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

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Copper(II) complexes were synthesized and their effects on the growth of *Escherichia coli* (*E. coli*) were studied by microcalorimetry. Based on the power-time curves of *Escherichia 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 activity³ 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 DNA⁵⁻⁷. 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 reductants⁸.

8-Hydroxy quinoline is also a well-known monoprotonic bidentate chelating agent which is able to combine with many primary, transition and rare-earth metal ions⁹⁻¹¹. 8-Hydroxy-quinoline and its derivatives exhibit substantial cytotoxic activity against cancer cells¹²⁻¹⁵. Some copper complexes show antimicrobial and antifungal properties, being constituents in antiseptic and disinfectant formulations, in preservatives and in agricultural fungicides¹⁴⁻¹⁵.

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

Escherichia 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 °C 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 reference¹⁶. 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 $[\text{Cu}(\text{phen})_3]\text{Cl}_2 \cdot 6\text{H}_2\text{O}$ (A), $[\text{Cu}(\text{phen})_2]\text{Cl}_2 \cdot 6\text{H}_2\text{O}$ (B), $(\text{C}_6\text{H}_6\text{NO})_n\text{Cu}$ (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_0) 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. $[\text{Cu}(\text{phen})_2]\text{Cl}_2 \cdot 6\text{H}_2\text{O}$ and $[\text{Cu}(\text{phen})_3]\text{Cl}_2 \cdot 6\text{H}_2\text{O}$ show similar activity on the growth of *E. coli*. Their concentrations are lower than those of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ and $(\text{C}_9\text{H}_6\text{NO})_2\text{Cu}$ at the same rate constant. This indicates their inhibiting action is stronger than $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ and $(\text{C}_9\text{H}_6\text{NO})_2\text{Cu}$. The inhibition action of $(\text{C}_9\text{H}_6\text{NO})_2\text{Cu}$ is stronger than that of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$.

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

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

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 ₉ H ₆ NO) ₂ Cu	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 ·6H ₂ O	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 ·6H ₂ O	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]Cl₂·6H₂O or [Cu(phen)₃]Cl₂·6H₂O are:

$$\text{CuCl}_2 \cdot 2\text{H}_2\text{O}: k = -9.264 c + 0.0223; R = 0.9152 \quad (5)$$

$$(\text{C}_9\text{H}_6\text{NO})_2\text{Cu}: k = -82.10 c + 0.0246; R = 0.9152 \quad (6)$$

$$[\text{Cu}(\text{phen})_2]\text{Cl}_2 \cdot 6\text{H}_2\text{O}: k = -1517 c + 0.0204; R = 0.9731 \quad (7)$$

$$[\text{Cu}(\text{phen})_3]\text{Cl}_2 \cdot 6\text{H}_2\text{O}: k = -2419 c + 0.0264; R = 0.9952 \quad (8)$$

The relationships between the growth rate constant (k) and the concentrations (c) of [Cu(phen)₂]Cl₂·6H₂O or [Cu(phen)₃]Cl₂·6H₂O are nearly linear. But those of CuCl₂·2H₂O and (C₉H₆NO)₂Cu is not linear.

Inhibition ratios (I) and half-inhibitory concentrations (IC₅₀)

The inhibitory ratio is defined as

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

k_c, k₀ 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 IC₅₀, which is used to reflect toxicity to the cell. Corresponding to the relationship between k and c, IC₅₀ is obtained and shown in Table-1. IC₅₀ of [Cu(phen)₂]Cl₂·6H₂O and [Cu(phen)₃]Cl₂·6H₂O 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]Cl₂·6H₂O and [Cu(phen)₃]Cl₂·6H₂O have the strongest antibacterial activity and CuCl₂·2H₂O 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 Cu²⁺ ions penetrate in cells and affect the activity of the enzymes because Cu²⁺ 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 ATPase²¹. The ligands, 1,10-phenanthroline and 8-hydroxy quinoline (C₉H₆NO), 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 cells²² and affect the growth of *E. coli*. So complexes containing 1,10-phenanthroline and 8-hydroxy quinoline (C₉H₆NO) showed stronger antibacterial activities than CuCl₂. Moreover, the ligand phen can insert and stack between the base pairs of DNA²³. The binding action will influence the nucleic acids metabolism of *E. coli* and finally inhibit its growth. The inhibition of [Cu(phen)₂]Cl₂·6H₂O and [Cu(phen)₃]Cl₂·6H₂O is stronger than that of (C₉H₆NO)₂Cu.

Considering the combined effect of the excessive accumulation of intracellular copper transported by phen or C₉H₆NO 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]Cl₂·6H₂O and [Cu(phen)₃]Cl₂·6H₂O > (C₉H₆NO)₂Cu > CuCl₂, which was according with microcalorimetric measurement.

REFERENCES

1. T.A. Yousef, G.M. Abu El-Reash, O.A. El-Gammal and R.A. Bedier, *J. Mol. Struc.*, **1029**, 149 (2012).
2. P. Gameiro, C. Rodrigues, T. Baptista, I. Sousa and B. de Castro, *Int. J. Pharm.*, **334**, 129 (2007).
3. C. Marzano, M. Pellei, F. Tisato and C. Santini, *Anti-cancer Agents Med. Chem.*, **9**, 185 (2009).
4. F. Arjmand, S. Parveen, M. Afzal and M. Shahid, *J. Photochem. Photobiol. B: Biol.*, **114**, 15 (2012).

5. D.S. Sigman, D.R. Graham, V. D'Aurora and A.M. Stern, *J. Biol. Chem.*, **254**, 12269 (1979).
6. R.S. Kumar and S. Arunachalam, *Polyhedron*, **26**, 3255 (2007).
7. V. Rajendiran, R. Karthik, M. Palaniandavar, H. Stoeckli-Evans, V.S. Periasamy, M.A. Akbarsha, B.S. Srinag and H. Krishnamurthy, *Inorg. Chem.*, **46**, 8208 (2007).
8. D.S. Sigman, *Biochemistry*, **29**, 9097 (1990).
9. J. Sary, *Anal. Chim. Acta*, **28**, 132 (1963).
10. P.K. Mishra, V. Chakravorthy and K.C. Dash, *Radiochim. Acta*, **47**, 235 (1989).
11. M. Albrecht, O. Osetska, R. Fröhlich, J.-C.G. Bünzli, A. Aebischer, F. Gumy and J. Hamacek, *J. Am. Chem. Soc.*, **129**, 14178 (2007).
12. J. Nordenberg, A. Novogrodsky and E. Beery, M. Patia, L. Wasserman and A. Warshawsky, *Eur. J. Cancer*, **26**, 905 (1990).
13. S. Zhai, L. Yang, Q.C. Cui, Y. Sun, Q.P. Dou and B. Yan, *Biol. Inorg. Chem.*, **15**, 259 (2010).
14. R.G.W. Hollingshead, The Antibacterial and Antifungal Action of Oxine, Its Derivatives and Chelates, Butterworth Scientific Publications, London, Vol. 4 (1954).
15. S.S. Block, Preservatives for Industrial and Miscellaneous Products, Lea & Febiger, Philadelphia, pp. 105-114 (1991).
16. H.M. Chao, Inorganic Compounds Synthesis Manual III, Chemical Industry Press, Beijing, pp. 52-76 (1988).
17. C.Y. Wang, F. Xu, L.X. Sun, Y.-J. Sun, S.-J. Qiu, Z.- Zhao, H.- Tan and S. Wang, *J. Therm. Anal. Calorim.*, **111**, 959 (2013).
18. L. Yi, Y. Chengnong, W. TianZhi, Z. Ruming, Q. Songsheng and S. Ping, *Thermochim. Acta*, **333**, 103 (1999).
19. J. Yao, Y. Liu, Z.-T. Gao, P. Liu, M. Sun, X. Zou, S.-S. Qu and Z.-N. Yu, *Chin. J. Chem.*, **20**, 746 (2002).
20. L.N. Yang, S.J. Qiu, F. Xu, L.X. Sun, Z.B. Zhao, J.G. Liang and C.G. Song, *J. Therm. Anal. Calorim.*, **89**, 875 (2007).
21. J.J. Ravia, R.M. Stephen, F.K. Ghishan and J.F. Collins, *J. Biol. Chem.*, **280**, 36221 (2005).
22. X.Q. Cai, N. Pan and G.L. Zou, *BioMetals*, **20**, 1 (2007).
23. L.N. Ji, Q.L. Zhang and J.G. Liu, *Sci. China Ser. B*, **44**, 246 (2001).