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Mössbauer studies of hemoglobin in erythrocytes exposed to neutron radiation

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Abstract We studied radiation effects on the stability of various states of hemoglobin (Hb) in red blood cells (RBC) irradiated with a very low dose of neutron rays, 50 μGy. We investigated RBCs isolated from blood of healthy donors. Mössbauer spectroscopy was applied to monitor different forms of Hb. Our results show, for the first time, that oxyhemoglobin (OxyHb) and deoxyhemoglobin (DeoxyHb) are two Hb forms sensitive to such a low neutron radiation. Both Hbs change into a new Hb form (Hb_{irr}). Additionally, OxyHb transfers into $HbOH/H_2O$, which under our experimental conditions is resistant to the action of neutron rays.

Keywords Read blood cell · Neutrons · Mössbauer spectroscopy

1 Introduction

All living organisms are continuously exposed to an ionizing radiation. The average dose rate of the background radiation (radioelements in the soil, rocks, cosmic radiation or medical procedures) is around 2.5 mSv per year, i.e. 5 nSv/min at the

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sea level. The primary effect of ionizing radiation is the production of free radicals and reactive oxygen species (ROS). All molecules in living organisms are potential targets.

Within the frame of this work we studied the influence of neutron radiation. The applied dose of 50 μGy is several orders of magnitude lower than those used in radio-immunotherapy (∼keV) or in prevention of posttransfusion-associated graft-versus-host disease (25–50 Gy) [1]. It is even three orders of magnitude lower than doses at which hormesis phenomena were reported (5–200 mGy). Moreover, the rate dose of the radiation (1.1 mGy/min) is only about 200 times higher than that one estimated for the background radiation. It is known, that neutrons can produce observable damage at lower absorbed doses than β- , γ- or X-radiation because they show higher relative biological effectiveness (RBE) which depends on the energy transfer (LET) being a function of the radiation type, energy and the properties of the target [2–4]. It was observed that μGy doses of ionizing radiation, independently of its type, may cause serious oxidative stress in living organisms. They may damage lipids and proteins, especially enriched in -SH groups [5, 6]. Therefore we wanted to check if there is a detectable influence of ionizing irradiation at an exposure time of a few minutes and at a very low dose rate of ionizing radiation on the hemoglobin (Hb) states in red blood cells (RBCs). We have chosen RBCs because they can serve as a good model system for studying the influence of ionizing radiation on the biomembranes of mammalian cells. Their structure is well known and mature RBCs have no nucleus or any other cellular organelles. We applied Mössbauer spectroscopy to follow different Hb states in untreated and irradiated RBCs. This method was shown to be a sensitive tool in the investigations of the valence and spin states of iron as well as properties of its binding sites [7–9].

2 Materials and methods

Erythrocytes were isolated from blood via centrifugation in phosphate buffer (pH 7.4) using a standard method [10]. The washed RBCs were suspended in the phosphate buffer at the cell concentration of $8{\cdot}10^{10}$ red cells / ml in a volume of about 1.5 ml. These samples were kept in a frozen state at −80°C before the measurement.

^{239}Pu-Be was our source of neutron radiation with two energetic maxima at 877 keV and 2.9 MeV. An average energy of the emitted neutrons was 4 ± 2MeV. Energy of the accompanying γ-rays was about 59 keV. The activity of this neutron source at the position of the sample was $1{,}36{\cdot}10^4$ Bq, what was equivalent to about 1.1 μGy/min or about 2 μSv/min [2].

In the Mössbauer experiments 50 mCi ^{57}Co(Rh) was a source of the 14.4 keV γ-radiation. The measurements were performed at 85 ± 0.1 K. Experimental data were fitted using Recoil Mössbauer Spectral Analysis Software [11].

3 Results and discussion

We studied the influence of a low dose of neutron radiation on the stability of Hb in RBCs isolated from two different representative healthy donors. The Mössbauer spectra of non-irradiated and irradiated samples are presented in Fig. 1A–D, respec-

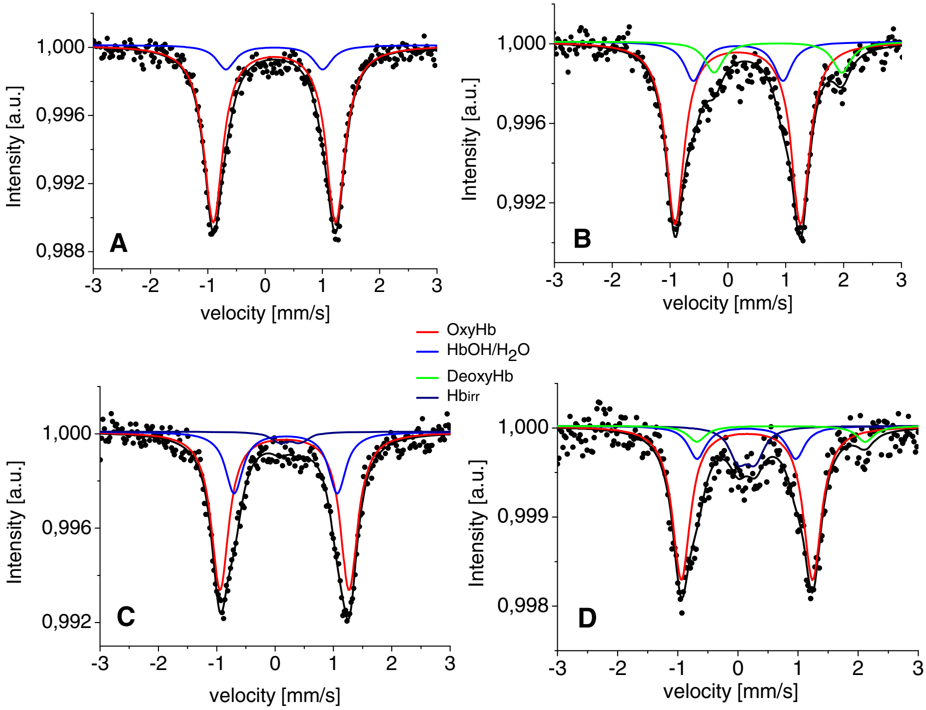

Fig. 1 Mössbauer spectra of non – irradiated (**A**, **B**) and irradiated (**C**, **D**) with 50 μGy neutron rays RBCs from healthy donors (**A**, **C** – sample 1; **B**, **D** – sample 2)

tively. Values of hyperfine parameters (IS – isomer shift, QS – quadrupole splitting) fitted to the experimental spectra obtained for both cases are collected in Table 1.

The Mössbauer spectra of untreated RBCs can be decomposed into two (sample 1) or three (sample 2) components characterized by different hyperfine parameters. The subspectrum with the largest QS of about 2.17 mm/s is characteristic for a diamagnetic state in oxygenated haemoglobin (OxyHb). At the moment, there are two models which may explain a diamagnetism of OxyHb: (i) the Pauling model suggesting an interaction between a low spin ferrous atom and singlet O_2 (a hydrophobic amino acids in the heme iron neighbourhood may help keep Fe^{2+} from becoming oxidized to Fe^{3+}) or (ii) the Weiss model assuming a strong antiferromagnetic coupling between a paramagnetic low spin ferric atom and a paramagnetic superoxide radical anion $O_2^{\cdot-}$ [7, 12–15]. Large quadrupole splitting comparable to that one observed for a high spin ferrous state results from a negative principal electric field gradient [7]. In OxyHb, the movement of the iron atom toward the porphyrin plane was caused by the contraction of the coordination bonds between iron and four nitrogens of the porphyrin.In consequence the proximal histidine (His) comes closer to the heme plane by about 0.5 Å. There is a hydrogen bond between distal His and the terminal oxygen atom of the coordinated molecular oxygen which stabilizes the Fe-OO angle about 120 degree as shown in ref. [16]. The doublet with QS $\sim$ 1.70 mm/s is assigned to deoxyhemoglobin in which the OH/H_2O molecule is the 6th ligand of the heme-iron (HbOH/H_2O). In this case the water molecule is

Table 1 Hyperfine parameters fitted to the Mössbauer spectra obtained for non-irradiated and irradiated RBCs isolated from healthy donors

Hyperfine parameters	Component 1 OxyHb	Component 2 HbOH/H_2O	Component 3 DeoxyHb	Component 4 Hb_{irr}
Sample 1 non-irradiated				
IS [mm/s]	0.16 ± 0.01	0.16 ± 0.04		
QS [mm/s]	2.14 ± 0.02	1.69 ± 0.15		
A [%]	88.0 ± 1.5	12.0 ± 1.5		
Sample 1 irradiated 45 min				
IS [mm/s]	0.16 ± 0.01	0.18 ± 0.02		0.25 ± 0.04
QS [mm/s]	2.21 ± 0.03	1.77 ± 0.07		0.35 ± 0.22
A [%]	69.0 ± 2.0	26.8 ± 2.0		4.2 ± 2.0
Sample 2 non-irradiated				
IS [mm/s]	0.17 ± 0.01	0.17 ± 0.04	0.87 ± 0.04	
QS [mm/s]	2.17 ± 0.02	1.55 ± 0.05	2.21 ± 0.08	
A [%]	72.3 ± 2.0	15.4 ± 2.0	12.3 ± 2.0	
Sample 2 irradiated 45 min				
IS [mm/s]	0.15 ± 0.01	0.14 ± 0.10	0.72 ± 0.17	0.14 ± 0.07
QS [mm/s]	2.18 ± 0.04	1.65 ± 0.21	2.80 ± 0.34	0.28 ± 0.12
A [%]	65.9 ± 2.0	14.3 ± 2.5	6.8 ± 2.5	13.3 ± 2.5

IS – isomer shift, QS – quadrupole splitting. The line width was 0.20 ± 0.02 mm/s

additionally bound to distal His via a hydrogen bridge [17]. One may consider the sixth Fe ligand as a hydroxide or water molecule depending on the delocalization of the hydrogen charge toward the His nitrogen or oxygen atom, respectively. In this case the heme-iron could be in a mixed valence state (Fe^{2+}/Fe^{3+}) having mixed spin states [7]. The third component with IS $\sim$ 0.9 mm/s and large QS comes from deoxygenated hemoglobin (DeoxyHb in sample 2) [7, 16]. In DeoxyHb the prophyrin is domed and the iron atom is displaced from the heme plane toward the proximal His. A second iron axial ligand is formed between Fe and distal His. In this case the heme iron is in a high spin state (S = 2). The large quadrupole splitting is due to the asymmetric charge distribution resulting from an extra electron in the xy plane [7, 16]. Irradiation of RBCs results in an occurrence of a new component with IS $\sim$ 0.20 mm/s and QS $\sim$ 0.30 mm/s. We call it Hb_{irr} and it can be assigned to five (six) -coordinated heme iron with CN(s)-group (low spin Fe^{3+} or high spin Fe^{3+} in a strong iron-ligand bonds with almost spherical electric field symmetry), which could be formed due to His imidazole ring radiolysis [18, 19]. However, the hyperfine parameters are also characteristic for a low spin ferrous state of the heme-iron bound to CO [7, 16]. Radiolysis of the protein-lipid matrix may cause the formation of carbon monoxide [19, 20]. The contribution of Hb_{irr} is low in the spectrum obtained for sample 1 ($\sim$4%) and much higher in the case of sample 2 ($\sim$13%). At the same time the content of OxyHb decreases in the spectra of both samples. Additionally, in the case of sample 1 the contribution of HbOH/H_2O increases by $\sim$50% whereas in the case of sample 2 the content of this component remains unchanged but the DeoxyHb contribution decreases by $\sim$50%.

Our results show that there are two Hb forms sensitive to the neutron irradiation of RBCs at a very low dose of 50 μGy. OxyHb and DeoxyHb change into Hb_{irr}. Additionally, OxyHb may transfer into HbOH/H_2O, which is resistant to the action of neutrons under our experimental conditions.

4 Conclusions

Mössbauer spectroscopy allowed us to gain an insight into possible changes of Hb in the RBC irradiated with neutrons at a very low dose of 50 μGy. We observed that only two Hb forms (OxyHb and DeoxyHb), which are physiologically active in O_2 transport, are sensitive to this neutron radiation. Inactive HbOH/H_2O seems to be resistant to such a low dose of neutron radiation.

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References

1. Treleaven, J., Gennery, A., Marsh, J., Norfolk, D., Page, D., Parker, A., Saran, F., Thurston, J., Webb, D.: Guidelines on the use of irradiated blood components prepared by the British Committee for Standards in Haematology blood transfusion task force. Br. J. Haematol. **152**(1), 35–51 (2011)
2. Blue, T.E., Gupta, N., Woollard, J.E.: A calculation of the energy dependence of the RBE of neutrons. Phys. Med. Biol. **38**, 1693–1712 (1993)
3. Chambers, D.B., Osborne, R.V., Garva, A.L.: Choosing an alpha radiation weigthing factor for doses to non-human biota. J. Environ. Radioact. **87**,1–14 (2006)
4. Raju, M.R., Eisen, Y., Carpenter, S., Inkert, W.C.: Radiobiology of a particles. III. Cell inactivation by a-particle traversals of the cell nucleus. Rad Result. **128**, 204–209 (1991)
5. Petkau, A., Chelack, W.S.: Radioprotective effect of superoxide dismutase on model phospholipid membranes. Biochim. Biophys. Acta **433**, 445–456 (1976)
6. Petkau, A., Chelack, W.S., Pleskach, S.D.: Protection of post-irradiated mice by superoxide dismutase. Int. J. Radiat. Biol. **29**, 297–299 (1976)
7. Lang, G., Marshall, W.: Mössbauer effect in some hemoglobin compounds. Proc. Phys. Soc. **87**, 3–34 (1966)
8. Burda, K., Hrynkiewicz, A., Kołoczek, H., Stanek, J., Strzałka, K.: Mixed valence state in iron-porphyrin aggregates. Biochim. Biophys. Acta **1244**, 345–350 (1995)
9. Kaczmarska, M., Kopyściańska, Z., Fornal, M., Grodzicki, T., Matlak, K., Korecki, J., Burda, K.: Influence of low doses of gamma rays on the stability of erythrocytes. Acta Biochim. Pol. **58** (2011, paper in press No 14610)
10. Burda, K, Lekki, J., Dubiel, S., Cieślak, J., Lekka, M, Stanek, J., Stachura, Z.: Molecular mechanism of hemolysis caused by organometallic compounds. Appl. Organomet. Chem. **10**, 148–154 (2002)
11. Rancourt, D.G., Ping, J.-Y.: Voigt-based methods for arbitrary-shape static hyperfine parameter distributions in Mössbauer spectroscopy. Nucl. Instrum. Methods B **58**, 85–87 (1991)
12. Pauling, L.: Nature of the iron-oxygen bond in oxyhaemoglobin. Nature **203**, 182–183 (1964)
13. Weiss, J.J.: Nature of the iron-oxygen bond in oxyhaemoglobin. Nature **202**, 83–84 (1964)
14. Collman, J.P., Gagne, R.R., Reed, Ch.A., Robinson, W.T., Rodley, G.A.: Structure of an Iron (II) dioxygen complex: a model for oxygen carrying hemeprotein. Proc. Natl. Acad. Sci. USA **71**, 1326–1329 (1974)
15. Suzdalev, J.P.: Gamma resonance spectroscopy of proteins in model systems (in Russian). Moscow, Nauka (1988)
16. Chen, H., Ikeda-Saiko, M., Shoik, S.: Nature of the Fe-O_2 bonding in oxy-myoglobin: effect of the protein. J. Am. Chem. Soc. **30**, 14778–14790 (2008)
17. Stryer, L., Kendrew, J.C., Watson, H.C.: The mode of attachment of the azide to sperm whale metmyoglobin. J. Mol. Biol. **8**, 96–104 (1964)

18. Rhynard, D., Lang, G., Spartalian, K., Yonetani, T.: Moessbauer studies of low-symmetry crystal fields in low-spin ferric heme complexes. J. Chem. Phys. **71**, 3715–3719 (1979), FEBS Lett. **186**, 158–162 (1985)
19. Oshtrakh, M.I., Semionkin, V.A.: Moessbauer effect study of gamma-irradiated human oxyhemoglobin. Radiat. Envir. Biophys. **30**, 33–44 (1991)
20. Prusakov, V.E., Stukan, R.A., Davidov, R.M., Gersonde, K.: Non-equilibrium state of a monomeric insect haemoglobin induced by γ-irradiation and detected by Moessbauer spectroscopy. FEBS Lett. **186**, 158–162 (1985)