Microcalorimetric Study of the Action of Yb^{3+} Ion on the Growth of *Staphylococcus aureus*

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A microcalorimetric method was used to evaluate the action of Yb^{3+} ions onthe growth metabolism of *Staphylococcus aureus*. The power-time curves of the growth metabolism of *Staphylococcus aureus* and the action of Yb^{3+} ions were obtained by using stopped-flow method at 37 ℃. For evaluation of the action, the growth rate constants (k_1 and k_2) for the log phase 1, log phase 2, and the total heat effect (Q_{total}) for *Staphylococcus aureus* were determined. The results show that Yb^{3+} ions at low concentrations have the stimulatory effect on *Staphylococcus aureus* and that Yb^{3+} ions at higher concentration could inhibit its growth.

Keywords microcalorimetry, *Staphylococcus aureus*, Yb^{3+}, duplex functions, thermokinetics

Introduction

Staphylococcus aureus,[1] as a kind of spheric gram-positive bacteria, can be widely found in nature and food resources. It belongs to drug-resistant strain and pollutes food easily. The enterotoxin could cause food toxin after being taken with food. Meanwhile, it is a significant pathogenetic fungi and source of infection which leads to cross infection in hospital. It has been treated as potential danger in sanitary bacteriology. Therefore, the research on inventing efficient antiseptic drugs is important for drug-development.

Chen *et al*.[2-5] adopted various methods to investigate the inhibitory function of cistine, red sage root ketone and their complexes, and Chitosan, sodium selenite on *Staphylococcus aureus*. To evaluate the effect of *D*-Glucosamine Shiff bases and their complexes on *Staphylococcus aureus* by microcalorimetry method was reported.[6] Systematical research on the affection of lanthanide ions with *Escherichia coli* was reported.[7]

Scientists have paid much attention to the bioactivity of lanthanide complexes for their antibacterial, *anti*-inflammation action and antineoplastic function.[8] Ytterbium belongs to heavy rare earth series, and plays an important role in inorganic biochemistry.[8,9] It has been reported[8] that the Ytterbic ions could penetrate through the pericellular membrane into the interior of a cell, and too many ions could destroy cell organs and result in cell aptosis. Yb^{3+} ions, as a kind of hard-acid, are easily to combine with hard-base ligands of biological molecules (such as COOH, OH, C=O, P=O, P—O^-) to form Ytterbic biological complexes. The formed complexes could affect the metabolism of organs, and even destroy the bioactivity function absolutely. Study on the interaction of Yb^{3+} ions with *Staphylococcus aureus* by microcalorimetric method is very important for us to understand the charactertics of ytterbium on bioactivity especially on cells, and its pharmacology and toxicology studies. Moreover, these information could lead us to discover and develop high-efficiency and low harmful drugs. Up to now, the research about the effection of lanthanide ion on *Staphylococcus aureus* has not been reported.

In this paper, the power-time curves of the growth metabolism of *Staphylococcus aureus* under the action of Yb^{3+} ions of different concentration were determined by LKB-2277 Bioactivity Monitor. From these power-time curves, the growth rate constants (k_1 and k_2), and the total heat effect (Q_{total}) for *Staphylococcus aureus* were obtained. The results show that Yb^{3+} ions have duplex functions, *i.e*, Yb^{3+} ions have stimulating effect on the metabolism of microorganism at low concentration, on the other hand, Yb^{3+} ions have inhibitory effect on the metabolism of microorganisms at high concentration.

Experimental

Reagents

All chemicals were of analytical reagent grade. Ytterbium nitrate pentahydrate was obtained from Aldrich Chemical Company. Peptone and yeast extract were of biological reagents (Oxoid).

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Bacteria

Staphylococcus aureus (CCTCC AB910393) was provided by the Chinese Center for Type Culture Collections, Wuhan University, China.

Growth media

Staphylococcus aureus was grown on a peptone culture medium. The peptone culture medium contained NaCl (5 g), peptone (10 g), yeast extract (5 g), per 1000 mL (pH = 7.0—7.2). It was sterilized in high-pressure steam at 120 ℃ for 30 min.

Instruments

LKB-2277 Bioactivity Monitor (Thermometric AB, Sweden) was employed in our research works. Its performance and the details of its assembly was described previously.[10,11] The LKB 2210 recorder was used in whole experiment process, which allowed continuous recording of the power-time curves for the *Staphylococcus aureus* growth. All measurements were carried out at 37 ℃.

Microcalorimetric measurements

The stopped-flow method was used for the microcalorimetric measurement. The procedure was as follows: Sterilized distilled water, HCl (0.1 mol/L), alcohol (75%), NaOH (0.1 mol/L) and sterilized water were pumped subsequently by an LKB-2132 Micro Perplex peristaltic pump through the cell for 15 min at a flow rate of 50 mL/h, respectively. Once the system was cleaned and sterilized and the baseline had been stabilized, the bacterial suspension containing 1×10^6 cells/mL and Yb^{3+} ions were pumped into the microcalorimeter at a flow rate of 50 mL/h. When the measuring cell of 2277-204 microcalorimeter (measuring volume, 0.6 mL) was filled fully, the pump stopped, monitor and computer were started to record the power-time curves.

Results and discussion

Power-time curves of growth of Staphylococcus aureus

Fig. 1 shows the power-time curve of *Staphylococcus aureus* at 37 ℃, which is a typical growth curve and could be divided into four parts, those are, lag phase, log phase, stationary phase and decline phase. *Staphylococcus aureus* has two log phases (log phase 1 and log phase 2). Fig. 2 shows ln *P*-*t* curve for the growth of *Staphylococcus aureus* at 37 ℃. Fig. 3 shows power-time curves of *Staphylococcus aureus* growth at 37 ℃ in the presence of different concentration of Yb^{3+} ions. However, the power-time curves in the presence of lower concentration of Yb^{3+} ions form four peaks, and they are different from the control curve. The power-time curves are similar to the control curve while the concentration of Yb^{3+} ions increases, and they also could be divided into four phases, in which the lag phase, log phase, and decline phase are very similar to each other, but the stationary phase is significantly different from those of *Staphylococcus aureus* without Yb^{3+} ions, and a broad peak appears. During the lag phase and the log phase for *Staphylococcus aureus*, Yb^{3+} ion has the capacity to inhibit its growth to some extent, and the inhibitory effects increase with the increase of Yb^{3+} ions concentration. The time of the lag phase suggested that the retarding time of *Staphylococcus aureus* be longer with the increasing concentration of Yb^{3+} ions. During the stationary phase of *Staphylococcus aureus* in the presence of Yb^{3+} ions, the heat output is greater than that of *Staphylococcus aureus* in the absence of Yb^{3+} ions.

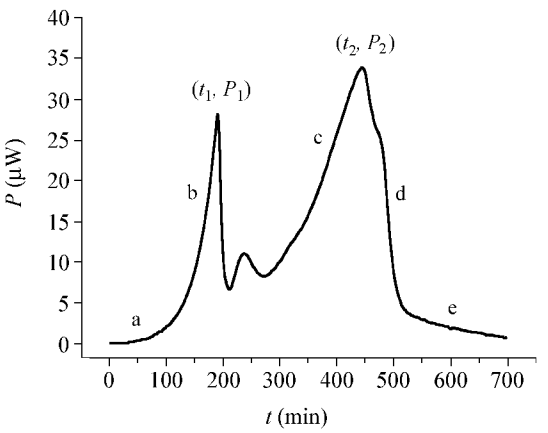

Fig. 1 Power-time curve for the growth of *Staphylococcus aureus* at 37 ℃. a, lag phase; b, log phase 1; c, log phase 2; d, stationary phase; e, decline phase.

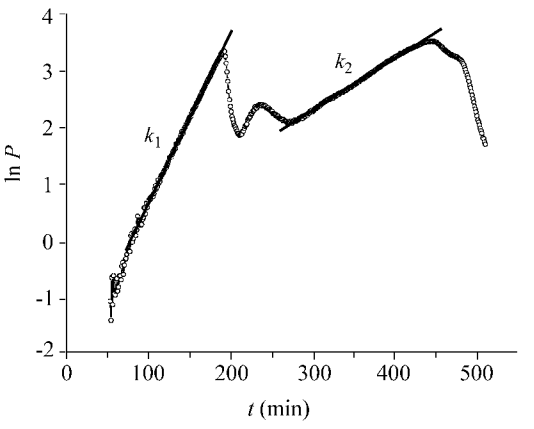

Fig. 2 ln *P*-*t* curve of *Staphylococcus aureus* growth at 37 ℃. k_1 and k_2: growth rate constants.

Growth rate constants (k) of Staphylococcus aureus

By analyzing of the power-time curves for *Staphylococcus aureus* in Fig. 1 and Fig. 3, the results show that the heat power increased exponentially during the log phases. So, it could be given the following equations:

$$P_t = P_0 \exp(kt)$$

or $$\ln P_t = \ln P_0 + kt$$

Using this equation, growth rate constants (k) of all experiments were calculated, and the results are listed in Table 1.

Table 1 Growth rate constant (k) of *Staphylococcus aureus* under the action of Yb^{3+} ions of different concentration

Concentration (μg/mL)	k_1 (min^{-1})	k_2 (min^{-1})
Control	0.02997	0.00909
13.4	0.03246	0.01329
26.8	0.02640	0.00792
80.4	0.02440	0.00709
134	0.02758	0.00664
268	0.02406	0.00579
402	0.02658	0.00493
536	0.02210	0.00277
670	0.02101	0.00192
938	0.01360	0.00145
1206	0.00323	0

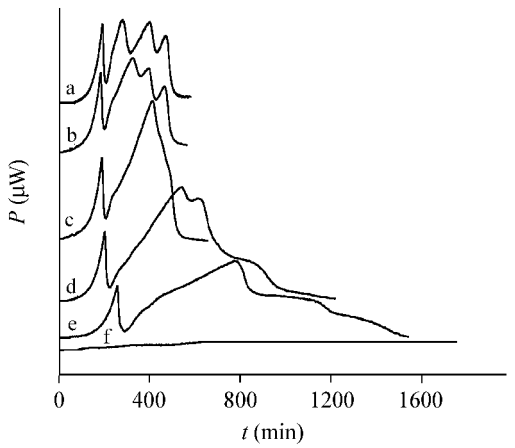

Fig. 3 Power-time curves for the growth of *Staphylococcus aureus* in the presence of Yb^{3+} ions. Concentrations of Yb^{3+} (μg/mL): a, 13.4; b, 26.8; c, 80.4; d, 402; e, 670; f, 1206.

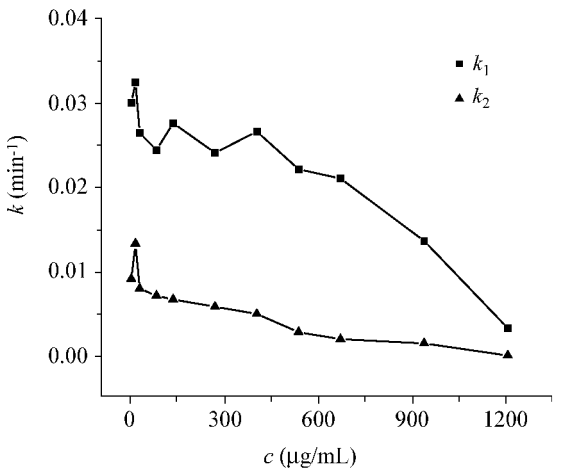

Fig. 4 Relation of k_1 and k_2 with c.

From Table 1 and Fig. 4, it was found that the growth rate constants (k_1 and k_2) decreased with the increase of concentration of Yb^{3+} ions, except for Yb^{3+} ions $\leqslant$ 13.4 μg/mL. Furthermore, k_1 decreased more quickly than k_2 with concentration, which indicated that metabolism of first log phase of the *Staphylococcus aureus* was more sensitive than that of the second log phase under actions of Yb^{3+} ions. When the concentration of Yb^{3+} ions reached at about 1200 μg/mL, very small k_1 or k_2 was obtained, Yb^{3+} ions exerted almost complete inhibitory effect on the growth of *Staphylococcus aureus*. In this case, lower heat effect could be observed in the power-time curve during the experiments.

From the data in Table 1 and Fig. 4, the relationship between k_1 or k_2 and c could be expressed as follows by linear regression:

$$k_1 = 0.03832 - 2.800c$$
$$R = -0.9881 \quad (402-1206\ \mu g/mL)$$

$$k_2 = 0.00749 - 6.7833 \times 10^{-6} c$$
$$R = -0.9741 \quad (26.8-1206\ \mu g/mL)$$

where R is the correlation coefficient. From equations above, it is a linear relationship between k_1 or k_2 and c.

Heat output (Q)

In order to interpret these results quantitatively, the heat output quantity (Q_1, Q_2) in the log phases, the heat output ($Q_{stat} = Q_{total} - Q_2$) in the stationary phases and the total heat effects (Q_{total}) from power-time curves of *Staphylococcus aureus* at different concentrations of Yb^{3+} ions were calculated. The values of Q_{stat} and Q_{total} are shown in Table 2. The trends of heat outputs (Q_{total}, Q_1, Q_2, Q_{Stat}) vs. Yb^{3+} ions concentration are shown in Fig. 5, which clearly shows that the heat output Q_1 decreases slowly while the heat output Q_2 increases sharply with the increase of concentration of Yb^{3+} ions when Yb^{3+} ions concentration is below 134 μg/mL, followed by a plateau during 134—938 μg/mL and a sharp drop above 938 μg/mL. As the concentration of Yb^{3+} ions rises, the total heat effect Q_{total} increases, which reaches a maximum value while the concentration of Yb^{3+} ions is about 536 μg/mL, and, subsequently, it decreases slowly and reaches approximately 0 with the concentration of Yb^{3+} ions increasing. From above analysis of trends of heat outputs (Q_{total}, Q_1, Q_2, Q_{stat}) vs. Yb^{3+} ions concentration, obviously, the contribution from the stationary heat effect (Q_{stat}) to the Q_{total} is upto 40% at all concentration of Yb^{3+} ions except 80.4 μg/mL. Therefore, the domain contribution to Q_{total} is from Q_{stat}.

From the data in Table 2 and Fig. 5, the relationship between Q_1, Q_2, Q_{stat}, Q_{total} or c could be expressed as follows:

Table 2 Ratio of Q_{stat} to Q_{total}

c (μg/mL)	0	13.4	26.8	80.4	134	268	402	536	670	938	1206
Q_{stat} (J)	0.1067	0.3399	0.2955	0.2105	0.3098	0.4941	0.6205	0.6144	0.5718	0.3861	0.1140
Q_{total} (J)	0.4117	0.5110	0.5750	0.6999	0.8051	1.0364	1.2181	1.2746	1.2079	0.9839	0.1740
$Q_{stat}/Q_{total} \times 100\%$	25.92	66.52	51.39	30.07	38.46	47.67	50.94	48.20	47.34	39.24	65.52

$$Q_2 = 0.1319 + 4.51 \times 10^{-3} c$$
$$R = 0.9886 \ (13.4\text{—}80.4\ \mu g/mL)$$

$$Q_2 = 0.4486 + 0.3816 \times 10^{-3} c$$
$$R = 0.9936 \ (80.4\text{—}536\ \mu g/mL)$$

$$Q_2 = 0.7410 - 0.1535 \times 10^{-3} c$$
$$R = -0.9982 \ (536\text{—}938\ \mu g/mL)$$

$$Q_{stat} = 0.1290 + 1.270 \times 10^{-3} c$$
$$R = 0.9925 \ (80.4\text{—}402\ \mu g/mL)$$

$$Q_{stat} = 1.0547 - 0.7559 \times 10^{-3} c$$
$$R = -0.9862 \ (536\text{—}1206\ \mu g/mL)$$

$$Q_{total} = 0.5575 + 1.70 \times 10^{-3} c$$
$$R = 0.9958 \ (26.8\text{—}402\ \mu g/mL)$$

$$Q_{total} = 2.2496 - 1.60 \times 10^{-3} c$$
$$R = -0.9388 \ (536\text{—}1206\ \mu g/mL)$$

where R is the correlation coefficient.

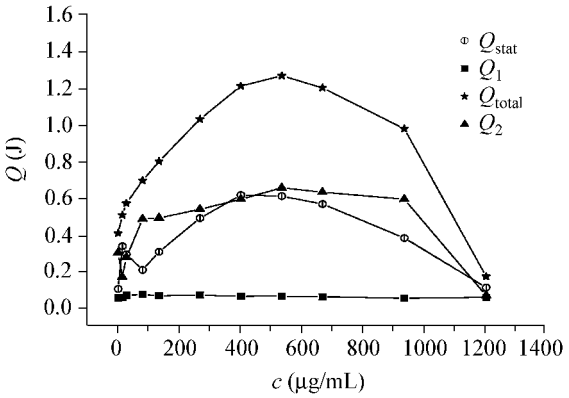

Fig. 5 Relationship between heat-output (Q) and c (Yb^{3+}) Q: heat output (J); Q_1: heat output from t_0 to t_1; Q_2: heat output from t_0 to t_2; Q_{total}: total heat; Q_{stat}: stationary heat.

Discussion

Yb^{3+} ion[12,13] could combine with enzymes within some cells, since it may penetrate the cell membrane and accumulate inside the cells by pinocytosis or phagocytosis. Takata[14] and Ohmichi[15] investigated the effect of the lanthanide elements on proteins and demonstrated that lanthanide elements could increase the stability of protein or the complex between enzyme and substrate. Based on the above results, the characteristics of interaction of lanthanide with bacteria may be an intracellular accumulation, so *Staphylococcus aureus* metabolizes vigorously and gives more heat. Apparently, the results obtained from our experiment could explain if the lanthanide elements could enhance the metabolic activity of the enzymes in *Staphylococcus aureus*.

In conclusion, lanthanide ions have duplex biological functions, and Yb^{3+} ions have stimulating effect on microorganism at low concentration, however, at high concentration, Yb^{3+} ions have inhibitory effect on the metabolism of microorganisms. Microcalorimetric investigations on the stimulatory effects of the lanthanide elements on microorganisms are potential and promising. Microcalorimetry is a powerful and accurate technique for studying the detailed mechanism of metabolism of microorganisms in the presence of the lanthanide elements and could provide significant information for microbiology research.

References

1 Shen, P. *Microbiology*, Beijing, **2001**, p. 38; 130 (in Chinese).
2 Chen, Y. K.; Xu, J.; Qu, Y. B.; Xi, G. P.; Zhiang, M. *J. Biochem. Phara.* **2001**, *22*, 29 (in Chinese).
3 Li, J. Q.; Zhan, Q. X.; Gao, J. C.; Lin, Z.; Guo, S. G. *J. Shanxi Med. Univ.* **1999**, *30*, 92 (in Chinese).
4 Zheng, L. Y.; Zhu, J. F.; Sun, K. S. *Materials Sci. & Eng.* **2000**, *2*, 22 (in Chinese).
5 Li, Y. M.; Shao, S. H.; Gu, S. H.; Zhang, Z. F. *J. Jilin Med. Univ.* **1999**, *19*, 3 (in Chinese).
6 Liu, Y.; Feng, Y.; Xie, C. L.; Qu, S. S. *J. Wuhan Univ.* (*Nat. Sci. Edn.*) **1995**, *4*, 434 (in Chinese).
7 Zhao, R. M. *Ph.D. thesis*, Wuhan University, Wuhan, **2002** (in Chinese).
8 Ni, J. Z. *Bioinorganic Chemistry of Rare-Earth Elements*, Beijing, **1995**, p. 285 (in Chinese).
9 Wang, K. *Trace Elements In Life Science*, Beijing, **1996**, p. 467 (in Chinese).
10 Xie, C. L.; Tang, H. K.; Qu, S. S. *Thermochim. Acta* **1988**, *123*, 33.
11 Xie, C. L.; Xu, G. D.; Qu S. S. *Acta Phys. Chim. Sin.* **1988**, 363 (in Chinese).
12 Du, X. L.; Zhang, T. L.; Li, R. C.; Wang, K. *J. Inorg. Biochem.* **2001**, *84*, 67.
13 Ni, J. Z. *Bioinorganic Chemistry of Rare-Earth Elements*, Beijing, **1995**, p. 197 (in Chinese).
14 Takata, M.; Pickard, W. F.; Lettvin, Y. T.; Moore, J. W. *J. Gen. Physiol.* **1966**, *50*, 461.
15 Ohmichi, T.; Sugimoto, N. *Biochem.* **1997**, *36*, 3514.

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