in a different way and may be effective against either a broad spectrum or a specific type of disease-causing agent, so selectively using antibiotics has become more importance, and more advanced information on antibiotics need to be better understood.

As a non-specific technique, microcalorimetry was confirmed to be valid as an alternative method in the study of metabolism of the cell and the effect of drugs on cell metabolism [8–11]. It is a useful tool for investigating the biological processes because it permits the continuous monitoring of the activity of a living process in situ without disturbing the system, and the heat evolved or adsorbed is strictly proportional to the rate of the biological processes [12–15]. With its abundant thermodynamic and kinetic information, microcalorimetry has been widely applied in clinical analysis, pharmacology, ecology, biotechnology and agriculture [16–21].

In the present work, we attempt to investigate the inhibitory effects of the cephalosporins (cefazolin sodium and cefonicid sodium) on *E. coli* metabolism by microcalorimetry. From the power–time curves, the growth rate constant (k), the maximum power output (P_m) and the time corresponding to the maximum power output (t_m) were calculated. According to the relationships between k, P_m, t_m and cephalosporin concentration (C), the effects of the inhibition of the two kinds of cephalosporins on the metabolism of *E. coli* were deeply studied. The results could provide more information to the clinic application.

* Author for correspondence: fenxu@dicp.ac.cn

1388–6150/$20.00

Akadémiai Kiadó, Budapest, Hungary
Springer, Dordrecht, The Netherlands

Experimental

Materials

Escherichia coli (*E. coli*) strain DH5α was used as the test organism, obtained from the Biomass Conversion Technology Group (Dalian Institute of Chemical Physics, CAS, Dalian 116023, P.R. China). The *E. coli* DH5α was routinely cultivated on Luria–Bertani (LB) culture medium consisting of tryptone 1 mass%, yeast extract powder 0.5 mass%, NaCl 1 mass% (pH=7.0–7.2), and the procedures have been reported previously [22]. The LB culture medium was sterilized by autoclaving at 121°C for 20 min before the experiment.

The cefazolin sodium and the cefonicid sodium were kindly afforded by Dalian Institute of Drug Detection, P.R. China.

Methods

Microcalorimetric experiments were performed on a TAM Air Isothermal Calorimeter (Thermometric AB, Sweden), which equipped with eight twin calorimetric channels with one side for the sample and the other for a static reference. Measurements were carried out in sealed 20 mL glass ampoules. The signal generated was recorded in-situ by a computer. The structure and operation of the instrument have been described previously [10, 23].

The microcalorimetric measurement was performed using ampoule method. The cephalosporins-containing LB culture mediums with a volume of 10 mL were placed in 20 mL glass ampoules which have been sterilized beforehand. Then 200 μL *E. coli* suspension (optical density is about 0.5 at λ=600 nm) were inoculated into each ampoule. After that, the ampoules were sealed with a cap and placed into the microcalorimeter. The microcalorimeter was controlled at 37°C by thermostat and the power–time signals were recorded at an interval of 1 min.

Results and discussion

Thermokinetics

In the logarithmic phase of growth, the power–time curves of *E. coli* obey the exponential equation [24]:

$$\ln P_t = \ln P_0 + kt \quad (1)$$

where P_0 and P_t are the power output at time 0 and t, respectively, k is the constant of cell growth rate. According to Eq. (1), the rate constant k of cell growth could be calculated according to the data P_t and t obtained from the power–time curves, and these values are given in Table 1.

The inhibitory ratio (I) is a good indicator of the inhibition of cephalosporin on metabolism of *E. coli*, and it can be defined as:

$$I = [(k_0 - k_c)/k_0] \cdot 100\%$$

where k_0 and k_c represent respectively the rate constant of *E. coli* growth without and with effect of the cephalosporin. When the inhibitory ratio (I) is 50%, the corresponding half-inhibitory concentration of the cephalosporin is expressed as IC_{50}. It can be regarded as the inhibiting concentration causing a 50% decrease of the *E. coli* growth rate constant. The calculated results are also exhibited in Table 1.

Table 1 Parameters of *E. coli* growth at different cephalosporins

Cephalosporins	C/μg mL^{-1}	k/10^{-3} min^{-1}	P_m/μW	t_m/min	I/%	IC_{50}/μg mL^{-1}
Cefazolin sodium	0	1.85	542.7	2271	0	
	0.1	1.55	498.7	2347	16.3	
	1.0	1.20	558.6	2462	35.1	
	1.5	1.14	630.4	2692	38.4	2.3
	2.1	1.05	702.2	4240	43.2	
	3.0	0	–	–	100	
Cefonicid sodium	0	1.85	542.7	2271	0	
	0.1	1.18	507.5	2286	43.8	
	0.3	0.71	386.5	2856	61.6	
	0.4	0.66	421.9	3189	64.6	0.2
	0.5	0.52	357.7	4026	71.9	
	0.6	0.49	273.3	4824	73.6	
	1.0	0	–	–	100	

k – growth rate constants, P_m – maximum power output, t_m – the time of P_m, I – inhibitory %

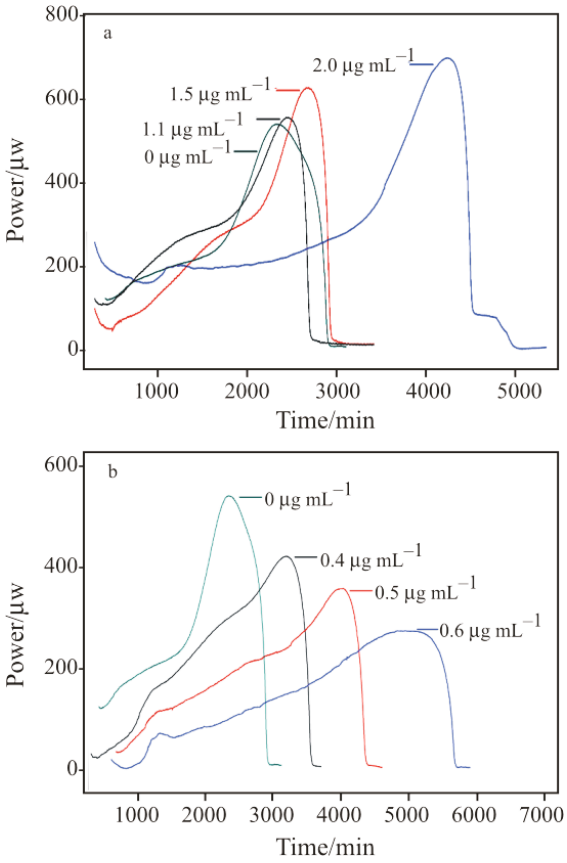

Fig. 1 Power–time curves for growth of *E. coli* affected by various concentrations of cephalosporins: a – cefazolin sodium, b – cefonicid sodium

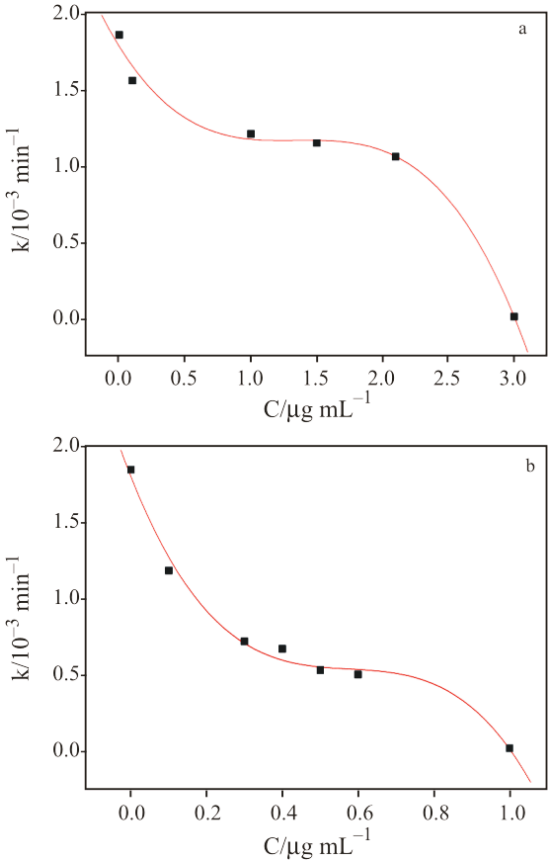

Fig. 2 Change of the growth rate constant (k) with change of cephalosporins concentration (C): a – cefazolin sodium, b – cefonicid sodium

Power–time curves

The power–time curves from the two kinds of cephalosporins (cefazolin sodium and cefonicid sodium) are plotted in Fig. 1. Difference of the inhibitory action of the two antibiotics on *E. coli* growth can be clearly seen. The heights of the peaks of the curves in Fig. 1a ascend with the cefazolin sodium concentration increasing, and the shape of the curves changed little after the addition of high concentration of cefazolin sodium. From Fig. 1b, it shows the heights of the peaks gradually descend with increasing the concentration. The time t_m corresponding to the maximum power output are all prolonged with an increase in the concentration of the cephalosporins.

Relationship between k and C

Table 1 and Fig. 2 demonstrate the effects of various concentrations of cephalosporins (cefazolin sodium and cefonicid sodium) on the heat production rate in *E. coli* cells. The k of the *E. coli* growth gradually declines with the increase of the C. This is mainly because after addition of the cephalosporins into the *E. coli* suspension, some cells were inhibited or killed, but the survivors do metabolize continuously which maintain a lower level of the heat production rate, and this level is directly depending on the concentration of cephalosporins. When the concentration reached 3.0 and 1.0 μg mL^{-1} for cefazolin sodium and cefonicid sodium, respectively, k decrease steeply to 0 and no growth were observed in the experimental period. Figures 2a and 2b show that their k change trends are similar. The k values decrease sharply in the low and high concentration, but in the middle concentration range (1.0–2.1 μg mL^{-1} for cefazolin sodium, 0.3–0.6 μg mL^{-1} for cefonicid sodium), it reduces slowly. The result suggests that cefazolin sodium and cefonicid sodium may have the same inhibition mechanism on the *E. coli* growth. The correlations between k and C could be formulated according to the following equations:

For cefazolin sodium:

$$k = -0.2587C^3+1.043C^2-0.3934C+1.773$$
$$R^2=0.9918$$

For cefonicid sodium:

$$k = -6.238C^3+10.79C_2-6.362C+1.802$$
$$R^2=0.9917$$

According to the relation between k and C for cefazolin sodium and cefonicid sodium, the inhibitory ratios (I) were calculated (Table 1). And the IC_{50} of cefazolin sodium and cefonicid sodium are 2.3 and 0.2 μg mL^{-1}, respectively. The result indicated that cefonicid sodium has a stronger inhibition effect than that of cefazolin sodium on *E. coli* growth.

Relationship between t_m and C

Experimental results for the t_m as function of C are presented in Fig. 3. An increase in the values of t_m is observed when C is increasing. The main reason for this could be that after the treatment with higher concentration of cephalosporins, there was a partial inhibition of the cell and the rest survivors were maintained growing and metabolizing at slower rates. For the cefazolin sodium, when its concentration range is below 1.5 μg mL^{-1}, the t_m ascends mildly; when the C reaches 2.1 μg mL^{-1}, the postponement of the t_m is largish. And for cefonicid sodium, the t_m increases with the C increase. It implied that its inhibition toward *E. coli* growth is more stable than that of cefazolin sodium. From the data in Table 1, the relationship between t_m and C can be described as:

For cefazolin sodium:

$$t_m=807.3C^3-1823C^2-1211C+2259$$
$$R^2=0.9998$$

For cefonicid sodium:

$$t_m=8494C^2-853.9C+2280$$
$$R^2=0.9963$$

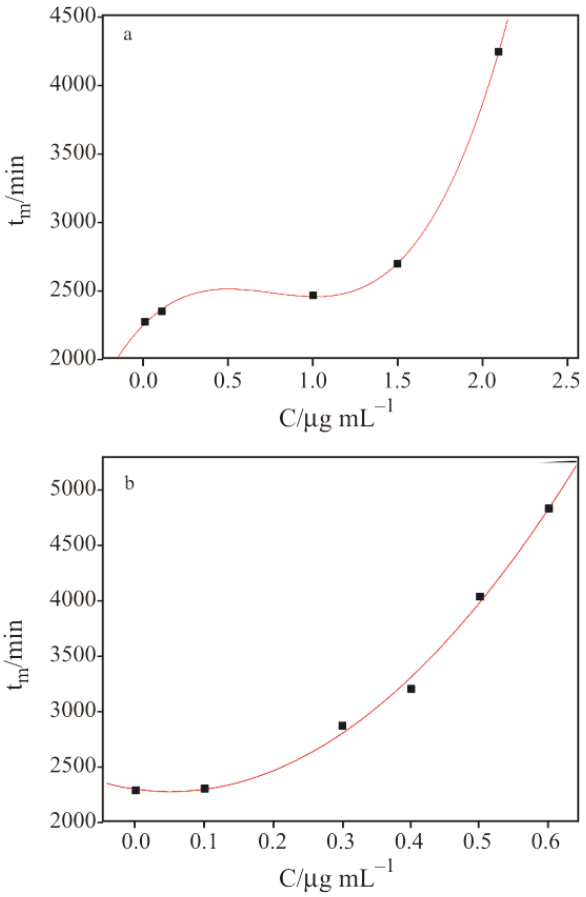

Fig. 3 The relation of the time corresponding to the maximum power output (t_m) and cephalosporins concentration (C): a – cefazolin sodium, b – cefonicid sodium

Relationship between P_m and C

The values for the P_m obtained from the power–time curves are summarized in Table 1. As seen from the table, with the increasing of cefazolin sodium concentration, the P_m elevates gradually except a lower value in the concentration of 0.1 μg mL^{-1}. The explanation for this phenomenon may be that the inhibition is only temporary, and the bacterial can start growing again, resulting in an increased metabolism with the higher maximum power output [25]. In the case of cefonicid sodium, a decrease in the P_m values with increasing C was observed. This may attribute to that the number of the survivors is small and decreases with increasing the cefonicid sodium concentration. Above results also indicated that cefonicid sodium has a stronger inhibition on the growth of *E. coli* than that of cefazolin sodium.

Conclusions

As shown in this work, the inhibitory effects of two different generations of cephalosporins on the growth of *E. coli* were studied by microcalorimetry. Comparison of the C–k curves of the two kinds of cephalosporins, it can be concluded that the two cephalosporins have the same inhibition mechanism on the metabolism of *E. coli*. Moreover, according to the relationship between t_m, P_m and C, one can know that they have their own characteristics. In addition, the IC_{50} of cefazolin sodium and cefonicid sodium are 2.3 and 0.2 μg mL^{-1}, and their lethal doses are 3.0 and 1.0 μg mL^{-1}, respectively. These values suggest that the cefonicid sodium has a stronger inhibition effects than that of cefazolin sodium on *E. coli* growth, which is in good agreement with the clinical manifestations. The work also proved that the microcalorimetry is a useful technique that can be applied to study microbial growth and estimate the efficiency of drugs.

Acknowledgements

The authors gratefully acknowledge the National Natural Science Foundation of China for financial support to this work under Grant No. 50671098, 20473091 and 20573112.

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Received: August 6, 2007
Accepted: September 12, 2007
OnlineFirst: January 27, 2008

DOI: 10.1007/s10973-007-8680-9