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In vivo synthesis of nano-selenium by *Tetrahymena thermophila* SB210

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ABSTRACT

Nano-selenium has a great potential to be used in chemical, biological, medical and environmental fields. Biological methods for nano-selenium synthesis have attracted wide interests, because they can be operated at ambient temperature and pressure without complicated equipments. In this work, a protozoa, *Tetrahymena thermophila (T. thermophila)* SB210, was used to *in vivo* synthesize nano-selenium. The biosynthesized nano-selenium was characterized using transmission electron microscopy, energy dispersive X-ray spectroscopy and Raman spectroscopy. The synthesized amorphous spherical selenium nanoparticles had diameters of 50–500 nm with the coexistence of irregular nano-selenium. The expressions of glutathione (GSH) synthesis related gene *glutathione synthase*, cysteine-rich protein metallothionein related gene *metallothionein-1* and [2Fe-2S] cluster-binding protein related gene were up-regulated in the nano-selenium producing group. Also, the subsequent GSH detection and *in vitro* synthesis experimental results suggest the three proteins were likely to be involved in the nano-selenium synthesis process.

1. Introduction

Nano-selenium is widely used due to its unique optical, spectral and other properties [1]. In biological and medical fields, nano-selenium has a great potential for practical applications. For example, nano-selenium showed an excellent antibacterial activity against *Staphylococcus aureus* compared with commercially available drug Ampicillin [2–4], and antifungal activity against several important clinical test strains [5]. In recent years, nano-selenium have been used for cancer therapy [6,7] and cancer-targeted nano-drug delivery [8]. Furthermore, nano-selenium also plays a positive role in wound healing, anti-oxidant and anti-Dengue virus [9].

To date, nano-selenium with desired sizes can be synthesized by chemical approaches [10], but they are usually expensive, environmentally contaminative, and require complicated equipments [11]. However, biological methods for nano-selenium synthesis are relatively simple, environmentally friendly and easier to be operated at ambient temperature and pressure [3]. Various types of organisms including bacteria [12,13], fungi [14] and plants [15] have been used for producing nano-selenium under mild conditions. Different species produced nano-selenium with distinct shapes and various sizes. However, there is no report about the synthesis of nano-selenium by animals yet.

Tetrahymena is a genus of protozoa, the simplest animals. It has cellular structural and functional complexity comparable to that of human [16], and has been proven to be a very valuable biological model in molecular biology, genetics [17–19] and toxicological studies [20]. Additionally, *Tetrahymena* has a rapid growth rate with a doubling time of less than two hours, which enables quick results and low maintenance costs [21]. These advantages make *Tetrahymena* an excellent surrogate model for animal research [22]. As a cost-effective animal, *Tetrahymena* is an ideal model to be explored for the synthesis of nano-selenium. The complete genome sequence of *T. thermophila* SB210 is available, enabling us to conduct mechanism studies on nano-selenium synthesis.

Therefore, in this work, nano-selenium was synthesized using *T. thermophila* SB210 in a simple, cost-effective and green way. Also, the prepared nano-selenium inside cells was characterized with various methods. Additionally, real-time polymerase chain reaction (PCR) and *in vitro* synthesis experiments were carried out to explore the underlying mechanism behind the nano-selenium synthesis in *Tetrahymena*.

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0141-0229/

Table 1
Primers for targeting genes.

Target gene	Primer sequence	Product size (bp)
Tubulin	Forward 5′-GTTCGGGAATGGG-3′ Reverse 5′-TTGAATAACTAGGAGCA-3′	396
Glutathione Synthase	Forward 5′-ATAAGAAGCAGGGTAG-3′ Reverse 5′-TCTCAAGGAAAGGGT-3′	215
mtt-1	Forward5′-GCGTAAGTAAGACTGATAAT-3′ Reverse 5′-AAGCAGCAGGGTTTAG-3′	145
2Fe2Sp	Forward 5′-CCACCATCATCACGC-3′ Reverse 5′-GCAGAAAGGACCATAAGT-3′	235

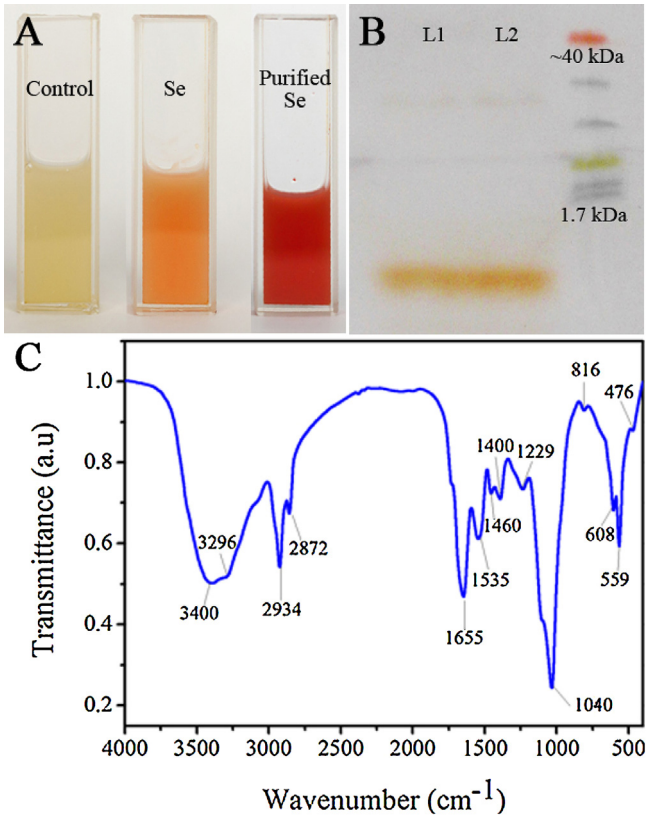

Fig. 1. Biosynthesis of the nano-selenium by *T. thermophila* SB210. (A) Control, selenite-dosing cells and purified nano-selenium; (B) SDS-PAGE electrophoresis of the purified nano-selenium; and (C) FTIR of purified nano-selenium.

2. Materials and methods

2.1. Nano-selenium synthesis and purification

T. thermophila (SB210) was kindly provided by Prof. Miao from the Institute of Hydrobiology, Chinese Academy of Sciences, China. The cells were grown in proteose peptone medium containing 1% (w/v) proteose peptone (BD, USA), 0.1% yeast extract (OXOID, UK), 0.2% glucose (Sinopharm Chemical Reagent Co., China), 0.003% ethylenediaminetetraacetic acid ferric sodium salt (Sigma-Aldrich, USA), and maintained at 27 °C in an orbital shaker (160 rpm).

When cells grew into the late log phase, 150 μM Na_2SeO_3 (Sigma-Aldrich, USA) was added into the medium. After additional 48-h cultivation, cells were centrifuged at 12,000 rpm for 5 min. The precipitate was washed twice with 10 mM Tris-HCl (pH 7.4) and re-suspended in the lysis buffer containing 2% (w/v) SDS (sodium dodecyl sulfate) and 0.2 M NaOH. Then, the cells were further treated by ultrasonic cell disruptor (Ningbo Scientz Biotechnology Co., China) for 10 min at a power output of 120 w in ice bath in order to release nano-selenium from cell components as much as possible. Finally, the homogenate was centrifuged at 12,000 rpm for

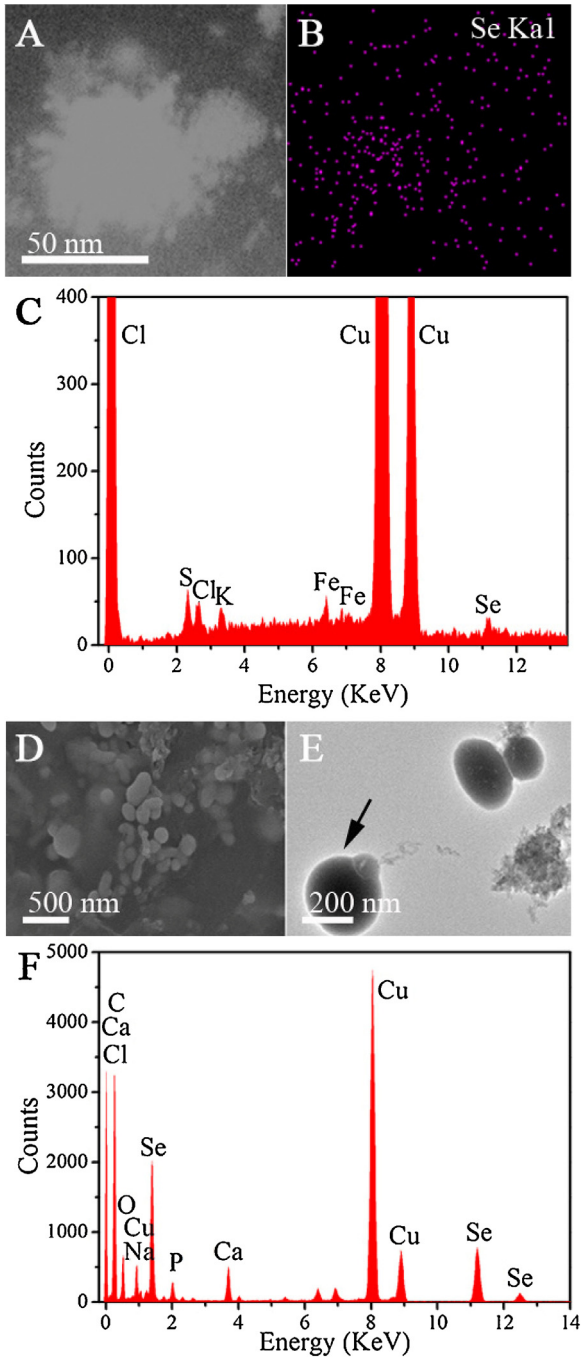

Fig. 2. Electron microscopy observation of the nano-selenium synthesized by *T. thermophila* SB210. (A) *In situ* dark-field electron microscopy; (B) corresponding Se element mapping of (A); (C) EDX of the nano-selenium; (D) SEM of the purified nano-selenium; (E) TEM of the purified nano-selenium; (F) EDX of purified nano-selenium indicated by arrow in (E).

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