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THE ACTION OF THE SELENOMORPHOLINE COMPOUNDS ON *ESCHERICHIA COLI* GROWTH BY MICROCALORIMETRY

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Abstract

The action of three kinds of the selenomorpholine compounds on a strain of *Escherichia coli* was studied by microcalorimetry. Differences in their capacities to affect the metabolism of this bacterium were observed. The extent and duration of the effect on the metabolism as judged from the rate constant (k) of *Escherichia coli* (in log phase) varied with the different drugs. The kinetics show that selenomorpholine compounds had an effect on the metabolism process of *Escherichia coli.* The k of *Escherichia coli* in the presence of the drugs increased with the increasing concentrations of the drugs (C) at low concentration; but at high concentration, the rate constant decreased with the increasing concentrations of the drugs. The experimental results reveal that the sequence of antibiotic activity of selenomorpholines is: N-selenomorpholinemethyl succinimide and its hydrochloride> N-(α-selenomorpholinebenzyl) succinimide.

Keywords: effect, *Escherichia coli,* metabolism, microcalorimetry, selenomorpholine, thermokinetics

Introduction

Selenium is one of the fourteen known essential biotrace elements [1]. The bioactivity of selenium has developed rapidly since selenium was found to be an active center of glutathione peroxidase (GSH-Px),which can catalyze and decompose lipid hydroperoxide or hydrogen peroxide [2, 3]. The antibacterial and antifungal activities of selenium have also been studied. Our previous studies showed that the antimicrobial activity of some selenomorpholine compounds is better than sodium selenite [4]. The purpose of this paper is to investigate the action of N-(α-selenomorpholinebenzyl)

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succinimide, N-selenomorpholinemethyl succinimide and its hydrochloride on *Escherichia coli* by means of microcalorimetry.

Microcalorimetry provides a general analytical tool for the characterization of the microbial growth process. It has been extensively used to investigate drug and the microbial cell interaction and has furnished much useful information [5, 6]. One of the most prominent features of the microbial growth process is the production of heat. If the heat is monitored by microcalorimeter, much useful information, both qualitative and quantitative, may be obtained. Each type of microbial has a unique power- time trace, as recorded 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, not only thermodynamic data but also kinetic data can be obtained.

In this paper, the power-time curves produced by *Escherichia coli* and *Escherichia coli* under the action of three kinds of selenomorpholine in 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. According to the k–C relationship, we could evaluate the antibacterial activity of the selenomorpholine compounds.

Materials, methods and instrument

Materials

Escherichia coli (CCTCC AB91112) was provided by China Center of Type Culture Collection, Wuhan University, P. R. China.

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

Selenomorpholine complexes were synthesized and characterized by the Department of Chemistry, Wuhan University [7]. Their structure is shown in Fig. 1.

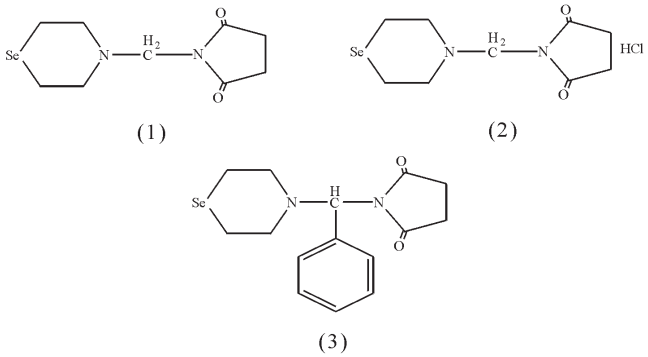

Fig. 1 The selenomorpholine compounds: (1) N-selenomorpholinemethyl succinimide, (2) N-selenomorpholinemethyl succinimide hydrochloride, (3) N-(α-selenomorpholinebenzyl) succinimide

Instrument

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°C. The voltage signal was recorded by means of an LKB-2210 recorder (1000 mV range). The baseline stability was 0.2 μW/24 h. For details of the performance and structure of the instrument [8].

Methods

In the calorimetric experiment, the flow cell was completely cleaned and sterilized. The procedure processed as followed: sterilized distilled water, 0.1 mol L^{-1} NaOH, 75% alcohol solution, 0.1 mol L^{-1} HCl and sterilized distilled water were pumped in sequence by a LKB-2132 microperplex peristaltic pump through the cell, each for 15 min at a flow rate of 50 mL h^{-1}.

Once the system was cleaned and sterilized and the baseline had been stabilized, the bacterial suspension, initially containing $2 \cdot 10^6$ bacteria/mL and compound (1), (2), (3), was pumped through the calorimetric cell with an LKB-2132 perplex peristaltic pump at a flow rate of 50 mL h^{-1}. 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 [8]).

In this type of experiment, the bacteria used were suspended in the peptone culture medium. Selenomorpholines were added from the beginning of the experiment, i.e., they were introduced as soon as the bacteria were inoculated in the peptone culture medium. The solutions of the selenomorpholine drugs were prepared in the sterilized distilled water, and were prepared freshly every time.

Results

Thermogenesis curves

Figure 2 shows the power-time curves obtained when a culture of the test bacteria was inoculated with selenomorpholine compound (2) at different concentrations. The power-time curve from the control experiment with no antibiotics is shown in Fig. 3.

From power-time curve, it can be seen that the shapes of the metabolic thermogenesis curves changed little when low concentration of selenomorpholine compounds was added into the suspension of *Escherichia coli*. But when high concentration of 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 an effect on the growth of *Escherichia coli*. This result is in agreement with the previous report [9].

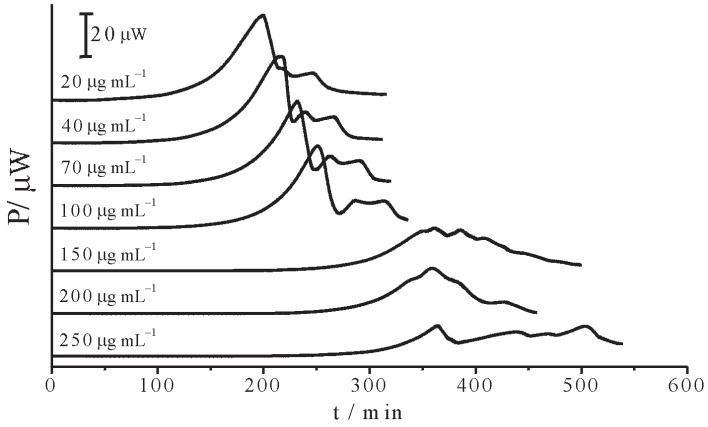

Fig. 2 The power-time curves of *Escherichia coli* growth in the presence of compound (2) at different concentration

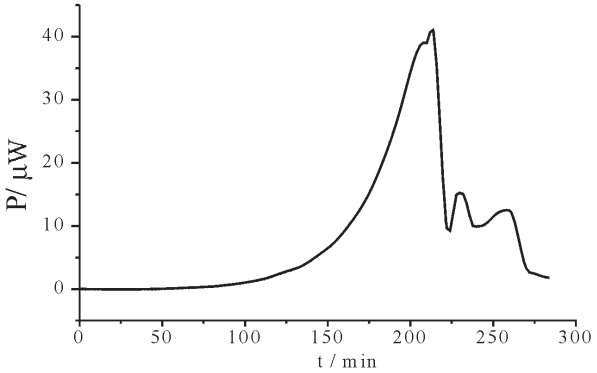

Fig. 3 The power-time curve of *Escharichia coli* at 37.00°C

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 experiments were calculated and the generation times (G), which equal (ln2)/k, were also obtained. Corresponding k and G are shown in Table 1 and Table 2.

From data in Table 1, it is apparent that k=0.03617±0.00073 min^{-1}. All of the correlation coefficients are larger than 0.9950, indicating a good reproducibility and correlation.

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

	Experiment number							
	1	2	3	4	5	6	7	8
k/min^{-1}	0.03519	0.03735	0.03579	0.03675	0.03503	0.03697	0.03667	0.3557
R[a]	0.9969	0.9992	0.9969	0.9974	0.9993	0.9986	0.9982	0.9959

[a]Correlation coefficient

Inhibitory ratio I

Inhibitory ratio I is defined as:

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

where k_0 is the rate constant of the control, k_C is the rate constant of bacterial growth inhibited by inhibitor, the concentration of which is C. The values of I are also shown in Table 2.

Table 2 Values of k, G and I of *Escharichia coli* in different drugs at 37.00°C

Drug	C/μg mL^{-1}	k/min^{-1}	G/min	I/%
Control	0	0.03617	19.2	–
Compound (1)	10	0.03779	18.3	–4.5
	20	0.03890	17.8	–7.5
	40	0.03731	18.6	–3.2
	70	0.03584	19.3	0.9
	100	0.03309	20.9	8.5
	150	0.03007	23.1	16.9
	200	0.02900	23.9	19.8
	250	0.02682	25.8	25.9
	300	0.02498	27.7	30.9
Compound (2)	20	0.03177	21.8	12.2
	40	0.03424	20.2	5.3
	70	0.03523	19.7	2.6
	100	0.03260	21.3	9.9
	150	0.03001	23.1	17.0
	200	0.02900	23.9	19.8
	250	0.02808	24.7	22.4
Compound (3)	10	0.03896	17.8	–7.7
	50	0.03703	18.7	–2.4
	100	0.03590	19.3	0.7
	300	0.03458	20.0	4.4
	500	0.03216	21.6	11.1
	700	0.03038	22.8	16.0
	900	0.02701	25.7	25.3
	1100	0.02604	26.6	28.0

Relationship between the growth rate constant (k) and the concentration of the selenomorpholine compounds (C)

Data in Table 1 show that the growth rate constant changes with the increase in the mass of the selenomorpholine compounds, as Fig. 4 shows. For compound (1) and (2), k increases with the increase of the concentration at low concentration and then decreases with the increase of concentration. But for compound (3), the growth rate constant decreases with the increase of concentration. Values of k are correlated to the concentration of Na_2SeO_3 (C) as:

Compound (1): $k = 0.03898 - 4.950 \cdot 10^{-5}\ C \quad R = -0.9869 \quad (20\sim300\ \mu g\ mL^{-1})$
Compound (2): $k = 0.03684 - 3.800 \cdot 10^{-5}\ C \quad R = -0.9521 \quad (70\sim250\ \mu g\ mL^{-1})$
Compound (3): $k = 0.03791 - 1.127 \cdot 10^{-5}\ C \quad R = -0.9895 \quad (10\sim1100\ \mu g\ mL^{-1})$

Discussion

In the present study, the time of the lag-phase suggests that the lag phase of bacteria growth was longer with increasing concentrations of compound (1), (2) and (3). It might be that selenomorpholine compounds killed some of the bacteria so it took longer to generate a detectable signal.

Analyses of the thermogenesis curves of *Escherichia coli* effected by selenomorpholine indicated that selenomorpholine compounds had an effect on the growth of *Escherichia coli*. The factors that determine the characteristics of a dose C response relationship 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 [2, 3]. 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

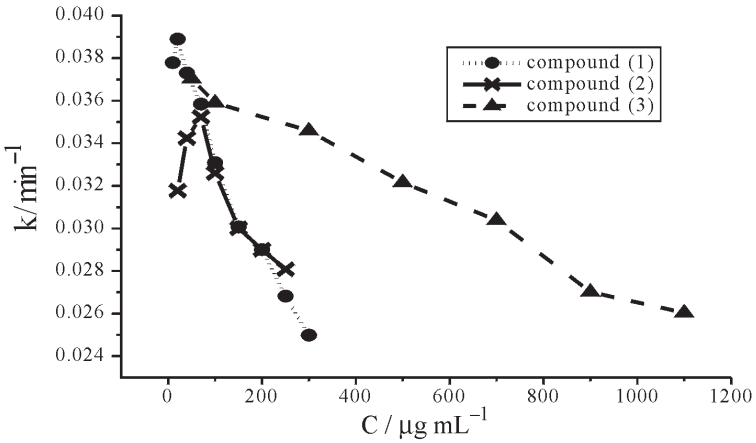

Fig. 4 Plot of k for the growth of bacterial *vs*. C for the selenomorpholine compounds

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.

Considering the rate constant, it could be concluded that compound (1) and (2) gave the best inhibitory effect on *Escherichia coli,* compound (3) was second. The effect of the drugs on the bacteria also depends on the structure of the drugs. Because compound (3) had a phenyl and it is difficult to enter into the bacterial cell, the inhibition of compound (3) on the bacteria is smaller than that of compound (l).

Conclusions

In conclusion, microcalorimetry offers means for the study of the kinetics of the antibacterial effect of antibiotics and for the estimation of the relative bioactivity of antibiotics. It provides a lot of kinetic and thermodynamic information that cannot be obtained by conventional bacteriological techniques, and all of this information is very significant for the synthesis of antibiotics. These results are very important on the studies of toxicology and pharmacology. This experimental result pointed out that the sequence of antibiotic activity of the selenomorpholine compounds is compound (1) and (2)>(3).

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