

Study of the Action of Se and Cu on the Growth Metabolism of *Escherichia coli* by Microcalorimetry

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Abstract The biological effect of Se and Cu^{2+} on *Escherichia coli* (*E. coli*) growth was studied by using a 3114/3236 TAM Air Isothermal Calorimeter, ampoule method, at 37°C. From the thermogenesis curves, the thermokinetic equations were established under different conditions. The kinetics showed that a low concentration of Se (1–10 $\mu\text{g}/\text{mL}$) promoted the growth of *E. coli*, and a high concentration of Se (>10 $\mu\text{g}/\text{mL}$) inhibited the growth, but the Cu^{2+} was always inhibiting the growth of *E. coli*. Moreover, there was an antagonistic or positive synergistic effect of Se and Cu^{2+} on *E. coli* in the different culture medium when Se was 1–10 $\mu\text{g}/\text{ml}$ and Cu^{2+} was 1–20 $\mu\text{g}/\text{ml}$. There was a negative synergistic effect of Se and Cu^{2+} on *E. coli* when Se was higher than 10 $\mu\text{g}/\text{ml}$ and Cu^{2+} was higher than 20 $\mu\text{g}/\text{ml}$. The antagonistic or synergistic effect between Se and Cu^{2+} on *E. coli* was related to the formation of Cu–Se complexes under the different experimental conditions chosen.

Keywords Microcalorimetry · Se · Cu · *E. coli* · Growth metabolism

Introduction

Selenium (Se) and copper (Cu) are essential elements for animals and human [1, 2]. The physiological functions of Se mainly play an antioxidant in the form of glutathione peroxidase (GSH-Px). The activity of glutathione peroxidase and antioxidant enzyme in body both decreases when Se is deficient in body. With Se concentration increasing, the

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activity of GSH-Px is elevated while the mutagenic effect decreases [3, 4]. Recent research revealed that Se has been not only the element for life but also the defensive reagent that can protect organism from toxicants' damage. For example, Hg discharged in the muscle and kidney in fish increases when Se is supplemented as a nutrition for fish [5]. As a toxicant, Cu will disturb the function of indispensable elements in body [6]. Abdel Rhaim [7] studied the effect of Cu^{2+} (1.3–200 mg/kg body weight) on the Se in food fed to rat according to experiments. The result revealed that there is an antagonism between Se and Cu. However, the interaction mechanism of Se and Cu is not clear completely.

Se and Cu are also the component part of a kind of biofluorescence probe Cu–ZnSe and CdSe etc. quantum dots (QDs) [8–10]. These QDs, e.g., CdSe QDs, with their broad excitation spectra, sharp emission, and easily tunable emission properties, have been widely used in light-emitting diodes, thin-film transistors, solar cells, especially in biodetection assays for replacing conventional fluorescent markers, etc. [11–13]. In the long run, this material will enter the environment, even the human body. So the study on the effect of Cu and Se is important for the toxicology of QDs.

Microcalorimetry can be used to study the metabolism of cell and the effect of the toxicants on cellular metabolism [14–17]. Beer demonstrated microcalorimetric measurements of bacterial growth revealed by temporal details not observed by other techniques [18]. One of the most prominent features of the microbial growth process is the production of heat. If heat is monitored by microcalorimeter, much useful information, both qualitative and quantitative, may be obtained [19].

Microbe has a unique power–time curve, as recorded by the microcalorimeter, under given growth conditions. Any substance that can affect the metabolic growth processes of microbe, including cells, will change the power–time curve obtained from the microcalorimeter. From the power–time curves, we can obtain corresponding kinetic data by studying the effect of toxic agents on microbe. The purpose of this paper was to report the effect of Se and Cu^{2+} on the growth of *E. coli* respectively by microcalorimetry, and meanwhile investigate the antagonism and synergism between Se and Cu^{2+} on the growth of *E. coli*.

Materials and Methods

Cells and Reagents

E. coli (CCTCC AB91112) was provided by China Center of Type Culture Collection, Wuhan University, China. Analytical reagent grade $\text{Cu}(\text{NO}_3)_2$ was supplied by Nankai Chemical Reagent Factory of Tianjin. Selenium morpholine was synthesized and characterized by the Department of Chemistry, Wuhan University.

Culture Medium

E. coli was grown in the peptone culture medium containing NaCl 5 g, peptone 10 g, beef extract 3 g in 1,000 mL de-ionized water, the pH of the medium was adjusted to 7.0 with NaOH and HCl, finally the medium was sterilized by autoclaving at 120°C for 30 min.

Instrument and Operating Principle

In this paper, the 3114/3236 TAM Air Isothermal Calorimeter, manufactured by Thermometric Corporation of Sweden, was used to research the action of Se and Cu of

different concentrations on the growth metabolism of *E. coli*. The working principle of the TAM Air Isothermal Calorimeter could be obtained from Liu P [20]. The production heat of *E. coli* growth at 37°C was measured by the isothermal calorimeter. From these thermogenesis curves (log phase), the growth rate constant, k , was calculated. According to the k - c relationship, we discussed and compared the action of Se and Cu^{2+} on *E. coli*.

Methods

The *E. coli* was cultivated under different conditions.

First, the *E. coli* was cultivated at control experiment; the values of k of *E. coli* growth metabolism at different control experiment times can be obtained (Table 1).

Second, the *E. coli* was cultivated only in the presence of different amounts of Se or Cu^{2+} . The thermogenic curves and the values of k of *E. coli* growth metabolism can be obtained (Figs. 1, 2, Tables 2, 3).

Finally, the *E. coli* was cultivated at different amounts of Se and Cu^{2+} . Two methods were applied in the experiment. One (method 1) was that the bacteria was cultivated in the peptone culture medium in the absence of Se. The values of k and the relationship curves of the k of *E. coli* can be obtained [Table 4, Fig. 3a]. The other (method 2) was that the bacteria was cultivated in the peptone culture medium in the presence of Se [Table 5, Fig. 3b]. The detailed procedures were basically similar to those described by Ref. [15].

Results

Thermogenesis Curves and the Growth Rate Constant of *E. coli*

The thermogenesis curves of *E. coli* growth metabolism affected by Se, Cu^{2+} , were obtained by microcalorimetry. These experiments had good reproducibility (Fig. 1).

In the log phase of growth, the cell number and culture time correspond to exponential law [21]. If the cell number is n_0 at time 0, and n_t at time t , then

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

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

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

We write $P_0 = N_0 w$ and $P_t = n_t w$ giving

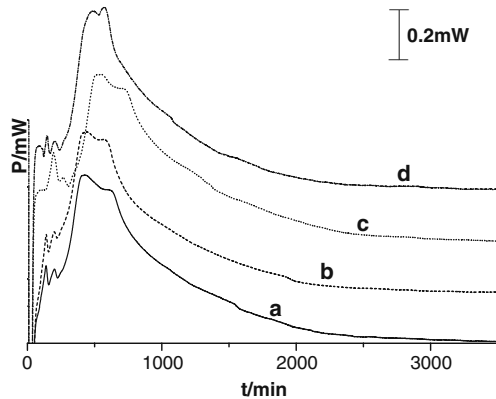
$$P_t = P_0 \exp(kt) \quad \text{or} \quad \ln P_t = \ln P_0 + kt \quad (3)$$

Table 1 Values of k of *E. coli* Growth Under Different Control Experiment Times

Times	1	2	3	4	5	6
k/min^{-1}	0.00757	0.00672	0.00619	0.00666	0.00757	0.00652
k/min^{-1}			0.00687			
R^a	0.9990	0.9959	0.9999	0.9993	0.9994	0.9958

R^a correlation coefficient

Fig. 1 The power–time curves of *E. coli* growth under different concentrations of Se. **a** *E. coli*+ 1 $\mu\text{g/ml}$ Se. **b** *E. coli*+4 $\mu\text{g/ml}$ Se. **c** *E. coli*+10 $\mu\text{g/ml}$ Se. **d** *E. coli*+20 $\mu\text{g/ml}$ Se



The thermogenetic curves of the log phase of growth obey Eq. 3. So, making use of the data $\ln P_i$ and t taken from the curves to fit a linear equation, can obtain the growth rate constant (k).

The power–time curves of the *E. coli* growth only containing Se of different concentrations were shown in Fig. 1. When the culture of *E. coli* containing different Se and Cu^{2+} was inoculated, the power–time curves were shown in Fig. 2. The values of rate constant (k) of *E. coli* growth under different experiment conditions were shown in Tables 1, 2, 3, 4, and 5.

Discussion and Conclusion

The Effect of Se on the Growth of *E. coli*

As seen from Table 2, the growth rate of *E. coli* in the presence of Se increased with the Se concentration increasing. When Se was 10 $\mu\text{g/mL}$, the maximal growth rate of *E. coli* was obtained. Subsequently, the growth rate of *E. coli* decreased with increasing of Se concentration. When Se ranged from 1 to 10 $\mu\text{g/mL}$, Se was mainly used to sustain the integrality of cell, and to act on the enzyme needed in biological body. The results showed

Fig. 2 The power–time curves of *E. coli* growth under different concentrations of Se and Cu^{2+} . **a** *E. coli*+20 $\mu\text{g/ml}$ Se. **b** *E. coli*+ 20 $\mu\text{g/ml}$ Se+10 $\mu\text{g/ml}$ Cu^{2+} . **c** *E. coli*+20 $\mu\text{g/ml}$ Se+20 $\mu\text{g/ml}$ Cu^{2+} . **d** *E. coli*+20 $\mu\text{g/ml}$ Se+ 50 $\mu\text{g/ml}$ Cu^{2+}

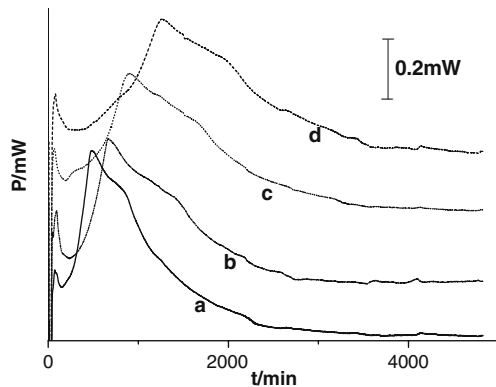


Table 2 Values of k of *E. coli* Growth Under Different Concentrations of Se

Drug	$\frac{C}{(\mu\text{g/mL})}$	k/min^{-1}
Se	0	0.00634
	1	0.00698
	4	0.00733
	10	0.00976
	20	0.00646
	40	0.00885

that the low-concentration (1–10 $\mu\text{g/mL}$) Se promoted the growth of bacteria, high-concentration ($>10 \mu\text{g/mL}$) Se inhibited the growth. The biochemistry function of Se is mainly showed in the interaction between Se and enzyme [22–24].

In the investigation of effect of trace elements on activity of *E. coli* formate dehydrogenase, Se is one of the indispensable trace elements to sustain the activity of the enzyme [25]. Furthermore, Se is one of the necessary component of formate dehydrogenate of anaerobion, and it exists in the form of Se-cysteine. If the suitable amount of Se is added to peptone culture medium, not only can one enzyme without Se be formed, but also the enzymes of much higher activity containing Se be produced. Under this condition, the growth rate of a lot of microbes can increase evidently. When the concentration of Se is much higher, however, Se compounds have toxic action on microbe by attacking the special dehydrogenate system, especially amber acid dehydrogenate, including methionine adenosine transferase and cytochrome oxidase. The reason of the toxic action of Se compounds is that Se inhibits the activity of enzymes and other functional proteins with the mitochondria through the catalysis of oxidation reactions of S–H groups to S–S or S–Se–S bonds. Consequently, the growth rate of *E. coli* decreases because the normal division or multiplication of the cell is inhibited. The changes of the growth rate of the microbe are closely related to Se.

The Effect of Cu on the Growth of *E. coli*

Cu is an essential trace element for human and animals. It is a necessary component for over 30 kinds of metal enzymes in the biological body, and one of the indispensable trace elements to sustain the activity of some enzymes. It has been proven that tyrosine hydroxylase, cytochrome C oxidase, and peroxide dismutase are vital enzymes containing Cu [26]. In the absence of Cu, the activity of the above kinds of enzymes will decrease

Table 3 Values of k of *E. coli* Growth Under Different Concentrations of Cu^{2+}

Drug	$\frac{C}{(\mu\text{g/mL})}$	k/min^{-1}
Cu^{2+}	0	0.00634
	10	0.00511
	20	0.00454
	30	0.00407
	50	0.00208
	70	0

Table 4 Values of k of *E. coli* Growth at Different Se and Cu^{2+} Concentrations (Method 1)

$c_{Cu^{2+}}$ ($\mu\text{g/mL}$)	c_{Se} ($\mu\text{g/mL}$)				
	0	4	10	20	40
10	0.00511	0.00574	0.00657	0.00653	0.00631
20	0.00454	0.00335	0.00622	0.00477	0.0049
30	0.00407	0.00211	0.00430	0.00428	0.00377
50	0.00208	0	0.00176	0.00235	0.00042
70	0	0	0	0	0

obviously. As a toxicant, Cu can inhibit the part activity of enzymes. Subsequently, the many-sided dysfunction also comes into being.

As seen from Table 3, the growth rate of *E. coli* decreased with increasing of Cu-only containing Cu. During this process, Cu inhibited the growth of *E. coli*, which might result

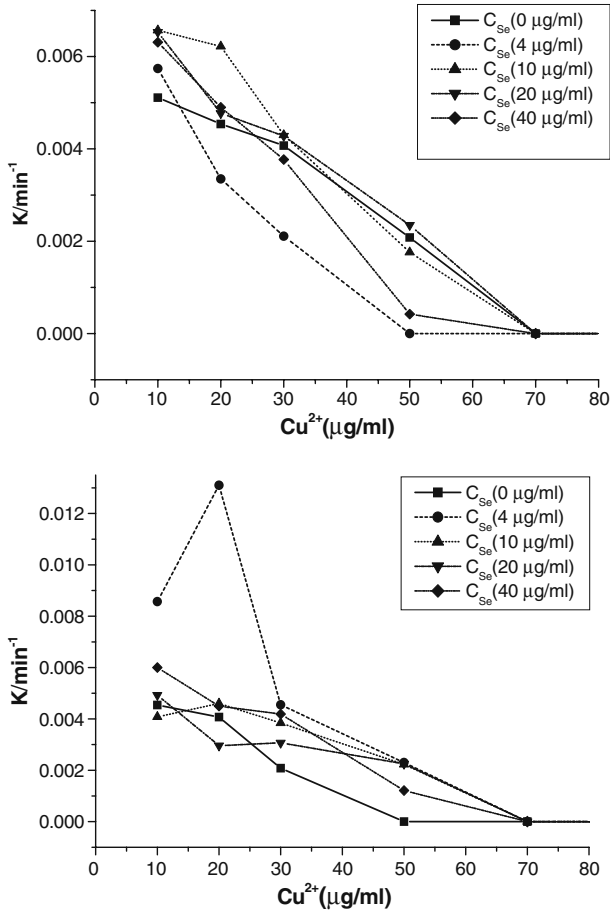


Fig. 3 The relationship curves of the growth rate constants of *E. coli* growth under different concentrations of Se and Cu^{2+} **a** culture medium in the absence of Se **b** culture medium in the presence of Se

Table 5 Values of k of *E. coli* Growth Under Different Concentrations of Se and Cu^{2+} (Method 2)

$c_{\text{Cu}^{2+}}$ ($\mu\text{g/mL}$)	c_{Se} ($\mu\text{g/mL}$)				
	0	4	10	20	40
10	0.00511	0.00857	0.00408	0.00493	0.00600
20	0.00454	0.01310	0.00460	0.00296	0.00450
30	0.00407	0.00455	0.00384	0.00307	0.00419
50	0.00208	0.00230	0.00223	0.00225	0.00121
70	0	0	0	0	0

from the action of Cu^{2+} on the enzymes in *E. coli*. Moreover, heavy metal ions, such as Hg^{2+} and Ag^+ , have the higher affinity to the ligand containing sulfur, which can result in the inhibiting action of heavy metal ions on S–H enzyme.

The Antagonism and Synergism Between Se and Cu^{2+} on the Growth of *E. coli*

The effect of Se and Cu^{2+} on some living organism has been studied. Research revealed there was antagonistic or synergistic action between Se and Cu^{2+} in living organism [27]. Cu^{2+} can inhibit the toxicity effect of Se on mice. The endurance ability of mice to Cu will decrease when the amount of Se is deficient in mice body. From Fig. 2, we found the shapes of the power–time curves of *E. coli* growth were almost similar. The higher the concentration of Cu is, the later the metabolism peak of *E. coli* adverts when the Se keeps invariability.

Comparing Table 3 with Tables 4 and 5, the strong antagonism of Se to Cu^{2+} was found in method 1 (Fig. 3a) when the concentration of Se was 1–10 $\mu\text{g/ml}$ and Cu^{2+} was 1–10 $\mu\text{g/ml}$. The inhibiting action of Cu^{2+} treated by Se on the growth of *E. coli* was less than that of Cu alone. However, the positive synergism of Se to Cu^{2+} was found in method 2 (Fig. 3b) when the concentration of Se was 1–10 $\mu\text{g/ml}$ and Cu^{2+} was 1–20 $\mu\text{g/ml}$. Thus, when Se was 1–10 $\mu\text{g/ml}$ and Cu^{2+} was 1–20 $\mu\text{g/ml}$, the growth rate of *E. coli* increased, and the k value 0.0131 is even much higher than the average value 0.00687 of the control experiment (Table 1). When the concentration of Se was higher than 10 $\mu\text{g/ml}$ and the concentration of Cu was higher than 20 $\mu\text{g/ml}$, the growth rate of *E. coli* always decreased. This showed there was a negative synergism of Se and Cu^{2+} on *E. coli*. What is more, from Fig. 3, we saw that the all growth rate constant values of *E. coli* in the culture medium presence of Se were higher obviously than that of *E. coli* in the culture medium absence of Se. This showed the effect of Se and Cu^{2+} on *E. coli* growth was related to the different experimental conditions.

The results also revealed that the Se and Cu^{2+} had toxic action on the metabolism of *E. coli* under above conditions. The injury induced by Cu^{2+} could not be prevented completely by adding Se to Cu^{2+} under any circumstances. The effect of Se was thought to be related to either the formation of Cu–Se complexes in association with metallothioneins, or changes of the distribution of Cu^{2+} in tissue. According to the results, the protective effect of Se resulted from the production of Se–Cu complex compounds. Cu^{2+} can combine with the S–H of proteins and enzymes to change their activity. Se is a softer alkali than sulfur. According to the hard and soft acids and bases rule, Se can have the advantage over sulfur to combine with soft acid Cu^{2+} and produce the Se–Cu complex compounds. Accordingly,

the Cu^{2+} becomes the stable and nontoxic Se–Cu complex compounds. The complex compounds may be vented through the body by metabolizing. The S–H target molecule can be protected from Cu^{2+} . So, the organisms can be kept from being injured.

This study shows that microcalorimetric method is a straight-forward and simple method in the studies of interaction between trace elements and organism. The microcalorimetric method requires only an observable difference between the power production in the treated and controlled incubations. Unlike many other procedures, transparent solution is not required. Colored or turbid solutions, even suspensions can be put into the calorimeter [28]. That approximates more closely the vivo state than many other techniques do. Through the technique and other methods, the reasonable effect concentrations of Se and Cu^{2+} on the growth of *E. coli* and the maximum growth rate constant k of *E. coli* are obtained easily by microcalorimetry. It also shows that the study is important for the toxicology detection of QDs. All of these are significant to synthesize and select drug, ecotoxicological tests, and biocompatibility estimate, and so on.

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