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Effects of Copper(II) Complexes on the Growth of *Escherchia coli* by Microcalorimetry

PING SHEN

School of Biological Engineering, Wuhan Polytechnic, Wuhan 430074, P.R. China

Corresponding author: Tel: +86 13886004617; E-mail: sping99@126.com

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Copper(II) complexes were synthesized and their effects on the growth of *Escherchia coli* (*E. coli*) were studied by microcalorimetry. Based on the power-time curves of *Escherchia coli* growth under the action of these copper(II) compounds, thermodynamics and kinetic data of metabolism of *E. coli* were calculated. The results revealed that there were differences in their capacities to inhibit the metabolism of this bacterium. The growth rate constant decreased with increasing concentrations of the copper complexes. Judged from the half-inhibitory concentration (IC_{50}) $[Cu(phen)_2]Cl_2{\cdot}6H_2O$ and $[Cu(phen)_3]Cl_2{\cdot}6H_2O$ have the strongest antibacterial activity and $CuCl_2{\cdot}2H_2O$ has the weakest antibacterial activity.

Key Words: Microcalorimetry, Copper(II) complex, Bioinorganic chemistry, Metabolism.

INTRODUCTION

Copper(II) complexes are known to play a significant role in biological activities including antibacterial activity[1,2], anti-tumor activity[3] and the interaction with DNA[4,5]. Among these copper(II) complexes, those containing phenanthroline and its derivatives, have attracted much attention for their various biological activities due to their ability to cleave DNA[5-7]. These redox-active copper(II) complexes inhibit the designated activities of DNA or RNA polymerase and induce strand scission of DNA in the presence of H_2O_2 or other reductants[8].

8-Hydroxy quinoline is also a well-known monoprotic bidentate chelating agent which is able to combine with many primary, transition and rare-earth metal ions[9-11]. 8-Hydroxyquinoline and its derivatives exhibit substantial cytotoxic activity against cancer cells[12-15]. Some copper complexes show antimicrobial and antifungal properties, being constituents in antiseptic and disinfectant formulations, in preservatives and in agricultural fungicides[14-15].

In this paper, the antibacterial activity of $CuCl_2{\cdot}2H_2O$, $(C_9H_6NO)_2Cu$, $[Cu(phen)_2]Cl_2{\cdot}6H_2O$, $[Cu(phen)_3]Cl_2{\cdot}6H_2O$ were investigated by microcalorimetry. On the basis of power-time curves of bacterial growth under these copper complexes, key parameters of microbiology, such as the growth rate constant (k), the generation time (t_G) and inhibition ratio (I), were calculated. According to the k-c relationship, we could evaluate the antibacterial activity of these copper(II) complexes.

EXPERIMENTAL

Escherchia coli (*E. coli,* AB91112) was provided by the China Center for Type Culture Collection, Wuhan University, P.R. China.

The peptone culture medium per 1000 mL contained 5 g NaCl, 10 g peptone and 3 g beef extract (pH = 7). It was sterilized in high pressure steam at 120 ºC for 0.5 h.

$CuCl_2{\cdot}2H_2O$ was purchased from Shanghai Kechang Fine Chemical Industry Co., Ltd. $(C_9H_6NO)_2Cu$ was purchased from Sinopharm Chemical Reagent Co., Ltd. $[Cu(phen)_2]Cl_2{\cdot}6H_2O$ and $[Cu(phen)_3]Cl_2{\cdot}6H_2O$ were synthesized according to reference[16]. Their structures are shown in Fig. 1. All chemicals were commercially available in AR grade and were used without further purification.

TAM Air 3114/3236 (Thermometric AB, Sweden) is an eight-channel isothermal heat conduction calorimeter operating in the milliwatt range. Measurements were recorded continuously and in real time through an 8-channel data logger connected to a computer. The metabolic power-time curves of bacteria were measured by isothermal heat conduction calorimeter. The technical details and functions of this instrument were described by Wang *et al.*[17].

Initially, *E coli* were inoculated in the prepared 5 mL culture medium. Then, a certain amount of concentration of the copper(II) complexes were added to the culture media. Finally, the medium was put into the TAM. The growth of *E. coli* in the presence of the complex was monitored by TAM.

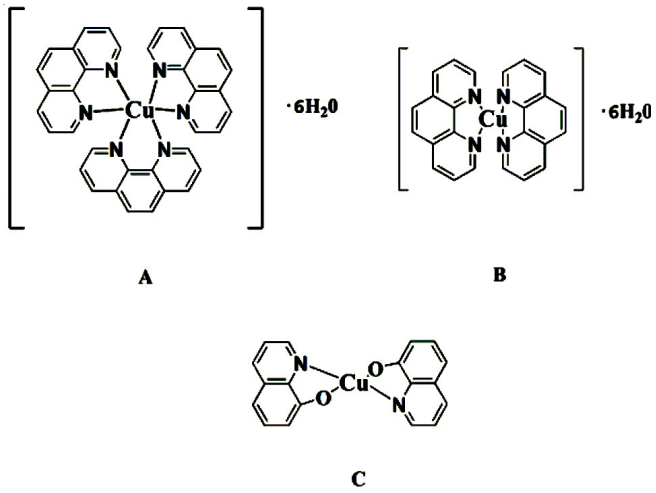

Fig. 1. The structures of $[Cu(phen)_3]Cl_2·6H_2O$ (A), $[Cu(phen)_2]Cl_2·6H_2O$ (B), $(C_9H_6NO)_2Cu$ (C)

The power-time curves of *E. coli* were recorded in real time. The experimental temperature was controlled at 37 °C.

RESULTS AND DISCUSSION

Thermokinetics: In the log phase of growth, the cell growth is exponential. If the cell number is n_0 at time 0 and n_t at time t, then

$$n_t = n_0 \exp(kt) \quad (1)$$

k is the growth rate constant. If the power output of each cell is w, then

$$n_t w = n_0 w \exp(kt) \quad (2)$$

$$P_0 = n_0 w \text{ and } P_t = n_t w$$

$$P_t = P_0 \exp(kt) \text{ or } \ln P_t = \ln P_0 + kt \quad (3)$$

when $P_t = 2P_0$,

$$t_G = \ln 2/k \quad (4)$$

t_G is defined as the generation time.

The growth *E. coli* curves (Fig. 2) of the log phase correspond to eqn. 3. In accordance with the data $\ln P_t$ and t taken from the curves to fit a linear equation, the growth rate constant (k) and the generation time (t_G) were obtained[18,19]. The results are shown in Table-1.

Relationship between k and concentrations of copper(II) complex: The values of the growth rate constant show that the growth of *E. coli* were affected by these copper(II) complexes. The growth rate constants (k) decrease along with the increase of concentrations of copper complexes. But the effect of these complexes on the growth of *E. coli* is different. $[Cu(phen)_2]Cl_2·6H_2O$ and $[Cu(phen)_3]Cl_2·6H_2O$ show similar activity on the growth of *E. coli*. Their concentrations are lower than those of $CuCl_2·2H_2O$ and $(C_9H_6NO)_2Cu$ at the same rate constant. This indicates their inhibiting action is stronger than $CuCl_2·2H_2O$ and $(C_9H_6NO)_2Cu$. The inhibition action of $(C_9H_6NO)_2Cu$ is stronger than that of $CuCl_2·2H_2O$.

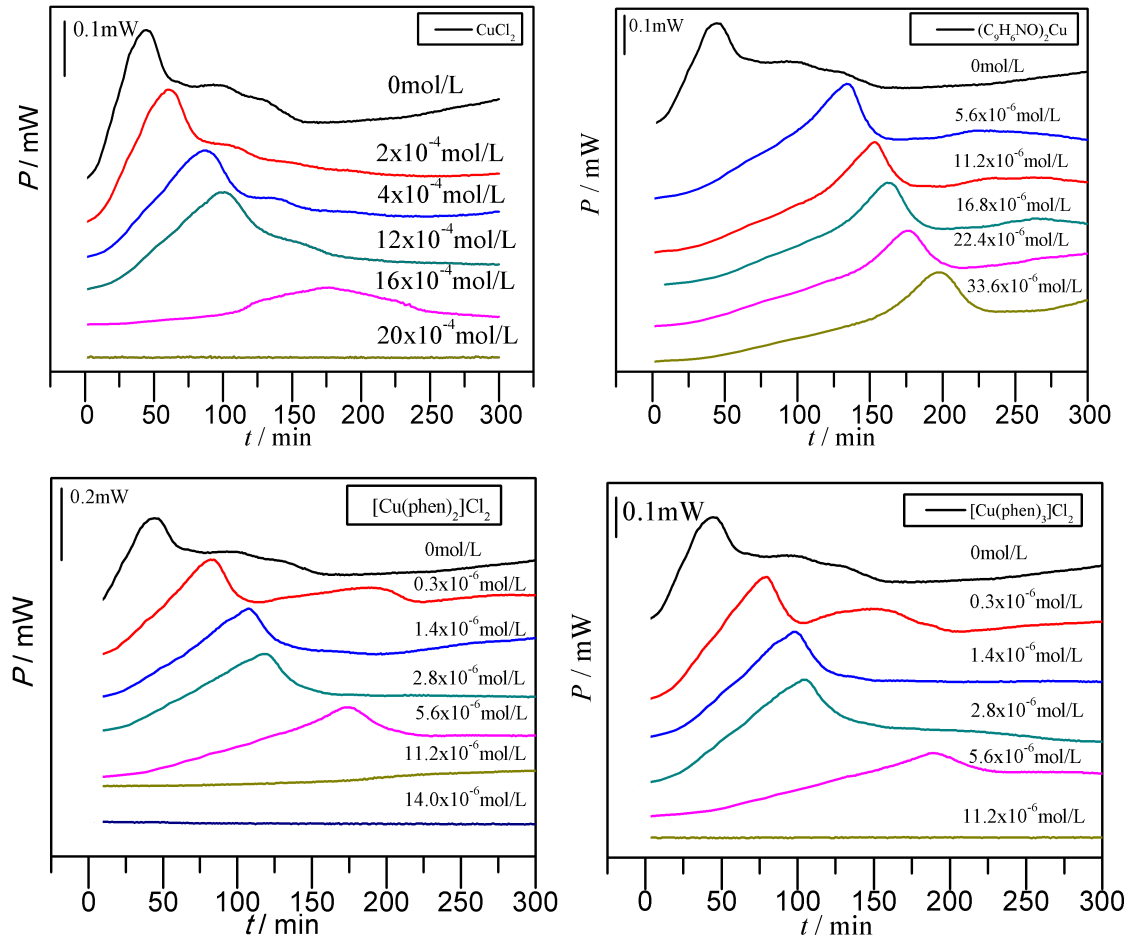

Fig. 2. Metabolic thermogenic curve of *Escherichia coli* in the presence of copper(II) complexes at 37 ºC

TABLE-1
THERMOKINETIC PARAMETERS OF *E. coli* IN THE PRESENCE OF COPPER(II) COMPLEXES AT 37 ºC

Complex	C (mol L^{-1})	k (min^{-1})	R	I (%)	t_G (min)	IC_{50} (mol L^{-1})
Control	0	0.02541	0.99945	0	27.28	
$CuCl_2$	2.0×10^{-4}	0.01936	0.99759	23.81	35.80	1.4×10^{-3}
	4.0×10^{-4}	0.01730	0.99564	31.93	40.08	
	12.0×10^{-4}	0.01538	0.99895	39.46	45.06	
	16.0×10^{-4}	0.00965	0.99523	62.01	71.81	
	20.0×10^{-4}	0	–	100	–	
$(C_9H_6NO)_2Cu$	2.8×10^{-6}	0.02499	0.99964	8.56	27.80	2.4×10^{-4}
	5.6×10^{-6}	0.02343	0.99975	12.67	29.65	
	11.2×10^{-6}	0.02332	0.99984	15.41	29.81	
	22.4×10^{-6}	0.02312	0.99987	16.78	30.06	
	39.2×10^{-6}	0.02124	0.99926	20.55	32.71	
$[Cu(phen)_2]Cl_2 \cdot 6H_2O$	2.8×10^{-7}	0.02278	0.99963	10.35	30.43	5.5×10^{-6}
	1.4×10^{-6}	0.01874	0.99772	26.25	36.98	
	2.8×10^{-6}	0.01328	0.99740	47.74	52.19	
	5.6×10^{-6}	0.01020	0.99895	59.86	67.95	
	11.2×10^{-6}	0.00373	0.99521	85.32	185.83	
	14.0×10^{-6}	0	–	100	–	
$[Cu(phen)_3]Cl_2 \cdot 6H_2O$	2.8×10^{-7}	0.02563	0.99950	-0.8	8.58	5.7×10^{-6}
	1.4×10^{-6}	0.02416	0.99954	4.94	30.31	
	2.8×10^{-6}	0.01947	0.99898	23.38	37.62	
	5.6×10^{-6}	0.01127	0.99913	55.66	65.01	
	11.2×10^{-6}	0	–	100	–	

The simple linear regression between the growth rate constant (k) and the concentrations (c) of $[Cu(phen)_2]Cl_2 \cdot 6H_2O$ or $[Cu(phen)_3]Cl_2 \cdot 6H_2O$ are:

$$CuCl_2 \cdot 2H_2O: k = -9.264\ c + 0.0223;\ R = 0.9152 \quad (5)$$

$$(C_9H_6NO)_2Cu: k = -82.10\ c + 0.0246;\ R = 0.9152 \quad (6)$$

$$[Cu(phen)_2]Cl_2 \cdot 6H_2O: k = -1517\ c + 0.0204;\ R = 0.9731 \quad (7)$$

$$[Cu(phen)_3]Cl_2 \cdot 6H_2O: k = -2419\ c + 0.0264;\ R = 0.9952 \quad (8)$$

The relationships between the growth rate constant (k) and the concentrations (c) of $[Cu(phen)_2]Cl_2 \cdot 6H_2O$ or $[Cu(phen)_3]Cl_2 \cdot 6H_2O$ are nearly linear. But those of $CuCl_2 \cdot 2H_2O$ and $(C_9H_6NO)_2Cu$ is not linear.

Inhibition ratios (I) and half-inhibitory concentrations (IC_{50})

The inhibitory ratio is defined as

$$I = [(k_0 - k_c)] / k_0 \times 100\ \% \quad (9)$$

k_c, k_0 are the growth rate constants in the presence or absence of copper(II) complexes. The results of I is also shown in Table-1.

When the inhibitory ratio is 50 %, the concentration corresponding is the half inhibition concentration IC_{50}, which is used to reflect toxicity to the cell. Corresponding to the relationship between k and c, IC_{50} is obtained and shown in Table-1. IC_{50} of $[Cu(phen)_2]Cl_2 \cdot 6H_2O$ and $[Cu(phen)_3]Cl_2 \cdot 6H_2O$ on *E. coli* approximates that of some antibiotics such as amoxicillin sodium (4×10^{-6} mol/L) and cefuroxime sodium (4×10^{-6} mg/L) [20]. According to the values of the half inhibition concentration, $[Cu(phen)_2]Cl_2 \cdot 6H_2O$ and $[Cu(phen)_3]Cl_2 \cdot 6H_2O$ have the strongest antibacterial activity and $CuCl_2 \cdot 2H_2O$ has the weakest antibacterial activity.

The action of the copper(II) complexes on the *E. coli* depends on the structure of these complexes. When copper(II) ions meet the bacteria, the cations can combine with the membrane due to the electrostatic incorporation and make the membrane protein froze. Moreover, some Cu^{2+} ions penetrate in cells and affect the activity of the enzymes because Cu^{2+} ions are easy to combine with sulfur ligands. This could be one of the reasons why these four copper (II) compounds all showed toxicity to *E. coli* to different extents.

The toxicity of complex also depends on the ligands' structure. Copper(II) ions are hydrophilic. This makes the ions hard to cross cell membrane. Biological uptake of copper relies on the presence of copper transporters named copper ATPase[21]. The ligands, 1,10-phenanthroline and 8-hydroxy quinoline (C_9H_6NO), have aromatic macro ring, which have lipophilicity and help copper to transport through biological membranes. This could lead to the excessive accumulation of copper in cells[22] and affect the growth of *E. coli*. So complexes containing 1,10-phenanthroline and 8-hydroxy quinoline (C_9H_6NO) showed stronger antibacterial activities than $CuCl_2$. Moreover, the ligand phen can insert and stack between the base pairs of DNA[23]. The binding action will influence the nucleic acids metabolism of *E. coli* and finally inhibit its growth. The inhibition of $[Cu(phen)_2]Cl_2 \cdot 6H_2O$ and $[Cu(phen)_3]Cl_2 \cdot 6H_2O$ is stronger than that of $(C_9H_6NO)_2Cu$.

Considering the combined effect of the excessive accumulation of intracellular copper transported by phen or C_9H_6NO and the binding action of complexes with the nucleic acids of *E. coli*, the sequence of the inhibition of these complexes on *E. coli* is: $[Cu(phen)_2]Cl_2 \cdot 6H_2O$ and $[Cu(phen)_3]Cl_2 \cdot 6H_2O$ > $(C_9H_6NO)_2Cu$ > $CuCl_2$, which was according with microcalorimetric measurement.

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