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Short communication

Microcalorimetric study about biological effect of a synthetic complex: $La(Glu)(Im)_6(ClO_4)_3{\cdot}4HClO_4{\cdot}4H_2O$

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ABSTRACT

A complex of lanthanum perchloric acid coordinated with glutamic acid, $La(Glu)(Im)_6(ClO_4)_3{\cdot}4HClO_4{\cdot}4H_2O$ was synthesized and characterized. The biological effect of the complex was evaluated by microcalorimetry on the growth of *E. coli* DH5α. Power–time curves of the growth metabolism of *E. coli* DH5α were studied using a TAM Air Isothermal Microcalorimeter at 37 °C. From the power–time curves, the parameters such as growth rate constants (k), inhibitory ratio (I), the maximum heat power (P_m) and the time of the maximum heat power (t_m) were obtained. The results show that the concentrations of the complex affect obviously the growth metabolism of *E. coli* DH5α. The stimulatory effect on growth of *E. coli* DH5α was observed when the concentration of the complex was kept in the range of (0–0.5 μg mL^{-1}). In contrast to the lower concentration, in the case of higher concentration of the complex (0.5–5.0 μg mL^{-1}), an inhibitory effect occurred.

1. Introduction

Complexes of rare earth ions with amino acids have many unique biochemical properties, which have been widely used as fertilizer, pesticide, antibacterial agent and so on in agriculture, stockbreeding, and medical treatment. Due to the extensive use in many areas, the rare earth elements spread through the food chain inevitably, and enter the bodies of human beings eventually. In order to study the long-term influence of rare earth elements on people and explore more extensively application of the complexes, the complexes of rare earth ions with amino acids have been synthesized and studied on their biological properties with some methods [1–5].

Microcalorimetry is a potential technique in the field of life sciences, which has the advantages of high sensitivity, high efficiency, non-invasive and non-destructive manner [6–8]. Therefore, it has been widely used as analytical tools in medicine, biotechnology, agriculture, forestry and ecology fields [9–13].

In this work, a complex of lanthanum perchlorate coordinated with glutamic acid and imidazole, $La(Glu)(Im)_6(ClO_4)_3{\cdot}4HClO_4{\cdot}4H_2O$ was synthesized. Microcalorimetry was applied to study the interaction between the complex and *E. coli* DH5α to elucidate the biological effects of the complex.

2. Experimental

2.1. Synthesis and characterization of the complex

The complex, $La(Glu)(Im)_6(ClO_4)_3{\cdot}4HClO_4{\cdot}4H_2O$, was synthesized in water solution. All the starting materials were analytical reagents from the Beijing Chemical Reagent Co. Rare earth oxide (La_2O_3) was dissolved in an excess amount of perchloric acid and the concentration of the solution was determined by EDTA titrimetric analysis. Then solid glutamic acid was added to the solution of La^{3+} in molar ratio of La^{3+}:Glu = 1:1. After the pH value of the reaction mixture was carefully adjusted to 6.0 by adding 0.1 mol L^{-1} NaOH slowly; imidazole was added as the same molar as glutamic acid. The reaction was performed at 60 °C in water-bath for 5 h. Then the solution was condensed at 80 °C for 5 h and put into desiccator in the fridge at −4 °C. The crystal was obtained after about one month [14].

An elemental analysis apparatus (Model PE-2400 II, USA) was used to measure the ratio of C, H, N in the complex. Content of La% was determined by EDTA titration. Found La, (9.423%), C (18.363%), N (12.964%) and H (2.736%), which are close to the theoretical values, La (9.057%), C (18.883%), N (12.452%) and H (2.805%). The sample formula was determined to be $La(Glu)(Im)_6(ClO_4)_3{\cdot}4HClO_4{\cdot}4H_2O$ and the purity, obtained from

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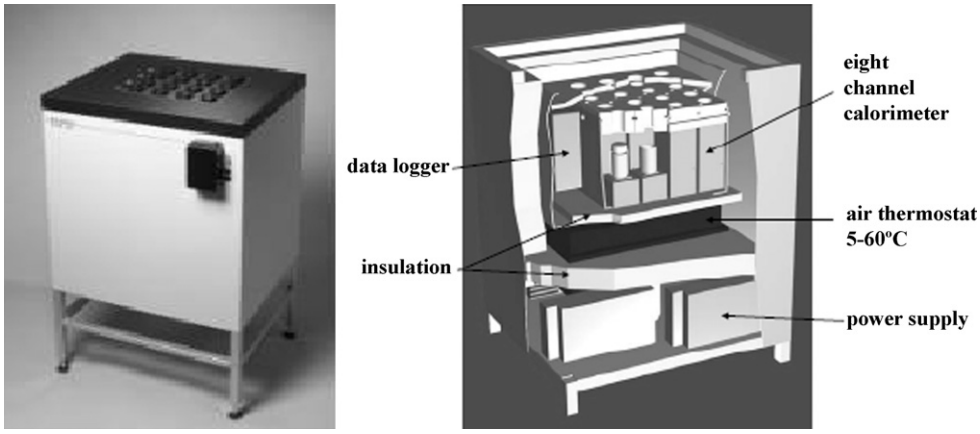

Fig. 1. Practicality and cutaway views of TAM Air 8-channel Isothermal calorimeter [16].

the EDTA titration under the same conditions was found to be 99.79%.

IR spectra were obtained using KBr pellets with a Tensor 27 (Bruker) spectrometer. It can be seen from the IR spectra of the complex that the symmetrical resonance frequencies, $\nu_{s(COO-)}$, shifted from 1431 to 1413 cm^{-1}, which suggested that the carboxyl group of Glu have coordinated to the metal ions [14].

2.2. The E. coli

The *E. coli* DH5α was provided by Biomass Conversion Technology Group, Dalian Institute of Chemical Physics, CAS, Dalian 116023, PR China. The strain of *E. coli* DH5α was stored in 10% glycerol solution at −20 °C.

The *E. coli* DH5α used in the present paper was prepared as follows. A single colony of *E. coli* DH5α from LB plates was inoculated into a 10 mL LB culture medium and cultivated at 37 °C in a rotary shaker (220 rpm) for 12 h under aerobic condition. Then, 200 μL of the above suspension was inoculated into 10 mL LB culture medium at 37 °C in a rotary shaker (220 rpm) for 2.5 h once again. The value of OD (optical density) of the *E. coli* DH5α suspension was measured to be about 0.6 by spectrophotometry at λ = 600 nm.

Per 200 mL of LB (Luria-Bertani) culture medium contained tryptone 2 g, yeast extract powder 1 g and NaCl 2 g (pH 7.0–7.2). It was sterilized in high pressure steam at 120 °C for 20 min before experiment.

2.3. Microcalorimeter

TAM (Thermal Analysis Microcalorimetry) Air is a type of 8-channel twin isothermal microcalorimeters that originally was developed for the study of the hydration process of cement and concrete. TAM represents an ultra-sensitive heat flow measurement which is complementary to TA Instruments differential scanning calorimeters (DSC). "Air" means the thermostat type. However, the high sensitivity and excellent long-term stability makes TAM Air useful also for other applications such as stability testing of energetic materials, determining the shelf life of food and monitoring biological processes [15].

A TAM Air Isothermal Microcalorimeter (see Fig. 1) manufactured by Thermometric AB Company of Sweden (incorporated by TA Instruments in 2006) was used to measure the power–time curves of the metabolism of *E. coli* DH5α at 37 °C. The main structure of the instrument is eight channels, each of which consists of aluminium heat sink, vessel holder and thermocouple plate, and each two of them are twin. Glass reaction vessel of 20 mL was used in each channel. The working temperature in the instrument was 5–60 °C controlled by air thermostat. The deviation of the controlled temperature is within ±0.02 °C. A computer was employed to record the voltage–time signals continuously which were converted to power–time signals through calibration. Thermal power detection limit was stated to be ±2 μW. The development and theory of many kinds of multi-channel isothermal microcalorimeters have been studied by I. Wadsö [13].

The ampoule method was used for the microcalorimetric measurement in this work. LB (Luria–Bertani) culture medium (10 mL) containing object compound with different concentrations were put into eight sample ampoules, which had been cleaned and sterilized. Then, the *E. coli* DH5α suspension was inoculated into the above eight ampoules. At last the ampoules were sealed with ampulla cap by ampoule filler. The working temperatures of the calorimeter were set and controlled at 37 °C. The power–time signals were recorded at intervals of 1 min.

3. Results and discussion

3.1. Thermokinetics

In the log phase of growth, the growth of *E. coli* DH5α cells is exponential [17]. It can be expressed as follows:

$$n_t = n_0 \ \exp(kt) \tag{1}$$

where t is the incubation time, n_t is the cell number at time t, n_0 is the initial cell number and k is the constant of cell growth rate. If the power output of each cell is denoted as P_w, then

$$n_t P_w = n_0 P_w \ \exp(kt) \tag{2}$$

We define P_0 as the initiative power output, P_t as the power output at time t, then Eq. (2) can be rewritten as follows

$$P_t = P_0 \ \exp(kt) \tag{3}$$

or

$$\ln P_t = \ln P_0 + kt \tag{4}$$

Thermokinetic parameters, inhibitory ratio (I) is defined as:

$$I = \frac{(k_0 - k_c)/k_0}{100\%} \tag{5}$$

where k_0 is the rate constant of the control, k_c is the rate constant of *E. coli* DH5α growth inhibited by the complex with a concentration of C.

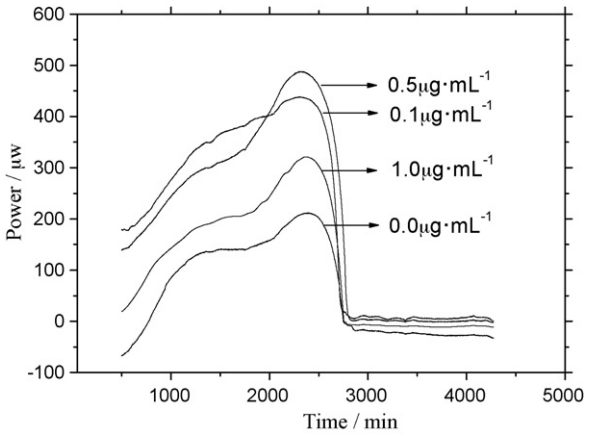

Fig. 2. Growth curves of *E coli* at different concentrations (0, 0.1, 0.5, 1.0 g mL^{-1}) of the complex at 37 °C.

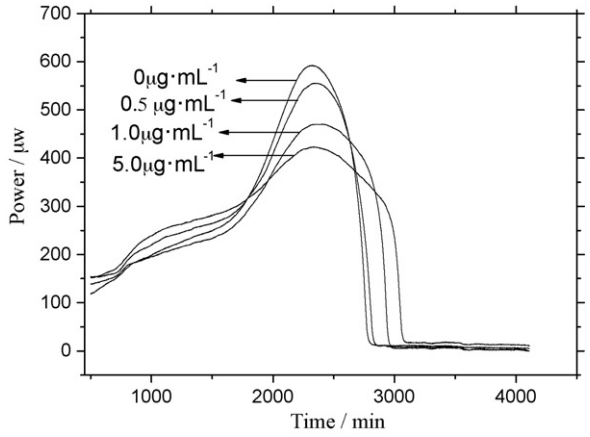

Fig. 3. Growth curves of *E coli* at different concentrations (0, 0.5, 1.0, 5.0 g mL^{-1}) of the complex at 37 °C.

3.2. Thermokinetic parameters

Figs. 2 and 3 show the growth power–time curves of *E. coli* DH5α under various concentrations (0–5.0 μg mL^{-1}) of the complex at 37 °C. It can be seen from the figures that growth curve of the *E. coli* DH5α can be divided into four typical phases, which are lag phase, log phase, stationary phase and decline phase. The thermokinetic parameters of growth, growth rate constants (k, 10^{-3} min^{-1}), the maximum heat power (P_m, μW) and the time of the maximum heat power (t_m, min) are derived from the curves by using the above Eqs. (1)–(5), and the results are listed in Tables 1 and 2.

The relationships among the concentration (C), the rate constant (k) and the maximum heat power (P_m) were plotted in Fig. 4 at different concentrations (0, 0.1, 0.5, 1.0 g mL^{-1}) based on the data of Table 1. It can be seen from the figure that the rate constant (k) and the maximum heat power (P_m) were both increasing with increase of the concentration from 0 to 0.5 μg mL^{-1}, which indicated that the complex could speed up the growth of *E. coli* DH5α. When the concentration (C) reached 0.5 μg mL^{-1}, the value of rate constant (k) showed a highest value. It demonstrated that the complex could promote clearly the growth of *E. coli* DH5α at this time. At the concentration (C) from 0.5 to 1.0 μg mL^{-1}, the line became decreasing, which was consistent with Fig. 5.

Fig. 5 was plotted from the data of Table 2, when the concentration (C) was from 0.5 to 5.0 μg mL^{-1}. It showed that the rate constant (k) and the maximum heat power (P_m) were both decreasing with the increase of the concentration (C), which indicated that the complex inhibited the growth of *E. coli* DH5α at this range of concentrations (C). When the concentration reached 5.0 μg mL^{-1}, the *E. coli* DH5α almost could not grow.

Table 1
Thermodynamic parameters of growth of *E. coli* at different concentrations of the complex.

C (μg mL^{-1})	k (10^{-3} min^{-1})	R	P_m (μW)	t_m (min)	I%
0	0.2	0.9992	211.9	2390	0
0.1	0.25	0.9967	438.2	2297	13.3
0.5	0.3	0.9989	488.0	2328	20.0
1.0	0.08	0.9981	379.6	2391	53.3

Table 2
Thermodynamic parameters of growth of *E. coli* at different concentrations of the complex.

C (μg mL^{-1})	k (10^{-3} min^{-1})	R	P_m (μW)	t_m (min)	I%
0	1.5	0.9993	592.3	2308	0
0.5	1.3	0.9961	555.4	2365	25
1.0	1.2	0.9985	470.5	2422	50
5.0	0.7	0.9981	423.8	2332	80

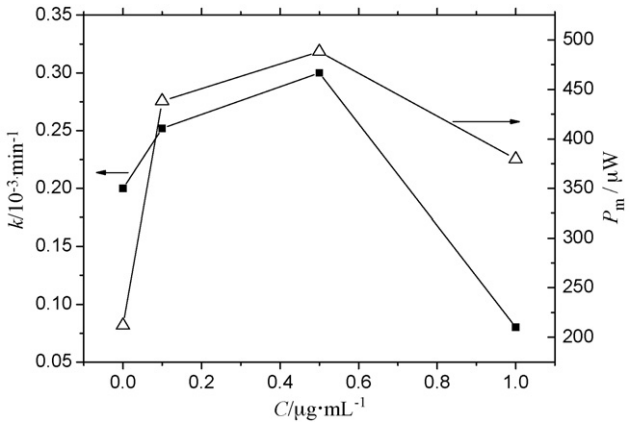

Fig. 4. *K–c* curves of *E coli* at different concentrations (0, 0.1, 0.5, 1.0 g mL^{-1}) of the complex at 37 °C.

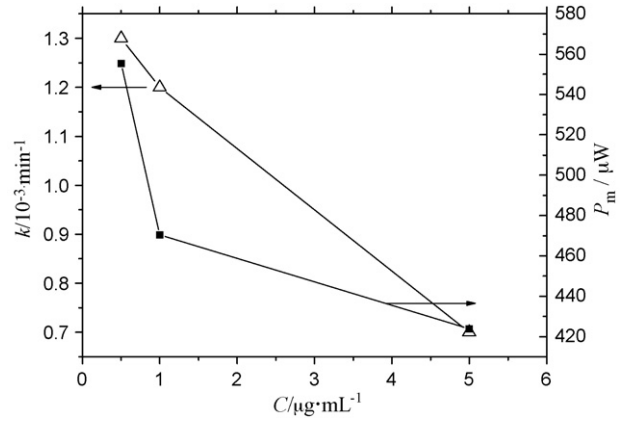

Fig. 5. *K–c* curves of *E coli* at different concentrations (0, 0.5, 1.0, 5.0 g mL^{-1}) of the complex at 37 °C.

4. Conclusions

In this paper, a new complex, $La(Glu)(Im)_6(ClO_4)_3{\cdot}4HClO_4{\cdot}4H_2O$ was synthesized and characterized. The microcalorimetric method was used to study the influence of synthetic complex on the growth of *E. coli* DH5α. The thermokinetic parameters of growth, such as

growth rate constants (k, 10^{-3} min^{-1}), the maximum heat power (P_m, μW) and the time of the maximum heat power (t_m, min) were derived. It was concluded that the complex has a stimulatory effect on the growth of *E. coli* DH5α below the concentration 0.5 μg mL^{-1}, and the complex could inhibit its growth in the concentration range between 0.5 and 5.0 μg mL^{-1}.

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