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Differences of the ^{57}Fe hyperfine parameters in both oxyhemoglobin and spleen from normal human and patient with primary myelofibrosis

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Abstract Study of oxyhemoglobin in red blood cells and spleen tissues from normal human and patient with primary myelofibrosis was carried out using Mössbauer spectroscopy with a high velocity resolution. The ^{57}Fe hyperfine parameters were evaluated and small variations in quadrupole splitting were revealed for both normal human and patient's oxyhemoglobin and both normal human and patient's spleen.

Keywords Mössbauer spectroscopy with a high velocity resolution · Quadrupole splitting · Oxyhemoglobin · Spleen · Primary myelofibrosis

1 Introduction

Study of iron containing biomolecules in hematological malignances is of interest for analysis of molecular nature of diseases. Previous studies of oxyhemoglobin (HbO_2) from patients with blood system malignant diseases [1–3] and spleen tissue (spleen contains iron storage protein ferritin mainly) from leukemia chicken [4] showed small variations of the ^{57}Fe hyperfine parameters in comparison with those for

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normal human HbO_2 and normal chicken spleen tissue, respectively. An increase in velocity resolution in Mössbauer spectroscopy permits to achieve better adjusting to resonance, more precise spectra measurement with decrease in instrumental error on velocity scale and better fit of complicated spectra due to increase in spectral points number (see [5–9]). New studies of normal HbO_2 with different molecular structure and HbO_2 from patients with blood system malignant diseases as well as chicken liver and spleen tissues with increased velocity resolution demonstrated some small differences in the ^{57}Fe hyperfine parameters [8, 10–12]. To continue these studies we chose a case with a very rare blood system malignant disease named primary myelofibrosis (PMF). PMF is a myeloproliferative neoplasm characterized by stem cell-derived clonal myeloproliferation, reactive bone marrow fibrosis, anemia, splenomegaly, etc. [13]. We studied HbO_2 in red blood cells (RBC) and spleen tissue from patient with PMF in comparison with normal human HbO_2 and spleen tissue using Mössbauer spectroscopy with a high velocity resolution.

2 Experimental

Samples of human normal and patient's spleen tissues and patient's red blood cells were obtained at the Hematological Division of the Sverdlovsk Regional Clinical Hospital No 1 (Ekaterinburg). The diagnosis and classification of PMF was made according to the World Health Organization criteria [13]. The spleen samples were obtained from a patient with primary myelofibrosis with myeloid metaplasia after splenectomy and a healthy man after traumatic spleen lesion. Spleen samples were washed from blood using physiological solution then lyophilized. For further investigations lyophilized spleen samples were powdered. Concentrated patient's RBC were obtaining by twice washing of venous fresh blood with physiological solution and then oxygenated. Concentrated normal human RBC were obtained from Hematological Research Center (Moscow) and oxygenated. The samples of oxygenated RBC were immediately frozen in liquid nitrogen. The sample thickness was about 0.9 mg Fe/cm^2. Samples of lyophilized spleen contained 1200–1800 mg of powder.

Mössbauer spectra were measured using an automated precision Mössbauer spectrometric system built on the base of the SM-2201 spectrometer with a saw-tooth shape velocity reference signal formed using 4096 bits. Details and characteristics of this spectrometer and the system were given elsewhere [9, 14, 15]. The 1.8×10^9 Bq ^{57}Co in rhodium matrix (Ritverc GmbH, St. Petersburg) was used at room temperature. The Mössbauer spectra were measured in transmission geometry with moving absorber in the cryostat at 295 K (spleen) and 90 K (RBC and spleen) and recorded in 4096 channels. For their analysis, the spectra of spleen and RBC samples were converted into 1024 channels by a consequent summation of four neighboring channels. Statistical count rate in the normal spleen spectrum was 6.4×10^6 counts per channel with a signal-to-noise ratio of 8 while that for patient's spleen spectra were 5.7×10^5 counts per channel with a signal-to-noise ratio of 22 at 295 K and 3.7×10^6 counts per channel with a signal-to-noise ratio of 58 at 90 K (spectra were measured up to four weeks). Statistical count rates in the spectra of HbO_2 in RBC were $\sim 1.3 \times 10^6$ counts per channel for normal sample and $\sim 2.9 \times 10^6$

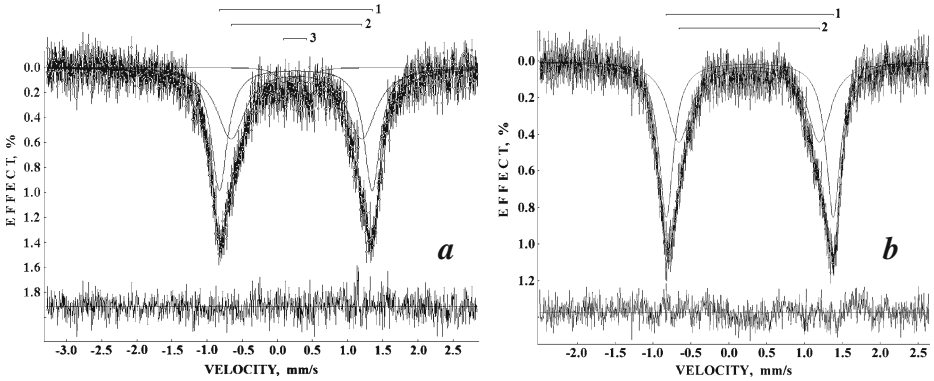

Fig. 1 Mössbauer spectra of oxyhemoglobin in normal human red blood cells (**a**) and in red blood cells from patient with primary myelofibrosis (**b**) measured at 90 K and presented in 1024 channels. Indicated components are the results of the better fits within the second model (1—^{57}Fe in α-subunits, 2—^{57}Fe in β-subunits, 3—probably carboxyhemoglobin)

counts per channel and the signal-to-noise ratio 17 and 20, respectively (spectra were measured up to two weeks). The spectra were computer fitted with the least squares procedure using UNIVEM-MS program with a Lorentzian line shape. The spectral parameters such as: isomer shift, δ, quadrupole splitting, ΔE_Q, line width, Γ, relative subspectrum area, S, and statistical criterion, χ^2, were determined. An instrumental (systematic) error for each spectrum point was ± 0.5 channel (the velocity scale), the instrumental (systematic) error for the hyperfine parameters was ± 1 channel. If an error calculated with the fitting procedure (fitting error) for these parameters exceeded the instrumental (systematic) error we used the larger error instead. Values of δ are given relative to α–Fe at 295 K.

3 Results and discussion

Mössbauer spectra of HbO_2 in human normal and patient's RBC are shown in Fig. 1. These spectra look like usual HbO_2 spectra consisting of two main peaks with asymmetry of the absorption line shapes [16].

These spectra were fitted using two models (see [10–12, 17]): 1) equivalent Fe(II) electron structure in α- and β-subunits of HbO_2 with spectra fitting using one quadrupole doublet; 2) non-equivalent Fe(II) electron structure in α- and β-subunits of HbO_2 with spectra fitting using two quadrupole doublets with equal areas. It should be noted that in the spectrum of normal human RBC an additional minor component 3 ($S \sim 5$ %) with parameters similar to carboxyhemoglobin (HbCO) was observed. The values of Γ for the Mössbauer spectra of HbO_2 from normal human and patient with PMF obtained from the first model fit were 0.418 ± 0.012 and 0.361 ± 0.010 mm/s, respectively, and those obtained from the second model were for α-subunits: 0.284 ± 0.012 and 0.233 ± 0.010 mm/s, and for β-subunits: 0.474 ± 0.012 and 0.369 ± 0.018 mm/s, for HbO_2 from normal human and patient with PMF, respectively. Details of these parameters analysis and relation to α- and β-subunits of HbO_2 were given in [17]. The values of δ and ΔE_Q obtained for HbO_2

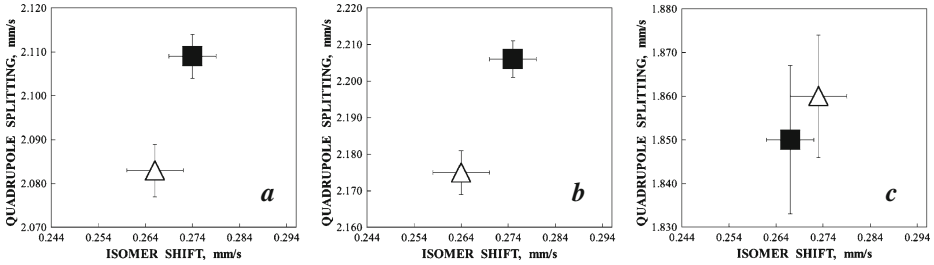

Fig. 2 Mössbauer hyperfine parameters for oxyhemoglobins from normal human (Δ) and patient with primary myelofibrosis (■) obtained from the spectra fit using model 1 (**a**) and model 2 for α-subunits (**b**) and β-subunits (**c**)

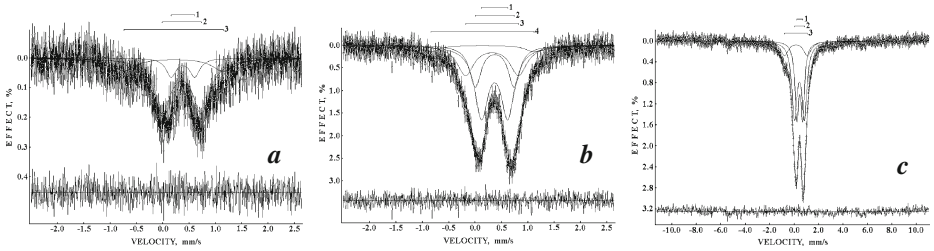

Fig. 3 Mössbauer spectra of normal human spleen (**a**) and spleen from patient with primary myelofibrosis (**b**, **c**) measured at 295 K (**a**, **b**) and 90 K (**c**) and presented in 1024 channels. Indicated components are the results of the better fits within the second model (component 3 (**a**, **c**) and component 4 (**b**) is related to residual hemoglobin in the oxidized form, other components are related to iron storage proteins)

using two models are shown in the plots of hyperfine parameters (Fig. 2). These results demonstrated some differences in the ^{57}Fe hyperfine parameters for normal and patient's HbO_2.

Mössbauer spectra of normal human spleen and spleen from patient with PMF measured at room temperature are shown in Fig. 3a,b. These spectra look like paramagnetic spectra of iron storage proteins with residual amount of oxidized hemoglobin (components 3 in Fig. 3a and 4 in Fig. 3b). These spectra were fitted using two models (see [18, 19]): 1) homogeneous iron core in iron storage proteins with spectra fitting using one quadrupole doublet; 2) heterogeneous iron core in iron storage proteins with spectra fitting using several quadrupole doublets. Using the fit within the first model it was possible to distinguish data for human normal spleen and spleen from patient with PMF in the plot of δ and ΔE_Q values (Fig. 4). The values of Γ for the Mössbauer spectra of spleen from normal human and patient with PMF obtained from the first model fit were 0.418 ± 0.018 and 0.412 ± 0.010 mm/s, respectively. Unfortunately, it was not possible to compare the results of these spectra fit using the second model due to a very small signal-to-noise ratio for the spectrum of human normal spleen. It was shown in [18] that it takes to reach a good signal-to-noise ratio in Mössbauer spectrum to use the second model well. Nevertheless, it was interesting to observe significant increase in the absorption effect in the Mössbauer spectrum of patient's spleen. Assuming the iron

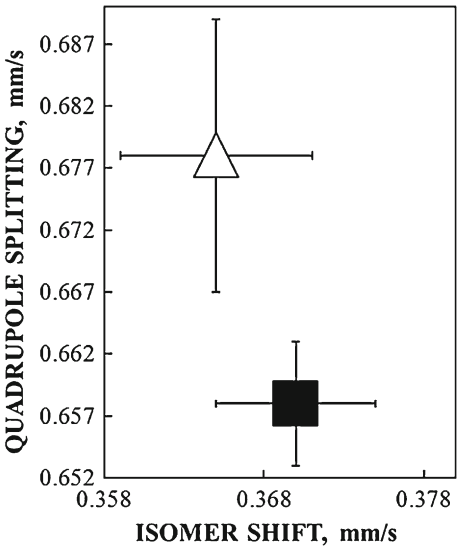

Fig. 4 Mössbauer hyperfine parameters for spleen from normal human (Δ) and patient with primary myelofibrosis (■) obtained from the spectra fit using model 1

overload in patient's spleen Mössbauer spectrum of this sample was additionally measured at 90 K (Fig. 3c). It is well known that in the case of the iron overload due to β-thalassemia an additional magnetic sextet was observed at 77 K in patients' spleen samples [20]. However, there was no magnetic component in the Mössbauer spectrum of spleen from patient with PMF at 90 K (it should be noted that in the case of the fit of this spectrum using additional minor sextet the obtained value of δ for this component was ~0.1 mm/s while the values of δ for both magnetic and paramagnetic components in the Mössbauer spectra of iron storage proteins should not be smaller than 0.4 mm/s at 90 K).

4 Conclusion

Study of oxyhemoglobin in red blood cells and spleen tissues from normal human and patient with primary myelofibrosis using Mössbauer spectroscopy with a high velocity resolution demonstrated some small variations in quadrupole splitting of studied samples indicating small structural differences in correspondent biomacromolecules in normal human and patient. On the basis of differences in the absorption effect it is possible to suppose a decrease in hemoglobin content in patient's red blood cells correlated with anemia in this disease and iron overload in spleen correlated with splenomegaly, increased red blood cells lysis with heme iron release and extramedullary hematopoiesis in patient's spleen.

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