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Nanoparticle Conjugates of Selenium Compounds: Preparation, Characterisation and Electron Transfer †

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Abstract: One of the important features influencing the biological applications of organoselenium compounds is their redox state, which in turn is affected by their interactions with nearby heteroatoms. To modulate the biological action of selenium in such compounds, researchers have designed new structural motifs and also developed new formulations using inorganic nanoparticles. Metal nanoparticles such as gold nanoparticles (GNPs) and magnetic nanoparticles (MNPs) like iron oxide (Fe_3O_4) have been extensively studied for conjugation with many heteroatoms (sulphur, nitrogen and oxygen) containing ligands. Selenium, being more polarisable than sulphur, can induce significant surface passivation, thereby providing easy modulations with physico-chemical properties. Considering this, we investigated the physico-chemical properties of a few selenium compounds conjugated to GNPs and MNPs. The GNP conjugates were characterised by spectroscopic and microscopic tools, such as optical absorption, Raman spectroscopy, dynamic light scattering (DLS), the zeta potential and transmission electron microscopy (TEM). The results confirmed that the selenium atom was covalently conjugated to GNPs and this conjugation not only increased their electron transfer ability, but also their antioxidant ability. In another study, asymmetric phenyl selenides were conjugated with MNPs and characterised byX-ray diffraction (XRD), TEM, DLS and zeta potential. The radical scavenging ability of the selenium compounds improved upon conjugation with the MNPs. Therefore, the above studies confirmed that the redox activities of selenium compounds can be modulated upon conjugation with inorganic nanoparticles, such as GNPs and MNPs, which in turn provides new avenues for delivering organoselenium compounds.

Keywords: selenium; gold nano particle; magnetic nano particle; nano-conjugates; redox reaction

1. Introduction

Selenium compounds have many applications in biology, medicine and material development. As amicronutrient, seleniumis present in humans in the form of selenoproteins such asglutathione peroxidase and thioredoxinreductaseenzymes, which help to prevent cellular damage from reactive oxygen species (ROS) and regulate the redox balance in the body [1–3]. Selenium has a very narrow toxicity profile for humans, ranging from being essential to toxic in the microgram concentration

region. In terms of the essential region, it is being explored for many medical applications, such as the treatment of cancer and many other endemic diseases. To explore this activity of selenium, researchers are exploring new methods for enhancing its redox activity by conjugating it to nanoparticle systems [2,3]. Among these systems, gold nanoparticles (GNPs) and magnetic nanoparticles (MNPs) are being excessively used due to their versatile properties [4–6]. GNPs display antimicrobial activity and have been studied to develop a potential drug and gene delivery system in cancer therapy. Additionally, they show photothermal effects, which provide an additional advantage. MNPs, in particular Fe_3O_4, are widely used as a carrier for the delivery of drugs and biomolecules due to their unique magnetic properties, low toxicity and biodegradability [5,6]. Both of the particles have reactive surfaces that can be easily functionalised with biocompatible molecules and pharmaceutical agents. Several ligands containing heteroatoms, such as nitrogen, sulphur, phosphorous, etc., have been extensively employed for the surface passivation of inorganic materials. In particular, molecules with thiol functional groups have demonstrated an excellent ability to bind with gold surfaces via chemisorption. Selenium is softer and more polarizable than sulphur [2]. Therefore, it is expected to interact more strongly. Selenium compounds attached to GNPs have been found to exhibit better anti-cancer activity than unconjugated selenium compounds [7–9]. GNPs have also been found to be very efficient substrates for the detection of selenium, even in a nanomolarrange, using surface enhanced Raman scattering(SERS) [7]. Recently, Gold-Se conjugates have been developed as efficient nanoprobes for the detection of signalling associated with cancer progression [8]. Therefore, preparing nanoconjugates with selenium compounds is emerging as an alternative and efficient approach for modifying their physico-chemical and biochemical properties. Considering this, in this study, a few selenium compounds were conjugated with GNPs and MNPs and evaluated for their radical scavenging activity. For GNP-selenium studies, we employed bis-2-ethanolselenide (EOH-Se-EOH) as a model Se compound and for MNP-selenium studies, we used various Se containing aminoacid derivatives such as selenoglycine derivative (SeG) and selenoserine derivative (SeS). The synthesis of selenium compounds has been reported in our earlier papers [10,11]. The chemical structure of the selenium compound used for the GNP interaction is given in Scheme 1.

Scheme 1. Chemical structure of bis-2-ethanolselenide(EOH-Se-EOH).

2. GNP-Selenium Studies

To understand the interaction of selenium compounds, GNPs were first prepared by reducing potassiumtetrachloroaurate(III) ($K[AuCl_4]$) with trisodium citrate in nanopure water under constant stirring and controlled heating. A deep wine red coloured solution was obtained after the formation of GNPs which exhibited characteristic optical absorption with a λ_{max} of 522–524 nm, due to the localised surface plasmon resonance. The average hydrodynamicdiameter of the GNPs, as obtained from dynamic light scattering (DLS), was found to be 15± 3 nm and the zetapotential of the particles was determined to be −39.5 ± 2.9 mV. These GNPs were treated with the symmetric selenoether—bis-2-ethanolselenide (EOH-Se-EOH). This led to a ligand exchange reaction at the surface of GNPs and a consequent shift in the absorption maximum to 650–680 nm (blue coloured). The changes in the optical properties of GNP upon binding with selenoether have been used to obtain the binding constant of the ligand [12]. Following the absorption changes at 520 nm, as a function of the EOH-Se-EOH concentration, fitting these data to a linear plot as per the Benesi–Hildebrand equation yielded a binding constant of $5.9 \pm 0.2 \times 10^2$ M^{-1}. Upon binding with selenother, the hydrodynamic size and zeta potential of the GNP changed to 26 ± 2 nm and −27.6 ± 0.8 mV, respectively.

SERS studies of GNP and GNP conjugated EOH-Se-EOH showed very interesting features. The C-Se-C stretching vibration of the compound at 551 cm^{-1}displayeda shift of 9.7 cm^{-1}upon binding to GNP. The CH_2 bending vibration, which exhibited multiple peaks from 1015 to 1558 cm^{-1}, also showed small shifts upon binding to GNP, indicating that both the selenium and the alkyl chain of

EOH-Se-EOH interact with GNP. This affects the electron density and redox potential of the selenium. To assess this, electron transfer reactions between EOH-Se-EOH with the $ABTS^{\bullet+}$ radical were studied in the presence and absence of GNP and were monitored by spectrophotometry [12]. $ABTS^{\bullet+}$ has a strong absorption band from400 to 800 nm and is a very long-lived radical. It can be prepared by the reaction of $ABTS^{2+}$with an oxidant, such as$K_2S_2O_8$. The absorption–time plot, as observed at 820 nm, did not show significant decay within the measured time scale, in the absence of any additive, and the estimated decay constant was 3.7×10^{-6} s^{-1}. In the presence of GNP, the decay constant increased to 1.1×10^{-4} s^{-1} and in the presence of EOH-Se-EOH, the decay constant was 8×10^{-5} s^{-1}. However, in the presence of the GNP-EOH-Se-EOH conjugate, the decay displayed a significant increase to 2.1×10^{-3} s^{-1}. This clearly confirmed that GNP conjugation increased the electron transfer ability of the selenium compound. The increase in the electron transfer ability upon conjugation to GNP has been attributed to an increase in the stability of the selenium-centred radical formed on the GNP surface in a separate study involving pulse radiolysis [12]. A schematic illustration of the binding of EOH-Se-EOH on gold nanoparticle surfaces and consequent changes in the colour of the nanoparticle suspension is given in Figure 1.

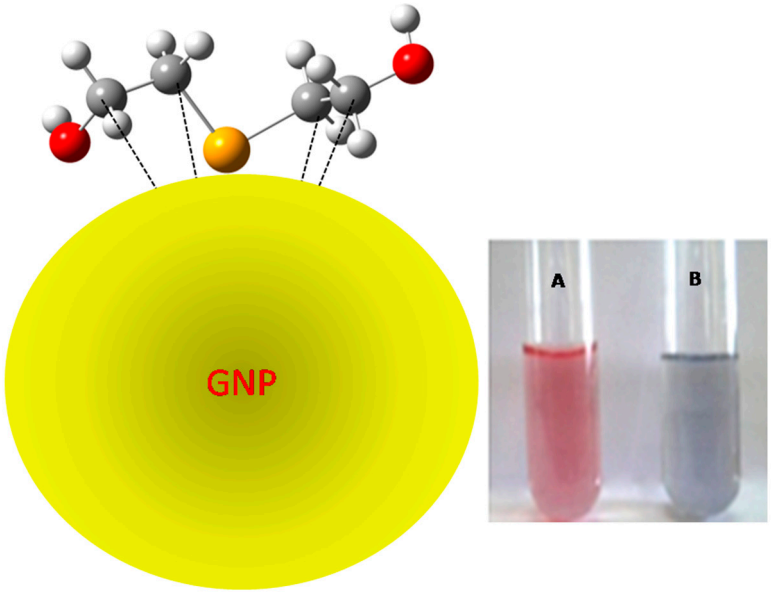

Figure 1. Schematic representation of gold nanoparticle(GNP)-bound EOH-Se-EOH and photographs of the GNP suspension before (**A**) and after (**B**) conjugation with the selenium compound.

3. MNP-Selenium Studies

In the second study, Fe_3O_4 magnetic nanoparticles (MNPs) coated with glycine were employed to conjugate selenium compounds. A glycine coating not only provides stabilisation to MNPs, but also leaves freely exposed amine groups, improving the water dispersibility. These MNPs (TEM ~10 nm) were prepared by the co-precipitation method, as previously reported [13], and separated from the supernatant using a permanent magnet. Since magnetic nanoparticles can be separated using a permanent magnet, this offers an attractive way to purify the suspension from any unbound molecules or reaction by-products. These MNPs were characterised by IR spectroscopy, which showed characteristic vibrations at 588 cm^{-1}(Fe-O), 1400 cm^{-1}($\upsilon_{sym}COO^-$) and 1586 cm^{-1}($\upsilon_{asym}COO^-$). The morphology of the particles were identified byTEM (roughly spherical particle with anaverage size of~10 nm), and the magnetisation studies indicated superparamagnetic behaviour, with a maximum magnetization of 65.2 emu/g at 300 K. The aminoacid-passivated MNPs were further conjugated with selenium containing N-acetyl alpha aminoacid compounds, $PhSeCH_2CONHCH_2COOH$ (SeG) and $PhSeCH_2CONHCH(CH_2OH)COOH$ (SeS),which were synthesised as reported elsewhere [11]. The conjugation of the selenium compounds to the MNPs was carried out by well-known bio-conjugation reaction EDC-NHS coupling between the carboxyl

group of the Se compound and amine functional group on the MNP surface [13]. Figure 2 shows a schematic illustration of the conjugation of selenium containing N-acetyl alpha aminoacids on glycine-passivated MNPs.

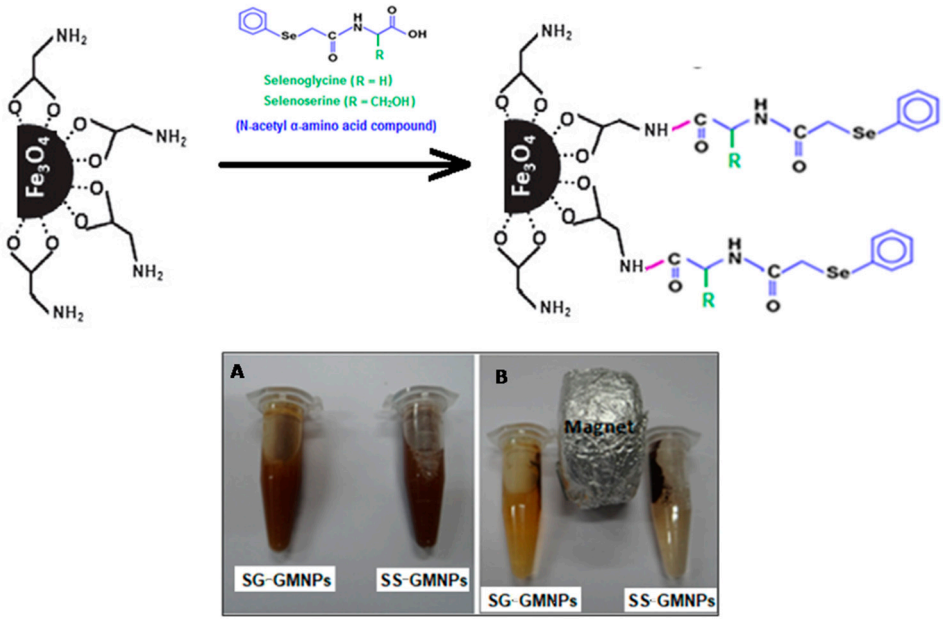

Figure 2. Schematic representation of the conjugation of N-acetyl α-amino acid compounds on glycine coated MNPs (GMNPs). Photographs of an aqueous suspension of SG-GMNPs and SS-GMNPs in the absence (**A**) and presence (**B**) of a magnetic field (field strength of table top magnet: 0.25 kOe). The agglomeration of the particles in the presence of a magnet can be clearly seen in the photograph.

The conjugation of SeG and SeS on the surface of MNPs did not affect the crystal structure of Fe_3O_4, as assessed by XRD. From the ICP-AES analysis, it was found that about 80 and 70 μg/g of SeG and SeS, respectively, could be loaded onto MNPs. DLS measurements indicated that the average hydrodynamic diameter of the particles was104 and 115 nm and the zeta potential was −20 and−25 mV at pH 7.4 for SeG-MNPs and SeS-MNPs, respectively. Furthermore, both SeG-MNPs and SeS-MNPs exhibited a good magnetic field responsivity under an external magnet.

The redox behaviour of the MNP-loaded selenium compounds was evaluated by the DPPH radical scavenging assay. The DPPH radical is a stable radical and gets reduced by compounds that have the ability to donate an electron or hydrogen atom. The reaction is followed by monitoring the absorbance at 517 nm due to the DPPH radical, which becomes pale yellow upon reduction. When SeG-MNP and SeS-MNP were evaluated with the DPPH scavenging assay, the results indicated that the selenium compounds not only retained their original DPPH scavenging activity, but also improved marginally upon binding to MNP.

4. Conclusions

In conclusion, we have demonstrated the ability of various inorganic nanocarriers for the binding of organoselenium compounds and the impact on electron transfer behaviour. In particular, the above studies indicate that selenium compounds can be easily conjugated to different types of nanoparticles, such as GNPs or MNPs. Binding with nanoparticles such as GNPs significantly improves their electron transfer ability. This can provide a novel way of improving their antioxidant activity. Similarly, organoselenium compounds can also be conjugated to Fe_3O_4 MNPs. Althoughsuch conjugation did not significantly increase their electron transfer ability, glycine conjugation provideswater solubility and itsmagnetic nature provides target specificity. Overall, these studies provide new avenues formodulating the biological properties of selenium compounds using inorganic nanocarriers.

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Conflicts of Interest: The authors declare no conflicts of interest.

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