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The Effect of Gold Nanoparticles and β-Carotene on the States of Hemoglobin Present in Erythrocytes

J. Fiedor[a,*], A. Siniarski[b,c], J. Kiecana[c], G. Gajos[b,c], J. Korecki[d] and K. Burda[a]

[a] *AGH University of Krakow, Faculty of Physics and Applied Computer Science, Reymonta 19, 30-059 Kraków, Poland*
[b] *Jagiellonian University, Medical College, Department of Coronary Artery Disease and Heart Failure, Faculty of Medicine, Prądnicka 80, 31-202 Kraków, Poland*
[c] *The John Paul II Hospital, Interventional Cardiology Clinic, Prądnicka 80, 31-202 Kraków, Poland*
[d] *Jerzy Haber Institute of Catalysis and Surface Chemistry, Polish Academy of Sciences, 30-239 Kraków, Poland*

Doi: 10.12693/APhysPolA.150.S69 *e-mail: fiedor@agh.edu.pl

Gold nanoparticles (AuNPs), because of their unique properties, are gaining increasing interest in various fields of science. Significant attention is paid to their potential biomedical applications. They exhibit advantageous properties — such as being inert, non-toxic, and biocompatible — which make them, among other things, very promising drug carriers and agents for photothermal therapy, diagnostics, and bioimaging. Carotenoids constitute a large group of natural antioxidants that are delivered to the human body through food. They can accumulate in the blood, particularly in the plasma and in the hydrophobic regions of the erythrocyte membranes. The widely studied model carotenoid, β-carotene, was already demonstrated to affect the structural and functional properties of erythrocytes. Here, the preliminary results of the studies on the effect of AuNPs on hemoglobin present in isolated red blood cells are reported. Data were obtained using Mössbauer spectroscopy applied to monitor the state of hemoglobin subjected to AuNPs treatment with or without β-carotene. The results demonstrate that, at the concentration used, AuNPs lead to significant changes in the hemoglobin state, causing a prominent increase in the methemoglobin form. The addition of β-carotene reduced the effect of AuNPs on hemoglobin.

topics: β-carotene, gold nanoparticles, heme iron, Mössbauer spectroscopy

1. Introduction

Gold nanoparticles (AuNPs) exhibit properties that make them suitable for a range of medical applications such as drug delivery, photomedicine, tissue engineering, biosensing, antimicrobial agents, or cellular probes [1]. They can differ in size and shape, and their physicochemical properties can easily be tuned to achieve certain biochemical behavior [2–4]. The widespread use of AuNPs raises the question of their impact on cell components. Here, we present preliminary results of the Mössbauer study on the effect of AuNPs on hemoglobin (Hb) present in red blood cells (RBCs). The applied spectroscopic technique allowed for a precise investigation of changes in forms of Hb, along with a description of the molecular parameters of heme iron (HFe) present in resolved Hb states. The experiments were also carried out in the presence of β-carotene (β-Crt), an important dietary antioxidant that is already known to act as a modulator of the structural and functional properties of RBCs [5].

2. Materials and methods

All procedures involving human blood were carried out according to the recommendations of the World Medical Association and approved by the local Bioethical Commission of Physicians (317/KBL/ OIL/2019).

2.1. Sample preparation

A fasting blood sample was obtained from a healthy non-smoking male donor at the John Paul II Hospital in Krakow, Poland. Blood was collected by vein puncture into a tube containing sodium heparin as an anticoagulant. RBCs were isolated by centrifugation and purified by triplicate suspension in a phosphate buffer (5 mmol/l, 0.15 mol/l NaCl, pH 7.4), following the procedure described in [5]. The final concentration of erythrocytes was set to be 1.65×10^9 cells/ml. The prepared solution was divided into three portions: (i) untreated RBCs that served as a control sample; (ii) RBCs incubated in the presence of 25 μl AuNPs (gold colloidal particles suspended in distilled water, 4000 ppm, GoldenGevity); and (iii) RBCs incubated in the presence of 25 μl AuNPs and 50 μmol/l β-Crt (95% all-*trans*, Sigma, suspended in ethanol). The incubation protocol described in [6] was applied to all samples. Briefly, samples were gently mixed for 10 min at room temperature in the dark, washed three times with phosphate buffer (pH 7.4), concentrated to a volume of $\simeq 1.5$ ml, frozen with liquid nitrogen, and stored at -80°C until further measurement.

2.2. Mössbauer spectroscopy

To explore the effect of AuNPs and β-Crt on the Hb present in RBCs, Mössbauer spectroscopy was applied. Measurements were carried out at 85 ± 0.1 K in a home-made cryostat that allows gas exchange. As the source of the 14.4 keV γ-radiation, the 50 mCi ^{57}Co(Rh) was used [6]. Spectra were recorded after 24 h of measurement and analyzed using Recoil software [7].

3. Results and discussion

Mössbauer spectroscopy is a powerful technique widely used in biomedical research. Due to the presence of the ^{57}Fe isotope in heme, this technique is efficiently used for the characterization of heme and, therefore, the states of Hb [8, 9]. In the course of the present study, Mössbauer spectroscopy was used to evaluate the effect of AuNPs on changes in the Hb states of Hb embedded in RBCs. Furthermore, the combined effect of AuNPs and dietary antioxidant (β-Crt) on Hb states was investigated. Measurements were carried out according to a well-established protocol [6]. Briefly, the samples were subjected to a high partial pressure of nitrogen, frozen with liquid nitrogen, and placed in a cryostat enabling gas exchange. The resulting Mössbauer spectra are presented in Fig. 1.

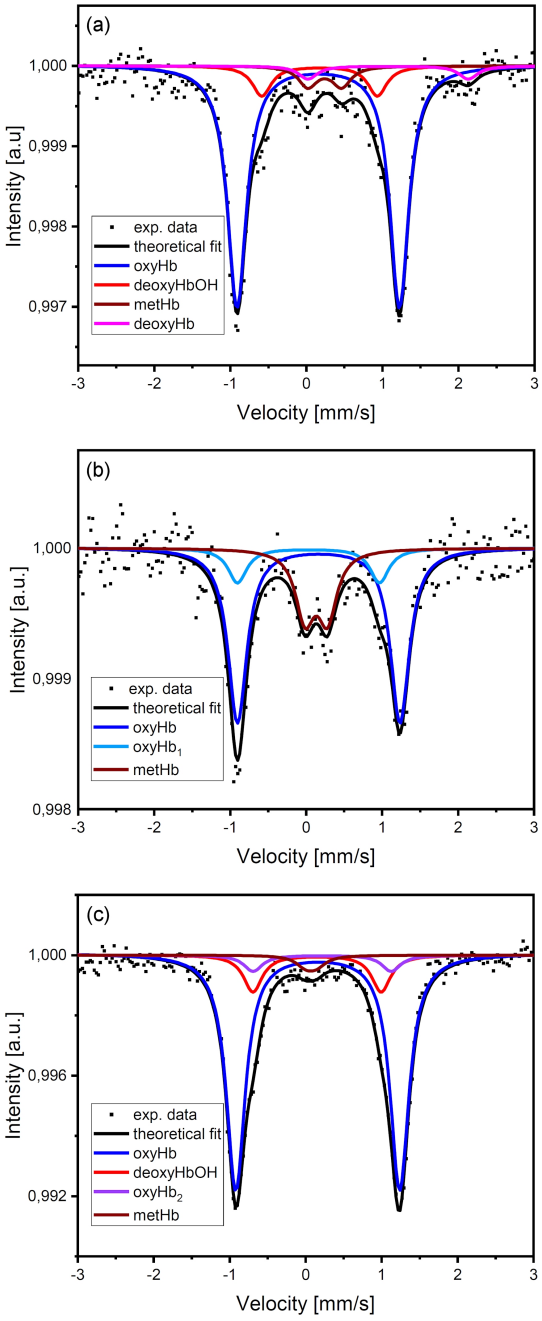

Fig. 1. Mössbauer spectra of RBCs: (a) untreated (control sample), (b) incubated in the presence of AuNPs, and (c) incubated in the presence of AuNPs and β-carotene. The experimental data (exp data) are indicated by points and theoretical curves by lines. The spectra were collected after 24 h of measurement.

The spectra shown in Fig. 1 have been fitted using four components (panels a and c) or three components (panel b). In all cases, oxyhemoglobin (oxyHb) represents the largest fraction of the spectra. It is characterized by a low-spin Fe^{2+} (see the hyperfine parameters given in Table I) [10]. Physiological deoxyhemoglobin (deoxyHb) with

TABLE I

Hyperfine interaction parameters of RBCs subjected to AuNPs and β-Crt treatment. Labels: A — contribution; IS — isomer shift; QS — quadrupole splitting.

Hb form	IS [mm/s]	QS [mm/s]	A [%]
control			
oxyHb	0.16 ± 0.01	2.13 ± 0.01	79.1
deoxyHb	1.07 ± 0.10	2.11 ± 0.10	4.3
deoxyHbOH	0.17 ± 0.04	1.52 ± 0.08	9.9
metHb	0.24 ± 0.05	0.44 ± 0.06	6.7
+ AuNPs			
oxyHb	0.17 ± 0.02	2.14 ± 0.01	63.3
Hb_1	0.03 ± 0.05	1.87 ± 0.07	12.5
metHb	0.13 ± 0.02	0.29 ± 0.04	24.2
+ AuNPs +β-Crt			
oxyHb	0.16 ± 0.01	2.16 ± 0.01	78.9
deoxyHbOH	0.15 ± 0.02	1.69 ± 0.05	12.4
Hb_2	0.22 ± 0.03	1.85 ± 0.04	5.5
metHb	0.07 ± 0.04	0.14 ± 0.04	3.2

a high-spin Fe^{2+} (IS of about 1 mm/s) [10] was detected exclusively in the control sample. Its contribution is about 4%. The presence of Fe^{3+}-containing metHb is observed in all samples, with a decrease in isomer shift (IS) and quadrupole splitting (QS) values in the following order: control > RBCs + AuNPs > RBCs + AuNPs + β-Crt (see Table I).

The proportion of metHb in the spectra is approximately four times higher in the sample treated with AuNPs alone, while in the case of RBCs + AuNPs + β-Crt, this proportion is half that of the control sample. The presence of a non-physiological deoxyhemoglobin (deoxyHbOH) with similar hyperfine parameters is visible in the spectra of the control sample and of RBCs incubated in the presence of AuNPs and β-Crt (see Table I) [10]. The content of deoxyHbOH in the spectra is also similar — it is $\simeq$ 10%. In the case of samples treated exclusively with AuNPs and AuNPs+β-Crt, additional components appeared. These components are Hb_1 and Hb_2, respectively. They were not observed in the control sample. Hb_1 and Hb_2 are also forms of deoxyHbOH [5]. They are characterized by a different ligand arrangement. They also differ in the strength of the ligand-HFe ion bond. This affects changes in its spin and/or valence states. An increase in bond asymmetry causes the QS value to rise to $\simeq$ 1.85 mm/s, whilst a change in the electron density within the HFe nucleus leads to a decrease (Hb_1) or an increase (Hb_2) in the isomeric shift. Additionally, it should be noted that the proportion of Hb_2 in the spectrum of RBCs treated with AuNPs in the presence of β-Crt is half that of Hb_1 in RBCs treated with AuNPs alone.

4. Conclusions

Data obtained using Mössbauer spectroscopy suggest that gold nanoparticles (AuNPs) can significantly impact the properties and functionality of red blood cells. The oxyHb content decreased by approximately 16% even at the low concentrations of AuNPs used, reaching a level of only about 63%. This was due to an almost four-fold increase in metHb, which is physiologically excluded from oxygen transport. Such alterations in the body could result in a state of hypoxia. The β-Crt impact on Hb bound in RBCs and its oxygen-binding properties has already been shown [5, 10]. It is very interesting, however, to observe that the simultaneous presence of AuNPs and even small amounts of β-Crt (50 μmol/l) can restore Hb's ability to bind oxygen (in this case, the oxyHb content increased to the level observed in the control, i.e., to about 79%). To fully understand this issue, further studies are needed.

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