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Kinetics of action of Schiff bases on *Aerobacter aerogenes* as studied by microcalorimetry

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Abstract

Microcalorimetry was used to study the kinetics of action of five kinds of Schiff base on a strain of *Aerobacter aerogenes*. Differences in their capacities to suppress the metabolism of this bacterium were observed. The extent and duration of the inhibitory effect on the metabolism as judged from the multiplication rate constant, k, varied with the different Schiff bases. The multiplication rate constants, k, of *Aerobacter aerogenes* (in log phase) in the presence of Co(III)–NG and Ni–NG (NG: D-glucosamine-β-naphthol aldehyde) decreased with the increasing concentrations of the compounds C, but the relationship between k and C was not of good linearity. For Fe(III)–NG, the multiplication rate constants are constant irrespective of variations in concentration. Similarly over the concentration range 50–200 $\mu g\ mL^{-1}$, there is nearly change in the inhibitory effects of NG on *Aerobacter aerogenes*, while they are slightly reduced beyond 200 $\mu g\ mL^{-1}$. The experimental results revealed that the sequence of antibiotic activity of Schiff base drugs is: Co(III)-NG > Ni–NG > NG > Fe(III)–NG.

Keywords: *Aerobacter aerogenes*; Kinetics; Microcalorimetry; Multiplication rate constant; Schiff base

1. Introduction

Since the first Schiff base metal complex was synthesized by H. Schiff in 1869, a great amount of research has been performed in this field to investigate their synthesis, structure, characteristics, bioactivity and biosimulations, etc. [1, 2]. At present related

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work is focused on the study of enzyme-simulation systems in the process of biochemistry [3] and antiphlogistics, antibiotics, anticancer drugs, etc. [4], as well as carrying of oxygen, nitrogen [5], etc.

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 [6, 7]. Microcalorimetry has a great advantage over many conventional bioassay procedures since the experimental record. P vs t, reveals not only thermal data but also kinetic data.

The purpose of this paper is to investigate the kinetic characteristic of *Aerobacter aerogenes*' growth, and the bioactivity of the Schiff base metal complexes on *Aerobacter aerogenes* by means of microcalorimetry. In this paper, the power–time curves produced by a strain of *Aerobacter aerogenes* and that of *Aerobacter aerogenes* under the action of five kinds of Schiff base drug in different concentrations were determined with an LKB-2277 Bioactivity Monitor. From these power–time curves (log phase) the multiplication rate constant, k, and the generation time, G, a classic parameter of microbiology, were calculated. According to the k–C relationship presented, we obtained the half-inhibitory concentrations from which the bioactivity of five Schiff base metal complexes was determined.

2. Material, methods and instrument

2.1. Materials

Aerobacter aerogenes (Chester CMCC(B) 45102) was provided by Department of Biology, Central China Normal University, Wuhan, P.R. China.

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

Schiff base metal complexes were synthesized and characterized by the Department of Chemistry, Central China Normal University [8–10]. Their structures are shown in Fig. 1.

2.2. Instrument

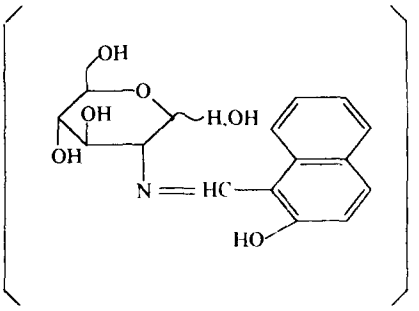
A microcalorimeter, LKB-2277 Bioactivity Monitor manufactured by LKB corporation of Sweden was used to obtain the metabolic power–time curves of the bacteria. The microcalorimeter was thermostated at 37.00°C. The voltage signal was recorded by means of an LKB-2210 recorder (1000 mV range). The baseline stability for the instrument was 0.2 μ W/24h. For details of the performance and structure of the instrument, see Ref. [11].

2.3. Methods

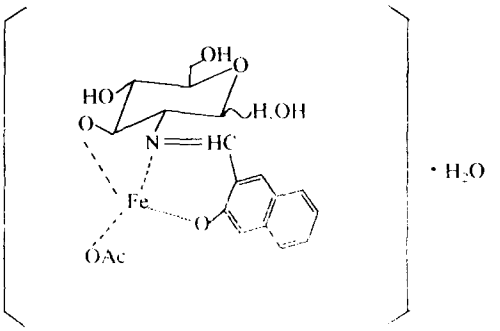
In the calorimetric experiment, the flow cell was firstly completely cleaned and sterilized. The procedure was: sterilized distilled water, 0.1 mol L^{-1} NaOH, 75%

alcohol solution, 0.1 mol L^{-1} HCl and sterilized distilled water were pumped in sequence by an LKB-2132 microperplex peristaltic pump through the cell, each for 15 min at a flow rate of 50 mL h^{-1}.

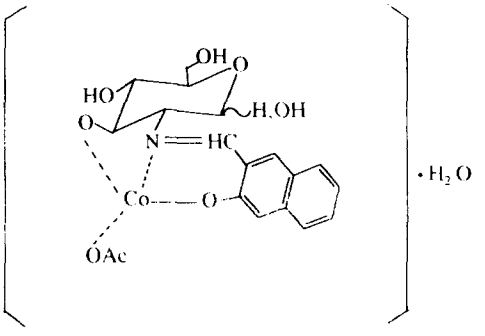
Once the system was cleaned and sterilized and the baseline had been stabilized, the bacterial suspension, initially containing 1.5×10^6 bacteria mL^{-1} and the Schiff base



NG

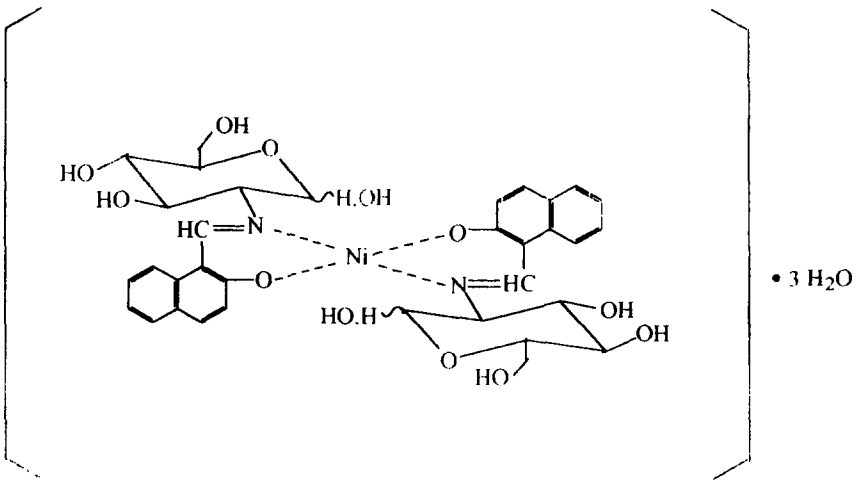


Fe(III)-NG



Co(III)-NG

Fig. 1. The structures of the Schiff base metal complexes.



Ni-NG

Fig. 1. (*Continued*).

drug, 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 curve of the bacterial growth (see the schematic diagram in Ref. [11]).

In this type of experiment, the bacteria used were suspended in the peptone culture medium. The Schiff base was added from the begining of the experiment, i.e., it was introduced as soon as the bacteria were inoculated in the peptone culture medium. The solutions of the Schiff base drugs were prepared in the peptone culture medium, and were prepared freshly every time.

3. Results

Figure 2 shows the power–time curves obtained when a culture of the test bacteria was inoculated with Co(III)–NG, Ni–NG, NG, Fe(III)–NG at concentrations of 50, 100, 150, 200, 250, 300 and 350 μg mL^{-1}. The power–time curve from the control experiment with no antibiotic is shown in Fig. 3.

In the log phase of growth, the power–time curve obeys the equation [11]:

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

Using this equation, the multiplication rate constants k of all experiments were calculated and the generation times G, which equal (ln 2)/k. were also obtained. Corresponding k and G are shown in Table 1.

As we can see from the Figs. 2 and 3, the shapes of the power–time curves obtained at 37.00°C are very similar except for that of the Co(III)–NG at higher concentrations, which completely inhibited the growth of the bacteria. But the maximum heat outputs

are lower than that of *Aerobacter aerogenes* control when the Schiff base drugs were added. In Table 1 the values of k and G reveal the same results. The experiment indicated that these five Schiff base drugs all have the capacity to inhibit the metabolic growth of *Aerobacter aerogenes* to different extents, and the inhibitory extent varied with different Schiff base drugs.

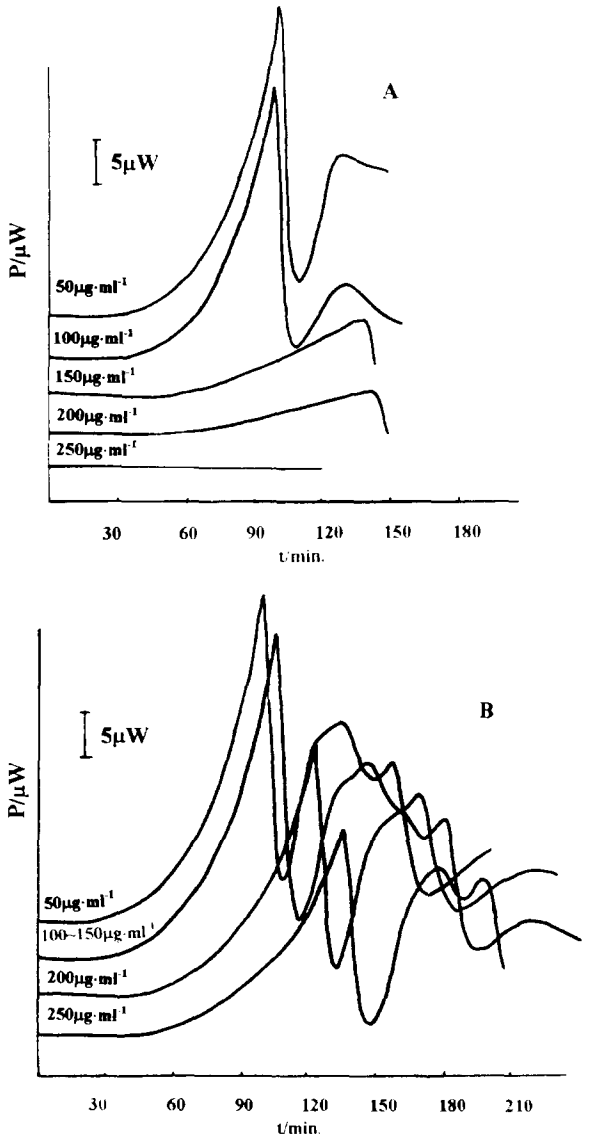


Fig. 2. The power–time curves of *Aerobacter aerogenes* in the presence of the Schiff base drugs at different concentrations. A: Co(III)–NG; B: Ni–NG; C: NG; D: Fe(III)–NG.

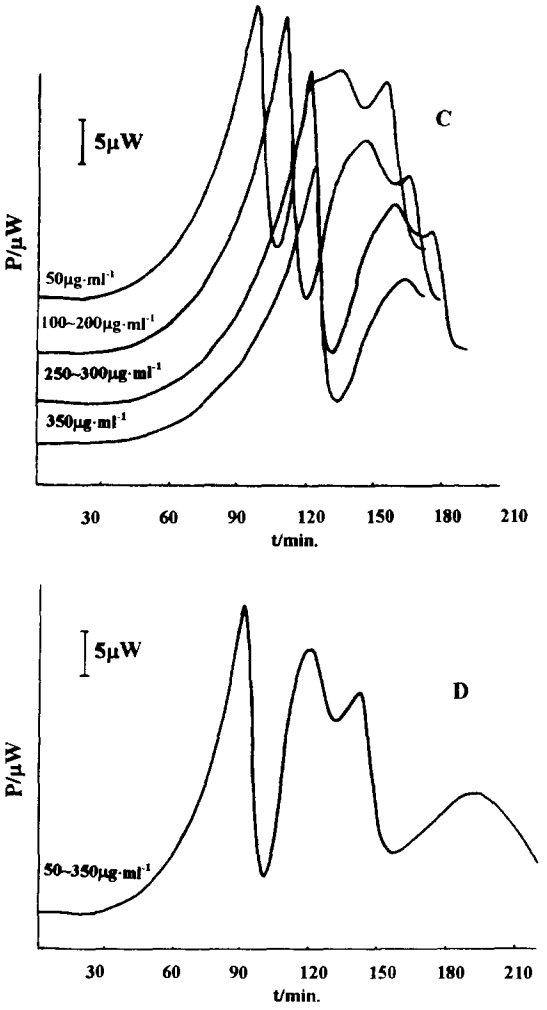


Fig. 2. (*Continued*).

The time of the lag-phase, that is between the start of the experiment and the ascending phase of the power–time curves shown in Figs. 2(A) and 2(B) suggested that retarding time of bacteria growth is longer with increasing concentrations of Co(III)–NG and Ni–NG. It might be that some of the bacteria are killed by the base so that it takes longer to generate a detectable signal. At a concentration of 250 μg mL^{-1}, the Co(III)–NG inhibited the metabolism of *Aerobacter aerogenes* completely, while the action of Ni–NG on bacteria is less than that of Co(III)–NG at the same concentration. The depressing effect on the rate constant was concentration-dependent, but the dose–rate constant relationship is not very linear for Co(III)–NG and Ni–NG over the concentration range 50–250 μg mL^{-1}, as shown in Fig. 4.

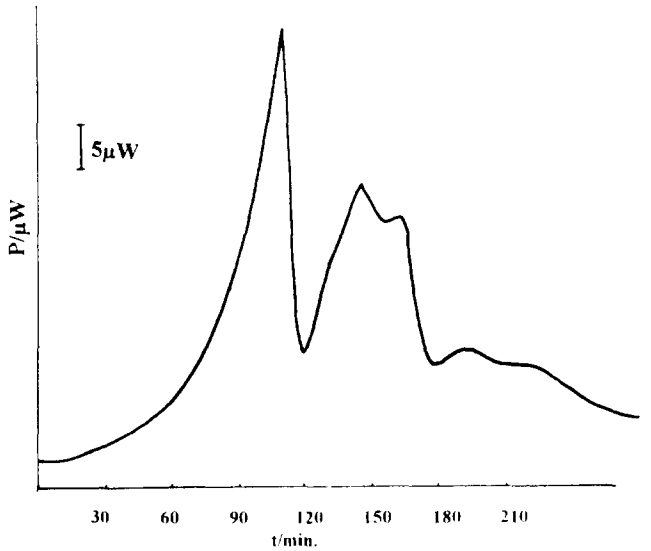


Fig. 3. The power–time curve of *Aerobacter aerogenes* at 37°C.

Table 1
Rate constants, k, and generation times, G, of *Aerobacter aerogenes* in different Schiff base drugs at 37.00°C

Drug	Conc. ($\mu g\ mL^{-1}$)	k/min^{-1}	G/min	R^a
Control	0	0.0400	17.3	0.99956
Co(III)–NG	50	0.0307	22.6	0.99580
	100	0.0286	24.2	0.99033
	150	0.0109	63.6	0.99901
	200	0.00814	85.2	0.99849
	250	0.0000		
Ni–NG	50	0.0301	23.0	0.99286
	100–150	0.0284	24.4	0.99175
	200	0.0227	30.5	0.99520
	250	0.0191	36.3	0.99717
Fe(III)–NG	50–350	0.0312	22.2	0.99580
NG	50	0.0300	23.1	0.99338
	100–200	0.0283	24.5	0.99478
	250–300	0.0250	27.7	0.99296
	350	0.0207	33.5	0.99504

[a] correlation coefficient.

It should be noted that the power–time curves of bacteria acted upon by Fe(III)–NG shown in Fig. 2(D) are the same irrespective of the concentrations used. The rate constants k shown in Table 1 remained at a fairly constant level were less reduced than those of the control group as the concentration vary. The results show that the

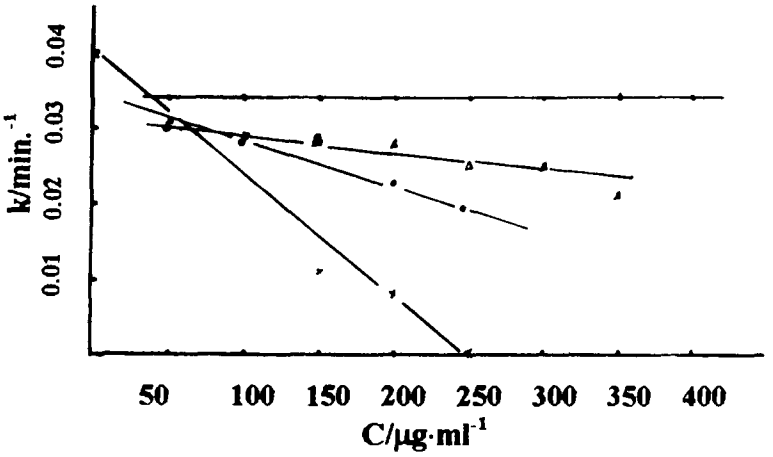


Fig. 4. Plot of k for the growth of bacteria vs C for Schiff base drugs.

Fe(III)–NG slightly inhibited the growth of *Aerobacter aerogenes* but the inhibitory effect did not change on increasing the studied concentrations. In addition, Table 1 shows that for NG there was no singnificant change in the rate constant between the concentrations of 50 and 200 $\mu g\ mL^{-1}$, and that k was slightly reduced when the concentration level was beyond 200 $\mu g\ mL^{-1}$.

From Fig. 4, we can obtain the k–C equations:

Co(III)–NG:	$k = -1.6 \times 10^{-4}C + 0.040$	(C: 0–250 $\mu g\ mL^{-1}$)
Ni–NG:	$k = -6.15 \times 10^{-5}C + 0.034$	(C: 50–250 $\mu g\ mL^{-1}$)
Fe(III)–NG:	$k = 0.0312$	(C: 50–350 $\mu g\ mL^{-1}$)
NG:	$k = -2 \times 10^{-5}C + 0.031$	(C: 50–350 $\mu g\ mL^{-1}$)

The half inhibitory concentration $C_{1/2}$ is defined as the concentration of Schiff base that reduces the rate constant of growth in the log-phase to half the control value. The half inhibitory concentrations of Co(III)–NG, Ni–NG and NG are 12 $\mu g\ mL^{-1}$, 227.6 $\mu g\ mL^{-1}$ and 550.0 $\mu g\ mL^{-1}$, respectively. Considering both the half inhibitory concentration and the rate constant, we conclude that among the Schiff base drugs added, Co(III)–NG gave the best inhibitory effect on *Aerobacter aerogenes*, Ni–NG was second, and NG and Fe(III)–NG followed sequentially i.e., the antibiotic activity of Schiff base drugs studied is Co(III)–NG > Ni–NG > NG > Fe(III)–NG.

4. Discussion

This experiment revealed that the action of Schiff base drugs on the bacteria differs from one another because of their different structures. Not all the inhibitory effects of the drugs on the bacterium, which are judged by the rate constants, depend on concentration, e.g. Fe(III)–NG. Even if they do, the relationship between the effect and the concentration is not always linear, this is not agreement with behaviour in the some papers [12, 13].

The action of Fe(III)–NG, in which Fe(III) is coordinated with ligand NG, on *Aerobacter aerogenes* is slightly smaller than that of ligand NG. We come to the same conclusion for another coordination compound of Fe(III), i.e., Fe(III)-2,4-L (2,4-L: 2,4-dihydroxybenzaldehyde glucosamine Schiff base) which has a little action on *Aerobacter aerogenes* and *S. aureus* [13]. It seems that Fe(III) is useful for the growth of *Aerobacter aerogenes*. On the contrary, the inhibitory effect of Co(III)–NG on the bacteria when Co(III) is coordinated with NG is stronger than that of NG. In our previous work [14], the results that Co(III)–2,4-L inhibited *Aerobacter aerogenes* are the same as that of Co(III)–NG. It may be concluded that Co(III) has stronger toxicity than Ni, NG and Fe(III). For the same reason the toxicity of Ni is second after that of Co(III) in our research. Therefore, these results reflected the difference of the metal ion structures of the Schiff base metal complexes which have the same ligand NG.

This experiment pointed out that microcalorimetry offers a means for study of the kinetics of the antibacterial action of antibiotics and for estimation for the relative bioactivity of antibiotics. It provides information that cannot be obtained by conventional bacteriological techniques. The information may be of use the conjunction with the pharmacokinetic data in establishing optimum doses and dose intervals in antibiotic therapy. It can also be used to study the relationship between the drug structure and the mechanism of microbial metabolism.

Acknowledgement

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