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Heme iron state in various oxyhemoglobins probed using Mössbauer spectroscopy with a high velocity resolution

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Abstract A comparative study of oxyhemoglobins from pig, rabbit, normal human and patients with blood system malignant diseases was performed using Mössbauer spectroscopy with a high velocity resolution at 90 K. Mössbauer spectra were fitted with the help of two models: using one quadrupole doublet (model of equivalent iron electronic structure in α- and β-subunits of hemoglobins) and superposition of two quadrupole doublets (model of non-equivalent iron electronic structure in α- and β-subunits of hemoglobins). The results obtained using both models demonstrated small variations of hyperfine parameters that were related to the heme iron state variation in different hemoglobins. These results were compared with structural and functional differences of the hemoglobins investigated.

Keywords Heme iron · Oxyhemoglobins · Mössbauer spectroscopy with a high velocity resolution · Quadrupole splitting

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Introduction

Heme iron electronic state plays an important role in regulating the structure and oxygen binding affinity of hemoglobins. The process of oxygen binding to and release by hemoglobin is accompanied by protein conformational transitions initiated by spin change of the heme iron. Humans and animals display a large number of known hemoglobin variants which differ in protein primary structure and oxygen affinity. The structure–function relationship of hemoglobin molecule including the role of heme iron stereochemistry has been discussed in details in many reviews (see, for instance, Weissbluth 1974; Perutz 1979; Banerjee 1983; Rifkind 1987; Perutz et al. 1987). It has been demonstrated that small stereochemical differences exist in the heme iron in different tetrameric hemoglobins as well as in non-identical α- and β-subunits within a tetramer. X-ray structures for various

hemoglobins confirmed this fact unequivocally. Mössbauer spectroscopy is one of the robust techniques for investigating iron electronic structure in biological molecules including hemoglobin and has been so for about 50 years (Gonser and Grant 1965; Lang and Marshall 1966; Spartalian and Lang 1980; Parak and Trautwein 1984; Oshtrakh 1999). Previous Mössbauer studies of myoglobin and hemoglobin (Trautwein et al. 1975, Eicher et al. 1976), normal adult and fetal hemoglobins (Oshtrakh and Semionkin 1985), normal hemoglobins and hemoglobins from patients with molecular diseases (Bill et al. 1982; Zeng and Lian 1992; Oshtrakh and Semionkin 1986, 1987, 1989) demonstrated small differences of Mössbauer hyperfine parameters in relation to the heme iron stereochemistry. Such small differences were further reiterated by analysis of isolated hemoglobin subunits and hemoglobin tetramer containing α-subunits partially enriched with ^{57}Fe (Trautwein et al. 1976) and iron non-equivalence in α- and β-subunits (Oshtrakh et al. 1989; Oshtrakh 1994, 1998; Oshtrakh and Semionkin 2004). The sensitivity of Mössbauer hyperfine parameters to small structural variations in the heme iron stereochemistry was considered by Oshtrakh (2004a, b). However, further development of the study of small variations of the heme iron electronic structure using Mössbauer spectroscopy is related to an improvement in velocity resolution and increase in stability, sensitivity and accuracy of Mössbauer spectroscopy. Recently we demonstrated advances of Mössbauer spectroscopy with a high velocity resolution in revealing small variations of hyperfine parameters and more reliable fitting of complicated Mössbauer spectra using our preliminary results (Oshtrakh et al. 2009a, b, 2010, 2011). Therefore, in the present work we investigated normal human, rabbit and pig oxyhemoglobins and oxyhemoglobins from patients with blood system malignant diseases using Mössbauer spectroscopy with a high velocity resolution in relation to the heme iron stereochemical and oxygen affinity variations.

Materials and methods

Hemoglobin samples

For probing heme iron state in normal hemoglobins we chose human adult, rabbit and pig hemoglobins. These hemoglobins have structural and functional differences (see Novy et al. 1973; Sinet et al. 1982, Rovida et al. 1983; Shaanan 1983; Uchida et al. 1998; Lu et al. 2000). The differences in amino acid composition in the α- and β-subunits in these hemoglobins are shown in Table 1. The structural differences in their protein subunits and hemes are shown in Fig. 1. These differences have been displayed using Protein Data Bank structures 2DN1, 2RAO and 1QPW, respectively, for the three hemoglobins mentioned above. Hemoglobin from patients with blood system malignant diseases were chosen in order to re-evaluate our previous results obtained using Mössbauer spectroscopy with a low velocity resolution (Oshtrakh and Semionkin 1986, 1987, 1989). Some functional variations of hemoglobins from patients were observed previously (Festa and Asakura 1979; Oshtrakh and Semionkin 1986) while there is no information about structure of such hemoglobins.

Concentrated normal human adult, rabbit and pig red blood cells were obtained by appropriate washing of outdated human and fresh arterial pig and rabbit blood at the Hematological Research Center (Moscow). Concentrated red blood cells from patients with chronic myeloleukemia and multiple myeloma were prepared from fresh venous blood at the Hematology

Table 1 Differences in amino acids composition of α- and β-subunits in human adult, rabbit and pig hemoglobins

	Human hemoglobin		Rabbit hemoglobin		Pig hemoglobin	
	α	β	α	β	α	β
Human hemoglobin	0	0	25	14	22	19
Rabbit hemoglobin	25	14	0	0	26	22
Pig hemoglobin	22	19	26	22	0	0

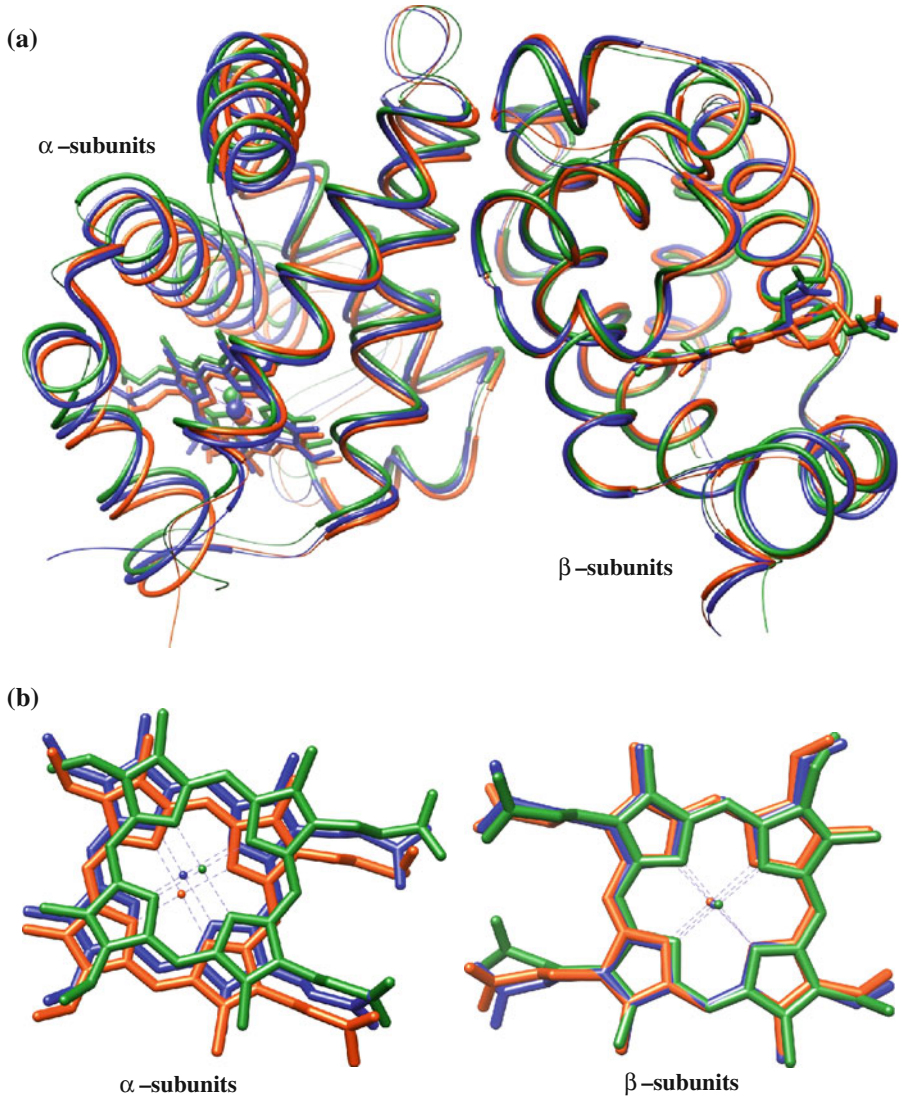

Fig. 1 Alignment of α- and *β*-subunits (**a**) and hemes (**b**) of human adult (*red*), rabbit (*blue*) and pig (*green*) hemoglobins

Department of the Sverdlovsk Regional Clinical Hospital No 1 (Ekaterinburg). Concentrated red blood cells were oxygenated and placed into special holders with about 2.5 ml of sample solution. Subsequently, the samples were immediately frozen with liquid nitrogen and stored at 77 K. One sample of rabbit red blood cells was frozen without additional oxygenation. The effective thickness of these samples was $\sim$0.9 mg Fe/cm^2.

Mössbauer spectra measurement

Mössbauer spectra of oxyhemoglobin samples were measured using an automated precision Mössbauer spectrometric system on the base of the spectrometer SM–2201 with a high velocity resolution and temperature variable liquid nitrogen cryostat with a moving absorber. Details and characteristics of this equipment and the system are described elsewhere (Oshtrakh et al. 2009c; Semionkin et al. 2010). The Mössbauer spectra were measured at 90 K in transmission geometry with a moving absorber in the cryostat and recorded in 4096 channels. For analysis, the spectra of oxyhemoglobins were converted into 1024 channels by a consequent summation of four neighboring channels. Statistical rate in the spectra varied from $\sim 1.0 \times 10^6$ to $\sim 3.5 \times 10^6$ counts per channel. The signal/noise ratio for obtained spectra was in the range from 15 to

29. The spectra were computer fitted with least squares procedure using the UNIVEM-MS program with Lorentzian line shape. Spectral parameters such as: isomer shift, δ, quadrupole splitting, ΔE_Q, line-width, Γ, relative subspectrum area, S, and statistical quality of the fit, χ^2, were thus determined. Instrumental error for the velocity scale or systematic error for the each spectra point was ± 0.5 channel, instrumental error for hyperfine parameters evaluation was ± 1 channel while instrumental error for Γ evaluation was ± 2 channels. It should be noted that spectrometer characteristics determined an integral velocity error (total mechanical and electronics systematic and random errors) which was several times less than a half of channel value in mm/s during spectra measurements using 4096 channels (Oshtrakh et al. 2009c). If error calculated with the fitting procedure (fitting error) for these parameters exceeded the instrumental (systematic) error, we used the larger error. Velocity resolution (velocity per one channel) was ~ 0.006 mm/s per channel for 1024 channels. The relative error for S did not exceed 10%. Criteria of the best fit were differential spectra, χ^2 values and physical meaning of parameters. The value of standard deviation (σ) of χ^2 was 0.044 for spectra presented in 1024 channels. Values of δ are given relative to α-Fe at 295 K.

Results and discussion

Normal hemoglobins

Mössbauer spectra of normal human adult, rabbit and pig red blood cells samples were measured twice at 90 K and presented in 1024 channels. Spectra of fully oxygenated samples and sample without additional oxygenation are shown in Fig. 2. These spectra are quadrupole doublets with asymmetry of the main absorption lines shape related to oxyhemoglobin. This asymmetry was observed earlier for various oxyhemoglobins in the region of liquid nitrogen temperature and explained on the basis of the iron electronic structure difference in α- and β-subunits (Oshtrakh 1994, 1998). Therefore, Mössbauer spectra of oxyhemoglobins were fitted in two ways. Firstly, spectra were fitted using one quadrupole doublet without accounting for the iron electronic structure difference in α- and β-subunits (the first model). Subsequently, all spectra were fitted using superposition of two quadrupole doublets with equal areas to account for the iron electronic structure difference in α- and β-subunits (the second model). It should be noticed that in the spectrum of normal human adult oxyhemoglobin (Fig. 2a) an additional minor component was observed while in the spectrum of rabbit red blood cells which were not additionally oxygenated an additional quadrupole doublet related to deoxyhemoglobin was clearly seen (Fig. 2d).

Model of equivalent iron electronic structure in α- and β-subunits of oxyhemoglobins

Mössbauer spectral parameters obtained using the first model are shown in Table 2. Quadrupole splitting and isomer shift for additional minor component in the spectrum of oxygenated human adult red blood cells are similar to carboxyhemoglobin (see Gonser and Grant 1965; Lang and Marshall 1966; Oshtrakh et al. 1989). It is possible that a small amount of carboxyhemoglobin, which formed and pre-existed due to a strong Fe(II)–CO bond, remained intact throughout the sample preparation process. The additional component in the spectrum of rabbit red blood cells which were not additionally oxygenated has quadrupole splitting and isomer shift corresponding to deoxyhemoglobin. It is possible that partial deoxygenation of oxyhemoglobin occurred during preparation of red blood cells. Comparison of Mössbauer hyperfine parameters of human adult, pig and rabbit oxyhemoglobins is shown in Fig. 3a in the plot of ^{57}Fe quadrupole splitting versus isomer shift. It is clearly seen that quadrupole splitting values for normal human adult, rabbit and pig oxyhemoglobins were distinguished well. This result may be explained by average variation of the heme iron electronic structure in different hemoglobins differing in amino acid composition that accumulated over the natural evolution process. The differences of the low-lying iron electronic terms energies on the basis of the scheme proposed by Bacci et al. (1979) may be used for explanation of the effect of small variations of the heme iron stereochemistry on the iron electron structure (Fig. 3b).

Model accounting for non-equivalent iron electronic structure in α- and β-subunits of oxyhemoglobins

Mössbauer spectra of oxyhemoglobins were fitted better using the second model. In this case, the

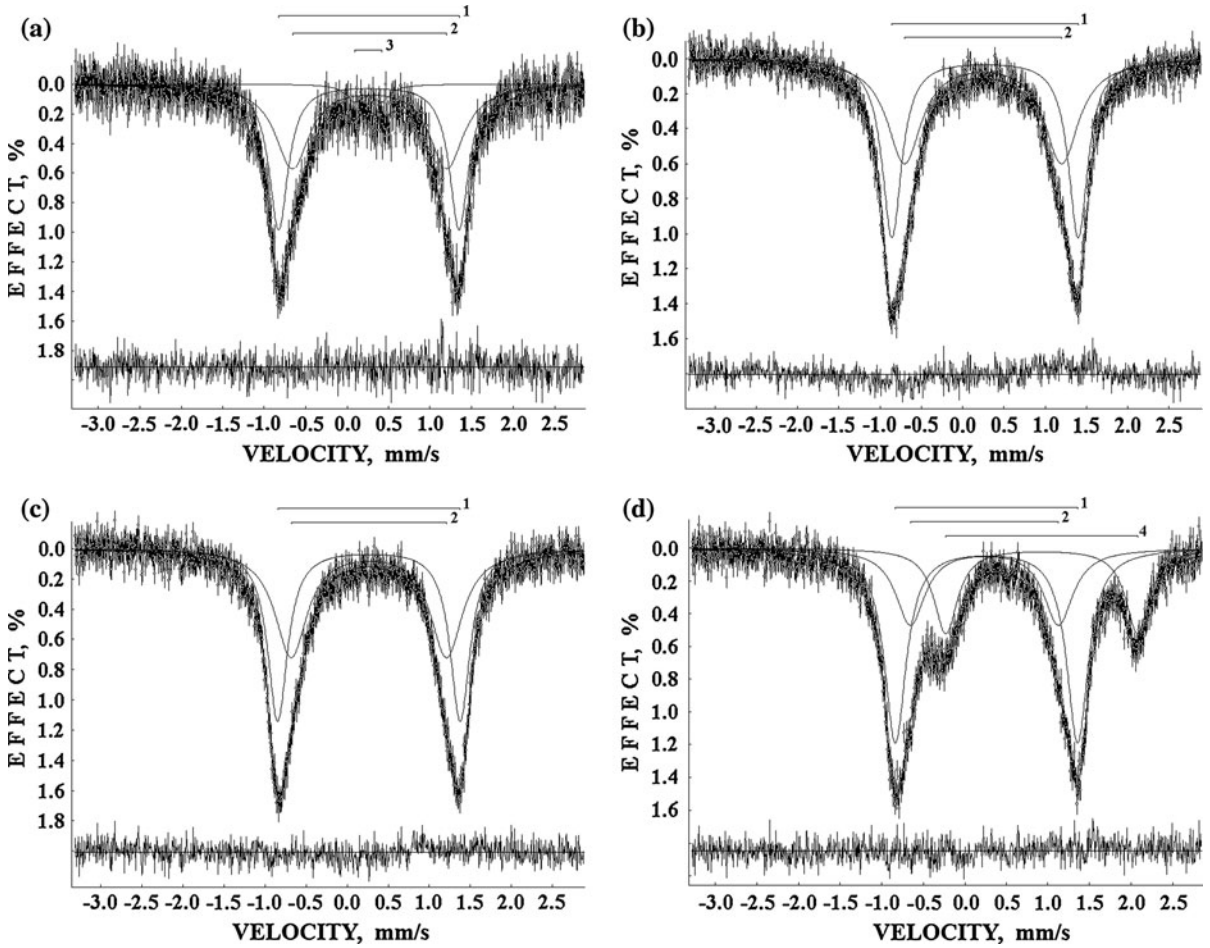

Fig. 2 Mössbauer spectra of normal human adult (**a**), pig (**b**) and rabbit (**c**, **d**) red blood cells samples measured at 90 K and presented in 1024 channels. *1* α-subunits in oxyhemoglobins, *2* β-subunits in oxyhemoglobins, *3* carboxyhemoglobin, *4* deoxyhemoglobin

Table 2 Mössbauer parameters of human adult, rabbit and pig red blood cells samples obtained using the first model

Sample	Γ, mm/s	δ, mm/s	ΔE_Q, mm/s	S, %	χ^2	Compound
Oxygenated human adult red blood cells	0.402 ± 0.012	0.266 ± 0.006	2.087 ± 0.006	92.3	1.051	Oxyhemoglobin
	0.583 ± 0.217	0.220 ± 0.039	0.363 ± 0.096	7.7		Carboxyhemoglobin
Oxygenated pig red blood cells	0.442 ± 0.012	0.268 ± 0.006	2.150 ± 0.006	100	1.520	Oxyhemoglobin
Oxygenated rabbit red blood cells	0.428 ± 0.012	0.267 ± 0.006	2.117 ± 0.006	100	1.259	Oxyhemoglobin
Washed rabbit red blood cells	0.424 ± 0.012	0.259 ± 0.006	2.109 ± 0.006	77.5	1.433	Oxyhemoglobin
	0.343 ± 0.012	0.925 ± 0.006	2.328 ± 0.007	22.5		Deoxyhemoglobin

asymmetry of the absorption line shape in oxyhemoglobin spectra was fitted using superposition of two quadrupole doublets. This fit led to differential spectrum improvement and decrease of χ^2 values. For instance, in the case of pig oxyhemoglobin spectrum fit using the first model, $\chi^2 = 1.520$ with χ^2 deviation of 11.8 σ while in the case of the same spectrum fit using the second model $\chi^2 = 1.049$ with

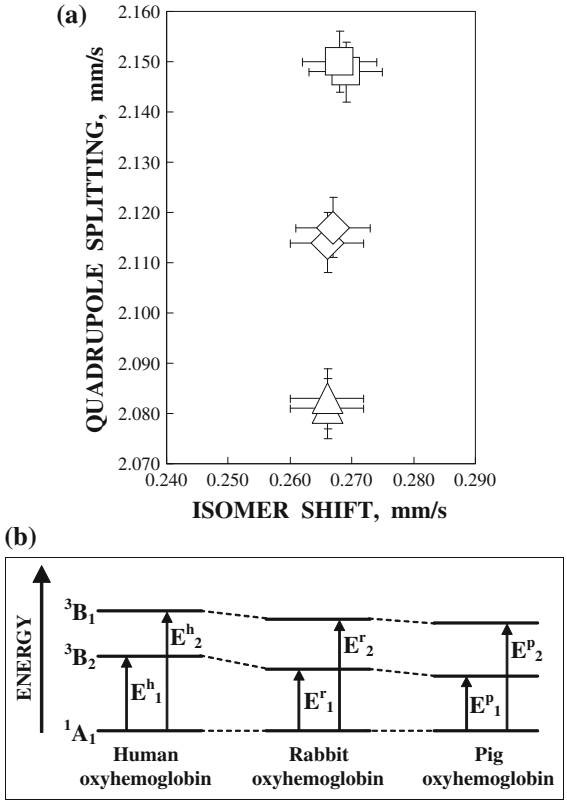

Fig. 3 Differences of quadrupole splitting for normal human adult (*open triangle*), rabbit (*open diamond*) and pig (*open square*) oxyhemoglobins obtained using the first model (**a**) and schemes of the ground and low-lying excited iron electronic terms for oxyhemoglobins with different quadrupole splitting (**b**)

χ^2 deviation of 1.1 σ. Similarly, in the case of rabbit oxyhemoglobin spectrum fit using the first model, $\chi^2 = 1.259$ with χ^2 deviation of 5.9 σ while in the case of the same spectrum fit using the second model $\chi^2 = 0.936$ with χ^2 deviation of 1.5 σ. The Mössbauer parameters obtained using the second model are shown in Table 3. In this case, line-width for the carboxyhemoglobin component became real and corresponded to the previously reported Γ value (Oshtrakh et al. 1989). Two quadrupole doublets for oxyhemoglobins had different Γ values and intensities. These differences were related to the possible formation of a hydrogen bond between terminal oxygen and ε–nitrogen atom of the distal *His* E7 imidazole ring in α-subunits while terminal oxygen is free for rotation around the Fe–O bond in β-subunits (see the results of X-ray structure analysis of human oxyhemoglobin (Shaanan 1983)). Based on this supposition quadrupole doublet 1 was related to the ^{57}Fe in α-subunits while doublet 2 was related to the ^{57}Fe in β-subunits of oxyhemoglobins. Differences of quadrupole splitting and isomer shift for both α- and β-subunits of human, rabbit and pig oxyhemoglobins are shown in Fig. 4. It was interesting to observe some small variations of Mössbauer hyperfine parameters in both α- and β-subunits of different hemoglobins. Linear correlations of quadrupole splitting and isomer shift are similar to a previously observed linear correlation of δ and ΔE_Q for isostructural iron compounds (Parwate and Garg 1984; Inoue et al. 1986). Such correlations were attributed to weakening or strengthening of the iron σ– and π–bonds with ligands. Some stereochemical differences of the heme iron in α- and β-subunits of normal human adult, rabbit and pig oxyhemoglobins calculated using their X-ray crystallographic structures are shown in Table 4 and Fig. 5. It is clearly seen that the heme iron stereochemistry in both subunits of three normal oxyhemoglobins was different. This fact may explain the different values of ^{57}Fe quadrupole splitting and isomer shift for corresponding subunits in different normal oxyhemoglobins.

The Mössbauer spectrum of rabbit red blood cells that were not additionally oxygenated (Fig. 2d) indicated that rabbit oxyhemoglobin was partially deoxygenated resulting in the presence of deoxyhemoglobin as component 4. Fitting of this spectrum using the second model with additional doublet for deoxyhemoglobin showed that relative areas of three doublets were as follows: S_1:S_2:S_4 = 50:26:24%. It was reported that oxygen affinity of α-subunits is three times larger than that of β-subunits (Perutz et al. 1998). Therefore, β-subunits release O_2 molecules relatively easily compared to α-subunits with further R → T transition (from oxy- to deoxy-conformation) thus decreasing of oxygen affinity of α-subunits. This transition is accompanied by different structural variations in the heme regions of α_1-, α_2- and β_1-, β_2-subunits. In the Mössbauer spectrum of partially deoxygenated rabbit oxyhemoglobin, the component of deoxyhemoglobin was about 25%. If we suggest that deoxygenation process starts when the O_2 molecules are released from β-subunits, this part of deoxy-form is expected to correspond to ~50% of fully deoxygenated β-subunits. In such a case oxygenated β-subunits should be transformed with the initiation of R → T transition. The values of ΔE_Q

Table 3 Mössbauer parameters of human adult, rabbit and pig red blood cells samples obtained using the second model

Sample	Γ, mm/s	δ, mm/s	ΔE_Q, mm/s	S, %	χ^2	Compound[a]
Oxygenated human adult red blood cells					0.935	Oxyhemoglobin:
	0.277 ± 0.012	0.264 ± 0.006	2.175 ± 0.006	47.6		α-subunits (1)
	0.492 ± 0.012	0.273 ± 0.006	1.860 ± 0.014	47.6		β-subunits (2)
	0.252 ± 0.012	0.250 ± 0.021	0.323 ± 0.034	4.8		Carboxyhemoglobin (3)
Oxygenated pig red blood cells					1.049	Oxyhemoglobin:
	0.286 ± 0.012	0.272 ± 0.006	2.254 ± 0.006	49.9		α-subunits (1)
	0.507 ± 0.014	0.247 ± 0.006	1.901 ± 0.016	50.1		β-subunits (2)
Oxygenated rabbit red blood cells					0.936	Oxyhemoglobin:
	0.291 ± 0.012	0.267 ± 0.006	2.224 ± 0.006	49.9		α-subunits (1)
	0.476 ± 0.014	0.267 ± 0.006	1.895 ± 0.018	50.1		β-subunits (2)
Washed rabbit red blood cells					1.041	Oxyhemoglobin:
	0.315 ± 0.012	0.262 ± 0.006	2.197 ± 0.006	50.0		α-subunits (1)
	0.428 ± 0.021	0.238 ± 0.006	1.786 ± 0.021	26.4		β-subunits (2)
	0.347 ± 0.012	0.926 ± 0.006	2.312 ± 0.007	23.6		Deoxyhemoglobin (4)

[a] Numbers in parentheses correspond to components numbers in Fig. 2

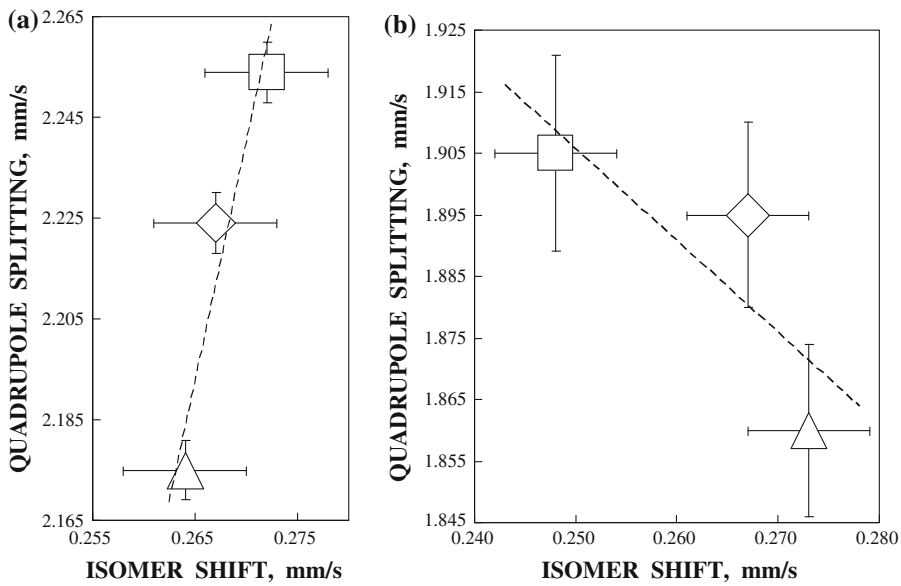

Fig. 4 Differences of Mössbauer hyperfine parameters for normal human adult (*open triangle*), rabbit (*open diamond*) and pig (*open square*) oxyhemoglobins obtained using the second model: **a** α-subunits, **b** β-subunits

evaluated for rabbit oxyhemoglobin α- and β-subunits in partially deoxygenated protein using the second model were less than those for fully oxygenated rabbit oxyhemoglobin subunits. This fact may be related to local stereochemical changes of the heme iron environment in partially deoxygenated protein after the initiation of R → T conformational transition. The values of isomer shift and quadrupole splitting for deoxygenated β-subunits indicate spin state transition ($S = 0 \rightarrow S = 2$) and heme iron stereochemical differences reflected deoxygenated β-subunits with T-like tertiary structure in the protein with quaternary R conformation.

Correlation of quadrupole splitting and oxygen affinity

Human adult, pig and rabbit hemoglobins demonstrate different oxygen affinity represented by their P_{50} values (Novy et al. 1973; Rovida et al. 1983;

Table 4 Heme iron stereochemical parameters in different oxyhemoglobins calculated from the corresponding X-ray crystallography data available in the Protein Data Bank

HbO_2, PDB code	Subunit	Bond length Fe–N_{porph}, Å	Bond length Fe–O1, Å	Bond angle Fe–O1–O2, °	Bond length Fe–N_ε (*His* F8), Å	Distance of O_2–N_ε (*His* E7), Å
Human, 2DN1	α	2.013	1.817	124.3	2.071	2.699
	β	2.019	1.775	125.9	2.063	3.018
Rabbit, 2RAO	α	2.064	1.668	131.5	2.175	2.750
	β	2.057	1.909	109.3	2.150	3.755
Pig, 1QPW	α	1.963	1.871	99.6	2.868	3.468
	β	1.968	1.846	127.1	2.848	2.863

[a] Fe–N_{porph} is average distance between heme iron and porphyrin nitrogen atoms, Fe–O1 refers to the bond between heme iron and the first oxygen atom (O1) in O_2 molecule, Fe–N_ε (*His* F8) represents the bond of heme iron and ε–nitrogen atom of proximal histidine F8 imidazole ring, O2–N_ε (*His* E7) is the distance between terminal oxygen atom (O2) in O_2 molecule and ε–nitrogen atom of distal histidine E7 imidazole ring

Heme iron stereochemistry is shown in Fig. 5

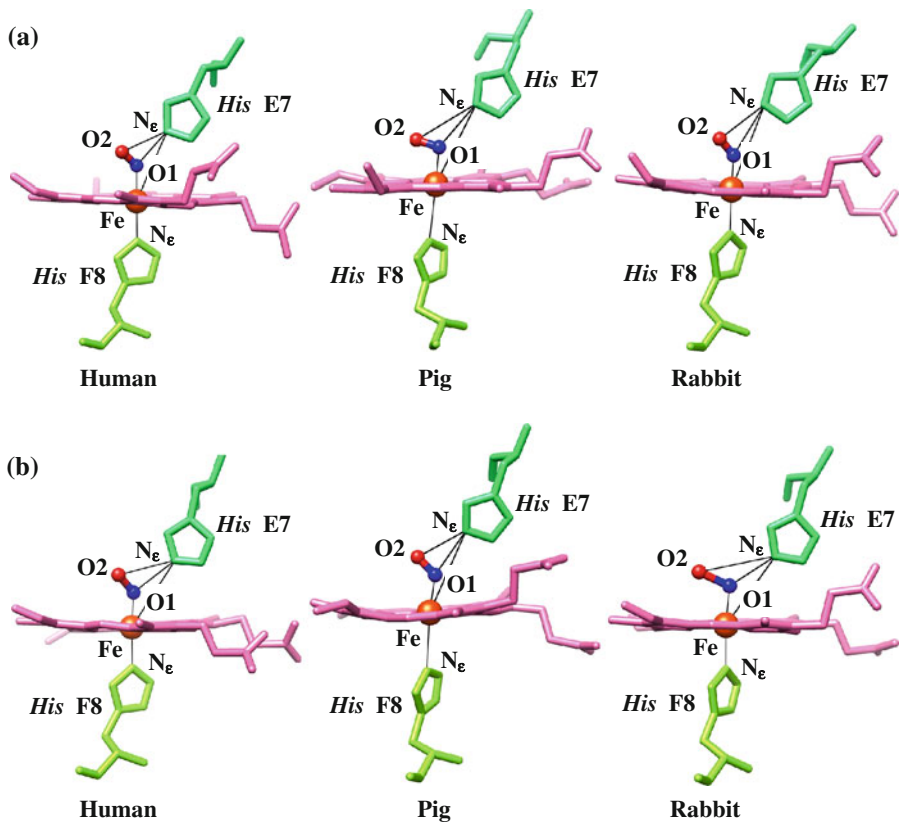

Fig. 5 Stereochemical differences of the heme iron in α-subunits (**a**) and in β-subunits (**b**) of normal human adult, pig and rabbit oxyhemoglobins. Some distances and angles are provided in Table 3

Uchida et al. 1998). Interestingly a correlation was observed between the values of quadrupole splitting obtained using both the first and the second models of Mössbauer spectra fittings and hemoglobin oxygen affinities (see Fig. 6). An increase of quadrupole splitting for oxyhemoglobins correlates with increase of P_{50} values. It is possible that increase in quadrupole splitting reflects changes of the heme iron interactions with ligands and probable decrease in strength of the Fe(II)–O_2 bonds in both α- and

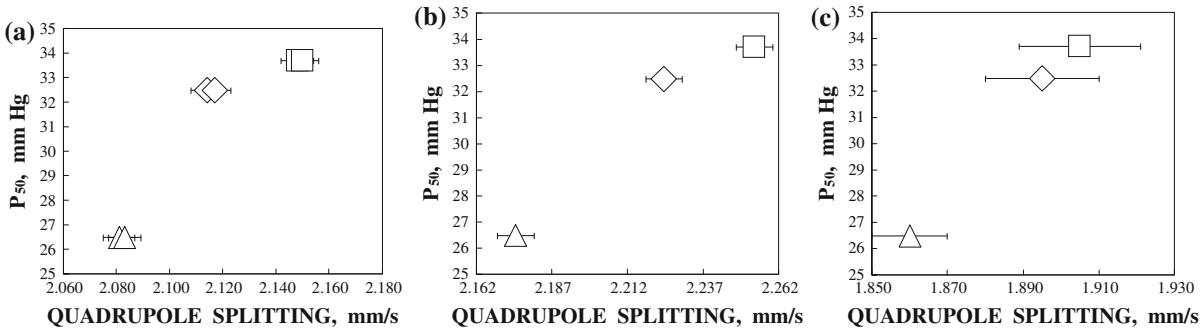

Fig. 6 Correlation of oxygen affinity for normal human adult (*open triangle*), rabbit (*open diamond*) and pig (*open square*) hemoglobins and quadrupole splitting for these oxyhemoglobins obtained using the first model (**a**) and the second model: α-subunits (**b**), β-subunits (**c**)

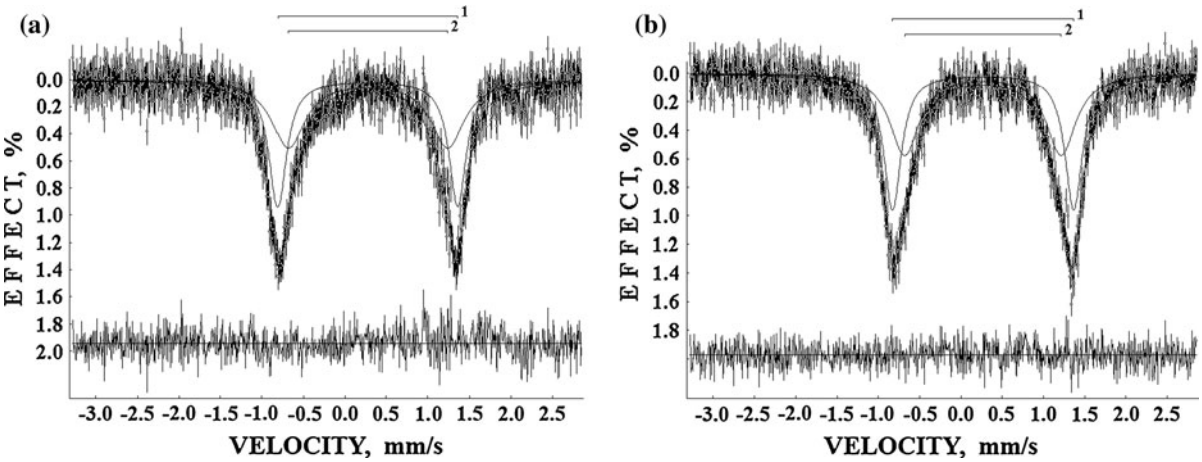

Fig. 7 Mössbauer spectra of oxyhemoglobins from patients with multiple myeloma (**a**) and chronic myeloleukemia (**b**) measured at 90 K and presented in 1024 channels. *1* α-subunits in oxyhemoglobins, *2* β-subunits in oxyhemoglobins

Table 5 Mössbauer parameters of red blood cells from patients with blood system malignant diseases obtained using the first model

Sample	Γ, mm/s	δ, mm/s	ΔE_Q, mm/s	S, %	χ^2	Compound
Oxygenated red blood cells from patient with multiple myeloma	0.355 ± 0.012	0.274 ± 0.006	2.099 ± 0.006	100	1.127	Oxyhemoglobin
Oxygenated red blood cells from patient with chronic myeloleukemia	0.379 ± 0.006	0.269 ± 0.006	2.103 ± 0.006	100	1.129	Oxyhemoglobin

β-subunits of the target oxyhemoglobins. As such, a weaker Fe(II)–O_2 bond may be related to the necessity of higher oxygen pressure for hemoglobin saturation with oxygen.

Oxyhemoglobins from patients

Mössbauer spectra of oxygenated red blood cells from patients with multiple myeloma and chronic myeloleukemia measured at 90 K and presented in 1024 channels are shown in Fig. 7. These spectra are similar to the Mössbauer spectra of other oxyhemoglobins (Fig. 2b, c) and do not contain any other components besides oxyhemoglobin. Mössbauer spectra were fitted using both the first and second models and the parameters obtained are shown in Tables 5 and 6. Interestingly, small differences of quadrupole splitting and isomer shift were observed for

Table 6 Mössbauer parameters of red blood cells from patients with blood system malignant diseases obtained using the second model

Sample	Γ, mm/s	δ, mm/s	ΔE_Q, mm/s	S, %	χ^2	Compound[a]
Oxygenated red blood cells from patient with multiple myeloma					1.055	Oxyhemoglobin:
	0.252 ± 0.012	0.274 ± 0.006	2.165 ± 0.006	49.9		α-subunits (1)
	0.484 ± 0.012	0.277 ± 0.007	1.918 ± 0.017	50.1		β-subunits (2)
Oxygenated red blood cells from patient with chronic myeloleukemia					0.997	Oxyhemoglobin:
	0.256 ± 0.021	0.269 ± 0.006	2.195 ± 0.013	49.9		α-subunits (1)
	0.439 ± 0.012	0.266 ± 0.006	1.896 ± 0.050	50.1		β-subunits (2)

[a] Numbers in parentheses correspond to components numbers in Fig. 7

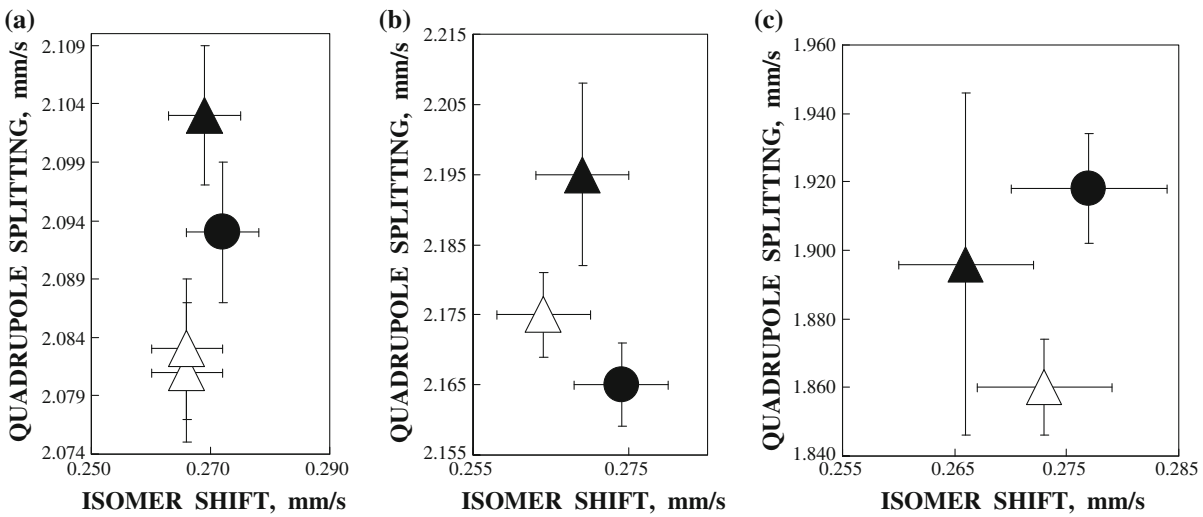

Fig. 8 Small differences of Mössbauer hyperfine parameters for normal human adult oxyhemoglobins (*open triangle*) and oxyhemoglobins from patients with multiple myeloma (*filled circle*) and chronic myeloleukemia (*filled triangle*) obtained using the first model (**a**) and the second model: α-subunits (**b**), β-subunits (**c**)

oxyhemoglobins from patients in comparison to the normal ones as clearly seen in the plots of δ versus ΔE_Q in Fig. 8 for both fitting models. Having compared Mössbauer hyperfine parameters for different normal oxyhemoglobins might be supposed that in the case of oxyhemoglobins from patients, small variations of the heme iron stereochemistry may also take place in comparison with human adult oxyhemoglobin. These changes may also affect the strength of the Fe(II)–O_2 bond and functional properties of such hemoglobins.

Conclusion

The comparative study of three normal oxyhemoglobins with different molecular structure and functions as well as two oxyhemoglobins from patients with blood system malignant diseases using Mössbauer spectroscopy with a high velocity resolution demonstrated small variations of Mössbauer hyperfine parameters which may be related to small variations of the heme iron stereochemistry in α- and β-subunits. These results demonstrate correlations of the changes in quadrupole splitting and isomer shift, iron electronic structure, heme iron stereochemistry and oxygen affinity for normal human adult, pig and rabbit oxyhemoglobins. It is possible that small variations of Mössbauer hyperfine parameters observed for oxyhemoglobin from patients are also related to some minor structural and functional variations. The ability to distinguish normal oxyhemoglobin and oxyhemoglobin from patients with

blood system malignant diseases using Mössbauer hyperfine parameters may be potentially useful for development of diagnostic tests. However, further studies in this direction are required.

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