Hyperfine Interactions (2004) 159:345–350
DOI 10.1007/s10751-005-9117-0

The Features of Mössbauer Spectra of Hemoglobins: Approximation by Superposition of Quadrupole Doublets or by Quadrupole Splitting Distribution?

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Abstract. Mössbauer spectra of hemoglobins have some features in the range of liquid nitrogen temperature: a non-Lorentzian asymmetric line shape for oxyhemoglobins and symmetric Lorentzian line shape for deoxyhemoglobins. A comparison of the approximation of the hemoglobin Mössbauer spectra by a superposition of two quadrupole doublets and by a distribution of the quadrupole splitting demonstrates that a superposition of two quadrupole doublets is more reliable and may reflect the non-equivalent iron electronic structure and the stereochemistry in the α- and β-subunits of hemoglobin tetramers.

Key Words: hemoglobins, Mössbauer spectra, quadrupole splitting.

1. Introduction

Mössbauer spectra of the oxy-form of hemoglobins (HbO_2) demonstrate non-Lorentzian asymmetric line shape in the range of liquid nitrogen temperature while Mössbauer spectra of the deoxy-form of hemoglobins (Hb) have Lorentzian line shape in this temperature range (see refs. [1, 2]). The approximation of HbO_2 Mössbauer spectra using one quadrupole doublet was not satisfactory in contrast to Hb Mössbauer spectra. Therefore, several approaches considered in [1, 2] were supposed to explain the features of Mössbauer spectra and to fit a non-Lorentzian line shape. The approximation of a non-Lorentzian line shape using a superposition of quadrupole doublets gives a better fit of HbO_2 Mössbauer spectra. This approximation implies the presence of different iron sites in hemoglobin. On the other hand, an approximation of HbO_2 Mössbauer

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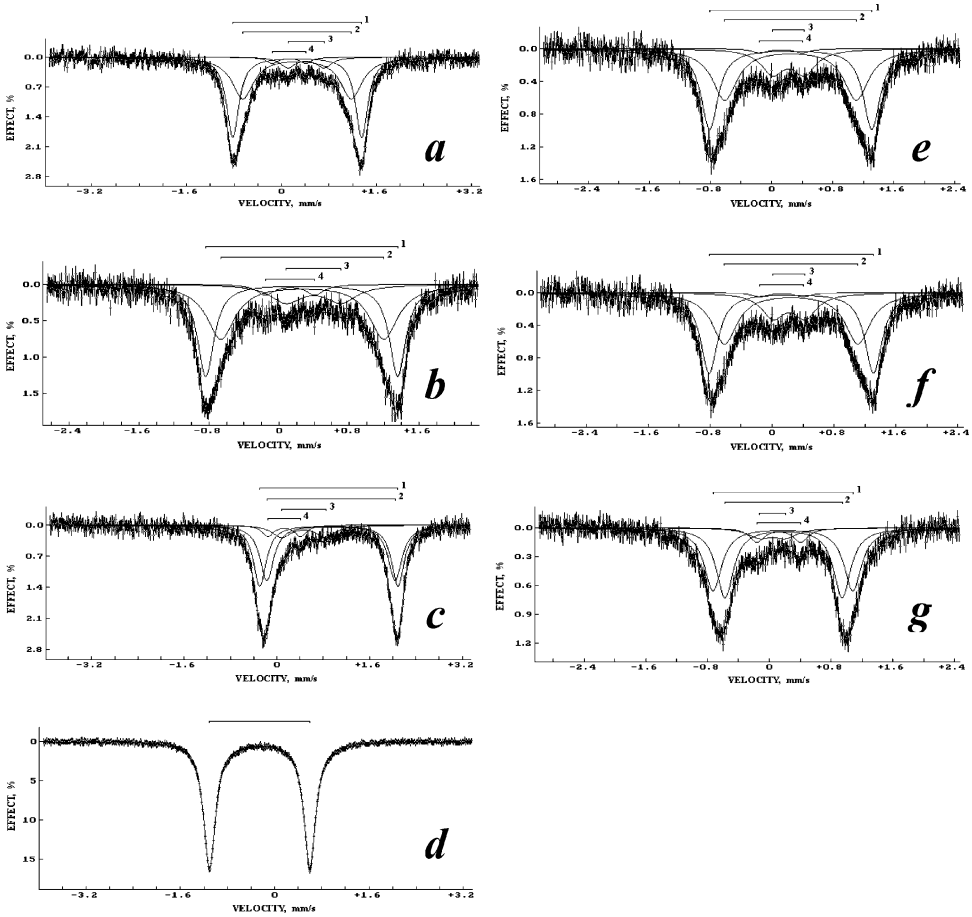

Figure 1. Mössbauer spectra of: ***a*** – HbO_2 (*FS*, *T* = 87 K), ***b*** – HbO_2 (*FS*, *T* = 87 K), ***c*** – Hb (*FS*, *T* = 87 K), ***d*** – SNP (295 K), ***e*** – Hb(PLP + GA)O_2 (*FS*, *T* = 87 K), ***f*** – Hb(PLP + GA)O_2 (*LF*, *T* = 87 K), ***g*** – Hb(PLP + GA)O_2 (*LF*, *T* = 295 K). 1 – α-subunits in tetramer, 2 – β-subunits in tetramer, 3 – unknown Fe^{3+} compound, 4 – Be(^{57}Fe).

spectra using a quadrupole splitting distribution was also used [3]. This approach may imply, for instance, a number of conformational substates of the hemoglobin molecule. In this work we compare both approximations for fitting of hemoglobin Mössbauer spectra measured with a high precision and sensitive spectrometer.

2. Materials and methods

The preparation of human HbO_2 and Hb in frozen solutions (FS) and oxygenated human hemoglobin modified by pyridoxal-5′-phosphate and glutaraldehyde (Hb(PLP + GA)O_2) in lyophilized form (LF) and frozen solution was described

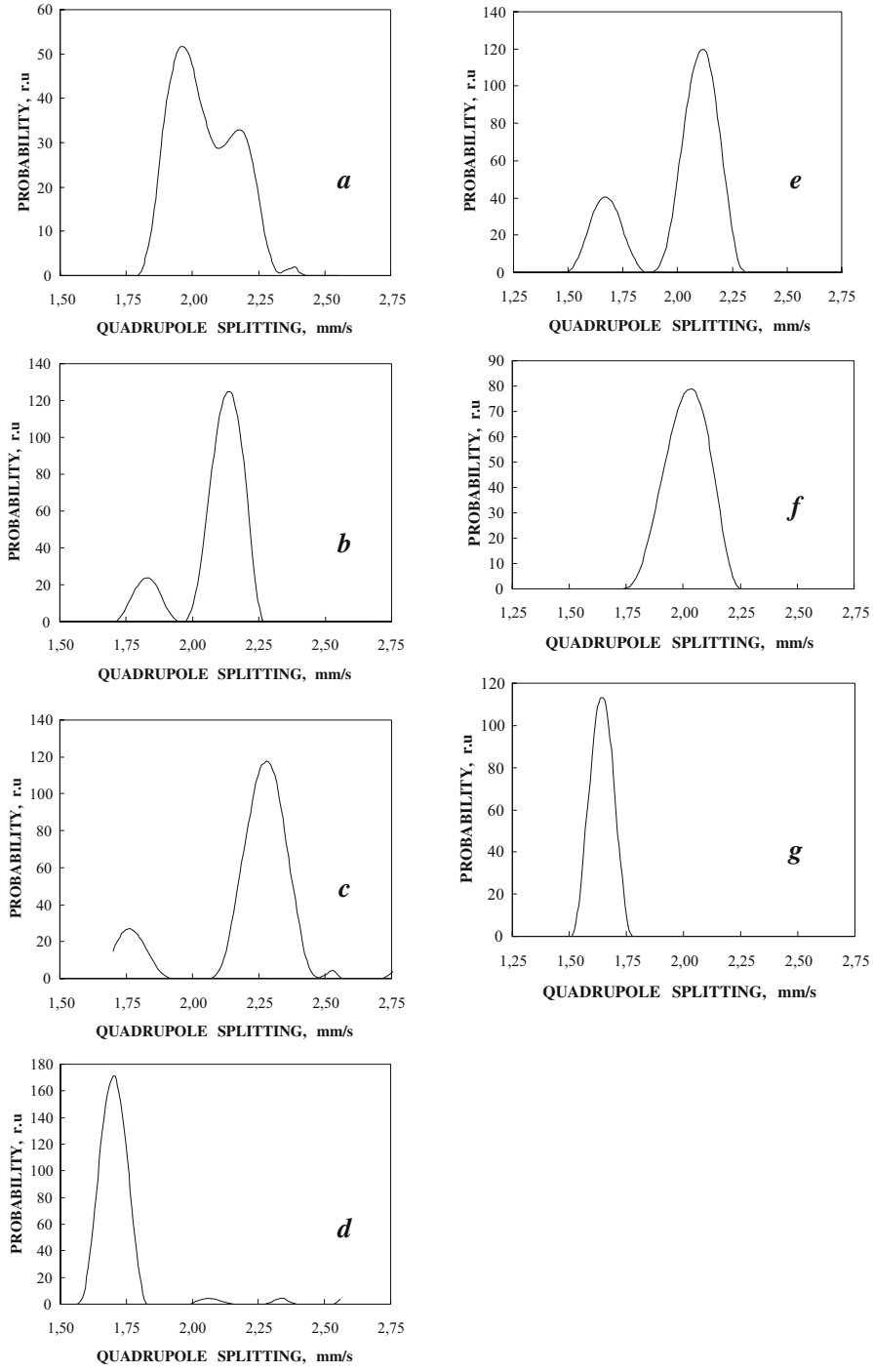

Figure 2. Distributions of quadrupole splitting for Mössbauer spectra of: ***a*** – HbO_2 (*FS*, *T* = 87 K), ***b*** – HbO_2 (*FS*, *T* = 87 K), ***c*** – Hb (*FS*, *T* = 87 K), ***d*** – SNP (295 K), ***e*** – Hb(PLP + GA)O_2 (*FS*, *T* = 87 K), ***f*** – Hb(PLP + GA)O_2 (*LF*, *T* = 87 K), ***g*** – Hb(PLP + GA)O_2 (*LF*, *T* = 295 K).

Table I. Results of the Mössbauer spectra fitting using 1 quadrupole doublet and a superposition of 2 quadrupole doublets[a]

Sample (spectrum in Figure 1)	Γ, (mm/s)	Γ_1, (mm/s)	Γ_2, (mm/s)	S_1, (%)	S_2, (%)	δ_1, (mm/s)	δ_2, (mm/s)	ΔE_{Q1}, (mm/s)	ΔE_{Q2}, (mm/s)
HbO_2, (***a***)	0.377	0.256	0.450	53	47	0.267	0.264	2.174	1.830
HbO_2, (***b***)	0.388	0.230	0.402	50	50	0.261	0.268	2.191	1.859
Hb (***c***)	0.335	0.270	0.291	51	49	0.899	0.936	2.385	2.215
SNP (***d***)	0.246	–	–	–	–	–	–	–	–
Hb(PLP+GA)O_2 (***e***)	0.425	0.253	0.422	44	56	0.256	0.252	2.137	1.776
Hb(PLP+GA)O_2 (***f***)	0.391	0.246	0.437	52	48	0.264	0.264	2.142	1.757
Hb(PLP+GA)O_2 (***g***)	0.383	0.280	0.270	48	52	0.182	0.190	1.814	1.519

[a] Experimental errors for Γ were from ± 0.019 to ± 0.028 mm/s, for δ and ΔE_Q were from ± 0.009 to ± 0.014 mm/s.

in [4]. Samples of these hemoglobins and the standard absorber of sodium nitroprusside (SNP) were measured using a high precision, sensitive and stable spectrometer SM-2201 with characteristics given in [4]. Hemoglobin samples were measured at 87 K, the lyophilized Hb(PLP + GA)O_2 sample was measured at room temperature as well. The standard absorber SNP was measured at room temperature. Mössbauer spectra were computer fitted with the least squares procedure using Lorentzian line shape and with the distribution of quadrupole splitting. Mössbauer parameters (quadrupole splitting ΔE_Q, isomer shift δ, line width Γ, subspectrum area S) were determined from the least squares fit. The values of Mössbauer parameters of the ^{57}Fe in the beryllium window of the scintillator detector Be(^{57}Fe) were determined from an independent measurement and fixed during the hemoglobin spectra fitting. The values of the isomer shift are given relative to α-Fe at 295 K.

3. Results and discussion

Mössbauer spectra of hemoglobin samples and SNP as well as distributions of quadrupole splitting are shown in Figures 1 and 2. Mössbauer parameters obtained from 1 and a superposition of two quadrupole doublets (except SNP) are given in Table I. The approximation by two quadrupole doublets with similar areas related to the small structural differences of the heme iron stereochemistry in α- and β-subunits of hemoglobin tetramers is illustrated in Figure 3.

Theoretical quantum chemical calculations [5] for the heme models in α- and β-subunits of Hb showed different ΔE_Q temperature dependencies which were in agreement with ΔE_{Q1} and ΔE_{Q2} differences at 87 K. We also pointed out that Γ values for HbO_2 and Hb Mössbauer spectra were higher than Γ for SNP (1 doublet fit). This broadening of HbO_2 and Hb Mössbauer spectra lines may also reflect a superimposed nature of these spectra. The larger values of Γ_2 may reflect an O_2 rotation in β-subunits and a distribution of Fe–O–O angles frozen at 87 K.

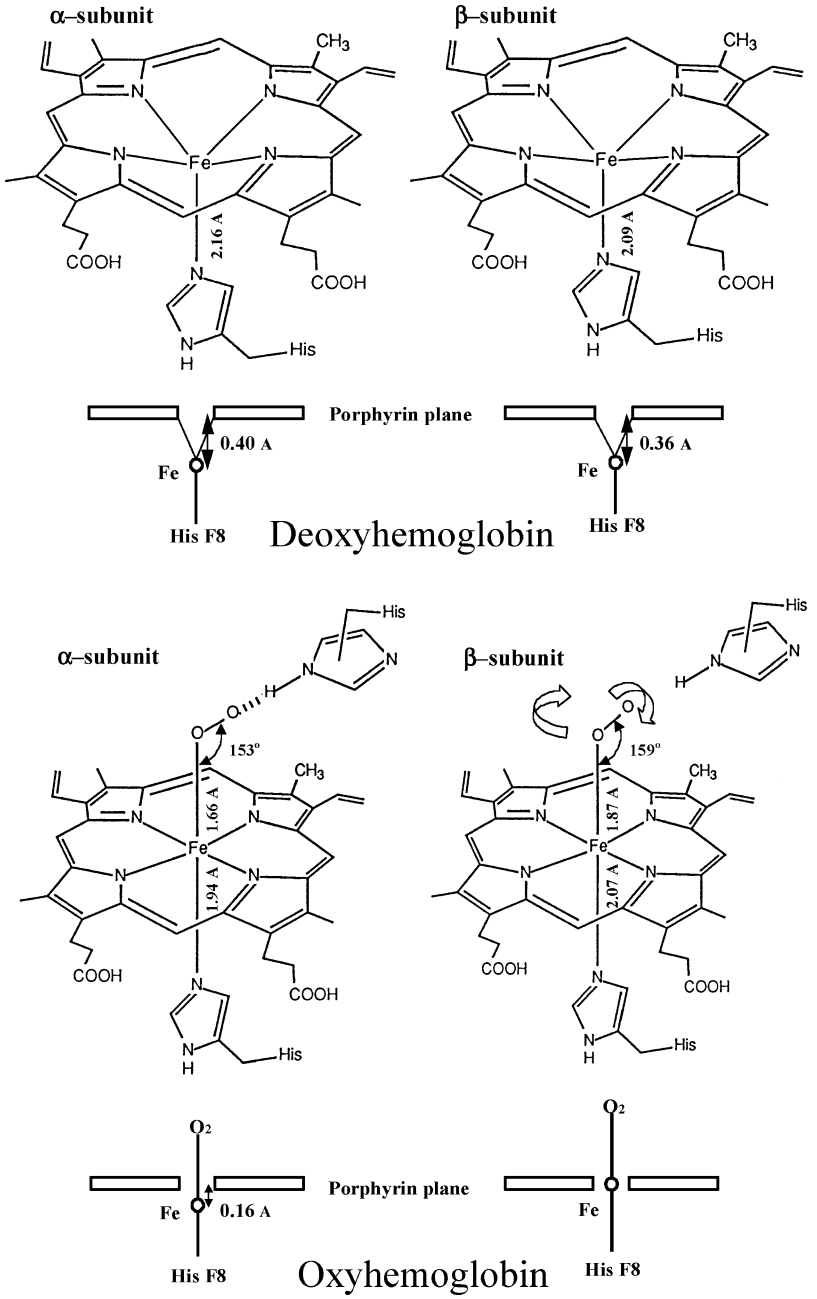

Figure 3. Differences of the heme iron stereochemistry in α- and β-subunits in deoxy- and oxyhemoglobins.

The distributions of ΔE_Q for HbO_2 Mössbauer spectra (*a*, *b*, *e* and *f*) with the same asymmetry revealed two-peak distributions with different peak intensities (*a*, *b* and *e*) and broad one-peak distribution (*f*). Mössbauer spectra of two

human HbO_2 samples in frozen solutions were identical while its distributions of ΔE_Q were different, although we cannot expect any differences of the conformational substates in these hemoglobins. On the other hand, similar distributions of ΔE_Q for Mössbauer spectra of one non–modified human HbO_2 (sample *b*) and sample of modified Hb(PLP + GA)O_2 in frozen solutions should not be considered as evidence of the similar conformational substates in native and cross-linked hemoglobins. Distributions of ΔE_Q for Mössbauer spectra of Hb, SNP and Hb(PLP + GA)O_2 (*c*, *d*, and *g*) with symmetrical lines were narrow (*d* and *g*) and broad (*c*) one-peak distributions. The distribution of a ΔE_Q for the Mössbauer spectrum of the lyophilized modified hemoglobin measured at room temperature appeared to be narrower than that of hemoglobin in frozen solution, although we can expect an increase of conformational substates of hemoglobin at room temperature. Thus, this fitting does not permit us to explain different distributions for similar spectra in terms of conformational substates of hemoglobins.

4. Conclusion

The comparison of various fittings of hemoglobin Mössbauer spectra shows that the approximation using a superposition of two quadrupole doublets with similar areas seems to be more reliable and correlates with differences of the heme iron electronic structure and stereochemistry in α- and β-subunits of hemoglobin tetramers.

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