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Microcalorimetrical Study of the Effect of Selenium and Cadmium on the Growth Metabolism of *Escherichia coli*

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A microcalorimetric technique was used to evaluate the influence of Se (selenium morpholine) and Cd^{2+} [$Cd(NO_3)_2$] on *Escherichia coli* growth. By means of 3114/3236 TAM Air isothermal calorimeter, ampoule method at 37 ºC, the thermogenic curves of *E. coli* growth were obtained under different conditions. In order to analyze the results, the maximum power P_m and the growth rate constants *k* were determined, which showed that values of P_m and k were linked to the concentration of Se and Cd^{2+}. The results showed that a low concentration of Se (1-10 µg/mL) promoted the growth of *E. coli* and a high concentration of Se (> 10 µg/mL) inhibited the growth, but the Cd^{2+} is always inhibiting the growth of *E. coli*. However, there was an antagonistic or positive synergistic effect of Se and Cd^{2+} on *E. coli* when Se was 1-4 µg/mL and Cd^{2+} was 1-3 µg/mL. There was a negative synergistic effect of Se and Cd^{2+} on *E. coli* when Se was higher than 4 µg/mL and Cd^{2+} was higher than 3 µg/mL. The effect of Se and Cd^{2+} on *E. coli* was related to the formation of the Se-Cd complex compounds under different conditions chosen.

Key Words: Microcalorimetry, Selenium, Cadmium, *E. coli*, Growth metabolism.

INTRODUCTION

Selenium is an essential trace element for humans and animals[1,2]. Selenium, as a component of glutathione peroxidase (GSH-Px), can clear or catching free radicals produced by Cd which promotes DNA synthesis. With increasing concentration of Se, the activity of GSH-Px was elevated while the mutagenic effect decreased[3]. Recent research revealed that Se has not been 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[4]. As a toxicant, Cd can disturb the function of indispensable elements in body. The antagonism of Se and Cd could partially alleviate the toxicities of Cd to rat kidney[5]. Wang research results revealed that there was an antagonism between Se and Cd[6]. However, the interaction mechanism of Se and the Cd is not clear completely. Furthermore, Se and Cd are the component part of a kind of biofluoresence probe CdSe quantum dots[7-9]. These quantum dots, *e.g.*, CdSe quantum dots, 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.*[10-12]. In the long run, this material will enter the environment, even the human body. So the study on effect of Se and Cd is important for the toxicology of quantum dots.

Microcalorimetry is a non-destructive and non-invasive technique. Therefore, it is valuable for monitoring a variety of processes, such as metabolism of microorganism. The thermogenic curves of the metabolism of *E. coli* and the effect of Se and Cd^{2+} on it were studied using 3114/3236 TAM air isothermal calorimeter. Compared to the metabolic thermogenic curves, we obtained some significant results. All the experimental results are very important to the study of the bioeffect of trace element.

EXPERIMENTAL

E. coli (CCTCC AB91112) was provided by the Chinese Center of Type Culture Collections of Wuhan University. $Cd(NO_3)_2$ was purchased from Tingxin Chemical Reagent Factory of Shanghai. Selenium morpholine was synthesized and characterized by the Department of Chemistry, Wuhan University, Wuhan 430072, P.R. China. All the chemicals used were of analytical grade and double distilled deionized water was used in all experiments.

Culture medium: Growth medium for *E. coli* was the peptone culture medium: added NaCl 5 g, peptone 10 g, beef extract 3 g in 1,000 mL of distilled water, then adjusted the solution to pH = 7.0 with NaOH and HCl, finally the solution was sterilized in high-pressure steam at 120 ºC for 0.5 h.

Instrument and methods: A 3114/3236 TAM air isothermal calorimeter (made in Sweden) was used to research the action of Se and Cd^{2+} on the growth metabolism of *E. coli* at different concentrations. The performance of this instrument and the details of its construction have been previously described[13-15]. The *E. coli* was inoculated in the prepared culture medium, which was put into the ampoule at once. The metabolic thermogenic curves of strain were recorded using ampoule method. One sealed ampoule contained a reference solution such as the cultural medium and the other ampoule contained the sample. The sample normally occupied position A in the monitor and reference occupied position. All calorimetric experiments were conducted at 37 ºC. From thermogenesis curves (log phase), the growth rate constant, *k*, was calculated.

Methods

***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 and the mean value $\bar{k}$ can be obtained (Table-1).

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

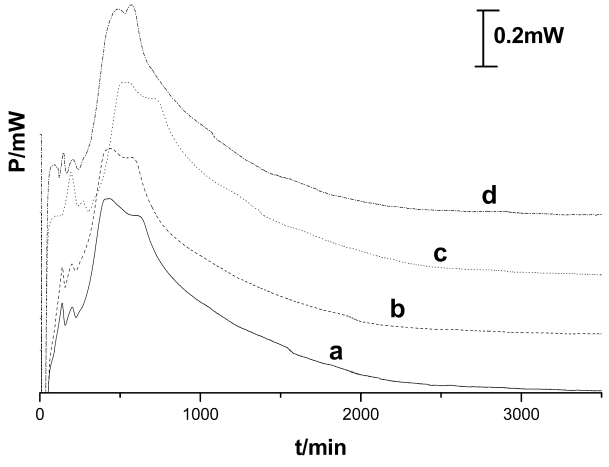

Fig. 1. Power-time curves of *E. coli* growth under different concentrations of Se a. *E. coli* + 1 μg/mL Se b. *E. coli* + 4 μg/mL Se c. *E. coli* + 10 μg/mL Se d. *E. coli* + 20 μg/mL Se

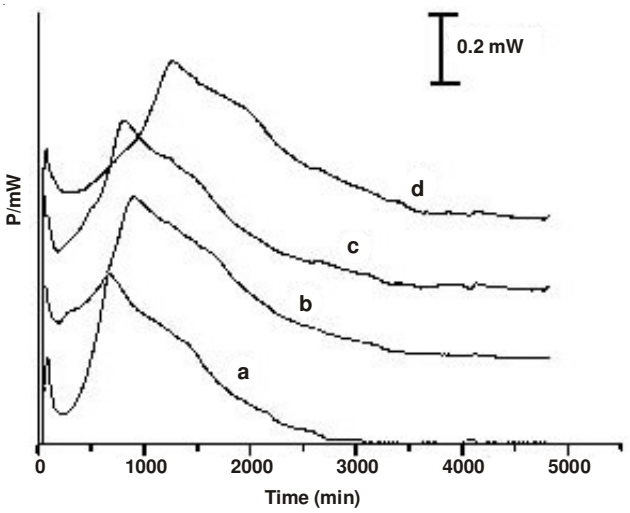

Fig. 2. Power-time curves of *E. coli* growth under different concentrations of Cd^{2+} (the Se concentration was 10 μg/mL) a. *E. coli* +10 μg/mL Se + 1 μg/mL Cd^{2+} b. *E. coli* + 10 μg/mL Se +3 μg/mL Cd^{2+} c. *E. coli* + 10 μg/mL Se + 5 μg/mL Cd^{2+} d. *E. coli* +10 μg/mL Se + 7 μg/mL Cd^{2+}

TABLE-2
VALUES OF k OF *E. coli* GROWTH UNDER DIFFERENT CONCENTRATIONS OF Se

Drug	c (μg/mL)	k (min^{-1})
Se	0	0.00634
	1	0.00698
	4	0.00733
	10	0.00976
	20	0.00646
	40	0.00885

TABLE-3
VALUES OF k OF *E. coli* GROWTH UNDER DIFFERENT CONCENTRATIONS OF Cd^{2+}

Drug	c (μg/mL)	k (min^{-1})
Cd^{2+}	0	0.00628
	1	0.00406
	3	0.00444
	5	0.00427
	7	0.00344
	10	0

Finally, the *E. coli* was cultivated at different amounts of Se and Cd^{2+}. Two methods were applied here. Initially, the bacteria was cultivated initially in the peptone culture medium and the culture medium is in the absence of Se. Then, the bacteria was inoculated in the experiment culture medium and put into the ampoule. After that, different concentrations of Se and Cd^{2+} were added into the ampoule. The values of *k* and the relationship curves of the *k* of *E. coli* can be obtained

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
$\bar{k}$ (min^{-1})				0.00687		
R^a	0.9990	0.9959	0.9999	0.9993	0.9994	0.9958

R^a: Correlation coefficient.

(Fig. 3a or Table-4). The other method was that the bacteria were cultivated in the peptone culture medium and the culture medium is in the presence of Se. The following procedure is same to the method one. The results were shown in Fig. 3b (or Table-5). The detailed procedures were basically similar to those described by the Li *et al.*[16].

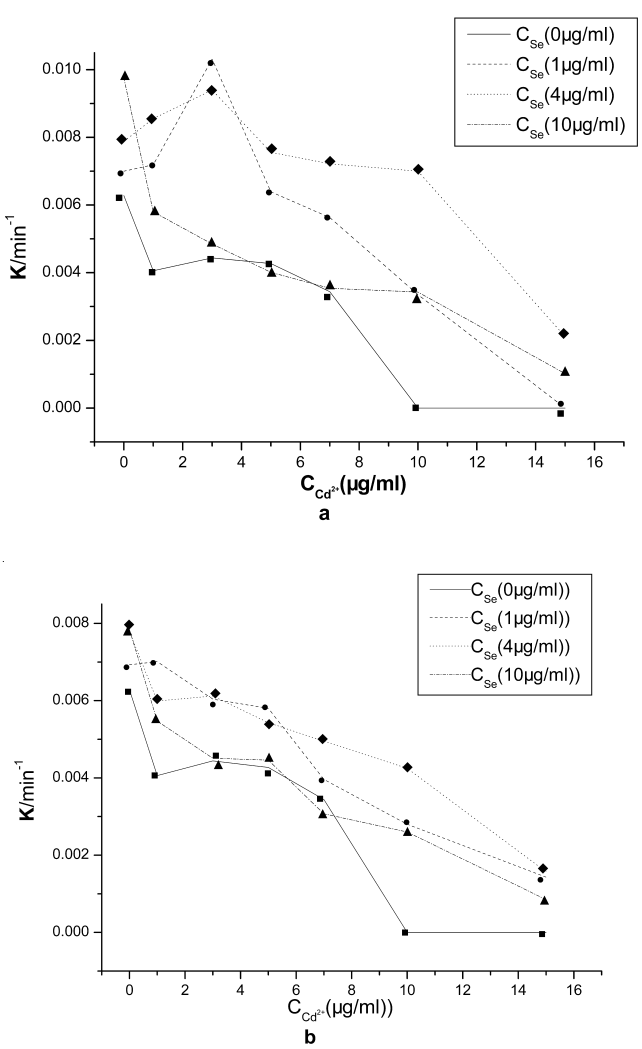

Fig. 3. Relationship curves of the growth rate constants of *E. coli* growth under different concentrations of Se and Cd^{2+} a. culture medium in the absence of Se b. culture medium in the presence of Se

TABLE-4
VALUES OF k Of *E. coli* GROWTH AT DIFFERENT Se AND Cd^{2+} CONCENTRATIONS (METHOD 1)

$c_{Cd^{2+}}$ (μg/mL)	c_{Se} (μgmL)			
	0	1	4	10
0	0.00628	0.00700	0.00788	0.00976
1	0.00406	0.00719	0.00855	0.00580
3	0.00444	0.01031	0.00940	0.00485
5	0.00427	0.00639	0.00756	0.00401
7	0.00344	0.00563	0.00722	0.00354
10	0	0.00335	0.00700	0.00343
15	0	0	0.00211	0.00102

RESULTS AND DISCUSSION

Thermogenesis curves and the growth rate constant of *E. coli*: The thermogenesis curves of *E. coli* growth metabolism affected by Se, Cd^{2+}, were obtained by microcalorimetry (Fig. 1). The curves would start from the same point and have been moved in the y-direction in the diagram.

TABLE-5
VALUES OF k OF *E. coli* GROWTH UNDER DIFFERENT CONCENTRATIONS OF Se AND Cd^{2+} (METHOD 2)

$C_{Cd^{2+}}$ (μg/mL)	C_{Se} (μg/mL)			
	0	1	4	10
0	0.00628	0.00693	0.00779	0.00784
1	0.00406	0.00701	0.00599	0.00547
3	0.00444	0.00604	0.00612	0.00451
5	0.00427	0.00580	0.00543	0.00446
7	0.00344	0.00396	0.00495	0.00307
10	0	0.00280	0.00425	0.00260
15	0	0.00143	0.00157	0.00085

In the log phase of growth, the cell number and culture time correspond to exponential law[15]. 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

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

The thermogenetic curves of the log phase of growth obey eqn. 3. So, making use of the data ln P_t 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 in the presence of Se at different concentrations were shown in Fig. 1 and the power-time curves obtained when a culture of the *E. coli* was inoculated containing Se and Cd^{2+} at different concentrations were shown in Fig. 2. The values of rate constant (*k*) of *E. coli* growth were shown in Tables 1-5. According to the *k*-c relationship, we discussed and compared the action of Se and Cd^{2+} on *E. coli*.

Effect of Se on the growth of *E. coli*: As can be seen from Table-2, when Se was at 1-10 μg/mL, the growth rate of *E. coli* in the presence of Se increased with increasing concentration of Se. When Se was 10 μg/mL, the maximal growth rate of *E. coli* was obtained. Subsequently, the growth rate of *E. coli* decreased with increasing Se concentration. The results showed, low concentration (1-10 μg/mL) Se promoted the growth of bacteria, but high concentration (> 10 μg/mL) Se can inhibited the growth. The biochemistry function of Se is mainly showed in the interaction between Se and enzyme[17-20]. Selenium was mainly used to sustain the integrality of cell and to act on the enzyme needed in biology body.

In the investigation of effect of trace elements on activation of *E. coli* formate dehydrogenase, Se was one of the indispensable trace elements to sustain the activation of the enzyme[20]. Furthermore, Se is one of the necessary component of formate dehydrogenate of anaerobion and it exists in the form of Se-cysteine. If 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. However, selenium 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. So, the changes of the growth rate of the microbe are closely related to Se.

Effect of Cd^{2+} on the growth of *E. coli*: Cadmium is a toxic trace element. It has a strong affinity on sulfur. It is apt to combine with S-H of cysteine and form a stable polynuclear chelate complex. Protein usually can act on the center enzyme in the organism. When S-H is in the center of activation, it can inhibit the activity of enzymes by reacting strongly with Cd^{2+}, which is the toxicity of Cd^{2+} to microbe[21].

As can be seen from Table-3, the growth rate of *E. coli* decreased with increasing concentration of Cd^{2+} in the presence of Cd^{2+} alone. During this process, Cd^{2+} inhibited the growth of *E. coli*, which may resulted from Cd^{2+} acting on the enzymes in *E. coli*.

Antagonism and Synergism of Se and Cd^{2+} on the growth of *E. coli*: The relationship between Se and Cd has been studied in some living organism. Wang[22] and Guo[23] found that Se could inhibit the carcinogenic effect of Cd on mouse and reduce the rate of death. Li and Zheng[24] indicated that Se decreased the micronuclear frequency of broad beans induced by Cd.

From Fig. 2, we found the shapes of the power-time curves of *E. coli* growth were almost similar. It can be seen that the lag phase, which is between the start of the experiment and the ascending phase of the power-time curve, became longer with increasing concentration of Cd^{2+} when Se keeps invariability.

Comparing Table-3 with Tables 4 and 5 (or Fig. 3), the strong antagonism of Se to Cd^{2+} was found when the concentration of Se was 1-4 μg/mL and Cd^{2+} was 1-3 μg/mL. The inhibiting action of Cd^{2+} treated by Se on the growth of *E. coli* was less than that of Cd^{2+} alone. Thus, when Se was 1-4 μg/mL and Cd^{2+} was 1-3 μg/mL, the growth rate of *E. coli* increased and the growth rate constant value even much higher than the mean value 0.00687 of control experiment (Table-1). When the concentration of Se was higher than 4 μg/mL and the concentration of Cd^{2+} was higher than 3 μg/mL, the growth rate of *E. coli* always decreased under method 1 and method 2 (Fig. 3).

This showed there was a negative synergism of Se and Cd^{2+} on *E. coli* under this experimental conditions. This also indicated that injury induced by Cd^{2+} could not be prevented completely by adding Se to Cd^{2+} under any circumstances. The effects of Se is thought to be related to either the formation of Se-Cd complexes in association with metallothioneins or changes the distribution of Cd^{2+} in tissue[25].

According to results presented here, it is thought that the protective effect of Se resulted from the production of Se-Cd complex compounds. Cd^{2+} can combined with the S-H of proteins and enzymes to change their activation. Selenium is more soft alkali than sulfate. According to the hard and soft acids and bases rule, Se can have advantage over sulfur to combine with soft acid Cd^{2+} and produce the Se-Cd complex. The complex may be vented through the body by metabolizing. The S-H target molecule can be protected from. Therefore, the organisms can be kept from being injured.

Microcalorimetric method is a straight-forward and simple method in the studies of interaction between trace elements and organism. Through the technique and other methods, we can study the kinetics and thermodynamics of biological sciences further and all of these are very significant to synthesize and select drug, understand ecotoxicological of trace elements and biocompatibility of nanomaterials.

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