Reactive Oxygen Species Scavenging Behavior of Selenium Upon Vinyl Chloride Interaction With Hemoglobin

Negar Hajimohammadi Oushani

Islamic Azad University Science and Research Branch

Masoumeh Valipour

Azarbaijan Shahid Madani University Faculty of Basic Sciences

Parvaneh Maghami (✉ maghami@srbiau.ac.ir)

Islamic Azad University Science and Research Branch <https://orcid.org/0000-0003-3086-1146>

Research

Keywords: Chemiluminescence, Reactive oxygen species, Hemoglobin, Vinyl chloride

Posted Date: September 7th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-838697/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background

Vinyl chloride (VC) a colorless gas with a pleasant odor that is capable of entering the body through oral or inhalation routes. Extensive studies on this compound indicated that it is a carcinogen, and Vinyl chloride exposure can result in a specific type of cancer in VC workers. Whereas hemoglobin plays a vital role in oxygen transfer throughout the body, the effect of VC on human hemoglobin has not been studied in a molecular aspect. Furthermore, selenium as an antioxidant is a vital factor for the health of humans and animals. Therefore, the effect of the antioxidant capability of *selenium* on the interaction between vinyl chloride and hemoglobin was studied.

Methods

The effect of the antioxidant capability of *selenium* on the interaction between VC and hemoglobin was investigated by different spectroscopy methods such as UV-visible, Fourier-transform infrared, chemiluminescence, and fluorescence spectroscopies and molecular docking

Results

The results indicated the destruction of hemoglobin structure in the presence of different concentrations of vinyl chloride, but in the presence of selenium, the damaging effect of VC on hemoglobin structure was decreased, relying on its antioxidant capability results.

Conclusions

According to our findings, vinyl chloride destroyed hemoglobin structure utilizing ROS production, and the presence of selenium as an antioxidant inhibits the destroying effect of vinyl chloride on hemoglobin

Background

Vinyl chloride (VC) or Vinyl chloride monomer (VCM) is a colorless gas that is a highly applied chemical in the plastic industry which has been considered an environmental pollutant. [1, 2] Vinyl chloride is mainly employed in the plastic industry and polyvinyl chloride (PVC).[3, 4] Furthermore, vinyl chloride is present in cigarette smoke and some underground waters that have been subjected to biodegradation by bacterial processes. [5–8] Since there are numerous reports on the incidence of liver disease in people exposed to this compound, vinyl chloride is considered a human carcinogen.[1] Exposure to vinyl chloride causes angiosarcoma of the liver and hepatocellular carcinoma. [9] Despite that liver is among the first organs in the body damaged by chemical and toxic substances, the liver cells are capable of being repaired.[10] Vinyl chloride enters the body through inhalation or oral routes, which can cause errors in transcription and consequent deletion or change in genetic information. Moreover, according to the results of numerous studies, exposure to significant amounts of vinyl chloride results in DNA single-strand breaks, which generally assumed that it is not accessible to repair [10]. Since the last 60 years, the

study of human hemoglobin (Hb) has increased more than any other molecule [7]; it was used better to assess the effects of VCM on the human body. Hemoglobin (Hb) is one of the most essential proteins in the body that plays a vital role in transmitting oxygen throughout the body, which is present in human and animal red blood cells. Regarding the role of hemoglobin in the transfer of oxygen to various tissues and organs, its concentration is high in the human blood [12]. Hb is a globular protein with a diameter of 5.5 nm and is a tetramer protein with four heme prosthetic groups for each polypeptide chain. In adults, hemoglobin is composed of two α chains and two β chains with comparable three-dimensional structures[12, 13] The α helices comprise 75–80% of the hemoglobin polypeptide chains structure which are adjacent to the heme in a way that prevents heme exposure to the water molecules and therefore the formation of a suitable packet for heme attachment to the oxygen.[14, 15] Selenium is a required trace element that is involved in the complex system of defense against oxidative stress via selenium-dependent glutathione peroxidases (GPx) and plays a pivotal role in human fertility.^[16] Additionally, it has a beneficial effect on the toxicity caused by chemical factors such as heavy metals.[14, 15, 17, 18] Selenium (Se) is a useful mineral element for humans and animals whose deficiency results in various diseases such as reduced immune function. Because cell reproduction and death are related to cell cycle control and apoptosis, selenium is considered as a cell cycle and apoptosis regulating agent and consequently important in the prevention of cancer.[17] As mentioned above, a specific type of liver cancer occurs as a result of vinyl chloride exposure. However, according to the studies, many cancers can be prevented and controlled using appropriate antioxidants in the diet.[18] Based on a previous study, selenium plays a moderating role in the effect of the pollutant on the liver and prevention of hepatic necrosis, [19] the current study attempted to address the moderating effect of selenium on the destruction of hemoglobin structure in the presence of vinyl chloride.

Materials

Vinyl chloride monomer (VCM) (100 mg, 99.5% purity) was purchased from Sigma as a colorless liquid. Hemoglobin was isolated and purified from human blood in the laboratory of biochemistry and Biophysics University of Tehran. The protein was maintained in a sterile vial in a -50°C freezer for future uses. Dipotassium hydrogen phosphate (K_2HPO_4), hydrochloric acid (HCl), Luminol, and Hydrogen peroxide 30%, used in the chemiluminescence assay, were obtained from Merck (Germany).

Methods

Purification of human hemoglobin

Adult hemoglobin (HbA) was isolated from red blood cells. To separate plasma, the blood containing heparin was centrifuged. The red blood cells were washed three times using 0.9% saline followed by lysis of red blood cells through osmosis using double distilled water and destruction of cells membrane. The membrane components were separated employing 3000 rpm centrifugation. The hemoglobin solution was centrifuged two times at 10,000 rpm to remove the insoluble parts. The 20% ammonium sulfate was

added to the supernatant. Then it was incubated for 15 min followed by centrifugation at 20,000 rpm at 2°C for 2 h.^[20] Diphosphoglycerate was removed using Benesch et al. method.^[21] The obtained hemoglobin was dialyzed against 50 mM phosphate buffer (pH 7.4). Finally, the purified hemoglobin was maintained in the freezer to prevent destruction.^[22]

UV-visible spectroscopy

The T92 + UV Spectrophotometer (PG INSTRUMENTS, England) was employed in the current study. Phosphate buffer (0.5 M, pH 7.4), hemoglobin solution (1.3 mg/ml), and selenium (1 ppb) were prepared and used. The effect of various concentrations of vinyl chloride on the structure and absorption of hemoglobin was studied. The experiment at first was established in finding hemoglobin structure, then titrations of hemoglobin solution with different concentrations of vinyl chloride. The titrations were tested in the presence of selenium, and the effect of selenium on the interaction between hemoglobin and vinyl chloride was investigated in the range of 200–700 nm. The absorbance values were explained between 250 and 600 nm.

Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR)

ATR is used in infra-red spectroscopy to increase accuracy. ATR accessory in FTIR eliminates the need for producing a pellet for analysis. Thermo Nicolet Fourier Transform Infrared Spectrophotometer (Thermo Fisher Scientific, USA) was used for ATR-FTIR measurements. In this test, the desired amount of vinyl chloride was added to the hemoglobin solution in the presence or absence of selenium.

Fluorescence spectroscopy

Cary Eclipse Fluorescence Spectrophotometer (Varian, Australia) was used in fluorescence measurements. Briefly, the vinyl chloride solution with the desired concentration was added to the hemoglobin solution (1.3 mg/ml in 10 mM phosphate buffer pH 7.4) in the presence or absence of selenium (1ppb). The excitation wavelength was 280 nm, and the emission intensities were recorded between 300–500 nm.

Chemiluminescence assay

Chemiluminescence assay was applied to measure and calculate the level of reactive oxygen species (ROS). Synergy H4 Hybrid Multi-Mode Plate Reader (BioTek Instrument, USA) was employed in the present study. In this essay, the amount of produced ROS upon the interaction of hemoglobin with vinyl chloride in the presence or absence of selenium was measured. Briefly, luminol was solubilized in the phosphate buffer and the desired concentration of 0.0005 M was added to all the solutions in the plate. The amount of emission was recorded by the instrument. Finally, the level of reactive oxygen species was calculated according to the H₂O₂ standard curve.

Results

UV-visible analysis

Figure 1 shows UV-Visible spectra (250 to 350 nm) of hemoglobin before and after vinyl chloride addition to the protein solution. As the data shows, the absorbance of Hb samples was gradually increased by vinyl chloride addition. Figure 2 represents the changes of Soret region absorbance in the absence and presence of different vinyl chloride concentrations. The absorbance of Soret band (around 412 nm) was deduced after vinyl chloride titration to Hb samples. The absorbance of the Q band is observed in Fig. 3. The intensity of the protein samples was increased as a result of vinyl chloride addition.

The effect of Se on Hb conformation upon interaction with vinyl chloride is indicated in Figure 4. The experiment was done applying the concentration of 1 ppb Se and 200 μ l vinyl chloride. The corresponding graph shows the protective role of Se on Hb conformational changes induced by vinyl chloride.

ATR-FTIR analysis

Based on the results of the ATR-FTIR test demonstrated in Fig. 5, no displacement was seen in the spectra in the regions of helices and β -sheets after Se and vinyl chloride addition. Higher intensity of spectra was seen for Se, and vinyl chloride treated samples.

Fluorescence spectroscopy analysis

Figure 6 shows tryptophan fluorescence intensity excited at 280 nm, and emission spectra were gathered from 300 to 400 nm (A). The maximum emission intensity was recorded at 340 nm and plotted in Fig. 6B. Data did not present a detectable fluorescence intensity change in the presence of selenium, while adding 1.4 μ M vinyl chloride in the absence of Se caused the increased intensity of tryptophan emission. The following two graphs present the effects of vinyl chloride and Se.

ROS measurements

Chemiluminescence assay was carried out to measure the level of reactive oxygen species. According to the results presented in Fig. 7, the induced changes in the amount of reactive oxygen species are evident after the addition of Se to the hemoglobin solution in the presence of vinyl chloride.

Molecular docking

According to our results derived from the docking studies, the ligand (vinyl chloride) binds to the protein through its hydrophobic region. Hb and vinyl chloride complex were bound to each other by -2.4 kcal/mol binding energy. The schematic illustration of the Hb- vinyl chloride complex is presented in Fig. 8. Interaction sites between Hb and ligand are shown in Fig. 9A. Furthermore, hydrophobic interactions and the amino acids involved in Hb- vinyl chloride interaction have been presented in Fig. 9B.

Discussion

Vinyl chloride is an important thermoplastic substance and is produced in millions of tons every year. [23, 24] This compound with a pleasant odor that is applied in huge amounts is very toxic, flammable, and carcinogenic, such that it was ranked fourth by the Agency for Toxic Substances and Disease Registry in 2015 as a threatening agent for human health.[25] Exposure to vinyl chloride can lead to cardiovascular, liver, and immune diseases. Therefore, vinyl chloride has been considered the first group of carcinogenic agents for humans by the International Agency for Research on Cancer. [24–28] In this study, the effect of vinyl chloride on Hb is examined. Hb is a specific protein in electron transfer. Adult human hemoglobin is composed of four polypeptide chains of two α subunits and two β subunits; each has an iron atom in the center. The capability of hemoglobin to bind oxygen is dependent on the heme prosthetic group. Besides, this group is responsible for the red color of blood. [29–31] Therefore, preserving the hemoglobin structure is necessary to transfer oxygen to the organs and cells in the body. Concerning UV-Vis experiments shown in Fig. 1, it is clear that the absorbance of the protein samples was increased at 280 nm after vinyl chloride addition. The absorbance at 280 nm is due to the presence of aromatic amino acids (Trp, Tyr, and Phe). These amino acids, especially Trp residue of Hb are the main reporters of the protein environment. So the increase of spectra absorbance at 280 nm stands for Hb environment alterations. To evaluate whether the heme prosthetic group of Hb is affected by vinyl chloride, the absorbance was detected at 412 nm. Figure 2 indicates the gradual reduction of solet band (412 nm) by vinyl chloride titration to the Hb samples. The decreased absorption in the peak at around 412 nm demonstrated the loss of interaction between heme group and globin.[32, 33] Based on our observation in Fig. 2, it is noticeable that Hb tertiary structure has been changed upon interaction with vinyl chloride. Figure 3 represents the hyperchromicity effect of vinyl chloride in the region 500 to 600 nm or Q bands of Hb, which approve the binding of oxygen to the heme group. The increased absorbance of Q bands in the presence of vinyl chloride is related to the alterations of oxy-hemoglobin form upon interaction with vinyl chloride.[34] To reduce the destructive effects of vinyl chloride on Hb, Se as an antioxidant was selected to be studied. Se has been shown to act as general preventive medicine in oncology.[35] It is incorporated in antioxidant enzyme as selenocysteine in the structure of glutathione oxidase and regulates glutathione activity in times of oxidative stress and increased free radicals and causes detoxification.[36–39] Se is also able to reduce oxidative stress induced by cadmium in plants.[40] This study aims at finding out the effect of Se on Hb titrated by vinyl chloride from a molecular point of view in vitro. It was revealed that Se alone did not induce changes in the hemoglobin structure. Besides, the inhibitory effect of Se on vinyl chloride induced hemoglobin structural destruction was obvious (Fig. 4). Moreover, secondary structure changes of hemoglobin were studied using ATR-FTIR, the two regions of FTIR spectra, namely amid I and II bands, are suitable to detect protein secondary structure. Amide I represent for C = O bond stretch around 1650 cm^{-1} , and amide II stands for N-H bending vibrations (around 1550 cm^{-1}) under the effect of neighboring CO, CC, and NC groups. These bonds take part in protein secondary structure by the ability to form hydrogen bonds. Their vibration pattern demonstrated as amid I and amide II bands give us insight into protein secondary structure. [41, 42] ATR-FTIR data shown in Fig. 5 indicates no changes in Hb secondary structure content because wavelength shift was not seen in the presence of vinyl chloride and Se. Results monitored by fluorescence spectroscopy (Fig. 6) showed the increased intensity of Hb in the presence of vinyl chloride. It means that Trp emission wavelength is affected by the polarity of the

micro-environment. So the interaction between vinyl chloride and Trp residue induces changes in Hb conformation. This result is in the line of soret band absorbance measurement in Fig. 2. It can be concluded from Fig. 2 and Fig. 6 that vinyl chloride was able to alter Hb conformation. However, the addition of Se to the protein solution showed a protective effect on Hb conformational change induced by vinyl chloride (Fig. 4 and Fig. 6A). Assessed by chemiluminescence experiments, adverse effects of vinyl chloride on Hb were related to ROS generation. Oxidative stress conditions may damage macromolecules such as proteins via protein oxidation. This will cause protein conformational diseases like Alzheimer's, Parkinson and etc.[43–45] Furthermore, Fig. 7 showed that the presence of Se vastly reduced the ROS production of Hb- vinyl chloride solution. So that the antioxidant effect of Se could protect the integrity of Hb in the presence of vinyl chloride. Finally, molecular docking examination was done to determine the type of interaction involved in the Hb- vinyl chloride complex (Fig. 8). Results in Fig. 9A and B show that Hb and vinyl chloride bind via hydrophobic interactions. Val, Ala, Leu, Phe, and Gly are the main residues that take part in the mentioned hydrophobic interaction.

Conclusion

In this work, the investigation of the antioxidant activity of Se upon the interaction between human hemoglobin and vinyl chloride was elucidated. According to our findings, vinyl chloride destroyed hemoglobin structure utilizing ROS production, and the presence of selenium as an antioxidant inhibits the destroying effect of vinyl chloride on hemoglobin. As mentioned in the previous section, hemoglobin is one of the essential proteins in the human body, transferring oxygen throughout the body. Since oxygen-binding depends on the heme prosthetic group, any change in heme structure led to lower oxygen binding, which means a lower oxygenation rate of the various tissues and organs. Furthermore, hemoglobin structure change increases the possibility of blood disorders like anemia. Thus, Selenium is a suitable ROS scavenger in the case of the Hb-vinyl chloride study. However, more studies are needed to discover the generality of Se deleterious effect in molecular level studies.

Abbreviations

VC: vinyl chloride

Declarations

Ethical Approval and Consent to participate

This study involved a search and review of publicly available online information only; it did not require ethics approval or consent to participate.

Consent for publication

All authors agree to publish this manuscript

Availability of data and materials

All relevant data included in this manuscript

Competing interests

The authors declare that they have no competing interests.

Funding

This study was unfunded.

Acknowledgments

This paper and the research behind it would not have been possible without the exceptional support of my colleges at Zakariaye Razi Laboratory Complex, Science and Research Branch, Islamic Azad University

Authors' information:

Affiliations:

Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran

Parvaneh Maghami ,Negar Hajimohammadi Oushani

Department of Biology, Faculty of Science, Azarbaijan ShahidMadani University, Tabriz, Iran

Masoumeh Valipour

Authors' contributions

Maghami: conceptualized and designed the study, collected the data, interpreted and analyzed the data, provided contributions and critically revised the manuscript, and gave final approval of the version to be published

Hajimohammadi Oushani: collected the data, interpreted and analyzed the data, drafted the manuscript and provided contributions and critically revised the manuscript, and gave final approval of the version to be published

Valipour: interpreted and analyzed the data, critically revised the manuscript, and gave final approval of the version to be published

Corresponding author:

Correspondence to Parvaneh Maghami

References

- [1] Anders LC, Lang AL, Anwar-Mohamed A, Douglas AN, Bushau AM, Falkner KC, Hill BG, Warner NL, ArteelGE, Cave M, McClain CJ. Vinyl chloride metabolites potentiate inflammatory liver injury caused by LPS in mice. *Toxicol Sci.* 151: 312-323(2016)
- [2] ReitzeJr AW, Lowell R. Control of hazardous air pollution. *BC Env'tl Aff L Rev.* 28:229 (2000)
- [3] Dogliotti E. Molecular mechanisms of carcinogenesis by vinyl chloride. *ANNALI-ISTITUTO SUPERIORE DI SANITA.* 42:163(2006)
- [4] OEHHA CA. Chloropicrin Acute Toxicity Summary (1999)
- [5] IARC Working Group on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans. IARC. (1979)
- [6] Kielhorn J, Melber C, Wahnschaffe U, Aitio A, Mangelsdorf I. Vinyl chloride: still a cause for concern. *Environ Health Perspect.* 108:579-588(2000)
- [7] Swenberg JA, Lu K, Moeller BC, Gao L, Upton PB, Nakamura J, Starr TB. Endogenous versus exogenous DNA adducts: their role in carcinogenesis, epidemiology, and risk assessment. *Toxicol Sci.* 120(suppl_1) S130-145(2011)
- [8] Anders LC, Yeo H, Kaelin BR, Lang AL, Bushau AM, Douglas AN, Cave M, Arteel GE, McClain CJ, Beier JI. Role of dietary fatty acids in liver injury caused by vinyl chloride metabolites in mice. *ToxicolApplPharmacol* 311:34-41(2016)
- [9] Wong RH, Chen PC, Du CL, Wang JD, Cheng TJ. An increased standardised mortality ratio for liver cancer among polyvinyl chloride workers in Taiwan. *Occup Environ Med*; 59:405-409(2002)
- [10] Lang AL. Vinyl chloride enhances diet-induced liver injury via metabolic dyshomeostasis: critical role of mitochondria (2018).
- [11] Clewell HJ, Gentry PR, Gearhart JM, Allen BC, Andersen ME. Comparison of cancer risk estimates for vinyl chloride using animal and human data with a PBPK model. *Sci Total Environ.* 274:37-66(2001)
- [12] Nagai M, Mizusawa N, Kitagawa T, Nagatomo S. A role of heme side-chains of human hemoglobin in its function revealed by circular dichroism and resonance Raman spectroscopy. *Biophys Rev.* 10:271-84(2018)
- [13] Perutz MF. Myoglobin and haemoglobin: role of distal residues in reactions with haem ligands. *Trends Biochem Sci.* 14:42-44 (1989)
- [14] Vuletich DA, Falzone CJ, Lecomte JT. Structural and dynamic repercussions of heme binding and heme-protein cross-linking in *Synechococcus* sp. pcc 7002 hemoglobin. *Biochemistry.* 45:14075-

14084(2006)

- [15] Liong EC, Dou Y, Scott EE, Olson JS, Phillips Jr GN. Waterproofing the heme pocket: role of proximal amino acid side chains in preventing heme loss from myoglobin. *J Biol Chem.* 276:9093-9100(2001)
- [16] Pieczyńska J, Grajeta H. The role of selenium in human conception and pregnancy. *J Trace Elem Med Biol.* 29:31-38(2015)
- [17] Zeng H. Selenium as an essential micronutrient: roles in cell cycle and apoptosis. *Molecules.* 14:1263-1278(2009)
- [18] Saha SK, Lee SB, Won J, Choi HY, Kim K, Yang GM, Dayem AA, Cho SG. Correlation between oxidative stress, nutrition, and cancer initiation. *Int J Mol Sci.* 18:1544(2017)
- [19] Schwarz K, Foltz CM. Selenium as an integral part of factor 3 against dietary necrotic liver degeneration. *Nutr Rev.* 36:338-340(1978)
- [20] Hink WF, Briggs JD. Bactericidal factors in haemolymph from normal and immune wax moth larvae, *Galleria mellonella*. *J Insect Physiol.* 14:1025-1034 (1968)
- [21] Benesch RE, Benesch R, Yu CI. Oxygenation of hemoglobin in the presence of 2, 3-diphosphoglycerate. Effect of temperature, pH, ionic strength, and hemoglobin concentration. *Biochemistry.* 8:2567-2571 (1969)
- [22] Antonini E, Brunori M. Hemoglobin. *Annu Rev Biochem.* 39:977-1042.(1970)
- [23] Hao JN, Xu XY, Lian X, Zhang C, Yan B. A luminescent 3d-4f-4d MOF nanoprobe as a diagnosis platform for human occupational exposure to vinyl chloride carcinogen. *Inorg Chem.* 56:11176-11183.(2017)
- [24] GAVRILĂ DE, STOIAN V. Studies on thermal and radioactive degradation of unplasticized polyvinyl chloride. *Studies.* 1:7-24.(2016)
- [25] Beer J. Agency for Toxic Substances and Disease Registry (2013)
- [26] Kumar AK, Balachandar V, Arun M, Ahamed SA, Kumar SS, Balamuralikrishnan B, Sankar K, Sasikala K. A comprehensive analysis of plausible genotoxic covariates among workers of a polyvinyl chloride plant exposed to vinyl chloride monomer. *Arch Environ Contam Toxicol.* 64:652-658 (2013)
- [27] Ward E, Boffetta P, Andersen A, Colin D, Comba P, Deddens JA, De Santis M, Engholm G, Hagmar L, Langard S, Lundberg I. Update of the follow-up of mortality and cancer incidence among European workers employed in the vinyl chloride industry. *Epidemiology.* 1:710-718.(2013)
- [28] Huang PC, Liu LH, Shie RH, Tsai CH, Liang WY, Wang CW, Tsai CH, Chiang HC, Chan CC. Assessment of urinary thiodiglycolic acid exposure in school-aged children in the vicinity of a petrochemical complex

in central Taiwan. *Environ Res.* 150:566-572 (2016)

[29] Emadi M, Maghami P, Khorsandi K, Hosseinzadeh R. Biophysical study on the interaction of cartap hydrochloride and hemoglobin: heme degradation and functional changes of protein. *J Biochem Mol Toxicol.* 33: e22325(2019)

[30] Chakraborty M, Paul S, Mitra I, Bardhan M, Bose M, Saha A, Ganguly T. To reveal the nature of interactions of human hemoglobin with gold nanoparticles having two different morphologies (sphere and star-shaped) by using various spectroscopic techniques. *J Photochem Photobiol B, Biol* 178:355-366(2018)

[31] Minai-Tehrani D, Toofani S, Yazdi F, Minai-Tehrani A, Mollasalehi H, Ziabari KB. Inhibition of pseudoperoxidase activity of human red blood cell hemoglobin by methocarbamol. *Int J Biol Macromol.* 94:788-792(2017)

[32] Valipour M, Maghami P, Habibi-Rezaei M, Sadeghpour M, Khademian MA, Mosavi K, Ahmad F, Moosavi-Movahedi AA. Counteraction of the deleterious effects of reactive oxygen species on hemoglobin structure and function by ellagic acid. *J Lumin.* 182:1-7(2017)

[33] Ariaeenejad S, A Moosavi-Movahedi A, Kavousi K, Reza Dayer M, Hong J, Yousefi R, Sheibani N, Habibi-Rezaei M. The species and heme pocket properties of Sturgeon Hemoglobins upon interaction with N-dodecyl trimethylammonium bromide. *Protein Pept Lett.* 21:171-178(2014)

[34] Hosseinzadeh R, Moosavi-Movahedi AA. Human hemoglobin structural and functional alterations and heme degradation upon interaction with benzene: a spectroscopic study. *Spectrochim Acta A Mol Biomol Spectrosc.* 157:41-49(2016)

[35] Cary L, Naveau A, Migeot V, Rabouan S, Charlet L, Foray N, Séby F, Bueno M, Jury C, Parmentier M, Louart J. From water-rock interactions to the DNA: a review of selenium issues. *Procedia Earth Planet Sci* 17:698-701(2017)

[36] El-Demerdash FM, Nasr HM. Antioxidant effect of selenium on lipid peroxidation, hyperlipidemia and biochemical parameters in rats exposed to diazinon. *J Trace Elem Med Biol* 28:89-93(2014)

[37] Safhi MM, Alam MF, Khuwaja G, Ashafaq M, Khan A, Islam F, Anwer T, Khan G, Sivakumar SM, Islam F. Selenium in combination with tellurium protects the toxicity of tellurium in the liver mitochondria of rats. *Bull Environ Pharmacol Life Sci.* 7:90-95(2018)

[38] Wrobel JK, Power R, Toborek M. Biological activity of selenium: revisited. *IUBMB life.* 68:97-105(2016)

[39] Hatfield DL, Berry MJ, Gladyshev VN, editors. *Selenium: its molecular biology and role in human health.* Springer Science & Business Media (2011)

- [40] Zimmerman MT, Bayse CA, Ramoutar RR, Brumaghim JL. Sulfur and selenium antioxidants: Challenging radical scavenging mechanisms and developing structure–activity relationships based on metal binding. *J Inorg Biochem.*145:30-40(2015)
- [41] Barth A. Infrared spectroscopy of proteins. *Biophys, Biochem Acta.* 1767:1073-1101(2007)
- [42] CALAbRò EM, MAgAzù S. Unfolding-induced in haemoglobin by exposure to electromagnetic fields: a FTIR spectroscopy study. *Orient J Chem.*30:31-35(2014)
- [43] Kim GH, Kim JE, Rhie SJ, Yoon S. The role of oxidative stress in neurodegenerative diseases. *Exp Neurobiol.* 24:325-340(2015)
- [44] Lévy E, El Banna N, Baille D, Heneman-Masurel A, Truchet S, Rezaei H, Huang ME, Béringue V, Martin D, Vernis L. Causative links between protein aggregation and oxidative stress: a review. *Int J Mol Sci.*20:3896(2019)
- [45] Uttara B, Singh AV, Zamboni P, Mahajan RT. Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. *Curr Neuropharmacol.* 7:65-74(2009)

Figures

Figure 1

UV- Vis spectra of 1.3 mg/ml Hb dissolved in phosphate buffer pH 7.3 at 25°C in the absence and presence of different vinyl chloride monomer (VCM) concentrations. The absorbance of the samples was scanned from 250 to 350 nm.

Figure 2

Soret band absorbance of Hb (1.3 mg/ml) before and after vinyl chloride monomer (VCM) addition. The samples were prepared in phosphate buffer pH 7.3 at 25°C. The absorbance of the samples was scanned from 350 to 500 nm.

Figure 3

Q band absorbance of Hb (1.3 mg/ml) before and after vinyl chloride monomer (VCM) addition. The samples were prepared in phosphate buffer pH 7.3 at 25°C. The absorbance of the samples was measured from 500 to 600 nm.

Figure 4

UV- Vis spectra of 1.3 mg/ml Hb samples including Hb alone, Hb + 0.4 μM Se, Hb + 1.4 μM vinyl chloride monomer (VCM) and Hb + 0.4 μM Se + 1.4 μM vinyl chloride monomer (VCM). All samples were dissolved in phosphate buffer pH 7.3 at 25°C The absorbance of the samples was scanned from 200 to 700 nm. Se represents selenium.

Figure 5

ATR-FTIR spectra of Hb before and after Se (selenium) and vinyl chloride monomer (VCM) addition. The spectra were gathered for samples prepared in phosphate buffer pH 7.3 at 25°C. 1.3 mg/ml Hb was dissolved in phosphate buffer. Se and vinyl chloride monomer (VCM) amount was 0.4 μM and 1.4 μM, respectively.

Figure 6

A: The change in Hb intrinsic fluorescence intensity, excited at 280 nm in the absence and presence of Se, vinyl chloride monomer (VCM), and Se- vinyl chloride monomer (Se +VCM). The emission spectra were collected between 300 to 400 nm. B: Hb tryptophan emission at 340 nm before and after Se, vinyl chloride monomer (VCM), and Se- vinyl chloride monomer (Se + VCM) addition. The samples were prepared in phosphate buffer at room temperature.

Figure 7

The changes in the level of ROS are based on the H₂O₂ standard curve, in the absence and presence of vinyl chloride monomer (VCM) and Se. All samples were prepared in phosphate buffer pH 7.3 and 25°C.

Figure 8

Schematic illustration of Hb and vinyl chloride complex. Secondary structure and CPK model of Hb and ligand.

A **B**

Figure 9

(A) Interaction sites between Hb and ligand. (B) Hydrophobic interactions and the amino acids involved in Hb- vinyl chloride interaction.