

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

K. I. Priyadarsini ^{1,*}, B.G. Singh ², P.P. Phadnis ³, K.C. Barick ³ and P. A. Hassan ³

¹ UM-DAE Centre for Excellence in Basic Sciences, University of Mumbai, Kalina Campus, Santacruz (E), Mumbai-400098, India

² Radiation & Photochemistry Division; beenam@barc.gov.in

³ Chemistry Division, Bhabha Atomic Research Centre, Trombay, Mumbai – 400085, India; phadnisp@barc.gov.in (P.P.P.); kckbarick@barc.gov.in (K.C.B.); hassan@barc.gov.in (P.A.H.)

* Correspondence: k.indira@cbs.ac.in

† Presented at the 1st International Electronic Conference on Catalysis Sciences, 10–30 November 2020; Available online: <https://eccs2020.sciforum.net>.

Published: 10 November 2020

Abstract: One of the important features of influencing the biological applications of organoselenium compounds is its redox state, which in turn is affected by its 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 like gold nanoparticles (GNP) and magnetic nanoparticles (MNP) like iron oxide (Fe₃O₄) have been extensively studied for conjugation with many heteroatoms (sulphur, nitrogen, oxygen) containing ligands. Selenium being more polarisable than sulphur can induce significant surface passivation thereby providing easy modulations in physico-chemical properties. With this aim, we investigated the physico-chemical properties of a few selenium compounds conjugated to GNP and MNP. The GNP conjugates were characterised by spectroscopic and microscopic tools like optical absorption, Raman spectroscopy, DLS, Zeta potential and TEM. The results confirmed that the selenium atom was covalently conjugated to GNP and this conjugation has not only increased their electron transfer ability but also their antioxidant ability. In another study asymmetric phenyl selenides were conjugated with MNP and characterised by XRD, TEM, DLS and zeta-potential. The radical scavenging ability of the selenium compounds improved on conjugation with the MNPs. Thus, the above studies confirmed that the redox activities of selenium compounds can be modulated on conjugating with inorganic nanoparticles like GNP and MNP, which in turn provide new avenues for delivering organoselenium compounds.

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

1. Introduction

Selenium compounds find many applications in biology, medicine and materials development. As a micronutrient, it is present in humans in the form of selenoproteins like glutathione peroxidase and thioredoxin reductase enzymes, that help in preventing cellular damage from reactive oxygen species (ROS) and regulating the redox balance in the body [1–3]. Selenium has a very narrow toxicity profile for humans, from being essential to toxic in microgram concentration region. In the essential region it is being explored for many medical applications like treatment of cancer and many other endemic diseases. To explore this activity of selenium researchers are exploring new methods to enhance its redox activity by conjugating to nanoparticle systems [2,3]. Among these gold nanoparticles (GNP) and magnetic nanoparticles (MNP) are finding excessive use due to their versatile properties [4–6]. GNPs show 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 additional advantage. MNPs, in particular Fe_3O_4 , are widely used as a carrier for delivery of drugs and biomolecules due to their unique magnetic properties, low toxicity, and biodegradability [5,6]. Both the particles have reactive surface that can be easily functionalized with biocompatible molecules and pharmaceutical agents. Several ligands containing heteroatoms like nitrogen, sulfur, phosphorous etc have been extensively employed for surface passivation of inorganic materials. In particular, molecules with thiol functional groups have shown excellent ability to bind with gold surfaces via chemisorption. Selenium is softer and more polarizable than sulfur [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 has also been found to be very efficient substrate for detection of selenium even in nanomolar range, using surface enhanced Raman scattering (SERS) [7]. Recently Gold-Se conjugates are being developed as efficient nanoprobes for detection of signaling associated with cancer progression [8]. Thus preparing nanoconjugates with selenium compounds is emerging as an alternative and efficient approach to modify their physico-chemical and biochemical properties. With this aim, a few selenium compounds have been conjugated with GNPs and MNPs and evaluated for radical scavenging activity. For GNP-Selenium studies, we have employed bis-2-ethanolselenide (EOH-Se-EOH) as a model SE compound and for MNP-Selenium studies we used various Se containing amino acid derivatives such as selenoglycine derivative (SeG) and selenoserine derivative (SeS). Synthesis of selenium compounds are reported in our earlier papers [10,11]. The chemical structure of selenium compound used for GNP interaction is given in scheme 1.



Scheme 1. Chemical structure of EOH-Se-EOH.

2. GNP-Selenium Studies

To understand the interaction of selenium compounds, first GNPs were prepared by reducing potassium tetrachloroaurate(III), $\text{K}[\text{AuCl}_4]$ with trisodium citrate in nanopure water under constant stirring and controlled heating. A deep wine red colored solution was obtained after formation of GNPs which show characteristic optical absorption with a λ_{max} of 522–524 nm, due to the localised surface plasmon resonance. The average hydrodynamic diameter of the GNPs, as obtained from dynamic light scattering (DLS) was found to be of 15 ± 3 nm and the zeta-potential 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 leads to a ligand exchange reaction at the surface of GNPs and a consequent shift in the absorption maximum to 650–680 nm (blue colored). 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 EOH-Se-EOH concentration and fitting these data to a linear plot as per Benesi-Hildebrand equation yielded a binding constant of $5.9 \pm 0.2 \times 10^2 \text{ M}^{-1}$. Upon binding with selenoether, 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} showed a shift of 9.7 cm^{-1} upon binding to GNP. The CH_2 bending vibration, which showed multiple peaks from 1015 to 1558 cm^{-1} , also showed small shifts on binding to GNP, indicating that both the selenium as well as the alkyl chain of EOH-Se-EOH interact with GNP. This would affect the electron density and redox potential of the selenium. To assess this, electron transfer reaction between EOH-Se-EOH with $\text{ABTS}^{\cdot+}$ radical were studied in the presence and absence of GNP and was monitored by spectrophotometry [12]. $\text{ABTS}^{\cdot+}$ has a strong absorption band from 400 to 800 nm and is a very long lived radical. It can be prepared by the reaction of ABTS^{2+} with an oxidant, like $\text{K}_2\text{S}_2\text{O}_8$. 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 $3.7 \times 10^{-6} \text{ s}^{-1}$. In presence of GNP, the decay constant increased to $1.1 \times 10^{-4} \text{ s}^{-1}$ and

in presence of EOH-Se-EOH the decay constant was $8 \times 10^{-5} \text{ s}^{-1}$. However, in the presence of GNP-EOH-Se-EOH conjugate, the decay showed a significant increase to $2.1 \times 10^{-3} \text{ s}^{-1}$. This clearly confirmed that GNP conjugation increased the electron transfer ability of the selenium compound. The increase in the electron transfer ability on conjugation to GNP has been attributed to 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 surface and consequent changes in the color of the nanoparticle suspension is given in Figure 1.

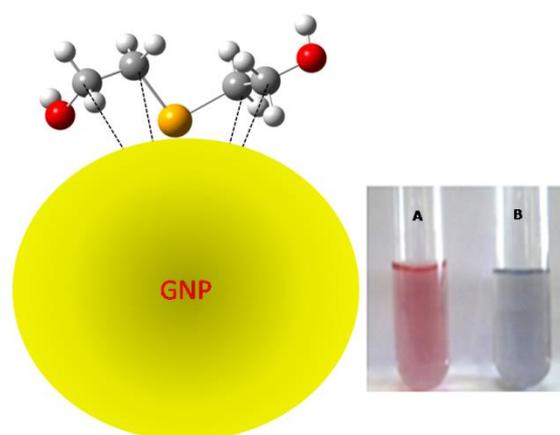


Figure 1. Schematic representation of GNP bound EOH-Se-EOH and photographs of the GNP suspension before (A) and after (B) conjugation with selenium compound.

3. MNP-Selenium Studies

In the second study, Fe_3O_4 magnetic nanoparticles (MNPs) coated with glycine were employed to conjugate selenium compounds. Glycine coating not only provides stabilization to MNPs but also leaves freely exposed amine groups, improving water dispersability. These MNPs (TEM $\sim 10 \text{ nm}$), were prepared by *co-precipitation method as reported earlier* [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 characterized by IR spectroscopy which showed the characteristic vibrations at 588 cm^{-1} (Fe–O), 1400 cm^{-1} ($\nu_{\text{sym}}\text{COO}^-$) and 1586 cm^{-1} ($\nu_{\text{asym}}\text{COO}^-$). The morphology of the particles were identified from TEM (roughly spherical particle of average size $\sim 10 \text{ nm}$), and the magnetisation studies indicate superparamagnetic behavior 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, $\text{PhSeCH}_2\text{CONHCH}_2\text{COOH}$ (SeG) and $\text{PhSeCH}_2\text{CONHCH}(\text{CH}_2\text{OH})\text{COOH}$ (SeS) which were synthesized as reported elsewhere [11]. The conjugation of the selenium compounds to the MNPs were carried by well-known bio-conjugation reaction EDC-NHS coupling between the carboxyl group of Se compound and amine functional group on MNP surface [13]. Figure 2 shows a schematic illustration of the conjugation of selenium containing N-acetyl alpha aminoacids on glycine passivated MNPs.

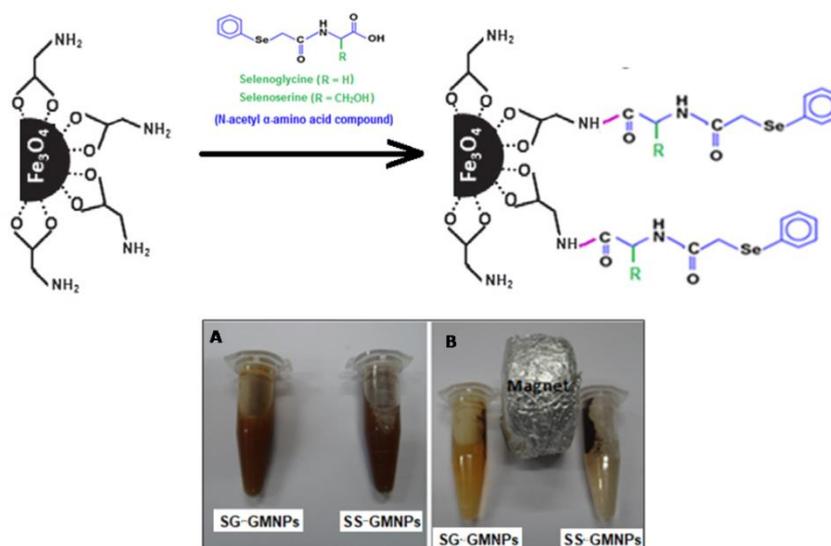


Figure 2. Schematic representation of conjugation of N-acetyl α-amino acid compounds on GMNPs. Photographs of aqueous suspension of SG-GMNPs and SS-GMNPs in the absence (A) and presence (B) of magnetic field (field strength of table top magnet: 0.25 kOe). The agglomeration of the particles in the presence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

Conjugation of SeG and SeS onto the surface of MNPs did not affect the crystal structure of Fe₃O₄ as assessed by XRD. From 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 was 104 and 115 nm and the zeta-potential of -20 and -25 mV at pH 7.4 for SeG-MNPs and SeS-MNPs, respectively. Further, both SeG-MNPs and SeS-MNPs showed good magnetic field responsiveness under external magnet.

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

4. Conclusions

In conclusion, we have demonstrated the ability of various inorganic nanocarriers for binding of organoselenium compounds and its impact on electron transfer behavior. In particular, the above studies indicate that selenium compounds can be easily conjugated to different types of nanoparticles such as GNPs or MNPs. Such binding with nanoparticles like GNP significantly improves their electron transfer ability. This can therefore provide novel way of improving their antioxidant activity. Similarly, organoselenium compounds can also be conjugated to Fe₃O₄ MNPs. Such conjugation although did not significantly increase their electron transfer ability, glycine conjugation gives water solubility and their magnetic nature provide targets specificity. Overall these studies provide a new avenues to modulate the biological properties of selenium compounds, using inorganic nanocarriers.

Author Contributions: “Conceptualization, K.I.P. and P.A.H.; methodology, B.G.S., P.P.P. and K.C.B.; data curation, B.G.S., P.P.P. and K.C.B.; writing—review and editing, K.I.P. and P.A.H.; supervision, K.I.P. All authors have read and agreed to the published version of the manuscript.”

Funding: This research received no external funding

Acknowledgments: The authors thank the Director, UM-DAE-CEBS, the Head, RPC Division BARC, and the Associate Director, Chemistry Group, BARC, for their support and encouragement. KIP thanks DAE for the Raja Ramanna Fellowship.

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

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