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Effect of glucosamine and chitooligomer on the toxicity of arsenite against *Escherichia coli*

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ABSTRACT

Escherichia coli was selected as the sample to study the toxicity of arsenite in the presence of saccharides. The effect of glucosamine, *N*-acetylglucosamine, glucose, lactose, sucrose, glucosamine and cyclodextrin on the toxicity of arsenite against *E. coli* was investigated by microcalorimetry. The glucosamine and the tested chitooligomer decreased the toxicity of arsenite on cells of *E. coli*, and the effect of glucosamine was stronger than that of the chitooligomer. These results suggest that the glucosamine and chitooligomer may be employed as the assistant antidote for arsenite.

1. Introduction

The toxic effects of trivalent arsenicals on organisms have been known for many years. Trivalent arsenic species are more toxic than the pentavalent form. Studies of the toxicity to humans are rather limited but arsenite is about 60 times more toxic than the oxidized arsenate (Jain & Ali, 2000). Despite its toxicity, arsenic has been used in Chinese medicine for thousands of years. Arsenic trioxide As_2O_3 has been approved by the FDA for the treatment of acute promyelocytic leukemia (PML) in humans which is unresponsive to conventional drugs like all trans-retinoic acid (Zhu, Chen, Lallemand-Breitenbach, & de Thé, 2002). Arsenic trioxide also appears to be a promising therapeutic agent for autoimmune diseases (Bobé, Bonardelle, Benihoud, Opolon, & Chelbi-Alix, 2006).

Many applications are controversial given the high toxicity of arsenic compounds. The acute toxicity of inorganic arsenicals is very high (LD50 of arsenite in mice is 35 mg/kg). The effective treatment of the solid tumors needs high dose of arsenite, but this carries significant risks (Gortzi et al., 2002). If the acute toxicity of arsenite is reduced, its usefulness would expand (Diepart et al., 2012). The organoarsenic compounds are about 100 times less toxic than inorganic arsenic compounds. Methylation of inorganic arsenic has actually been described as the most important detoxification process in the human body since it reduces the affinity of the compound for tissue (Li et al., 2012). Until now, there are no better organoarsenic compounds as the medicine than arsenite. Seeking for some substances, which can reduce the acute toxicity of arsenite but not reduce its therapeutic effect, is an interesting research topic.

Glucosamine is one of the most abundant monosaccharides. The amino sugar is part of the structure of chitosan and chitin, which compose the exoskeletons of crustaceans and other arthropods, cell walls in fungi and many higher organisms. Glucosamine is produced commercially by the hydrolysis of chitin. It has been used in various forms for osteoarthritis in the Europe for decades and has not demonstrated any significant toxicological effects (Vangsness Jr, Spiker, & Erickson, 2009). It has acquired substantial popularity as a dietary supplement in almost all countries throughout the world because of its safety and effectiveness (McAlindon, LaValley, Gulin, & Felson, 2000).

Chitooligomer is the oligomer of glucosamine and has good water-solubility and non-toxicity, and can be achieved by depolymerization of chitosan (Qin et al., 2006). Some researchers have reported that chitooligomers have various biological activities such as antitumor and immune enhancing effects (Kim & Rajapakse, 2005).

Our previous study shows that chitooligomers can decrease the inhibitory effect of selenite, arsenate and fenaminosulf on microorganism growth (Peng, Qin, & Li, 2009; Wang, Peng, Li, & Qin, 2010). *Escherichia coli* is often selected as the sample to study the effect of trivalent arsenicals on organisms with "thiol" enzymes (Stevenson, Hale, & Perham, 1978). In this paper, the researchers investigate the

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effect of glucosamine and chitooligomer on the toxicity of arsenite against *E. coli*.

2. Experimental

2.1. Materials and chemicals

Crude chitosan, D-glucosamine, and HCl were supplied by Golden-shell Biochemical Co., Ltd., China. The chitooligomer sample COS with weight–average molecular weight (M_w) 1.6×10^3, which is water-soluble at pH 3–11, was prepared in our laboratory (Qin et al., 2006), and the pH of 1% COS in distilled water (W/V) was 9. All other chemicals were of analytical grade.

Original 1.0 g/L As (III) water solution: 132.03 mg arsenic trioxide was added into 80 ml sodium hydroxide (106.8 mg) water solution, and diluted to 100.0 ml with volumetric flask.

E. coli (CCTCC AB91112) was provided by Chinese Center of Type Culture Collection, Wuhan University, China. The strain was grown to the stationary phase in nutrient broth at 37 °C.

LB medium, consisting of peptone 10 g, yeast extract 5 g, NaCl 5 g, per liter, pH 7.0, was sterilized by autoclaving for 20 min at 120 °C.

2.2. Effect of glucosamine and chitooligomers on the bacteriostatic action of arsenite

TAM air (an eight-channel isothermal batch calorimeter for heat flow measurements, whose limit of detectability is 2 μW) manufactured by TA Instruments-waters LLC of USA, was used to obtain the metabolic growth power–time curves of microorganism in 20 ml ampoules, and TAM Air Assistant was used to treat these data.

Before each batch experiment, 5.0 ml original 1.0 g/L As (III) solution was diluted to 50.0 ml by adding sterilized physiological saline and 1.0 mol/L HCl to obtain the 100 mg/L As (III) solution with pH 7.0 for test. The inocula were homogeneously distributed into 50 ml of LB medium at a concentration 10^6 CFU/ml for bacteria by gentle shaking to obtain the suspensions.

The weighed saccharides were added into the sterilized ampoules, respectively. The requested As (III) solutions were added by micropipette. Then, 5.0 ml of the suspensions was added into each ampoule. Sterilized physiological saline was used as a control instead of the sample. The pH of the mixture in each ampoule was adjusted to 7.0 by adding 1 mol/L HCl or NaOH. The liquor volume in each ampoule was adjusted to 5.5 ml by adding physiological saline. The ampoules were sealed tightly and shook for 3 min.

The ampoules were placed in the calorimeter and signals obtained during growth were detected. The experiments were run at 37.00 °C. Each batch experiments were carried out at least twice.

3. Results and discussion

One of the most prominent features of the microbial growth process is the production of heat. Microbial activity may be quantified by the detection of heat output accompanying all biochemical redox reaction. Microcalorimetry is a useful tool for quantitative evaluation of the growth activity of microbial cells based on detection of their metabolic heat (Wadsö, 2002). When the heat is monitored by microcalorimeter, much useful information, both qualitative and quantitative, may be obtained. Modern instruments allow heat quantities as small as a microwatt, e.g. evolved by bacteria, to be recorded. The method further provides information on the bacteriostatic and bactericidal effects of various chemicals.

Fig. 1 is the power–time curve for growth of the *E. coli* at 37.00 °C, which is a typical growth curve for *E. coli* and can be divided into four phases, that is, lag phase, log phase, stationary phase and decline phase. The calorimetric power, P, which reflects the multiplication of the cells, can be used as a parameter to characterize the growth of the cells. Since $P = dQ/dt$, the area under the curve records the heat output Q released during the experimental period. During the log phase, the power–time curves obeyed the following equation: $\ln P_t = \ln P_0 + kt$, where P_t is the power output at time t, P_0 is the initial power output, and k is the growth rate constant.

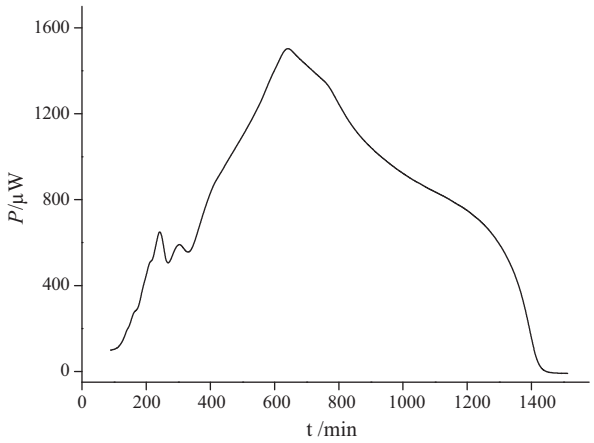

Fig. 1. Power–time curve for growth of *E. coli*.

Fig. 2 shows the power–time curves for the growth of *E. coli* at 37.00 °C in the presence of As (III) of different concentrations. Some similarities and differences were observed from a qualitative point of view. As (III) had the capacity to inhibit the growth of *E. coli* to a different extent, and the inhibitory effects increased with the concentrations of As (III) from 1.0 mg/L to 4.0 mg/L. When the concentration of As (III) increased to 4.0 mg/L, the power–time curve became a straight line, indicating that the microbial growth was totally inhibited.

Fig. 3 shows the effect of chitooligomer on arsenite (3.4 mg/L) against the growth of *E. coli*. The power–time curve in the presence of 2.0 g/L COS is very similar to that of the control, 2.0 g/L COS had no significant influence on the growth of *E. coli* in LB medium. LB medium is optimal medium for the growth of *E. coli*. Water-soluble chitooligomer (e.g. M_w 1.4×10^3) has no antimicrobial activity, but some molecules with higher degree of polymerization (e.g. M_w 2.8×10^3) may gently disturb some cells for the absorption of nutrition (Qin et al., 2006). However, the COS decreased the inhibitory effect of arsenite on the growth of *E. coli*. The inhibitory effect of arsenite on the growth of *E. coli* decreased with the increase of COS

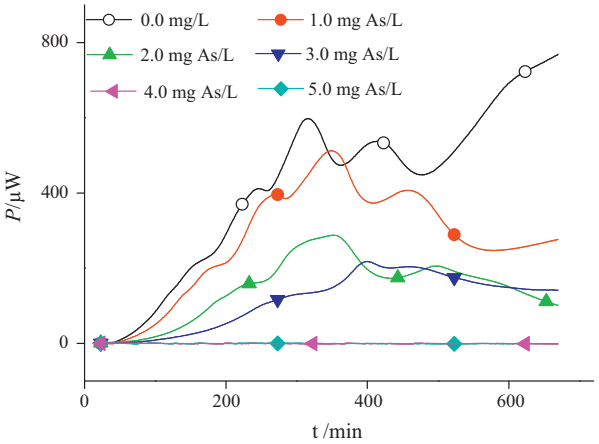

Fig. 2. Effect of arsenite on the growth of *E. coli*.

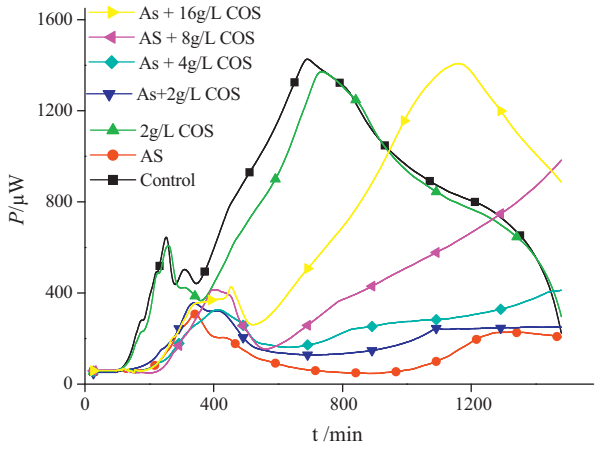

Fig. 3. Effect of chitooligomer on arsenite against the growth of *E. coli*.

Table 1
Influence of COS on 3.4 mg/L As (III) against the growth of *E. coli*.

COS (g/L)	0	2.0	4.0	8.0	16.0	Control
Q in 16 h (J)	6.08	9.18	10.41	13.47	21.97	45.19

from 2.0 g/L to 16.0 g/L (Table 1). The results suggest that the COS had the effect to lower the toxicity of arsenite on cells of *E. coli*.

Fig. 4 shows the effect of different saccharides (10.0 g/L) on arsenite (3.5 mg/L) against the growth of *E. coli*. Sucrose almost had no effect on the arsenite against the growth of *E. coli*, and lactose and *N*-acetylglucosamine had slight influence on the bacteriostatic action of arsenite. Glucose decreased the inhibitory effect of arsenite on the growth of *E. coli* to a much lower extent than did glucosamine. Cyclodextrin decreased the inhibitory effect of arsenite on the growth of *E. coli* after 7 h. In the presence of glucosamine, the peak-height of power curve *P* increased drastically, and released heat output *Q* increased drastically, and that indicated that the growth of *E. coli* increased fast. The glucosamine had the greatest effect on the arsenite against the growth of *E. coli* among the six saccharides. The control tests with only the saccharides without arsenite showed that these saccharides had no promoting effect for the growth of the *E. coli* (the power–time curves not showed). These results suggest that the glucosamine had significant effect to lower the toxicity of arsenite on cells of *E. coli*, and the effect of glucosamine was stronger than that of chitooligomer.

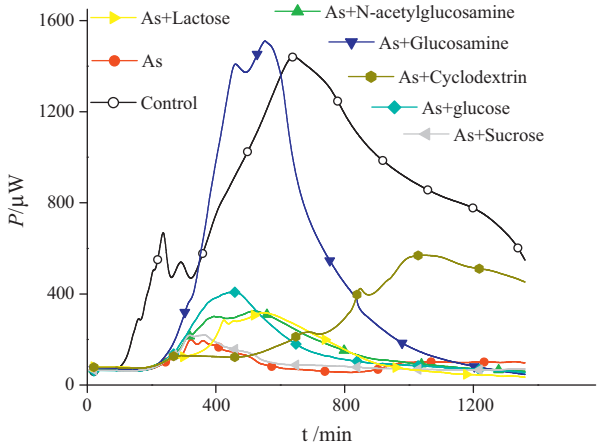

Fig. 4. Effect of different saccharides on arsenite against the growth of *E. coli*.

The pyruvate dehydrogenase multienzyme complex (PDH complex) is a nuclear-encoded mitochondrial matrix multienzyme complex that provides the primary link between glycolysis and the tricarboxylic acid (TCA) cycle by catalyzing the irreversible conversion of pyruvate into acetyl-CoA. These "thiol" enzymes widely exist in bacteria, plants and animals. PDH complex from *E. coli* is comprised of three enzymes: pyruvate dehydrogenase, lipoate acetyltransferase, and lipoamide dehydrogenase.

Chemically, arsenite reacts with sulfhydryl groups and exhibits very high affinity to vicinal thiols (Serves, Charalambidis, Sotiropoulos, & Ioannou, 1995). Many of the adverse effects of arsenite on biological systems may therefore be caused by its reaction with closely spaced cysteine residues on critical cellular proteins (Platanias, 2009). The resulting arsenic-thiol linkages are mainly responsible for the ability of arsenic to modulate the function of various key molecules, enzymes, and ion transporters inside cells.

The hydrolysis of arsenic trioxide in water produces arsenous acid H_3AsO_3. The first pK_a is 9.2. Raman spectral and NMR studies indicate that, unlike the phosphorous acid molecule, which has both H—P and H—O bonds, and hydrogen atoms in arsenous acid are linked to oxygen (Kolozsi et al., 2008). The addition of base converts arsenous acid $As(OH)_3$ to the arsenite ions $[AsO(OH)_2]^-$, $[AsO_2(OH)]^{2-}$, and $[AsO_3]^{3-}$ (Smedley & Kinniburgh, 2002).

The experimental results show glucosamine and chitooligomer have significant effect to decrease the toxicity of arsenite on cells of *E. coli*, indicating that the $-NH_2$ is the key factor for the effect. Organic arsenic compounds are less toxic than inorganic ones. H_3AsO_3 is tri-protonic acid, and it can interact with amino group. The anions of H_3AsO_3 may combine with one to three $-NH_3^+$ to form larger molecules, and the absorption by cells can be reduced.

The hydroxymethyl group of compounds, such as glucose, fructose, fructose-6-P, and dihydroxyacetone can react with arsenate in neutral aqueous solutions to form esters in the absence of an enzyme (Lagunas & Sols, 1968; Long & Ray, 1972). The H_3AsO_3 acids have three —OH that can react with alcohols, three series of esters such as $ROAs(OH)_2$, $(RO)_2As(OH)$ and $(RO)_3As$ could be formed, but these esters can hydrolyze in aqueous media (Committee on Medical and Biological Effects of Environmental Pollutants, 1977).

The monosaccharide can form complexes with arsenite (Ren, Deng, & Cheng, 1995), but disaccharide is not easy to form complexes with arsenite (Ren, He, Deng, & Cheng, 1993). The $-NH_2$ has stronger electron-donating ability than —OH, so glucosamine can form more stable complex than glucose. In addition, the spatial structure of ligands affects the stability of the forming complex. The glucosamine is monosaccharide, a molecule has a reducing end and a hemiacetal —OH groups in C-1. A chitooligomer molecule is composed of several glucosamine residue units, but it has only a reducing end group. Therefore, at the same mass, glucosamine has more reducing end groups than chitooligomer. The effect of glucosamine is stronger than that of chitooligomer for lowering the toxicity of arsenite, suggesting that the chelation of arsenite with vicinal $-NH_2$ in C-2 and —OH in C-1 may be stronger than that of arsenite with vicinal $-NH_2$ in C-2 and —OH in C-3. The physicochemical structure on interaction of arsenite with glucosamine should be studied in the future.

4. Conclusions

In this study, the glucosamine and chitooligomer were found to have significant effect to lower the toxicity of arsenite on cells of *E. coli*, and the effect of glucosamine was stronger than that of chitooligomer. These results suggest that the glucosamine and chitooligomer may be employed as the assistant antidote

for arsenite. Therefore, further studies are needed to investigate deeply the effect in vivo.

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