Action of the Selenomorpholine Compounds on the Bacterium Growth by Microcalorimetry

LI, Xi*,[a,b](李曦) LIU, Yi[b](刘义) WU, Jun[b](吴军) QU, Song-Sheng[b](屈松生)

[a] *State Key Laboratory of Advanced Technology for Materials Synthesis and Processing, Wuhan University of Technology, Wuhan, Hubei 430070, China*

[b] *Department of Chemistry, Wuhan University, Wuhan, Hubei 430072, China*

The action of β-(*N*-selenomorpholine) ethyl phenyl ketone hydrochloride and 4-(*N*-selenomorpholine)-2-butanone hydrochloride on *Escherichia coli* and *Staphylococcus aureus* was studied by microcalorimetry. Differences in their capacities to affect the metabolism of this bacterium were observed. The kinetics shows that the selenomorpholine compounds had action on the metabolism process of *Escherichia coli* and *Staphylococcus aureus*. The rate constant (k) of the studied bacterium in the presence of the drugs are concentration-dependant. The growth rate constants decrease with an increase in the mass of the selenomorpholine compounds, but their relationship is different. As deduced from the rate constant (k) of the studied bacterium (in log phase) and the half inhibitory concentration (IC_{50}), the experimental results reveal that the studied selenomorpholine compounds all have good antibiotic activity and better antibacterial activity on *Staphylococcus aureus* than on *Escherichia coli*.

Keywords selenomorpholine compounds, bacterium, metabolism, antibacterial activity, microcalorimetry

Introduction

Selenium is one of the fourteen known essential biotrace elements.[1] The bioactivity of selenium has developed rapidly[2-5] since it was found to be an active center of glutathione peroxidase (GSH-Px), which can catalyze and decompose lipid hydroperoxide or hydrogen peroxide.[6,7] The antibacterial and antifungal activities of selenium have also been studied. Our previous studies show that the antimicrobial activity of some selenomorpholine compounds is better than that of sodium selenite.[8] The purpose of this paper is to investigate the action of β-(*N*-selenomorpholine) ethyl phenyl ketone hydrochloride and 4-(*N*-selenomorpholine)-2-butanone hydrochloride on the bactrium by means of microcalorimetry.

Microcalorimetry provides a general analytical tool for the characterization of the microbial growth process. It has been used extensively to investigate the interaction between drug and the microbial cell leading to useful information.[9-12] One of the most prominent features of the microbial growth process is the production of heat. If the heat is monitored by microcalorimeter, useful information, both qualitative and quantitative, may be obtained. Each type of microbial has a unique power-time trace, recordable by the microcalorimeter under a defined set of growth conditions. Any substance that can modify the metabolic growth processes involved in cell will change the power-time curve obtained from the microcalorimeter. From the power-time curves, both thermodynamic data and kinetic data can be obtained.

In this paper, the power-time curves produced by *Escherichia coli* and *Staphylococcus aureus* under the action of the selenomorpholine compounds of different concentrations were determined with a LKB-2277 Bioactivity Monitor. From these power-time curves (log phase) the growth rate constant, k, and the generation time, G, classic parameter of microbiology, were calculated. Ac-

* E-mail: lixi68@sina.com

Received November 27, 2001; revised March 15, 2002; accepted May 6, 2002.

Project supported by the National Natural Science Foundation of China (Nos. 29973030 and 30170010) and Chenguang Project Foundation of Wuhan (No. 20025001009).

cording to the k-ρ relationship, the antibacterial activity of the selenomorpholine compounds was evaluated.

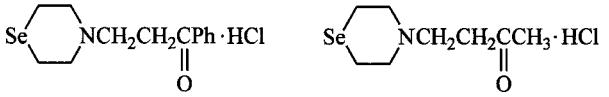
Experimental

Materials

Escherichia coli (CCTCC AB91112) and *Staphylococcus aureus* (CCTCC AB910393) were provided by China Center of Type Culture Collection, Wuhan University, China.

The peptone culture medium contained NaCl 5 g, peptone 5 g and beef extract 3 g per 1000 mL (pH = 7.0). It was sterilized in high-pressure steam at 120 ℃ for 30 min.

Selenomorpholine compounds were synthesized and characterized by Department of Chemistry, Wuhan University.[13] Their structures are shown in Fig. 1.



1 2

Fig. 1 Selenomorpholine compounds β-(*N*-selenomorpholine) ethyl phenyl ketone hydrochloride (**1**) and 4-(*N*-selenomorpholine)-2-butanone hydrochloride (**2**).

Instruments

A microcalorimeter, LKB-2277 Bioactivity Monitor manufactured by LKB corporation of Sweden, was used to obtain the metabolic growth power-time curves of the bacteria. The microcalorimeter was thermostated at 37.00 ℃. 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 were described previously.[14]

Methods

In the calorimetric experiment, the flow cell was completely cleaned and sterilized. The procedure was as follows: sterilized distilled water, NaOH (0.1 mol/L), 75% alcohol solution, HCl (0.1 mol/L) and sterilized distilled water were pumped subsequently 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 was cleaned and sterilized and the baseline had been stabilized, the bacterial suspension, initially containing 2×10^6 bacteria/mL and compound **1** or **2** was pumped through the calorimetric cell with an LKB-2132 perplex peristaltic pump at a flow rate of 50 mL/h. When the flow cell (volume 0.6 mL) was full, the pump was stopped and the monitor was used to record the power-time curves of the bacterial growth (see the schematic diagram in Ref. 14).

In this type of experiment, the bacteria were suspended in the peptone culture medium. The selenomorpholine compounds were added from the beginning of the experiment, *i.e.*, it was introduced as soon as the bacteria were inoculated in the peptone culture medium. The solutions of the selenomorpholine compounds were prepared in the sterilized distilled water, and were prepared freshly every time before use.

Results

Power-time curves

The power-time curves obtained when a culture of the test bacteria was inoculated with selenomorpholine compound **1** at different concentrations are shown in Fig. 2.

From power-time curve, it can be seen that the shapes of the metabolic thermogenesis curves changed little when the selenomorpholine compounds at low concentrations were added into the suspension of the bacterium. But when high concentration of the selenomorpholine compounds was added, the shapes changed obviously and the lag phase, which is between the start of the experiment and the ascending phase of the power-time curve, became longer. These curves show that the selenomorpholine compounds had inhibiting action on the bacterial growth.

Thermokinetics

In the log phase of growth, the power-time curve obeys the equation:[8]

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

Using this equation, the growth rate constants k of all the experiments were calculated and the generation

Table 1 Rate constants (k) for the growth of *Escharichia coli* at 37.00 ℃

Experiment number	1	2	3	4	5	6	7	8
k/min^{-1}	0.0352	0.0374	0.0358	0.0368	0.0350	0.0370	0.0367	0.0356
R[a]	0.9969	0.9992	0.9969	0.9974	0.9993	0.9986	0.9982	0.9959

[a] Correlation coefficient.

Table 2 Rate constants (k) for the growth of *Staphylococcus aureus* at 37.00 ℃

Experiment number	1	2	3	4	5	6
k/min^{-1}	0.0299	0.0302	0.0296	0.0295	0.0297	0.0306
R[a]	0.9994	0.9985	0.9953	0.9999	0.9985	0.9996

[a] Correlation coefficient.

Table 3 Parameters of *Escharichia coli* growth in the solution with different drugs at 37.00 ℃

Drug	ρ (μg/mL)	k (min^{-1})	G (min)	I (%)	IC_{50} (μg/mL)	IC_{50}(Se) (μg/mL)
Control	0	0.0362	19.2	—	—	—
1	10	0.0367	18.8	-2.2	67	17
1	20	0.0324	21.4	10		
1	30	0.0252	27.6	30		
1	50	0.0231	30.0	36		
1	70	0.0176	39.3	51		
1	100	0.0000	—	100		
2	10	0.0342	20.2	5.4	110	34
2	20	0.0321	21.6	11		
2	30	0.0303	22.8	16		
2	40	0.0271	25.6	25		
2	50	0.0255	27.2	30		
2	70	0.0233	29.8	36		
2	100	0.0195	35.6	46		

Table 4 Parameters of *Staphylococcus aureus* growth in the solution with different drugs at 37.00 ℃

Drug	ρ (μg/mL)	k (min^{-1})	G (min)	I (%)	IC_{50} (μg/mL)	IC_{50}(Se) (μg/mL)
Control	0	0.0299	23.2	—	—	—
1	5	0.0244	28.4	18	24	6.0
1	10	0.0213	32.6	29		
1	20	0.0157	44.1	47		
1	30	0.0130	53.2	56		
1	50	0.0106	65.5	65		
2	10	0.0271	25.5	9.3	58	18
2	20	0.0244	28.4	18		
2	30	0.0215	32.3	28		
2	50	0.0173	40.0	42		
2	70	0.0114	61.1	62		
2	100	0.0069	101	77		

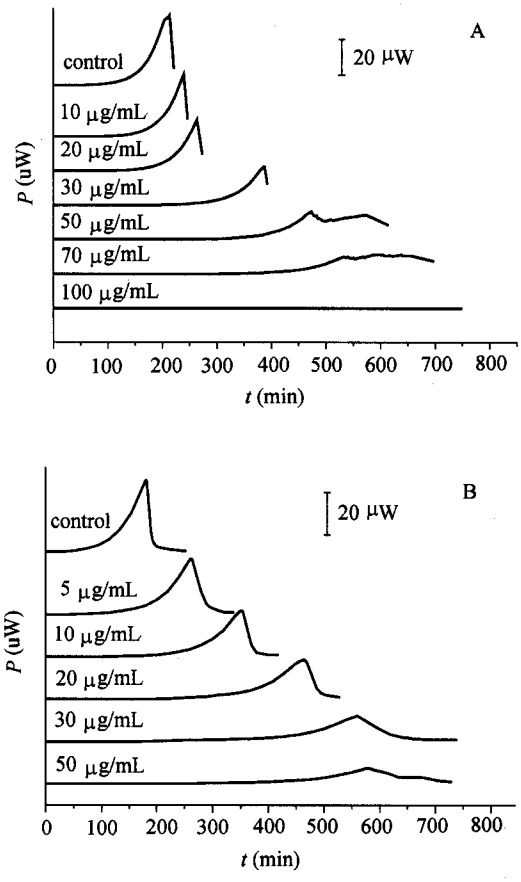


Fig. 2 Power-time curves of *Escherichia coli* growth (A) and *Staphylococcus aureus* growth (B) in the presence of compound **1** at different concentrations.

times (G), which equal ($\ln 2$)/k, were also obtained. Corresponding k and G are shown in Tables 1, 2, 3 and 4.

From the data in Tables 1 and 2, it is apparent that $k = (0.0362 \pm 0.0007)\ \text{min}^{-1}$ for *Escherichia coli* growth and $k = (0.0299 \pm 0.0004)\ \text{min}^{-1}$ for *Staphylococcus aureus* growth. All of the correlation coefficients are larger than 0.9950, indicating a good reproducibility and correlation.

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 with a concentration of ρ. When inhibitory ratio I is 50%, the corresponding concentration of inhibitor is called as the half inhibitory concentration IC_{50}. The values of I and IC_{50} are also shown in Tables 3 and 4.

Discussion

The power-time curves show that the time of the lag-phase becomes longer and the maximum power decreases with increasing concentrations of the selenomorpholine compounds. These results mean that with increasing concentrations of the selenomorpholine compounds it takes longer time to generate a detectable signal, and indicate that the selenomorpholine compounds have action on the growth of the test bacterium.

This can be verified by the growth rate constant of the bacterium. The data in Tables 3 and 4 show that the growth rate constants decrease with an increase in the mass of the selenomorpholine compounds as shown in Fig. 3. Fig. 3 also shows that the concentration-rate constant relationship is nearly linear for compound **2**, but for compound **1** the relationship is not linear. This is perhaps related to the different mechanism of the inhibition because of the different structures of the drugs. The experiments show that the selenomorpholine compounds have better antibacterial activity on *Staphylococcus aureus* than on *Escherichia coli*. But this result is different from selenomorpholine and sodium selenite,[8,15] indicating that the mechanism of the action of these compounds on

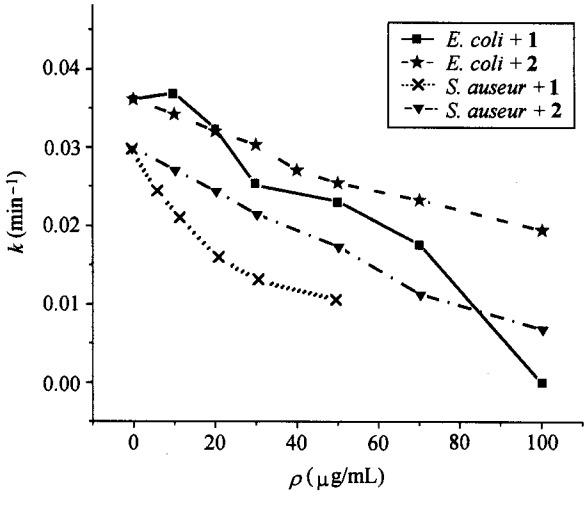


Fig. 3 Plot of k for the growth of bacterial vs. ρ for the selenomorpholine compounds.

the different bacteria is different. The mechanism needs further investigation.

The factors to determine the characteristics of a doseresponse relationship are the drug mode of action in cells, its number of target sites and its afinity for those target sites. Selenium is an active center of glutathione peroxidase (GSH-Px), which can catalyze and decompose lipid hydroperoxide or hydrogen peroxide.[1] Selenium can catalyze the production of reactive oxygen radical resulting in the oxidative damage. In this study, the growth of *Escherichia coli* 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.

The action of the drugs on the bacteria also depends on the structures of the drugs. The values of IC_{50} indicate that the antibacterial activity of the studied selenomorpholine compounds is better than that of selenomorpholine (for *Escherichia coli*, IC_{50} (Se) is 336 μg/mL;[8] for *Staphylococcus aureus*, IC_{50}(Se) is more than 1000 μg/mL[15]). This is probably because they have more hydrocarbyl group and hydrocarbyl group has affinity with the bacterial cell.

In conclusion, microcalorimetry provides a tool for studying the kinetics of the antibacterial action of antibiotics and for estimation of the relative bioactivity of antibiotics. It provides useful kinetic and thermodynamic information that can not be obtained by other bacteriological techniques, and the information is very significant for the synthesis of antibiotics. These results are important on the studies of toxicology and pharmacology. The experiment results illustrate that the studied selenomorpholine compounds have good antibiotic activity and they have better antibacterial activity on *Staphylococcus aureus* than on *Escherichia coli*.

References

1 Xu, H. B.; Huang, K. X. *Chemistry and Biochemistry of Selenium and Its Application in Life Sciences*, Huazhong University of Science and Technology Press, Wuhan, **1994** (in Chinese).

2 Bowie, G. L.; Sanders, J. G.; Riedel, G. F.; Gilmour, C. C.; Breitburg, D. L.; Cutter, G. A.; Porcella, D. B. *Water, Air, Soil Pollut.* **1996**, *90*, 93.

3 Russo, M. W.; Murray, S. C.; Wurzelmann, J. I.; Woosley, J. T.; Sandler, R. S. *Nutr. Cancer* **1997**, *28*, 125.

4 Campa, A.; Shor-Posner, G.; Indacoche, F. *J. Acquired Immune Defic. Syndr. Hum. Retrovirol.* **1999**, *20*, 508.

5 Miniero, R.; D'Archivio, M.; Olivieri, A. *Ann. Ist. Super. Sanita* **1998**, *34*, 349.

6 Rotruck, J. T.; Pope, A. L.; Ganther, H. E.; Swanson, A. B.; Hafeman, D. G.; Hoekstra, W. G. *Science* **1973**, *179*, 588.

7 Landenstein, R.; Epp, O.; Bartels, K.; Jones, A.; Huber, R.; Wendel, A. *J. Mol. Biol.* **1979**, *134*, 199.

8 Li, X.; Liu, Y.; Zhao, R. M.; Wu, J.; Shen, X. S.; Qu, S. S. *Biol. Trace Elem. Res.* **2000**, *75*, 167

9 Montanari, M. L. C.; Montanari, C. A.; Pilo-Veloso, D.; Beezer, A. E.; Mitchell, J. C.; Volpe, P. L. O. *Quant. Struct.-Act. Relat.* **1998**, *17*, 102.

10 Montanari, M. L. C.; Beezer, A. E.; Montanari, C. A. *Thermochim. Acta* **1999**, *328*, 91.

11 Qu, S.-S.; Feng, Y.; Zheng, C.-Y. *Thermochim. Acta* **1997**, *303*, 1.

12 Liu, Y.; Deng, F.-J.; Qu, S.-S. *Chemosphere* **2000**, *40*, 851.

13 Wu, J. *Ph. D. Dessertation*, Wuhan University, Wuhan, **1998** (in Chinese).

14 Yan, C. N.; Liu, Y.; Shen, P. *Chemosphere* **1999**, *38*, 891.

15 Li, X.; Liu, Y.; Wu, J.; Qu, S. S. *Thermochim. Acta* **2001**, *375*, 109.

(E0111275 LI, L. T.; DONG, L. J.)