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STUDY ON BIOLOGICAL EFFECT OF A KIND OF HETERO-BIMETALLIC SCHIFF- BASE ON *ESCHERICHIA COLI*

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The biological activity of a kind of hetero-bimetallic Schiff-base complex was studied using *Escherichia coli* (*E. coli*) cell as the target. By microcalorimetry, the difference of anti-bacterial activity between the binuclear Schiff-base and the ligand was determined and analyzed. To analyze the inhibition of the bacterial growth internally, the *E. coli* cells grown in the presence of hetero-bimetallic Schiff-base complex were observed by scanning electron microscopy. The images in high resolution revealed the damage of outer cell membrane caused the inhibitory effect on *E. coli*. Inductively coupled plasma-mass spectrometry results proved the absorption of the complex by cells, which confirmed the interaction between the Schiff-base and biological macromolecule.

Keywords: *anti-bacterial, coordination, inhibition, microcalorimetry, Schiff-base*

Introduction

The Schiff-base was characterized with its antibacterial and anti-tumor properties [1–4]. A series of Schiff-bases were synthesized to develop the potential drugs for purposes of diminishing inflammation and curing cancer. Recently, both transition metals and f-block metals with Schiff-base ligand have drawn wide attention because these complexes have exhibited excellent biological effect [5, 6]. The coordination ability of transition metals and f-block metals helps to enhance the biological effect of Schiff-base complexes. N,N'-ethylenebis (3-methoxysalideneiminato) zinc(II) holmium(III) nitrate was synthesized and characterized [7]. The structure of the Schiff-base ligand (H_2L) and the complex ($ZnHo(NO_3)_3L$) are shown in Fig. 1. This hetero-bimetallic complex contains both transition metal (zinc) and f-block metal (holmium) with Schiff-base ligand.

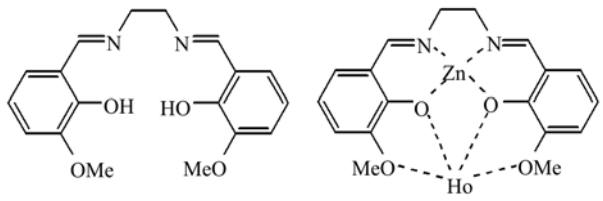

Fig. 1 The structure diagram of Schiff-base ligand (H_2L) and the complex ($ZnHo(NO_3)_3L$) with both transition and f-block metals. NO_3 was omitted for clarity

E. coli was employed in this study to investigate the biological effect of bimetallic Schiff-base complex. *E. coli*, a class of Gram-negative bacteria, has been studied extensively on the outer membrane [8, 9]. Lipopolysaccharide (LPS), a major component of the surface of Gram-negative bacteria, is composed of lipid A, an inner core and an outer core, and the units of O-antigen. The LPS is an important entity in determining outer membrane barrier function and the virulence of Gram-negative pathogens. LPS is often referred to as endotoxin, which is responsible for the pathophysiological phenomena associated with Gram-negative infections [10, 11]. There is considerable interest in understanding the properties of LPS and its interaction with complexes because LPS is involved in developing new anti-bacterial drug for treatment of pathology induced by Gram-negative bacteria [11].

Here we reported the growth curves of *E. coli* in the presence of binuclear Schiff-base complex by microcalorimetry. As a bioactivity monitor, microcalorimetry is a non-destructive and non-invasive method for monitoring a variety of processes, such as metabolism of microorganisms [12, 13]. Furthermore, scanning electron microscopy (SEM) observation and inductively coupled plasma-mass spectrometry (ICP-MS) analysis gave us addition insight into the biological effect of binuclear Schiff-base complex on *E. coli*.

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Experimental

Materials

Escherchia coli (K12) was provided by the Chinese Center for Type Culture Collections of Wuhan University. The LB culture was sterilized in high-pressure steam at 120°C for 30 min. N,N'-Ethylenebis(3-methoxysalideneiminato)zinc(II)holmium(III)nitrate was prepared according to literature procedure [7].

Methods

Microcalorimetry

LKB 2277 Bioactivity Monitor (Thermometric AB, Sweden), which is a type of heat conduction microcalorimeter, was designed to monitor continuously heat released or absorbed in a series of processes, such as the metabolism of cells. The performance and the details of this instrument have been described previously [12–14].

E. coli was inoculated in the prepared LB cultures containing different amount of H_2L or $ZnHo(NO_3)_3L$. Then the solutions were put into the calorimeter to monitor the growth of *E. coli* cells in the presence of the complex. The metabolic thermogenic curves were recorded in real time.

SEM observation

After *E. coli* was grown to stationary phase, the cells were washed and collected. Glutaraldehyde and osmium tetraoxide (OsO_4) were used to fix and dehydrate the protein (or lipid) in cells. Double fixation by oxidization after reduction made the most of the characteristics of two different fixatives, providing satisfactory results. Then the cells were dehydrated in a series of increasing concentration of ethanol (50, 60, 70, 80, 90, 95, 100%). After the *E. coli* cells were fixed and dehydrated, they were observed by SEM (LEO-1530).

Confirmation of the Schiff-base absorption by *E. coli*

E. coli was incubated in 2 pieces of LB cultures, one of them containing 5.0 μg mL^{-1} $ZnHo(NO_3)_3L$. After the cells grow to stationary phase at 37°C, they were centrifuged and collected. The cells were then re-suspended in a solution of 0.9% NaCl. The operation was repeated 4 times to remove the media completely.

The pellet was incubated in HNO_3 and $HClO_4$ until a clear solution was formed. The solution was diluted to the origin volume of bacteria solution for the determination of Ho^{3+}, Zn^{2+} concentration using ICP-MS (PE-SCITEX, API 365). Ultra-pure water was used for dilution.

Results and discussion

All biological processes coincide with heat release or absorption, e.g. the metabolism of bacteria. Microcalorimetry can monitor the bacteria growth by measuring very small heat flow as a non-destructive and non-invasive technique [13]. Figures 2a and b have shown the growth process of *E. coli* in the presence of H_2L and $ZnHo(NO_3)_3L$, respectively. According to thermogenic curves in Fig. 2, the presence of H_2L has a little effect on *E. coli* growth. Lag phase was prolonged with the increasing concentration of H_2L. No obvious change was observed about the maximum heat power and the metabolism in growth process. From Fig. 2b, the Schiff-base ligand with 3d,4f-bi-metals are observed to show strong inhibitory effect on *E. coli* growth. The growth rate of *E. coli* in the presence of $ZnHo(NO_3)_3L$ decreased significantly and generation time prolonged accordingly. With the increasing amount of $ZnHo(NO_3)_3L$ in culture, the heat output power of *E. coli* cells decreased. When the concentration increased to 7.5 μg mL^{-1}, the growth of bacteria was inhibited completely and no heat released from metabolism was detected.

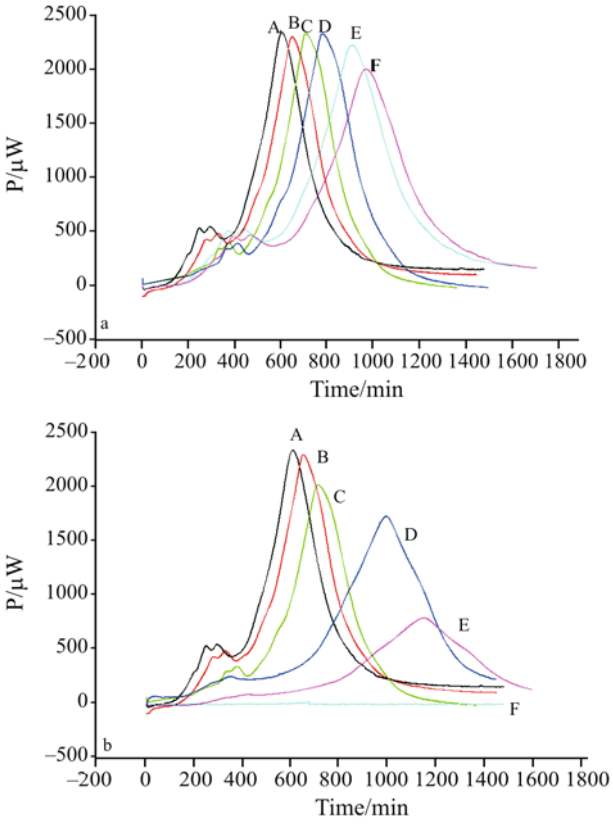

Fig. 2 The metabolic thermogenic curves of *E. coli* cells in the presence of a – H_2L and b – $ZnHo(NO_3)_3L$; A – control, B – 1.5, C – 3.0, D – 4.5, E – 6.0, F – 7.5 μg mL^{-1}

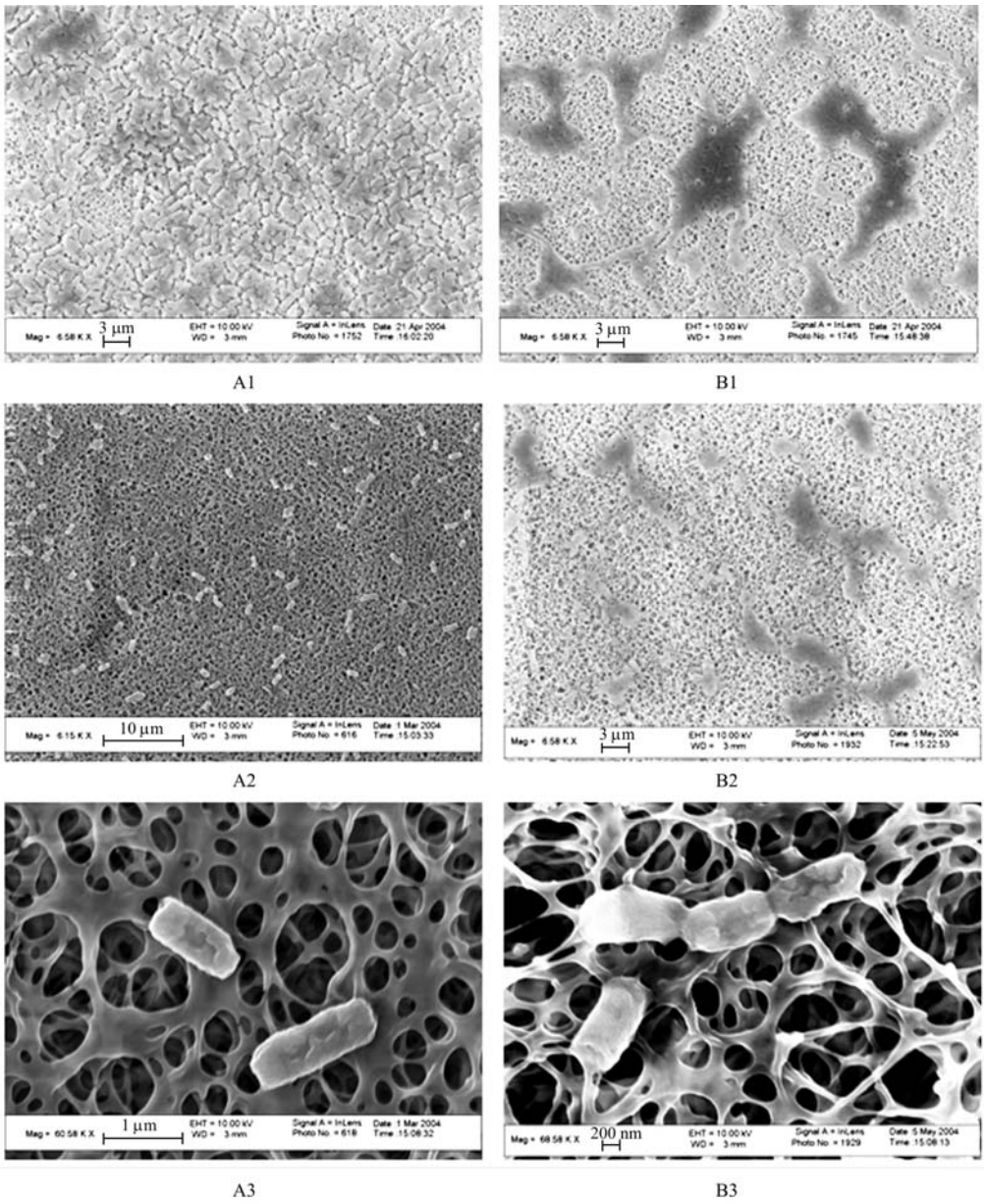

Fig. 3 SEM images of the native *E. coli* cells (column A) and the cells grown in the presence $ZnHo(NO_3)_3L$ (column B)

To analyze the mechanism that *E. coli* growth was inhibited in the presence of binuclear Schiff-base complex, *E. coli* cells were observed by SEM after grown to stationary phase. Columns A and B in Fig. 3 showed the native cells and cells grown in the presence of $ZnHo(NO_3)_3L$, respectively. From graph A1 and B1, it can be seen that the native cells were separated uniformly, while cells in the presence of $ZnHo(NO_3)_3L$ conglomerated. The cells were observed in different scanning area in images A2, A3 and B2, B3 after diluted to 10 tenth. The conglomeration of *E. coli* cells in the presence of $ZnHo(NO_3)_3L$ can be observed more clearly in image (B3). The outer membrane of cells was 'melted' to some extent. Cells were conglutinated by the melting LPS, which is the main component of the cell wall (outer membrane). According to the results of microcalorimetry and SEM, inhibition of *E. coli* growth can be attributed to the disassembly of the cell wall. The hetero-bimetallic complex has stronger inhibitory effect on bacteria than exclusively the ligand because both transition metals and f-block metals have strong affinity to coordinate with the macromolecule on cell surfaces [15, 16].

LPS on the surface of Gram-negative bacteria is called endotoxin because it causes fever and pathology when injected into animals [17]. The complication of Gram-negative infections is a common cause in debilitated patients. Considerable progress has been made in defining the mechanism by which endotoxin interacts with animal cells [18]. During infections, LPS can dissociate from bacteria and deliver to a surface protein of macrophages and other LPS-re-

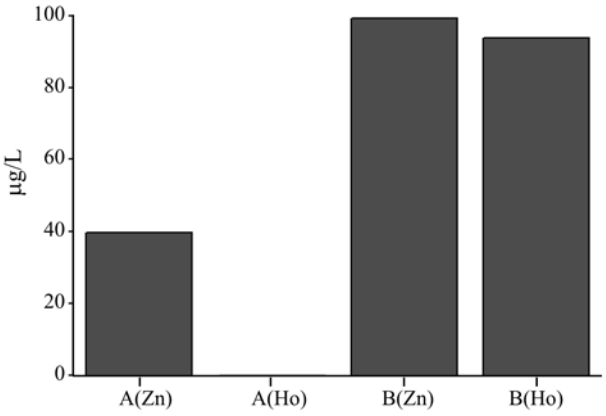

Fig. 4 The concentration of Zn and Ho in the two groups of *E. coli* cells; A – native cells, B – cells grown in the presence of $ZnHo(NO_3)_3L$

sponsive cells. One of the approaches to the treatment of endotoxin-induced shock is to block the activation of macrophages [19]. According to images in column B of Fig. 3, presence of $ZnHo(NO_3)_3L$ leads to the destroying of outer membrane (LPS) and conglomeration of cells, which is an evidence of interaction between $ZnHo(NO_3)_3L$ and LPS. The ability of both transition metals and f-block metals to coordinate with oxygen in LPS molecule may be the basis of the toxicity of the complex.

The concentration of Ho^{3+} and Zn^{2+} in two groups of *E. coli* cells were determined by ICP-MS to evaluate the effect of $ZnHo(NO_3)_3L$ after interaction with the outer membrane of *E. coli*. From the results shown in Fig. 4, both Ho^{3+} and Zn^{2+} were detected in the cells grown in the presence of $ZnHo(NO_3)_3L$. The presence of Ho^{3+} and Zn^{2+} in cells indicated that the complex can be absorbed by the bacteria, which confirmed the interaction between the Schiff-base and biological macromolecule. Thus the complex has the physiological functions and biological effects on the organelle and macromolecule in cytoplasm. Further work will be done to elucidate the role that binuclear Schiff-base complex plays in cells.

Conclusions

Schiff-base, a potential anti-bacterial and anti-cancer drug, has attracted great attention and interest of researchers. By coordination with transition metals and f-block metals, the Schiff-base increased its anti-bacterial activity due to the strong affinity of such metal to oxygen atom or nitrogen atom in macromolecule [20–23]. It is proposed that oxygen atom in LPS was drawn from 2,3-diacylglucosamine, which lead to serious damage of outer cell membrane (LPS). Consequently, the growth of bacteria was inhibited in the presence of hetero-bimetallic Schiff-base complex. Because LPS is involved in the endotoxin-induced shock of Gram-negative bacteria, serious damaging of LPS by Schiff-base complex will give insight into the treatment of pathology caused by Gram-negative bacteria.

Acknowledgements

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