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Thermochimica Acta 387 (2002) 57–61

thermochimica acta

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Microcalorimetric study of *Staphylococcus aureus* growth affected by selenium compounds

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Received 4 April 2001; received in revised form 21 August 2001; accepted 18 October 2001

Abstract

The effect of four kinds of the selenium compounds on *Staphylococcus aureus* growth was studied by microcalorimetry. The extent and duration of the inhibitory effect on the metabolism as judged from the rate constant (k), varied with the different drugs. The rate constant of *S. aureus* (in log phase) in the presence of the drugs decreased with increasing concentrations of the drugs. The experimental results reveal that the sequence of antibiotic activity of selenomorpholines is: *N*,*N*′-methylene bisselenomorpholine > selenomorpholine and Na_2SeO_3 > selenomorpholine hydrochloride.

Keywords: Selenium compound; *Staphylococcus aureus*; Inhibition; Microcalorimetry; Thermokinetics

1. Introduction

Selenium presents a nutrition conundrum through its dual status as an essential and highly toxic nutrient. From early in this century, selenium has been known to cause toxicity in animals producing conditions, such as blind staggers and alkali disease [1]. In 1957, Schwarz and Foltz demonstrated trace elements of selenium protected against liver necrosis in vitamin E-deficient rats and established nutritional essentiality [2]. Selenium deficiency has been indicated as one of the causes of two human diseases found in China with particularly low soil selenium, the cardiomyopathy, Keshan disease and the Kaschin–Beck disease, involving osteoarthropathy [3,4]. Meanwhile, selenium status has been implicated in a wide range of disorders, including heart disease, cancer and acquired immunodeficiency syndrome (AIDS) [5,6]. The appearance of Ebselen, an organoselenium compound with higher antiinflammatory activity, stimulated the study of biochemistry and pharmaceutical chemistry of organoselenium compounds [7]. For the comparability of organoselenium compounds and organosulphur compounds, many organoselenium compounds were synthesized and their antimicrobial activity was studied [8,9]. 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.

Since, morpholine compounds are typical bactericide and fungicide, the present study was undertaken to investigate the action of selenomorpholine compounds and sodium selenite on *Staphylococcus aureus*

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0040-6031/02/$ – see front matter
PII: S0040-6031(01)00825-5

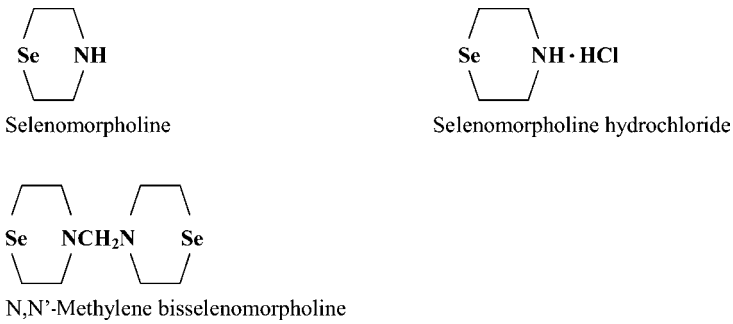

Fig. 1. The structure of the selenomorpholine compounds.

by microcalorimetry. By using an LKB-2277 Bioactivity Monitor, the fundamental power–time curves of *S. aureus* under different concentrations of four kinds of selenium compounds were gained. The curves reflect the dynamic changes of the growth process of *S. aureus* under the action of the selenium compounds, which can help to elucidate the effects of selenium on the biological processes.

2. Experimental

2.1. Materials

S. aureus (CCTCC AB910393) was provided by China Center of Type Culture Collection, Wuhan University, PR China. The peptone culture medium contained per 1000 ml (pH = 7.0): 5 g NaCl, 5 g peptone, 3 g beef extract. It was sterilized in high pressure steam at 120 °C for 30 min. Selenomorpholine compounds were synthesized and characterized by the Department of Chemistry, Wuhan University, PR China [10]. The structure of them are shown in Fig. 1. Sodium selenite was of analytical reagent grade.

2.2. Instruments

A microcalorimeter, LKB-2277 Bioactivity Monitor manufactured by LKB (Bromma, 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 per 24 h. For details of the performance and structure of the instrument, see [11].

2.3. Methods

In the calorimetric experiment, the flow cell was completely cleaned and sterilized. The procedure was as: 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 an LKB-2132 microperpex 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 2×10^6 bacteria ml^{-1} and selenium compounds, was pumped through the calorimetric cell with an LKB-2132 perpex peristaltic pump at a flow rate of 50 ml h^{-1}. When the flow cell (volume 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 [11]).

In this type of experiment, the bacteria used were suspended in the peptone culture medium. Selenium compounds were added at 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 selenium compounds were prepared in sterilized distilled water and were prepared freshly every time.

3. Results

3.1. The power–time curves of S. aureus

Similar was the shape of the power–time curves of *S. aureus* under the action of four kinds of selenium compounds. But, with the increase of the concentration

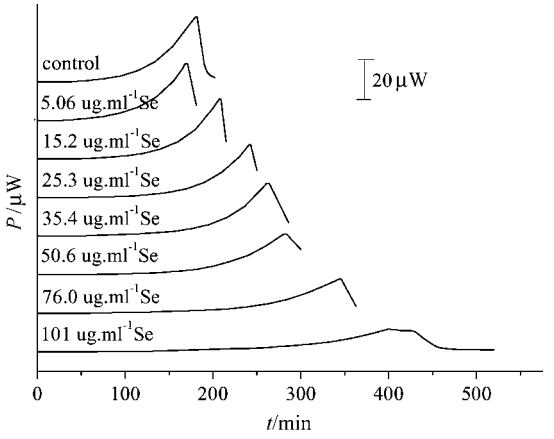

Fig. 2. The power–time curves of *S. aureus* in the presence of *N*,*N*′-methylene bisselenomorpholine at different concentration.

of the selenium compounds, lag phase, i.e. the period between the start of the experiment and the ascending phase of the power–time curves, became longer and the maximum heat production rate (P_m) decreased. Fig. 2 shows the lag and the log phases of the power–time curves obtained when a culture of *S. aureus* was inoculated with *N*,*N*′-methylene bisselenomorpholine at different concentrations.

3.2. Calculation of the growth rate constant of S. aureus

The growth curves of *S. aureus* showed that the log phase of growth obeys the equation [11]

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

where t is the time, P the calorimetric power at time t, P_0 the power at time $t = 0$ and k the growth rate constant. Using this equation, the growth rate constant (k) of *S. aureus* were calculated. The generation times (G), which are (ln 2)/k, were also obtained. Corresponding k and G are shown in Tables 1 and 2. From the data in Table 1, it is apparent that the mean growth rate constant in the absence of selenium is $k = 0.02991 \pm 0.00043\ \text{min}^{-1}$. All of the correlation coefficients are larger than 0.9950, indicating a good reproducibility and correlation.

3.3. Relationship between the growth rate constant (k) and concentration of selenium (c(Se))

The values of the growth rate constant (k) in Table 2 show that *N*,*N*′-methylene bisselenomorpholine, selenomorpholine and Na_2SeO_3 had a promoting action on the growth of *S. aureus* at a certain range of low concentrations, but at high concentration they had toxic action. At high concentrations, analyzing the values of the growth rate constant (k) and the corresponding concentration of selenium (c(Se)), we can find that the value of k declines with the increase of the concentration, as shown in Fig. 3. From Fig. 3, we can obtain the k–c(Se) equations:

Na_2SeO_3: $k = 0.03111 - 1.740 \times 10^{-5} c(\text{Se})$
and $R = -0.9949\ (45.7\text{–}502\ \mu\text{g/ml})$

Selenomorpholine: $k = 0.03483 - 2.273 \times 10^{-5} c(\text{Se})$
and $R = -0.9965\ (47.0\text{–}517\ \mu\text{g/ml})$

Selenomorpholine hydrochloride:
$k = 0.03059 - 1.218 \times 10^{-5} c(\text{Se})$
and $R = -0.9930\ (42.4\text{–}381\ \mu\text{g/ml})$

N, *N*′-Methylene bisselenomorpholine:
$k = 0.03060 - 1.851 \times 10^{-4} c(\text{Se})$
and $R = -0.9957\ (2.53\text{–}50.6\ \mu\text{g ml}^{-1})$

Table 1
Rate constants (k) for the growth of *S. aureus* at 37 °C in the absence of selenium

	Experiment					
	1	2	3	4	5	6
k (min^{-1})	0.02994	0.03019	0.02958	0.02951	0.02968	0.03056
R[a]	0.9994	0.9985	0.9953	0.9999	0.9985	0.9996

[a] Correlation coefficient.

Table 2
Values of k and G of *S. aureus* growth at different selenium compounds and concentrations at 37 °C

Drug	c (μg ml^{-1})	c(Se) (μg ml^{-1})	k (min^{-1})	G (min)	R^a
Control	0	0	0.02991	23.17	0.9984
Na_2SeO_3	10	4.57	0.02749	25.21	0.9960
	50	22.8	0.02837	24.43	0.9993
	100	45.7	0.03012	23.01	0.9969
	300	137	0.02869	24.16	0.9959
	500	228	0.02719	25.49	0.9996
	700	320	0.02612	26.53	0.9997
	900	411	0.02374	29.20	0.9992
	1100	502	0.02218	31.25	0.9953
Selenomorpholine	8.9	4.70	0.02989	23.19	0.9982
	44.6	23.5	0.03112	22.27	0.9961
	89	47.0	0.03411	20.32	0.9967
	268	141	0.03112	22.27	0.9959
	446	235	0.02973	23.31	0.9970
	625	329	0.02731	25.38	0.9981
	804	423	0.02495	27.78	0.9977
	982	517	0.02334	29.70	0.9983
Selenomorpholine hydrochloride	10	4.24	0.02830	24.49	0.9993
	50	21.2	0.02925	23.70	0.9990
	100	42.4	0.02990	23.18	0.9978
	300	127	0.02915	23.78	0.9973
	500	212	0.02829	24.50	0.9979
	700	297	0.02681	25.85	0.9973
	900	381	0.02591	26.75	0.9987
N,N′-Methylene bisselenomorpholine	10	5.06	0.03018	22.97	0.9973
	30	15.2	0.02816	24.61	0.9999
	50	25.3	0.02599	26.67	0.9996
	70	35.4	0.02280	30.40	0.9966
	100	50.6	0.02103	32.96	0.9985
	150	76.0	0.01668	41.56	0.9973
	200	101	0.01217	56.96	0.9998

[a] Correlation coefficient.

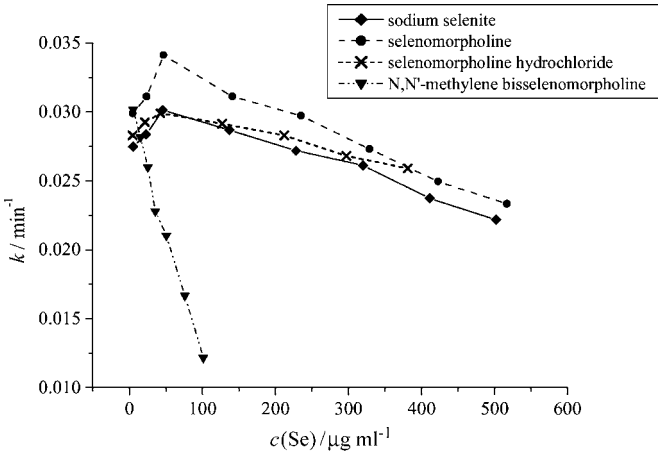

Fig. 3. Plot of k for the growth of *S. aureus* vs. c(Se) for selenium compounds.

4. Discussion

The result that the time of the lag phase of bacteria growth was prolonged with increasing concentrations of the selenium compounds indicates that the bacterial culture took longer time to produce a sufficient number of cells for a detectable signal and that excess selenium inhibited the growth of *S. aureus*. This probably resulted from the fact that selenium catalyzed the production of reactive oxygen radicals resulting in the oxidative damage. During this process, more active free radicals may be produced that further damage the membrane structure and functions of cells [8].

The power–time curves of *S. aureus* under the action of four kinds of selenium compounds showed that with increasing concentrations of selenium, the lag phase became longer and the maximum heat production rate (P_m) decreased. This indicates that these four selenium compounds all have the capacity to inhibit the growth metabolism of *S. aureus* to different extents and the inhibitory extent varied with the different drugs. This can be verified from Fig. 3 that shows that *N*,*N*′-methylene bisselenomorpholine gave the best inhibitory effect on *S. aureus*, the inhibitory action of Na_2SeO_3, selenomorpholine and selenomorpholine hydrochloride were almost equal at the experimental concentration. But considering the trends of the plots of k versus c, we can conclude that the antibacterial action of Na_2SeO_3 and selenomorpholine was better than that of selenomorpholine hydrochloride. The action of the drugs on the bacteria depended on the structure of the drugs. Because selenomorpholine hydrochloride was a hydrochloride and it was difficult to enter into the bacterial cell, the inhibition of selenomorpholine hydrochloride on the bacteria was smaller than that of selenomorpholine. The reason why the inhibition of *N*,*N*′-methylene bisselenomorpholine on the bacteria was much better than selenomorpholine was probably because *N*,*N*′-methylene bisselenomorpholine had more hydrocarbyl groups and a double amount of selenium. Hydrocarbyl groups have a higher affinity to the bacterial cell.

The depressing effect on the rate constants was concentration-dependent and the concentration rate constant relationship nearly linear, but of different shape. This is perhaps related to different mechanisms of inhibition because of the barging structures of the drugs. The mechanisms need further investigation.

Our experiments showed that microcalorimetry is a powerful tool for monitoring and controlling the growth process of microbes. It provides kinetic and thermodynamic information that cannot be obtained by conventional bacteriological techniques and all this information is significant for the synthesis of antibiotics. The results are important for the studies of toxicology and pharmacology. The experimental result points out that the sequence of antibiotic activity of the selenomorpholine complexes is *N*,*N*′-methy-lene bis-selenomorpholine $>$ selenomorpholine and Na_2SeO_3 $>$ selenomorpholine hydrochloride.

Acknowledgements

The authors are grateful to the National Natural Science Foundation of China (29973030, 30170010), Natural Science Foundation of Hubei Province (98J052) and Youth Academic Mainstay Foundation of Wuhan University for supporting this project.

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