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Thermochimica Acta 375 (2001) 109–113

thermochimica acta

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The effect of the selenomorpholine derivatives on the growth of *Staphylococcus aureus* studied by microcalorimetry

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Received 19 December 2000; received in revised form 22 March 2001; accepted 26 March 2001

Abstract

The effect of three kinds of the selenomorpholine derivatives on *Staphylococcus aureus* growth was studied by microcalorimetry. Differences in their capacities to inhibit the metabolism of this bacterium were observed. The growth rate constant, k, of *S. aureus* (in log phase) in the presence of the drugs decreased with increasing concentrations of the drugs, c. The relationship of k and c is nearly linear for selenium compounds. Judged from the rate constant k and the half-inhibitory concentration, Ic_{50}, the experimental results reveal that the sequence of antibiotic activity of selenomorpholines is *N*-selenomorpholinemethyl succinimide hydrochloride > *N*-selenomorpholinemethyl succinimide > *N*-(α-selenomorpholine-benzyl) succinimide

Keywords: Selenomorpholine derivatives; *Staphylococcus aureus*; Antibacterial effect; Microcalorimetry; Thermokinetics

1. Introduction

Selenium is a normally occurring trace element. It is essential for humans and animals but is very toxic at higher concentrations. 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 [1,2]. Meanwhile, the appearance of Ebselen, an organoselenium compound with higher anti-inflammatory activity stimulated the study of biochemistry and pharmaceutical chemistry of organoselenium compounds [3]. For the comparability of organoselenium compounds and organosulphur compounds, many organoselenium compounds were synthesized and their antimicrobial activity was studied [4–6]. Previous studies showed that the antimicrobial activity of organoselenium compounds is many times higher than that of the sulphur and oxygen analogs having isosteric elements. So, it is very significant for biochemistry and pharmaceutical chemistry to study the antibacterial and antifungal activity of organoselenium complexes. The purpose of this paper is to investigate the action of selenomorpholine derivates on *Staphylococcus aureus* 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 drug and the microbial cell interaction and has furnished much useful information [7,8]. One of the most prominent features of the microbial growth process is the production of heat. If the heat is monitored by

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0040-6031/01/$ – see front matter
PII: S0040-6031(01)00515-9

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 but also kinetic information can be obtained.

In this paper, the power–time curves produced by *S. aureus* alone and *S. aureus* under the action of three kinds of selenium compounds in different concentrations were determined with an LKB-2277 Bioactivity monitor. From these power–time curves (log phase) the growth rate constant, k and the generation time, G classic parameters of microbiology were calculated.

2. Experimental

S. aureus (CCTCC AB910393) was provided by China Center of Type Culture Collection, Wuhan University, PR China. The peptone culture medium per 1000 ml (pH = 7.0) contained: NaCl 5, peptone 5, beef extract 3 g. It was sterilized in high pressure steam at 120°C for 30 min.

Three Selenomorpholine derivatives were synthesized and characterized by the Department of Chemistry, Wuhan University [9], the structure of which are shown in Fig. 1.

compound (1): *N*-selenomorpholinemethyl succinimide,

compound (2): *N*-selenomorpholinemethyl succinimide hydrochloride,

compound (3): *N*-(α-selenomorpholinebenzyl succinimide).

A microcalorimeter, LKB-2277 Bioactivity monitor manufactured by LKB corporation, Sweden was used to obtain the metabolic growth power–time curves of the bacteria. The microcalorimeter was thermostated at 37°C. The voltage signal was recorded by means of an LKB-2210 recorder (1000 mV range). The baseline stability was 0.2 μW for 24 h. For details of the performance and structure of the instrument, see [10].

In the calorimetric experiment, the flow cell was completely cleaned and sterilized. The procedure was: sterilized, distilled water, 0.1 mol/l NaOH, 75% alcohol solution, 0.1 mol/l HCl and sterilized distilled water were pumped in sequence by a LKB-2132 microperpex 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 per ml and one of the selenomorpholine drugs was pumped through the calorimetric cell at a flow rate of 50 ml/h. When the flow cell (0.6 ml) was filled, the pump was stopped and the monitor was used to record the power–time curves of the bacterial growth (see the schematic diagram in [10]).In this type of experiment, the

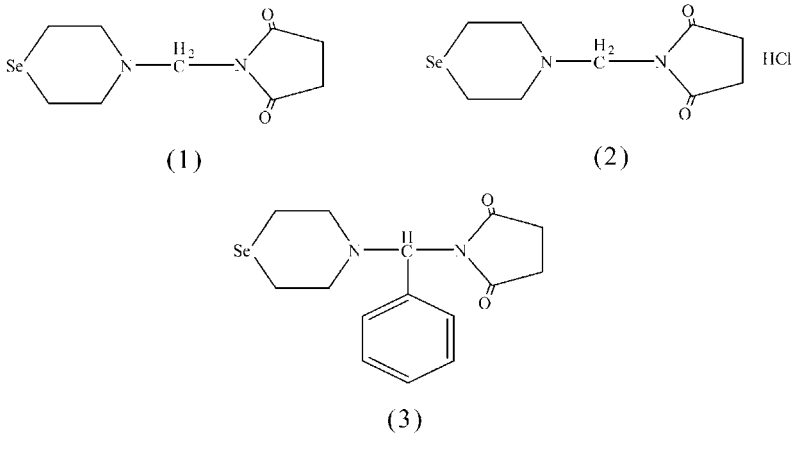

Fig. 1. The selenomorpholine compounds: (1) *N*-selenomorpholinemethyl succinimide; (2) *N*-selenomorpholinemethyl succinimide hydrochloride; (3) *N*-(α-selenomorpholinebenzyl) succinimide.

bacteria used were suspended in the peptone culture medium. The selenomorpholine was added at 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 drugs were prepared in sterilized distilled water, and were prepared freshly every time.

3. Results

The power–time curves (log phase) shown in Fig. 2 obtained when a culture of the test bacteria was inoculated with selenomorpholine compound (1) at different concentrations. It can be seen that the lag phase, which is between the start of the experiment and the ascending phase of the power–time curve, became longer with an increase of concentration.

These curves show that low concentrations of the selenomorpholine compounds had a promoting action on the growth of *S. aureus*, and high concentrations of the selenomorpholine compounds had an inhibitory action. This result is in agreement with the previous report [4,5].

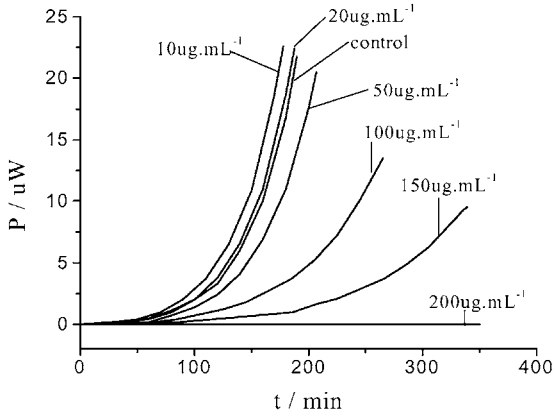

Fig. 2. The power–time curves (log phase) of *S. aureus* in the presence of compound (1) at different concentration.

In the log phase of growth, the power–time curve obeys the following equation [10]:

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

Using this equation, the growth rate constants k of all experiments were calculated and the generation times (G), which equal (ln 2)/k, were also obtained.

Table 1
Values of k, G, I and Ic_{50} of *S. aureus* in Different Drugs at 37°C

Drug	c (μg ml−1)	k (min)	G (min)	I(%)	Ic_{50}(μg ml^{-1})
Control	0	0.02991	19.2	–	–
Compound (1) ($M = 261$)[a]	10	0.03241	21.4	−8.4	135
	20	0.03067	22.6	−2.5	
	50	0.02652	26.1	11.3	
	100	0.01878	36.9	37.2	
	150	0.01309	53.0	56.2	
	200	0.0	–	100	
Compound (2) ($M = 297.5$)[a]	10	0.03189	21.7	−6.6	85
	20	0.03024	22.9	−1.1	
	50	0.02113	32.8	29.4	
	80	0.01655	41.9	44.7	
	100	0.01090	63.6	63.6	
	120	0.00848	81.7	71.6	
Compound (3) ($M = 337$)[a]	100	0.02999	23.1	−0.3	1666
	300	0.02681	25.9	10.4	
	500	0.02494	27.8	16.6	
	700	0.02322	29.9	22.4	
	900	0.02263	30.6	24.3	
	1100	0.02015	34.4	32.6	

[a] M: molecular mass.

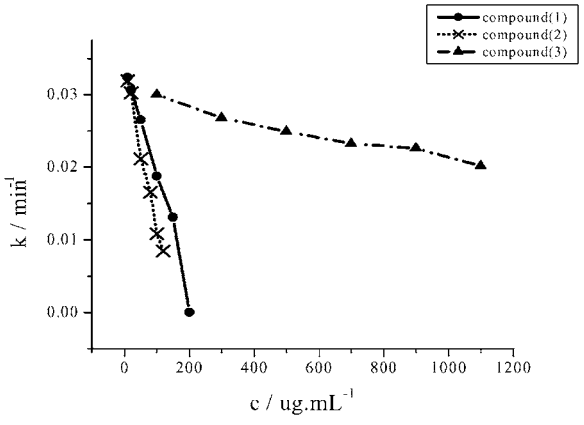

Fig. 3. Plot of k for the growth of *S. aureus* vs. concentration (c) of the selenomorpholine compounds.

The values of k and G are shown in Table 1. It shows that the growth rate constant(k) decreases with an increase in the mass of the selenomorpholine compounds and Fig. 3 shows that the concentration rate constant relationship is nearly linear. The relationship between k and c can be described as

$$\text{Compound}\,(1):\ k = 0.03241 - 1.612 \times 10^{-4}c,$$
$$R = -0.9910\,(10{-}200\,\mu\text{g ml}^{-1})$$

$$\text{Compound}\,(2):\ k = 0.03377 - 2.195 \times 10^{-4}c,$$
$$R = -0.9940\,(10{-}120\,\mu\text{g ml}^{-1})$$

$$\text{Compound}\,(3):\ k = 0.03006 - 9.066 \times 10^{-4}c,$$
$$R = -0.9822\,(100{-}1100\,\mu\text{g ml}^{-1})$$

Inhibitory ratio I is defined as

$$I = \left[\frac{k_0 - k_C}{k_0}\right] \times 100\%$$

where k_0 is the growth rate constant of the control, k_C is the rate constant of bacterial growth inhibited by inhibitor with concentration c. The values of I are also shown in Table 1.

When the inhibitory ratio I is 50%, the corresponding concentration of inhibitor is called the half inhibitory concentration Ic_{50}. The values of Ic_{50} of compound (1) and (2) obtained from Fig. 3 is 135 and 85 μg ml^{-1}, respectively. The value of Ic_{50} of compound (3) is 1666 μg ml^{-1} extrapolated from the relationship between the growth rate constant and concentration.

4. Discussion

The time of the lag-phase of bacteria growth was longer with increasing concentrations of the selenomorpholine drugs. This indicated that the bacteria took longer time to generate a detectable signal. This result, probably was due to excess selenium inhibited the growth of *S. aureus* or killed the bacteria. The experiment indicated that these three selenomorpholine derivates all have the capacity to inhibit the growth of *S. aureus* to different extents, and the inhibitory extent varied with different compounds. Considering the rate constant, Ic_{50} and the molecular mass, it could be concluded that compound (2) gave the best inhibitory effect on *S. aureus*, compound (1) was second, and compound (3) followed sequentially. The action of the drugs on the bacteria depends on the structure of the drugs. Because compound (3) has a phenyl group and it is difficult to enter the bacterial cell, the inhibition of compound (3) on the bacterial metabolism is smaller than that of compound (l).

From the power–time curves of *S. aureus* effected by the selenomorpholine, it can be seen that low concentrations of the selenomorpholine compounds have a promoting action on *S. aureus*, but high concentrations inhibit the growth of *S. aureus*. The factors that determine the characteristics of a dose–response curve 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 [1,2]. At low concentration, selenium can decompose reactive oxygen and hydroxyl radicals, and therefore prevent the oxidative damage but at high concentrations, selenium can catalyze the production of reactive oxygen radicals resulting in the oxidative damage. In this study, the growth of *S. aureus* was inhibited by excess selenite 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.

In conclusion, microcalorimetry offers a means for studying the kinetics of the antibacterial action of antibiotics and for estimation of the relative bioactivity of antibiotics. It provides kinetic and

thermodynamic information that can not 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. These experimental results pointed out that the sequence of antibiotic activity of the selenomorpholine compounds is compound *N*-selenomorpholinemethyl succinimide hydrochloride > *N*-selenomorpholinemethyl succinimide > *N*-(α-selenomorpholinebenzyl) succinimide.

Acknowledgements

The authors are grateful to the National Natural Science Foundation of China (29973030) and Young Mainstay Teachers Foundation of Chinese Educational Ministry for supporting this project.

References

[1] J.T. Rotruck, A.L. Pope, H.E. Ganther, A.B. Swanson, D.G. Hafeman, W.G. Hoekstra, Science 179 (1973) 588.
[2] R. Landenstein, O. Epp, K. Bartels, A. Jones, R. Huber, A. Wendel, J. Mol. Biol. 134 (1979) 199.
[3] M. Renson, N. Dereu, J. Pharm. Belg. 45 (1990) 322.
[4] Li. Xi, L. Yi, Z Ruming, Wu. Jun, S. Xuesong, Qu. Songsheng, Biol. Trace Elem. Res. 75 (2000) 167.
[5] H. Xu, K. Huang, Chemistry and Biochemistry of Selenium and its Application in Life Sciences, Huazhong University of Science and Technology Press, Wuhan, 1994.
[6] Y.D. Kulkami, A. Rani, A. Bishiioi, R.L. Shukla, Z.K. Anan, J. Indian Chem. Soc. 72 (1995) 261.
[7] G. Buckion, S.J. Russell, A.E. Beezer, Thermochim. Acta 193 (1991) 195–214.
[8] S. Hofuer, S. Svenson, A.E. Beezer, J. Antimicrob. Chemother. 25 (1990) 353.
[9] J. Wu, PhD thesis, Studies on the synthesis and properties of plant systemic organoselena compounds, Wuhan University, 1998.
[10] C. Xie, H. Tang, Z. Song, S. Qu, Thermochim. Acta 123 (1988) 33.