ELSEVIER

Thermochimica Acta 320 (1998) 121–126

thermochimica acta

A comparative study on half-inhibitory concentration of Schiff base-metal complexes reacting with bacteria[1]

Huang Zaiyin, Liu Yi, Qu Songsheng*, Feng Ying

(Department of Chemistry, Wuhan University, Wuhan 430072, China

Received 17 January 1998; accepted 2 June 1998

Abstract

The metabolic inhibition of the three series of SG, O-VG and 2,4-L totaling eleven kinds of Schiff base complexes and two kind of corresponding metal compounds on *Aerobacter aerogenes* and *Staphylococcus sureus* has been studied microcalorimetrically. The studies reveal how, with reaction of different complexes, the difference in the metabolic power–time curves of the two kinds of bacteria metabolism. According to the power-time curves, the multiplication rate constant, generation time and inhibitory ratio of bacterial growth have been calculated, characterizating quantitatively the inhibition of Schiff base-metal complexes on bacteria metabolism. The relationship between the bacterial growth rate constant k and Schiff base-metal complex concentration c is thereby established, and on the basis of the quantity of half-inhibitory $C_{1/2}$ of Schiff base-metal complexes, the relationship between the structure of Schiff base-metal complexes and its antibacterial activity has been further studied by comparing the half-inhibitory quantity. A discussion of the cases of Schiff base-metal complexes of the same ligand at different metal ions, and of the same ions but different ligand reacting with different bacteria, has also been made, respectively, in comparative depth. It is found that, as for different bacteria, the same complex has different antibiotic activities, reflecting the difference between different kinds of bacteria; the inhibition of the same kind of bacteria varies with different complexes, showing the difference in the structure of the complexes, and thus providing useful information for the synthetic designing and the reasonable clinical administration of medicine of Schiff base drugs. © 1998 Elsevier Science B.V.

Keywords: *Aerobacter aerogenes*; Half-inhibitory concentration; Microcalorimetry; *Staphylococcus sureus*; Schiff base-metal complexes

1. Introduction

Microcalorimetry has been extensively used in the study of the interactions between drugs and microbes with a great deal of useful information already obtained [1,2], and it is of great significance in drug designing, reveals the nature and mechanism of the interaction between drugs and microbes and in further studying the nature of cell membrane. The power–time curves of bacterial growth can be determined microcalorimetrically [3], and by analyzing the exponential growth phase of the power–time curves the parameters of the rate constant of bacterial growth, activation energy and generation time can be evaluated [3–5], thus characterizing the growth metabolism of bacteria.

Extensive research work has been carried out on Schiff base and its metal complexes due to the bioac-

*Corresponding author.

[1]This project was supported by the National Foundation of China.

PII S0040-6031(98)00415-8

tivities of antivirus, antibacteria and anticancer. The Complexes Chemistry Group of Central China Normal University has done much research in the synthesis, characterization and antibiotic activity in recent years [6–10]. Our group has studied microcalorimetrically the inhibitory effect of the eleven kinds of newly synthesized Schiff base drugs on *Aerobacter aerogenes* and *Staphylococcus sureus* respectively, using the half-inhibitory quantity to indicate quantitatively the inhibition of drugs on bacteria. On this basis, by comparing the half-inhibitory quantity of the eleven kinds of drugs reacting with bacteria, the relationship between the compositional structure of the drugs and bacteria metabolism has been further studied, thus providing useful information for the synthetic designing of drugs and the reasonable clinical administration of these drugs.

2. Instrument, methods and materials

2.1. Equipment

A microcalorimeter, LKB-2277 bioactivity monitor manufactured by LKB corporation of Sweden was used to obtain the metabolic power–time curves of bacteria, which is reliable in thermostability with constant temperature at $\pm 1\times10^{-4}$°C. The voltage single was recorded by means of LKB-2210 recorder (1000 mV range). The baseline stability for instrument was 0.2 μW/24 h. For details of the performance and structure of the instrument see Refs. [3,11].

2.2. Materials

Aerobacter aerogenes (Chester CMCC(B) 45102) and *Staphylococcus sureus* (CMCC(B) 26001) were provided by the Department of Biology, Central Normal University, Wuhan A30079, P.R. China.

The peptone culture medium contained, per 1000 ml (pH=7.2): NaCl 5 g, peptone 5 g and beef extract 10 g. It was sterilized in high-pressure steam at 120°C for 30 min.

Schiff base-metal complexes were synthesized and characterized at the Department of Chemistry, Central China Normal University, Wuhan 430079, P.R. China.

2.3. Methods

In the calorimetric experiment, the flow cell was, to start with, throughly cleaned and sterilized. The procedure was as follows: sterilized distilled water, 0.1 mol l^{-1} NaOH, 75% alcohol solution, 0.1 mol l^{-1}HCI and sterilized distilled water were pumped in sequence by an 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 the baseline had been stabilized, the bacterial suspension, initially containing 1.5×10^{6} bacteria ml^{-1} and the Schiff base drug, was pumped through the calorimetric cell with an LKB-2130 perplex peristaltic pump at a flow rate of 50 ml h^{-1}. When the flow cell was full, the pump was stopped and the monitor was used to record the power–time curves of bacterial growth.

3. Results

At 303K and with absence of oxygen the metabolic power–time curves of *Staphylococcus sureus* and *Aerobacter aerogenes reacting* with three strains: SG, 2.4-L and O–VG of different concentrations has been, respectively, determined. In the log phase of bacterial growth, the power–time curve obeys the following equation [11].

$$\ln P_t = kt + \ln P_0$$

According to this equation, the multiplication rate constant, k, of all experiments were calculated and the generation times, G, which equal $(m^2)/k$, were also obtained. As the rate constant k and the concentration are linearly related, and, when the rate constant, k, is defined as the concentration of the drug, where the rate constant k with bacteria being inhibited is equal to half of that of bacteria without being inhibited, we can obtain half-inhibitory concentrations, $C_{1/2}$, of bacteria brought about by different drugs, according to the $k \propto c$ formula, as shown in Tables 1 and 2.

3.1. Note

Schiff base 2,4-L reacting with *S. Sureus,* Cu (II)–SG, Cu (II)—2,4-L and Cu (II)–O–VG reacting with *Aerobacter aerogenes* have made the growth rate

Table 1
The comparison of the effect of half-inhibitory concentrations of the Schiff base-drugs on bacteria at 303 K

Schiff base	$C_{1/2}$(*S.sureus*)/(μg ml^{-1})	Order	$C_{1/2}$(*Aerobacter aerogenes*)/(μg ml^{-1})	Order
Zn–SG	91	1–3	202	1–4
Cu (II)–SG	148	2–6	k=0.0264	4–11
Co (III)–SG	273	3–8	685	3–10
SG	343	4–9	267	2–9
Cu (II)–2,4-L	59	1–1	k=0.0282	5–13
Co (III)–2,4-L	103	2–4	38	1–1
Zn–2,4-L	205	3–7	193	2–3
Fe (III)–2,4-L	712	4–10	257	4–7
2,4-L	k=0.0182	5–11	204	3–5
Cu(II)–O–VG	90	1–2	k=0.0277	2–12
Zn–O–VG	113	2–5	255	1–6
$Zn(Ac)_2{\cdot}2HO$			80	1–2
$Cu(Ac)_2{\cdot}H_{2O}$			268	2–8

Table 2
The compararison of the effect of half-inhibitory concentrations of the Schiff base-drugs on bacteria at 303 K

Schiff base	$C_{1/2}$(*S.sureus*)/((μg ml^{-1})	Order	$C_{1/2}$(*Aerobacter aerogenes*)/(μg ml^{-1})	Order
Zn–SG	91	1	202	3
Zn–O–VG	113	2	255	4
Zn–2,4-L	20	3	193	2
$Zn(Ac)_2{\cdot}2H_2O$			80	1
Cu (II)–2,4-L	59	1	k=0.0282	
Cu(II)–O–VG	90	2	k=0.277	
Cu(II)–SG	148	3	k=0.0264	
$Cu(Ac)_2.H_2O$			268	
Co(III)–2,4-L	103	1	38	1
Co(III)–SG	273	2	685	2
SG	343	1	267	2
2,4-L	k=0.0182	2	204	1

constant k of bacteria irrelevant to concentration c within the research concentration range. The table has provided the corresponding rate constant k, the rate constants of the control group being k (*S. sureus*)=0.0198 min^{-1}, and k (*Aerobacter aerogenes*)= 0.0293 min^{-1}, respectively.

4. Discussion

The ways in which different complexes react with bacteria vary due to the difference in the structure, and the inhibitory ability displayed also differs with the difference in bacteria. The half-inhibitory quantity can be employed to characterize the inhibitory effect. The following analysis can be made according to Tables 1 and 2.

4.1. Analysing Table 1: Ligand being the same with different metal ions

In Table 1, the half-inhibitory quantities of Schiff base-metal complexes with the same ligand but different metal ions are listed. Due to the difference in the structure of complexes, the difference in the power–time curves of action on the same bacteria, therefore,

reflects the difference in structures. According to the half-inhibitory quantities, the inhibition sequence of the effects of SG, 2,4-L and O–VG strains, acting, respectively, with *S. sureus* and *Aerobacter aerogenes* is listed. The general inhibitory sequence of the effect of the eleven kinds of complexes on *S. sureus* and *Aerobacter aerogenes* is shown in Table 1. The three strains of complexes and the two kinds of metal-ion compounds are discussed below.

4.1.1. SG strain analysis

(a) The inhibitory effect of ligand SG on *S. sureus* is a bit weak; however, after combining with metal ions, its inhibitory effect increases, the combination with different metal ions resulting in varying degrees of increases in its antibiotic effect, the increase being different with different metal ions, the sequence of inhibition as shown in Table 1. For the inhibition on *Aerobacter aerogenes*, however, after coordination with metal ions, the inhibitory effect of Zn (II) complexes increases, while that of Co (III) complexes decreases, and the inhibitory effect of Cu (II) complexes, being impervious to the change in concentration, is a bit weak [12].

(b) It is found by comparing the half-inhibitory quantities of the metal complexes of SG strain acting, respectively, with *S. sureus* and *Aerobacter aerogenes* that, in general, the inhibitory quantities of the complexes of SG strain acting with *Aerobacter Aerogenes* are larger than those of their action on *S. sureus*, an indication that *Aerobacter aerogenes* is stronger than *S. sureus* in antidrug ability (referring to anti-SG strain metal complexes), or in other words, that the antibiotic activity of SG strain metal complexes against *S. sureus* is stronger than that against *Aerobacter aerogenes*.

(c) It can be seen from Table 1 that Zn-SG has very strong antibiotic effect on both kinds of bacteria; that Cu (II)–SG has stronger inhibitory effect on *S. sureus* than on *Aerobacter aerogenes*; that Co (III)–SG has greater effect in *S. sureus* than on *Aerobacter aerogenes,* its effect on *S. sureus* being equal to that of SG on *Aerobacter aerogenes*; that the inhibition of SG on *Aerobacter aerogenes* is stronger than on *S. sureus*. All this shows that different kinds of bacteria, because of the difference in the composition of cell wall, have cell walls of different thicknesses, therefore, even the same metal complex react with the bacteria in different ways, thus reflecting the difference in different kinds of bacteria.

4.1.2. 2,4-L Strain analysis

(a) By comparing the half-inhibitory quantity of 2,4-L strain complexes acting with *S. sureus* and *Aerobacter aerogenes,*, it is found that there exists in the strain no such case, where the antibiotic inhibitory action of *Aerobacter aerogenes* (anti-SG strain complexes) in SG strain is stronger than that of *S. sureus*, namely that it cannot be judged from the half-inhibitory quantity that the antibiotic ability of *Aerobacter aerogenes* is stronger or weaker than that of *S. sureus* which denotes different effects of different strains on the bacteria. It is worth noting that there occurs an extreme case, where Cu (II)-2,4-L has very strong inhibitory and killing effect on *S. sureus,* but it has only a mild inhibitory effect on *Aerobacter aerogenes*, this being similar to the case of Cu (II)-SG discussed previously and Cu (II)–O–VG to be discussed, demonstrating completely different ways of the two kinds of bacteria and, at the same time, indicating the strong antibiotic effect of *Aerobacter aerogenes* on Cu (II) complexes. Co (III)-2,4-L has a very strong inhibitory and killing effect on *Aerobacter aerogenes*; the inhibition of Zn–2,4-L on the two kinds of bacteria is basically the same; the inhibition of Fe (III)–2,4-L on *Aerobacter aerogenes,* is stronger than that on *S. sureus* ; ligand 2,4-L has inhibitory effect on *Aerobacter aerogenes,* but on *S. sureus* the inhibitory effect being impervious to concentration.

(b) Ligand 2,4-L has comparatively weak inhibitory effect on *S. sureus,* but after coordinating with metal ions, its antibiotic ability increases, the degree of the increase being different with different metal ions, and the sequence of the inhibitory effect as shown in Table 1. Though ligand 2,4-L has inhibition on *Aerobacter aerogenes*, the antibiotic ability of same complexes increases, followed by some decreasing after coordinating with metal ions.

4.1.3. O–VG strain analysis

(a) It is found by comparing the two kinds of metal complexes of O–VG strain, that the inhibitory effect of both kinds of metal complexes on *S. sureus* is stronger than on *Aerobacter aerogenes*. Cu (II)–O–VG has very strong inhibitory effect on *S. sureus,* and its inhibitory

effect is stronger than that of Zn–O–VG, but its activity on *Aerobacter aerogenes* is fairly low with antibiotic ability being irrelevant to concentration.The antibiotic ability of Zn–O–VG is stronger than that of Cu (II)–O–VG.

(b) The half-inhibitory quantity of the two kinds of metal compounds on *Aerobacter aerogenes* shows that Zn $(Ac)_2 \cdot H_2O$ is violently piosonous to *Aerobacter aerogenes*, its antibiotic activity being stronger than that of $Cu(Ac)_2 \cdot H_2O$.

4.2. Analysis of Table 2: The same metal ions with different ligands

4.2.1. Zn (II) strain analysis

The antibiotic effect of both SG ligand and 2,4-L on *S.sureus* is comparatively weak while the antibiotic activity of SG is stronger than that of 2,4-L. The structural difference between the two kinds of ligands is that 2,4-L has one more hydroxyl ion than SG, and viewed from their action with *S. sureus*, the introduction of hydroxyl ion has decreased antibiotic effect of the ligands, namely, the introduction of hydroxyl fails to increase the antibiotic ability of the ligand, but after combining with Zinc (II) ions, the inhibitory effect has increased considerably, which shows that Zn (II) has a very important role to play in the inhibitory effect on *S. sureus,* and that it might promote the antibiotic effect of SG on bacteria, or a combination of metal ions and complexes increases the antibiotic ability, with SG and 2,4-L ligands having certain inhibitory effect upon *Aerobacter aerogenes*. In this case, the introduction of hydroxyl ions increases the antibiotic ability of ligands. Even though, the metal ion compounds of Zn (II) are poisonous to *Aerobacter aerogenes* (the half-inhibitory quantity being 80 $\mu g\ ml^{-1}$), its antibiotic ability increases slightly after combining with the two kinds of ligands, showing that after Zn (II) forms complexes, it might not be helpful to the absorption of *Aerobacter aerogenes*, but will be helpful to the absorption of *S. sureus* and, thereby, reflect the difference in the bacteria.

The inhibitory effect of Zn SG and Zn–O–VG on *S. sureus* is stronger than that on *Aerobacter aerogenes*, while the inhibition of Zn-2,4-L in the two kinds of bacteria is similar to each other.

4.2.2. Cu (II) Strain analysis

After combining with metal ions, the antibiotic ability of SG and 2,4-L ligands on *S. sureus* increases considerably, especially that of Cu (II)–2,4-L. Ligand 2,4-L almost does not react with *S. sureus*, and the considerable increase in the antibiotic ability after combination indicates that Cu (II) plays a very important role in the inhibitory effect of *S. sureus*. It is worth nothing that the three strains of Cu (II) compounds almost do not react with *Aerobacter aerogenes,* and though the ligands in themselves have certain antibiotic effect, they lose their antibiotic ability soon after combining with Cu (II), which shows that *Aerobacter aerogenes* has a very strong antibiotic effect on the metal complexes of Cu (II) strain, and changes its mode of reaction with bacteria after combining with ligands.

4.2.3. Co (III) strain analysis

After combining with metal ions Co (III), and also that of Cu (II), the antibiotic ability of ligands SG and 2,4-L increases, which shows that Co (III) ions play a very important role in its action with *S. sureus* and it might promote the action of ligand with bacteria. After combination with metal ions, Co (III), the antibiotic ability of ligand 2,4-L, reacting with *Aerobacter aerogenes*, increases markedly, showing that after the combination of Co (III) ion and 2,4-L, the mechanism and the pattern of action have changed and Co (III) might have promoted the action of ligand 2,4-L with bacteria. The antibiotic ability of ligand SG reacting with *Aerobacter aerogenes* after combining with metal ions Co (III) decreases, which shows that the coordination of Co (III) might check the action of ligand with bacteria.

The structural difference between SG, O–VG and 2,4-L is that 2,4-L has one more hydroxyl ion than SG and O–VG has one more methoxy than SG.

In Zn (II) strain compounds, the introduction of both hydroxyl and methoxy decreases the inhibitory effect of complexes on *S. sureus*: the introduction of methoxy also decreases the inhibitory effect of *Aerobacter aerogenes,* while the introduction of hydroxyl ion does not greatly influence the inhibitory effect of *Aerobacter aerogenes*. In Cu (II) strain compounds, the introduction of both hydroxyl and methoxy increases the antibiotic effect of complexes on *S. sureus* but without effect on *Aerobacter aerogenes*;

in Co (II) strain complexes, the introduction of hydroxyl ion also decreases the antibiotic effect of two kinds of bacteria with the same group introduced; the combination with different metal ions, therefore, results in different degrees of action with bacteria, reflecting the structural difference of metal ions. To sum up, after combining, respectively, with metal ions, the antibiotic ability of all ligands on *S. sureus* increases, while after the combination of the three kinds of ligands with metal ions reacting with *Aerobacter aerogenes,* the case of all ligands is basically similar to that of the ligands without combining with metal ions, except that the antibiotic ability of Co (III)-2,4-L increases considerably, and that of Co (III)-SG decreases with Cu (II) strain having no antibiotic ability, namely the case of Zn (II) strain reacting with *Aerobacter aerogenes* is basically the same as the case of separate ligands reacting with bacteria.

Generally speaking, the antibiotic activity of metal compounds is comparatively stronger than that of ligands and the combination of ligands with metal ions can reduce toxicity of metal ions and increase the antibiotic activity of ligands. For example, the inhibitory effect of SG and 2,4-L on *S. sureus* is a bit weak; however, its antibiotic activity increases after combining with metal ions, as shown in Table 1.

It was earlier reported by Zhu Xinde [10] that the biological activity of Schiff base compounds is related to coordination of metal ions, namely, the combination of ligands and metal ions increases their antibiotic activity. Viewed from the action of SG strain and 2, 4-L strain on *S. sureus*, the conclusion is correct; however, when viewed from the action of the two strains with *Aerobacter aerogenes,* the antibiotic effect of metal ions after combination might not increase. For instance, the antibiotic ability of Co (III)–SG and Cu (II)–SG is weaker than that of SG, the antibiotic ability of Fe (III)–2,4-L and Cu (II)–2,4-L being also weaker than that of ligand 2,4-L. Different complexes are different from each other in structure as well as in their mechanism of action. Though the complex is the same, it is characterized by a different nature while reacting with different kinds of bacteria.

It can be seen from the above analysis that microcalorimentry can quantitatively denote the biological activity of Schiff base complexes and the structure and function of complexes can be related to each other through analysis, it is bound to play a very important role in terms of medicine synthesis, drug selection, and reveal the mechanism of interaction of drugs.

References

[1] E. BeeZer, L.J. Ashby, S.M. de Morais, R. Bolton, M. Shafiq, N. Kfeldsen, Thermochim. Acta 172 (1990) 81.
[2] C. Xie, D.U. Sun, Z.H. Song, Songsheng Qu, Thermochim. Acta 197 (1992) 101.
[3] C. Xie, Houkuan Tang, Songsheng Qu, Thermochim. Acta 123 (1988) 33.
[4] C. Xie, Houkuan Tang, Songsheng Qu, Acta physico-chemica Sinica 3 (1987) 113.
[5] C. Xie, Houkuan Tang, Songsheng Qu, Acta physico-chimica Sinica 5 (1989) 608.
[6] Changjian Feng, Zhifeng Le, Zhifeng Le, Xiangcai Zhang, J. Central China Normal University 28(4) (1984) 499.
[7] Zishen Wu, Zhifeng Le, Zhenhuan Yan, Synth. React. Inorg. Met.-org. 18 (1993) 291.
[8] Xinde Zhu, Zhifeng Le, Zishen Wu, Chem. Res. Chinese University 12(8) (1991) 1066.
[9] Feng, Changjian, Master research paper, Central China Normal University, Wuhan, 1995.
[10] Xinde Zhu, Changgang Wang, Zhifeng Le, Synth. Reac. Inorg. Met. -org. 21(9) (1991) 1365.
[11] Changli Xie, Gueiduan Xu, Songsheng Qu, Acta physico-chimica Sinica 2(4) (1986) 363.
[12] Ying Feng, Yi Lu, Songsheng Qu, Acta physico-chimica Sinica 12(8) (1996) 746.