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# Optimization of selenylation modification for garlic polysaccharide based on immune-enhancing activity

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Pyridine (PubChem CID: 1049)
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Ascorbic acid (PubChem CID: 54670067)
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ABSTRACT

Garlic polysaccharide (GPS) was modified in selenylation respectively by nitric acid-sodium selenite (NA-SS), glacial acetic acid-selenous acid (GA-SA), glacial acetic acid-sodium selenite (GA-SS) and selenium oxychloride (SOC) methods each under nine modification conditions of $L_9(3^4)$ orthogonal design and each to obtain nine selenizing GPSs (sGPSs). Their structures were identified, yields and selenium contents were determined, selenium yields were calculated, and the immune-enhancing activities of four sGPSs with higher selenium yields were compared taking unmodified GPS as control. The results showed that among four methods the selenylation efficiency of NA-SS method were the highest, the activity of $sGPS_5$ was the strongest and significantly stronger than that of unmodified GPS. This indicates that selenylation modification can significantly enhance the immune-enhancing activity of GPS, NA-SS method is the best method and the optimal conditions are 0.8:1 weight ratio of sodium selenite to GPS, reaction temperature of 70 °C and reaction time of 10 h.



## 1. Introduction

Selenium (Se) is an essential trace element that is important to the development and maintenance of a healthy body and can only be obtained from food or other source of supplementation (Sanmartín, Plano, Sharma, & Palop, 2012; Zhang et al., 2009). It is a constituent of numerous selenoproteins that play critical roles in reproduction, thyroid hormone metabolism, DNA synthesis, and protection from oxidative damage and infection (Whanger, 2002). For adults, the daily intake of selenium must be achieved 60 μg for women or 70 μg for men (Kippa et al., 2015). The selenium content of food varies widely based on the selenium content of soil. For example, the average selenium content of cultivated soil in China was 265 μg $kg^{-1}$ (Tan et al., 2002). The average selenium content of rice in Guangzhou with Se-adequate soil was 58 μg $kg^{-1}$, in Suzhou with moderate Se-deficiency soil was 24 μg $kg^{-1}$, while in Keshan with high Se-deficiency soil was low to 6 μg $kg^{-1}$ (Gao et al., 2011). But Se-lacking regions are widely distributed in all

*Abbreviations:* GPS, garlic polysaccharide; sGPSs, selenizing garlic polysaccharide; Se-P, selenium polysaccharide; NA-SS, nitric acid-sodium selenite; GA-SA, glacial acetic acid-selenous acid; GA-SS, glacial acetic acid-sodium selenite; SOC, selenium oxychloride.

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over the world, e.g. China, New Zealand, Scandinavian and Balkan countries, and Atlantic Region of Canada (Oropeza-Moe, Wisloff, & Bernhoft, 2015).

Selenium deficiency will severely harm the health of organism and cause many disease in human and animals (Chen, 2012; Duncanson, 2013), such as immune dysfunction, cancer and viral diseases (Kohrle, 2000) In 1935 a fatal cardiomyopathy outbroken in Keshan County of China is caused by Se deficiency (Yang, Lin, Li, Guo, & Yin, 1988; Yang, Ge, Chen, & Chen, 1988). Therefore selenium-supplementing is imperative for human and animals in Se-lacking regions. The source of selenium supplement includes inorganic and organic selenium source. The organic selenium is more effective and safer than inorganic selenium as a dietary supplement (Rayman, 2000).

Selenium polysaccharide (Se-P) is one of the organic selenium compounds, possesses duple and higher biological activity in comparison with polysaccharide or selenium, and is more easily absorbed and utilized by the organism (Staaf, Yang, Huttunen, & Widmalm, 2000; Shang, Zhang, Wen, Li, & Cui, 2009). Its biological activities are involved in enhancing immunity (Qin, Chen, Wang, Hu, Wang, et al., 2013; Qin, Chen, Wang, Hu, Zhang, et al., 2013), anti-oxidation (Stapleton, 2000; Wang, Zhao, Wang, Yao, & Zhang, 2012), anti-cancer (Decker, Bianchi, Decker, & Morel, 2001), anti-metal poisoning (Das, Jacob, & Bouquegneau, 2002) anti-hyperglycemia (Liu et al., 2013) and so on. The natural Se-P normally resides in plants or microorganisms such as pumpkin, *Codonopsis pilosula*, *Ganoderma lucidum* and so on, but the selenium content value in Se-P is lower, even if the plants grown in rich-selenium region. For example, both *Astragalus membranaceus* and rivier giant arum tuber grown in Enshi district of Hubei Province of China, a selenium-rich region, contain Se-P, but the selenium contents in Se-P are only 1.06 $\mu g\,g^{-1}$ and 1.55 $\mu g\,g^{-1}$ respectively (Wu et al., 1994); the selenium contents of Se-polysaccharides extracted from Se-enriched fruit body of *Flammulina velutipes* and *Auricularia auricula* were 7.97 $\mu g\,g^{-1}$ and 4.97–7.08 $\mu g\,kg^{-1}$, respectively (Song & Du, 2010; Tie et al., 2008); which cannot meet the selenium-supplementing needs for human and animals. National Research Council (US) reported that the dietary requirement ranges of selenium in pigs were from 0.3 mg/kg (weanling pigs) to 0.15 mg/kg (fattening pigs and sows) (National Research Council, 2012).

Therefore, researchers have been exploring the methods to get Se-P. The bio-enrichment method is one of the reported methods to get Se-P, that is, inorganic selenium (sodium selenite) is added into the culture medium of fungi, algae and bacteria or other small plant, the selenium content is richened through growth, metabolism and biotransformation (Cui, Shang, & Zou, 2003; Song & Du, 2010). Some successful cases include *Catathelasma ventricosum* Se-P, *Astragalus membranaceus* Se-P, Ziyang green tea Se-P and so on (Li et al., 2014; Malinowska et al., 2009; Wang et al., 2013). However, this method is easily restricted by plant growth cycle, and the analysis and purification of the polysaccharide are more difficult.

In recent years, it has been found that artificial synthetic selenizing polysaccharide is a better way. Polysaccharides contain functional active groups, such as hydroxyl group, aldehyde group, ketone group and so on, which can react with other compounds and make selenium group graft into polysaccharide molecules (Liu et al., 2013; Qin, Xiao, Du, Shi, & Chen, 2002). Some methods have been reported such as nitric acid-sodium selenite (NA-SS) method (Li, Miu, & Liu, 2001; Yang, Huang, Jiang, Zhu, & Han, 2009), glacial acetic acid-selenite (GA-SA) method (Pang, Yang, & Zhao, 2009), glacial acetic acid-sodium selenite (GA-SS) method (Liang, Ma, Zhao, Xu, & Sun, 2011) and selenium oxychloride (SOC) method (Gong, 1997; Wang, Zhang, et al., 2009; Wang, Deng, Wan, Zuo, & Li, 2009).

Garlic is the squamous bulb of *Allium sativum* L. in liliaceae plants. It is not only an edible vegetable, but also a kind of herbal medicine being used in China. It contains abundant polysaccharide reaching up to 77% of dry weight (Koch & Lawson, 1996). In our previous research the Chinese angelica polysaccharide, lycium barbarum polysaccharide and garlic polysaccharide had been successfully modified in selenylation by NA-SS method, and the conclusion also had been confirmed that selenylation modification could significantly enhance the immune-enhancing and antioxidant activities of these polysaccharides (Qiu, Chen, Chen, et al., 2014; Stapleton, 2000; Wang et al., 2012).

In present study, garlic polysaccharide (GPS) was extracted, purified and modified in selenylation respectively by NA-SS method, GA-SA method, GA-SS method and SOC method, each according to $L_9(3^4)$ orthogonal design of three factors, ratio of selenic reagent to GPS, reaction temperature and time, at three levels and each to obtain nine selenizing GPSs (sGPSs), $sGPS_1$-$sGPS_9$. Their structures were identified, the yield and selenium contents were determined, the selenium yields were calculated, the effects of four sGPSs with higher selenium yields on lymphocytes proliferation, and the effects of $sGPS_5$ on cytokines production were compared taking the unmodified GPS as control. The purpose of this study is to validate whether selenylation modification can improve the immune-enhancing activity of GPS, choose out the high-efficiency modification method and optimal modification conditions, and offer the theoretical evidence for using selenylation modification to enhance the biological activity of polysaccharide.

## 2. Materials and methods

### 2.1. Reagents

Sodium selenite was bought from Tianjin Chemical Reagent Ltd.; Selenous acid, nitric acid, glacial acetic acid, pyridine, anhydrous sodium carbonate, ascorbic acid were the products of Sinopharm Chemical Reagent Ltd.; Selenium oxychloride (97%) Sigma; Hydrochloric acid, concentrated sulfuric acid, barium chloride were purchased from Nanjing Chemical Reagent Ltd.; Perchloric acid (guaranteed reagent) was the product of Deer Chemical Reagent Ltd.; KBr (spectral purity) was bought from Sinopharm Chemical Reagent Ltd.; Selenium standard solution (1000 $\mu g\,mL^{-1}$) was supplied by National non-ferrous metals and electronic materials analysis testing centers.

RPMI-1640 (Gibco) supplemented with 100 IU $mL^{-1}$ benzylpenicillin, 100 IU $mL^{-1}$ streptomycin and 10% fetal bovine serum was used for washing and re-suspending the cells, diluting the mitogen and culturing the cells. Hanks' solution, pH was adjusted to 7.4 with 5.6% sodium bicarbonate solution, supplemented with benzylpenicillin 100 IU $mL^{-1}$ and streptomycin 100 IU $mL^{-1}$. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, American Co.) was dissolved into 5 mg $mL^{-1}$ with calcium and magnesium-free (CMF) phosphate-buffered saline (PBS, pH7.4). These reagents were filtered through a 0.22 $\mu m$ syringe filter. Sodium heparin solution was stored at −20 °C, the others were at 4 °C and MTT solution was stored in a dark bottle. Dimethylsulfoxide (DMSO) was the product of Shanghai Lingfeng Chemical Reagent Ltd. Chicken ELISA kits were the products of Shanghai Langdun Biotechnology Inc.

### 2.2. Extraction and purification of GPS

Garlic was bought from a farm product market of Nanjing City, the product of Shandong Province of China. GPS was extracted by water decoction and ethanol precipitation method. 2500 g of peeled garlic was cut into small pieces about 0.5–2 $cm^3$, put in