Antibacterial Activities of Novel Diselenide-bridged *bis*(Porphyrin)s on *Staphylococcus aureus* Investigated by Microcalorimetry

Xiang-jiao Xu · Zhi Xue · Qi Xiao · An Xin Hou · Yi Liu

Abstract The actions of two novel diselenide-bridged *bis*(porphyrin)s (1 and 2) on *Staphylococcus aureus* growth was investigated by microcalorimetry at 37.00°C, compared with that of Na_2SeO_3. Differences in their capacities to inhibit the growth metabolism of *S. aureus* were observed. By analyzing the power–time curves, crucial parameters such as the rate constant of bacterial growth (k), inhibitory rate (I), and generation time (t_G) were determined. The growth rate constant (k) of *S. aureus* (in the log phase) in the presence of the drugs decreased with increasing concentrations of the drugs regularly. The relationship of k and c is nearly linear for diselenide-bridged *bis*(porphyrin) 2. The sequence of the antibacterial activities of these selenium compounds tested was $2>1>Na_2SeO_3$.

Keywords Diselenide-bridged *bis*(porphyrin)s · *Staphylococcus aureus* · Antibacterial activities · Microcalorimetry · Thermokinetics

Introduction

The research on the bioactivity of selenium has developed rapidly since selenium was found to be a key component of a number of functional selenoproteins required for normal health. The appearance of Ebselen, an organoselenium compound with higher anti-inflammatory and antimicrobial activity, stimulated the study of biochemistry and pharmaceutical chemistry of organoselenium compounds [1–3]. For the comparability of organoselenium compounds and organosulfur compounds, many organoselenium compounds were synthesized, and their antimicrobial activities were studied [4–7]. In our

X.-j. Xu · Z. Xue · Q. Xiao · A. X. Hou · Y. Liu (✉)
State Key Laboratory of Virology, College of Chemistry and Molecular Sciences, Wuhan University, Wuhan 430072, People's Republic of China
e-mail: liuyi@chem.whu.edu.cn

X.-j. Xu
College of Chemical Engineering and Technology, Wuhan University of Science and Technology, Wuhan, Hubei 430081, People's Republic of China

previous works, we have studied the antimicrobial activity of some selenium compounds [8–10]. Studies showed that the antimicrobial activity of organoselenium compounds is many time higher than that of sulfur analogs having isosteric elements. However, for all the selenium-containing compounds, their effects on cells are strictly dose and chemical form dependent. At low doses, selenium has beneficial effects, whereas at high doses, selenium is toxic and possibly carcinogenic.

As the doses of the main known dietary sources of selenium, such as selenomethionine, selenocysteine, and inorganic selenium, are limited by toxicity, synthetic derivatives have been developed. The interest in organoselenium biochemistry and pharmacology has increased in the last two decades due to a variety of organoselenium compounds that possess biological activity [11]. Accordingly, a number of novel pharmaceutical agents that are selenium based or which target specific aspects of selenium metabolism are under development [12–14].

With respect to the specific affinity for cells of porphyrins, we are prompted to design two novel diselenide bridged *bis*(porphyrin)s (1 and 2) [15]. The combination of porphyrin with selenium moiety might exert stronger antibacterial activity than porphyrin or selenium alone, with the aid of a successful transform of selenium moiety to cells.

Microcalorimetry as a technique has been widely used in life sciences because of its high sensitivity, high accuracy, and automaticity [16]. Microcalorimetry has a great advantage over studies of complex living systems, compared with the traditional microbiological techniques such as cell number counting or biomass methods, which are considered time consuming and less efficient, and provides a particularly useful tool for the characterization of the microbial growth processes [17]. The power–time curves of bacterial growth can be determined by means of microcalorimetry, and by analyzing the exponential growth phase of the power–time curves, kinetic parameters, such as the rate constant (k), for bacterial growth can be evaluated [18].

In this paper, by using a LKB-2277 Bioactivity Monitor, the inhibitory effects of diselenide-bridged *bis*(porphyrin)s (1 and 2) on *Staphylococcus aureus* at 37.00°C were investigated, which can help to elucidate the effects of selenium compounds on the biological process.

Materials, Instruments, and Methods

Materials

S. aureus (CCTCC AB910393) was provided by China Center of Type Culture Collection, Wuhan University, People's Republic of China. The peptone culture medium contained per 1,000 mL (pH=7.0): 5 g NaCl, 10 g peptone, and 6 g beef extract. It was sterilized in high-pressure steam at 120°C for 30 min. Novel diselenide-bridged *bis*(porphyrin)s (1 and 2) were synthesized and characterized by the Department of Chemistry, Wuhan University [15]. Their structures are shown in Fig. 1.

All other chemicals used are of analytical grade and are available locally.

Instruments

A heat conduction microcalorimeter, LKB-2277 Bioactivity Monitor, manufactured by LKB of Sweden, was used to obtain the metabolic growth power–time curves of bacteria. The microcalorimeter was thermostated at 37°C. The voltage signal was recorded by means
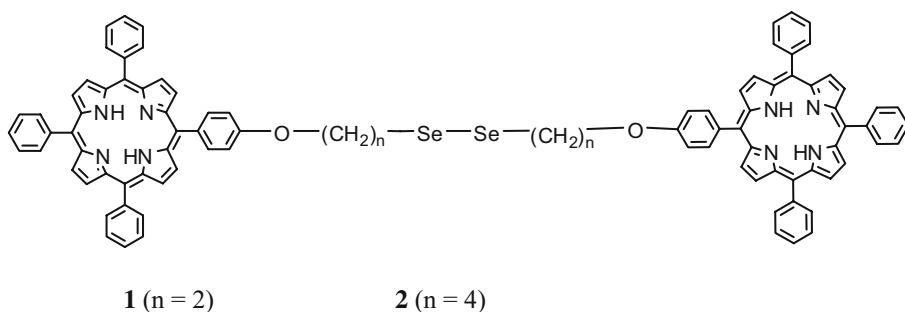
1 (n = 2)   **2** (n = 4)

Fig. 1 The structure of novel diselenide-bridged *bis*(porphyrin)s (1 and 2)

of a computer. The baseline stability was 0.2 µW/24 h. For details of the performance and structure of the instrument, see [17].

Methods

In the calorimetric experiment, the flow cell was completely cleaned and sterilized. The procedure was as follows: Sterilized distilled water, 0.1 mol/L NaOH, 75% alcohol solution, 0.1 mol/L HCl, and sterilized distilled water again were pumped in sequence by an LKB-2132 microperplex peristaltic pump 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 milliliter and diselenide-bridged *bis*(porphyrin)s (1 and 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 filled, the pump was stopped, and the monitor was used to record the power–time curves of the bacterial growth [17].

In this type of experiment, diselenide-bridged *bis*(porphyrin)s (1 and 2) were added from the beginning of the experiment, and they were introduced as soon as the bacteria were inoculated in the peptone culture medium. The solutions of diselenide-bridged *bis* (porphyrin)s (1 and 2) were prepared in the dimethylformamide solutions and were prepared freshly every time.
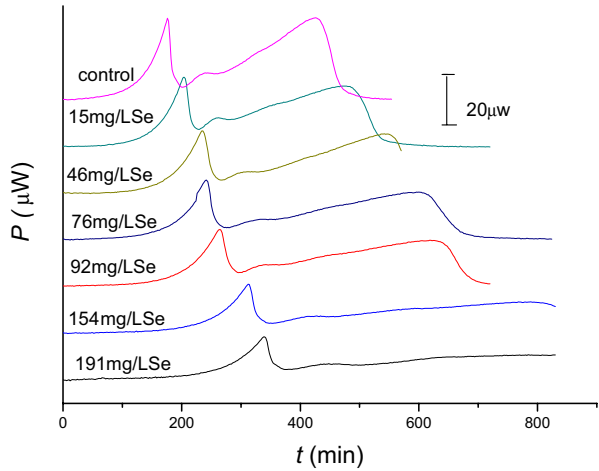
Results

The Power–Time Curves of *S. aureus*

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

From the power–time curves, it can be seen that the shapes of the metabolic thermogenesis curves changed little when low concentration of selenium compounds was added into the suspension of the bacterium. However, when a high concentration of selenium 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 selenium compounds had an obvious inhibitory action on *S. aureus* growth.

Thermokinetics

The growth power–time curves of S. *aureus* show that the log phase of growth obeys the equation [17]:

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

In this equation, P_0 and P_t are the heat output power at time 0 and t, respectively. Using this equation, the growth rate constant (k) of all the experiments is calculated by analyzing the date of the first peak. Furthermore, the generation time (t_G), an important parameter in microbiology, can be derived from the equation:

$$t_G = \ln 2 / kc \qquad (2)$$

In this equation, k_c represents the rate constant of the growth of S. *aureus* in the presence of a given complex at concentration c. In our experiments, t_G of the control agreed well

Fig. 3 The power–time curves of
S. *aureus* growth at 37.00°C
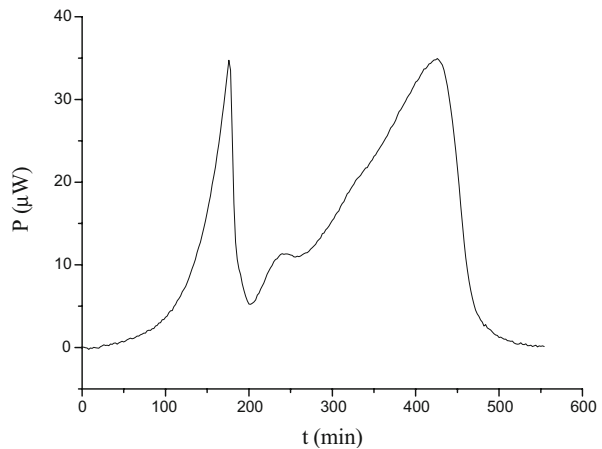
with the literature value [19]. The corresponding values between k and t_G are shown in Table 1.

Inhibitory Ratios

The growth inhibition ratio is calculated on the basis of the growth rate constant, and the inhibitory ratio can be defined as:

$$I = [(k_0 - k_c)/k_0] \times 100\% \tag{3}$$

In this equation, k_0 is the growth rate constant of the control for *S. aureus*. The values of I are also shown in Table 1.

Relationship Between Growth Rate and Concentration

Compared with Na_2SeO_3, the antibacterial effects of diselenide-bridged *bis*(porphyrin)s (1 and 2) were studied through the relationships between growth rate (k) and concentration (c). The data in Table 1 showed that the growth rate constant (k) changed with the increase in compound concentration (c), as Fig. 4 showed. As for 1 and Na_2SeO_3, k at a certain range of low concentrations was higher than or close to that of the control. This indicates that, at a certain range of low concentrations, these two chemicals stimulate the growth of *S. aureus*. For the antibacterial assay, we thus focused on concentrations at which the

Table 1 Parameters of *S. aureus* Growth in Solutions with Different Drugs at 37.00°C

Drug	c (mg/L)	c(Se) (mg/L)	k (min^{-1})	R^a	t_G (min)	I (%)
Na_2SeO_3[b]	0	0	0.02991	0.9984	23.17	0
	10	4.57	0.02749	0.9960	25.21	8.09
	50	22.8	0.02837	0.9993	24.43	5.15
	100	45.7	0.03012	0.9969	23.01	−0.70
	300	137	0.02869	0.9959	24.16	4.08
	500	228	0.02719	0.9996	25.49	9.09
	700	320	0.02612	0.9997	26.54	12.67
	900	411	0.02374	0.9992	29.20	20.63
	1,100	502	0.02218	0.9953	31.25	25.84
1	0	0	0.02846	0.9993	24.36	0
	161	18	0.02985	0.9997	23.22	−4.88
	483	54	0.03272	0.9986	21.18	−14.97
	646	74	0.03102	0.9985	22.35	−8.99
	807	92	0.02973	0.9998	23.31	−4.46
	968	111	0.02846	0.9992	24.20	−0.63
	1,610	184	0.02506	0.9979	27.66	11.95
2	0	0	0.02862	0.9995	24.22	0
	137	15	0.02721	0.9998	25.47	4.93
	411	45	0.02398	0.9997	28.91	16.31
	683	76	0.02249	0.9997	30.82	21.42
	820	92	0.02150	0.9995	32.24	24.88
	1,377	154	0.01732	0.9991	40.02	39.48
	1,707	191	0.01380	0.9993	50.23	51.78

[a] Correlation coefficient

[b] Na_2SeO_3, see [19]

Fig. 4 Relationship between k and c
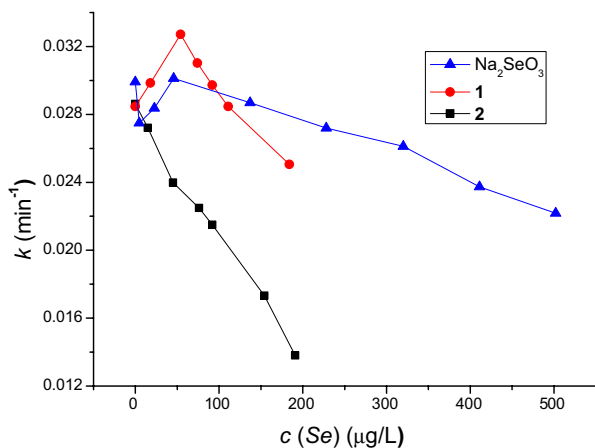
growth rate was less than that of the control. Thus, the relationship between the growth rate constant (k) and the corresponding concentration of selenium (c(Se)) could be established as follows:

$$Na_2SeO_3 : k = 0.03111 - 1.740 \times 10^{-5}\, c\,(Se)\,(45.7 - 502\ mg/L);\ R = -0.9949$$
$$1 : k = 0.03530 - 5.730 \times 10^{-5}\, c\,(Se)\,(54 - 184\ mg/L);\ R = -0.9898$$
$$2 : k = 0.02822 - 7.414 \times 10^{-5}\, c\,(Se)\,(0 - 191\ mg/L);\ R = -0.9962$$

The Relationship of Maximum Power, Maximum Time, and Concentration

The maximum microcalorimetric power (P_{max}) of 2 for the log phase of *S. aureus* decreased linearly in the concentration range of selenium (c(Se)) 0–191 mg/L. This phenomenon is in accordance with the relationship between k and c of 2. The experimental relationship between P_{max} and c is $P_{max} = 32.22 - 0.09583\, c$(Se), with $R = -0.9678$. Furthermore, from the data in Table 2, we can also see that with the increase in the concentration of 2, the maximum time of the log phase (t_{max}) increased. The relationship between t_{max} and c of 2 is as follows:

$$t_{max} = 192.7 + 1.027\, c\,(Se)\,(0 - 191\ mg/L);\ R = 0.9819$$

However, for 1 and Na_2SeO_3, analyzing the relationship of P_{max} vs c and t_{max} vs c, we cannot draw these concentration–dependence relationship. Therefore, the observation that

Table 2 Experimental Result of Effects of Diselenide-bridged *bis*(Porphyrin) 2 on *S. aureus* Growth

c(Se) (mg/L)	t_{max} (min)	P_{max} (µW)
0	179.5	35.2
15	207.1	29.7
45	239.8	27.1
76	270.6	24.3
92	315.8	21.0
154	349.3	18.3
191	376.5	14.9

P_{max} decreased and t_{max} increased rapidly in the case of 2 further confirms that 2 better inhibited the growth of *S. aureus*, compared with 1 and Na_2SeO_3.

Discussion

In the present study, the time of the lag phase of bacteria growth was prolonged with increasing concentrations of the selenium compounds. This indicated that the bacteria took a longer time to generate a sufficient number of cells for a detectable signal. This probably resulted from the fact that selenium can catalyze the production of reactive oxygen radical (O_2^-) resulting in the oxidative damage. In this study, the growth of *S. aureus* was inhibited by selenium compounds excess probably through the catalysis of oxidation reactions of thiol groups (SH) 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 [20–21].

The power–time curves of *S. aureus* under the action of three kinds of selenium compounds showed that with increasing concentrations of selenium, the lag phase became longer and the maximum heat production rate (P_{max}) decreased. This indicates that these 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. 4 that shows that 2 gave the best inhibitory effect on *S. aureus*, 1 was second, and Na_2SeO_3 followed. The action of the drugs on the bacteria also depended on the structure of the drugs. The reason why the inhibition of 1 and 2 on *S. aureus* was greater than that of Na_2SeO_3 was probably because 1 and 2 had a double amount of selenium and more porphyrin groups, which have a higher affinity to the bacterial cell. Compound 2 contained two more CH_2 units compared to 1, and the hydrophobicity and steric dimension of alkyl increased with the increasing number of carbon atoms [22]. Therefore, the lipophilicity of 2 was different from that of 1 resulting in a different antibacterial activity.

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 the information is very significant for the synthesis of antibiotics. These results are very important on the studies of toxicology and pharmacology. The experiment results illustrate that antibiotic activities of the studied diselenide-bridged *bis*(porphyrin)s (1 and 2) were better than that of Na_2SeO_3. The sequence of the antibiotic activity of these studied complexes was $2>1>Na_2SeO_3$.

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