# The Features of Mössbauer Spectra of Hemoglobin in Relation to the Quadrupole Splitting and Heme Iron Stereochemistry*

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Mössbauer spectra of various human ferrous hemoglobins in different ligand forms demonstrated some features such as non-Lorentzian line shape for oxyhemoglobins, narrower line width for carbonmonoxide, and variations of quadrupole splitting for different hemoglobins. These features were considered in relation to the variations of quadrupole splitting and heme iron stereochemistry in different hemoglobins as well as in non-equivalent subunits of hemoglobin tetramer.

*Key words:* Mössbauer Spectroscopy; Human Hemoglobins; Quadrupole Splitting; Heme Iron Stereochemistry.

## Introduction

Hemoglobin is a vitally important protein related with the oxygen transport in the body. Mammalian hemoglobins consist of two pairs of non-equivalent subunits ($\alpha$- and $\beta$-subunits in adult hemoglobin, $\alpha$- and $\gamma$-subunits in fetal hemoglobin, etc.) [1]. Each subunit contains a protein chain which binds an iron-porphyrin complex (heme) as the active site. The heme iron reversibly binds oxygen molecule. The various types of hemoglobins have a wide range of the oxygen affinity related with the hemoglobin structure including the heme iron electronic structure and stereochemistry [2].

The presence of iron ions at the active site of hemoglobin permits to apply Mössbauer spectroscopy for the analysis of the heme iron electronic structure. The first results of Mössbauer studies of hemoglobin were published more then 30 years ago [3 - 5]. The following three decades of Mössbauer spectroscopy of hemoglobin gave a lot of important information (see [6 - 11]). However, there are some features of Mössbauer spectra of hemoglobin whose reasons are not clear yet.

In the present work we consider some features of Mössbauer spectra of human ferrous hemoglobins measured in the liquid nitrogen temperature region, in relation with the heme iron electronic structure and quadrupole splitting.

## Materials and Methods

We have studied various human hemoglobins such as normal adult and fetal hemoglobins in deoxy-, oxy-, and carbonmonoxy- forms (HbA, HbF, $HbAO_2$, $HbFO_2$, HbACO, HbFCO, respectively), hemoglobin from patients with leukemia and erythremia in deoxy- and oxy- forms (HbL, $HbLO_2$, respectively) [oxy-forms were obtained in red blood cells (RBC), while other derivatives were prepared in hemolyzate], and $HbAO_2$ in concentrated solution (CS) and in lyophilized form (LF). The methods of sample preparation were described in [12 - 16].

The Mössbauer spectra measurements at 87 K were described in details in [12, 14]. The Mössbauer spectra were fitted by the least squares procedure using Lorentzian line shape. Mössbauer parameters such as quadrupole splitting ($\Delta E_Q$), isomer shift ($\delta$), line width ($\Gamma$), and relative area ($S$) were evaluated.

## Results

### *Mössbauer Spectra and their Features*

Mössbauer spectra of normal adult and fetal hemoglobins in various forms and lyophilized $HbAO_2$

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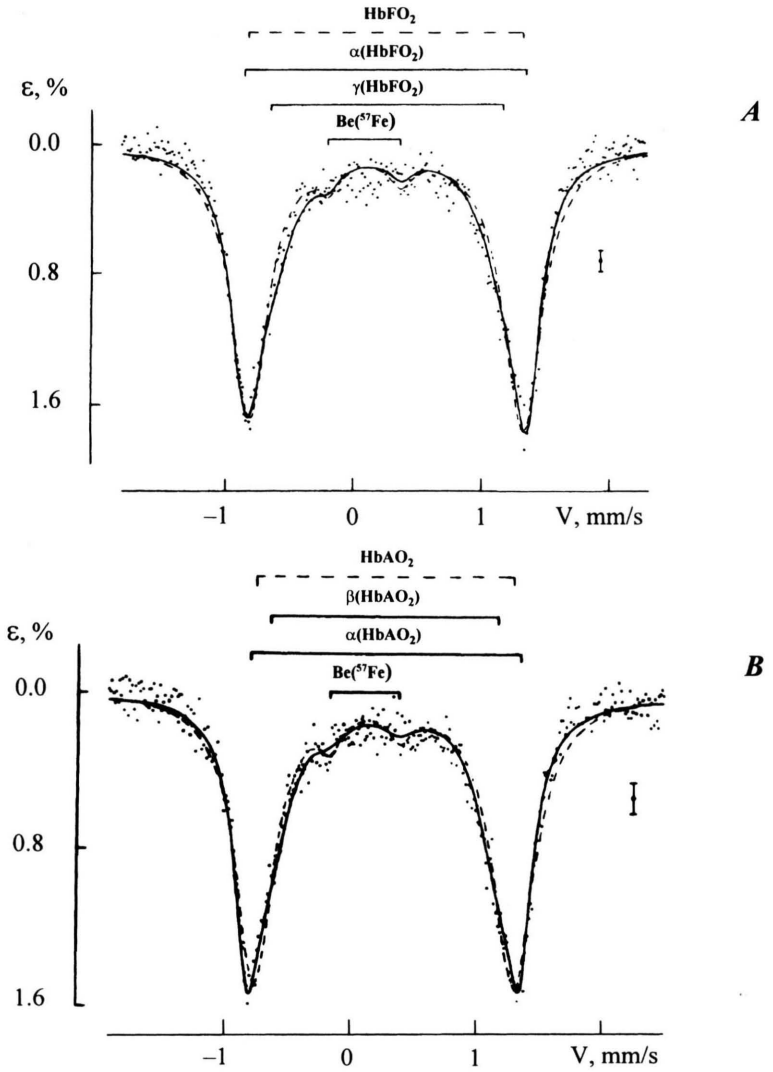


Fig. 1. Mössbauer spectra of $HbFO_2$ (A) and $HbAO_2$ (B). $T$ = 87 K. The dashed lines result from the least squares fit with one quadrupole doublet. The solid lines result from the least squares fit with two quadrupole doublets.

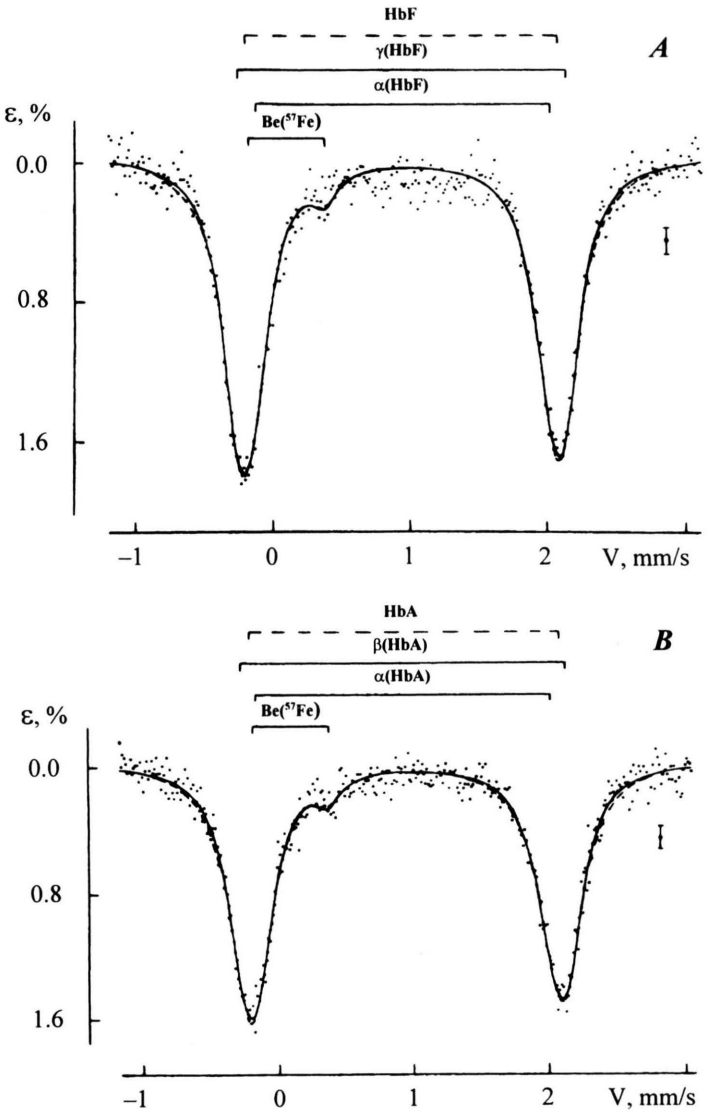


Fig. 2. Mössbauer spectra of HbF (A) and HbA (B). $T$ = 87 K. The dashed lines result from the least squares fit with one quadrupole doublet. The solid lines result from the least squares fit with two quadrupole doublets.

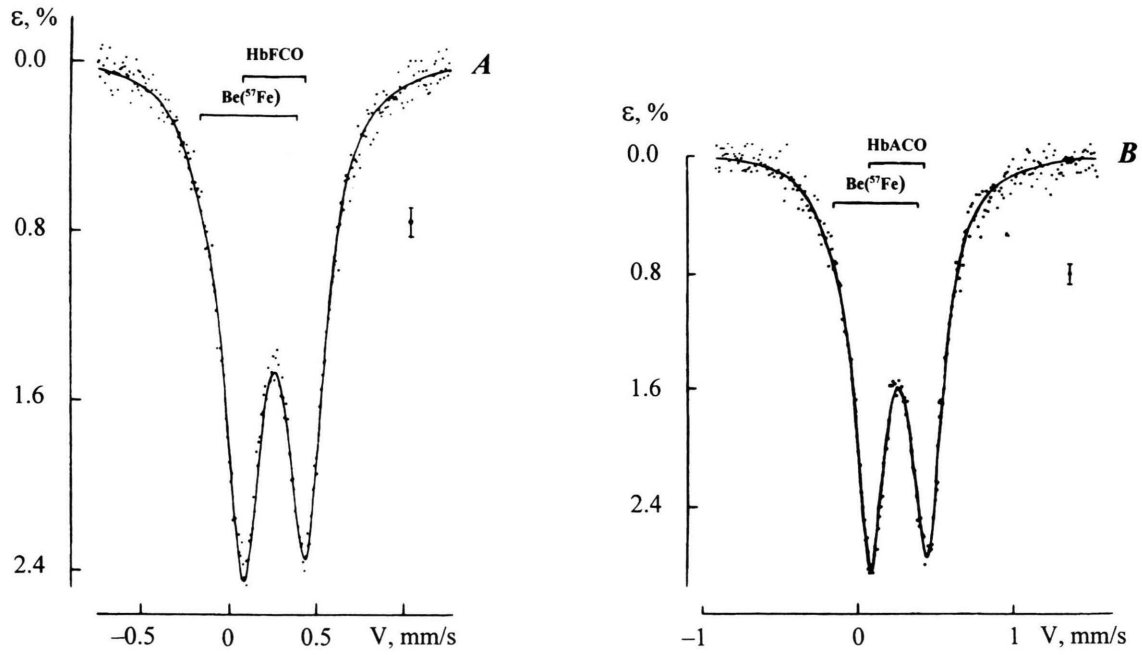


Fig. 3. Mössbauer spectra of HbFCO (A) and HbACO (B). $T = 87$ K. The solid lines result from the least squares fit with one quadrupole doublet.

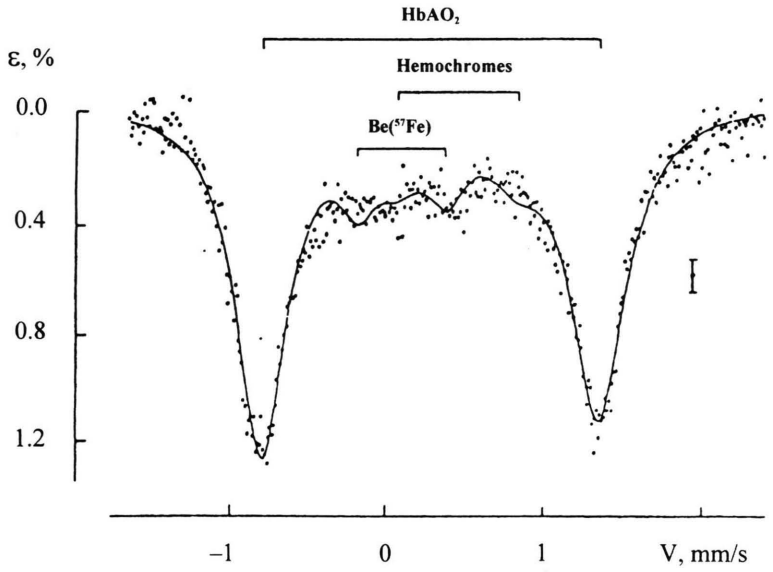


Fig. 4. Mössbauer spectrum of $HbAO_2$ in lyophilized form. $T = 87$ K. The solid line results from the least squares fit with one quadrupole doublet.

are shown in Figures 1 - 4. The Mössbauer spectra of the patient's hemoglobin look like those of corresponding normal samples [14, 15]. Mössbauer spectra of $HbAO_2$ in RBC and CS have different absorption effects ($\varepsilon$) due to the high hemoglobin concentration in solution only [16].

The Mössbauer spectra of hemoglobin were fitted using one quadrupole doublet (we excluded from consideration subspectra of $^{57}Fe$ nuclei in the scintillator detector beryllium window Be($^{57}Fe$) for all spectra, ferritin-like iron for spectra of hemoglobin from several patients with erythremia, and hemochromes impurity for the spectrum of $HbAO_2$ in LF). Mössbauer parameters are given in Table 1 in comparison with data for the standard absorber of sodium nitroprusside (SNP).

Table 1. Mössbauer parameters of hemoglobin derivatives and standard absorber resulting from one doublet approximation.

| Sample | $\Gamma_{left}$, mm/s | $\Gamma_{right}$, mm/s | $\Delta E_Q$, mm/s | $\delta$, mm/s | $\chi^2$ |
|---|---|---|---|---|---|
| $HbAO_2$ (RBC) | 0.370 | 0.349 | 2.078 | 0.244 | 1023 |
| $HbAO_2$ (CS) | 0.346 | 0.337 | 2.078 | 0.265 | 990 |
| $HbAO_2$ (LF) | 0.389 | 0.406 | 2.139 | 0.276 | 563 |
| $HbFO_2$ | 0.386 | 0.354 | 2.117 | 0.260 | 985 |
| $HbLO_2$ | 0.370 | 0.379 | 2.120 | 0.270 | 957 |
| HbA | 0.359 | 0.359 | 2.317 | 0.932 | 561 |
| HbF | 0.354 | 0.354 | 2.284 | 0.937 | 557 |
| HbL | 0.327 | 0.327 | 2.303 | 0.950 | 580 |
| HbACO | 0.243 | 0.259 | 0.369 | 0.264 | 550 |
| HbFCO | 0.263 | 0.264 | 0.366 | 0.261 | 582 |
| SNP | 0.260 | 0.260 | 1.7034 | –0.260 | 521 |

Maximal experimental error for $\Delta E_Q$ and $\delta$ was $\pm$ 0.013 mm/s. Maximal experimental error for $\Gamma_{left}$ and $\Gamma_{right}$ was $\pm$ 0.026 mm/s.

The first feature of the obtained Mössbauer spectra is the non-Lorentzian line shape of various oxyhemoglobins in RBC or CS, while spectra of other derivatives and $HbAO_2$ in LF have Lorentzian line shape. The asymmetry of Mössbauer spectra lines is clearly seen in Fig. 1 (the deviation of the dashed lines) and from Table 1 (high values of the statistical criteria $\chi^2$). The second feature is the same narrow line width for HbACO, HbFCO and SNP, while spectra of other hemoglobin samples have broader line width (see Table 1). The third feature is the variation of the $\Delta E_Q$ values for different hemoglobins in deoxy- and oxy- forms, while the $\Delta E_Q$ values are the same for the carbonmonoxy-form of hemoglobins (see Table 1).

## Discussion

*Variations of the Heme Iron Electronic Structure and Stereochemistry and Quadrupole Splitting as the Reason of the Features of Mössbauer Spectra*

The non-Lorentzian (asymmetrical) line shape of Mössbauer spectra of oxyhemoglobin in RBC and solution was observed in several studies [17 - 22]. However, this fact was not yet adequately explained. Some explanations of the non-Lorentzian line shape were critically considered in [12, 16], so we will not consider them here. We proposed that the non-Lorenzian line shape as well as other features of the spectra are related with the heme iron electronic structure and stereochemistry in various hemoglobins and their non-equivalent subunits.

Let us assume firstly the same iron electronic structure in both types of subunits of each studied hemoglobin ($\alpha$- and $\beta$-subunits in adult hemoglobin, $\alpha$- and $\gamma$-subunits in fetal hemoglobin, etc.) and consider the results of Mössbauer spectra fitting with one quadrupole doublet (Table 1). Slight differences of the $\Delta E_Q$ values for HbA, HbL and HbF, for $HbAO_2$, $HbFO_2$ and $HbLO_2$, for $HbAO_2$ in RBC or CS and $HbAO_2$ in LF are clearly seen. These differences of $\Delta E_Q$ could be the result of small variations of the heme iron electronic structure and stereochemistry in different hemoglobins. For instance, structural differences between HbA and HbF were found by X-ray structure analysis [23] and XANES spectroscopy [24]. Thus, possible variations of molecular structure of adult, fetal, patient's and lyophilized hemoglobins could be reflected by the small changes of the heme iron electronic structure and $\Delta E_Q$ value. We could suppose that the differences of the $\Delta E_Q$ values for adult and fetal hemoglobins in both deoxy- and oxy-forms indicate small structural differences in distal and proximal heme regions. On the other hand, the differences of $\Delta E_Q$ of normal adult and patient's oxyhemoglobins, and the same values of $\Delta E_Q$ for deoxyhemoglobins indicate small structural differences in the distal heme region only. This supposition is in agreement with the results of a comparative study of normal adult hemoglobin and anomalous hemoglobin Zürich with amino acid substitution in the distal heme region [25].

However, the $\Delta E_Q$ values for HbACO and HbFCO were the same. A small value of $\Delta E_Q$ for HbACO and HbFCO indicates a strong Fe(II)-CO bond with nearly spherical symmetry of the electric field which smooths away the contribution of the heme iron electronic structure distinctions resulting from the different heme iron stereochemistry. Therefore, the $\Delta E_Q$ values for the carbonmonoxy-form of various mammalian hemoglobins should be the same and HbACO and HbFCO Mössbauer spectra could be fitted with one quadrupole doublet because we may neglect the influence of the small variations of the heme iron stereochemistry in adult and fetal hemoglobins as well as in their non-equivalent subunits. It should be noted that the narrow line width of HbACO and HbFCO Mössbauer spectra (which was the same as that of the SNP Mössbauer spectrum) reflects also the absence of the influence of any structural variations due to a strong Fe(II)-CO bond. In this case, a broadening of the Mössbauer spectra of other hemoglobin deriva-

Table 2. Mössbauer parameters of hemoglobin derivatives resulting from two doublets approximation.

| Sample | $\Gamma_1$, mm/s | $\Delta E_{Q1}$, mm/s | $\delta_1$, mm/s | $\Gamma_2$, mm/s | $\Delta E_{Q2}$, mm/s | $\delta_2$, mm/s | $S_1$, % | $S_2$, % | $\chi^2$ |
|---|---|---|---|---|---|---|---|---|---|
| $HbAO_2$ (RBC) | 0.257 | 2.154 | 0.244 | 0.470 | 1.809 | 0.235 | 51 | 49 | 568 |
| $HbAO_2$ (CS) | 0.227 | 2.165 | 0.264 | 0.358 | 1.800 | 0.265 | 58 | 42 | 525 |
| $HbAO_2$ (LF) | 0.303* | 2.192 | 0.281 | 0.526 | 1.987 | 0.253 | 52 | 48 | 596 |
| $HbFO_2$ | 0.252 | 2.207 | 0.261 | 0.407 | 1.868 | 0.259 | 54 | 46 | 545 |
| $HbLO_2$ | 0.272 | 2.205 | 0.272 | 0.406 | 1.891 | 0.262 | 54 | 46 | 557 |
| HbA | 0.270* | 2.434 | 0.942 | 0.299* | 2.159 | 0.946 | 51 | 49 | 525 |
| HbF | 0.262* | 2.391 | 0.931 | 0.300* | 2.107 | 0.936 | 52 | 48 | 592 |

Maximal experimental error for $\Delta E_Q$ and $\delta$ was $\pm$ 0.013 mm/s. Maximal experimental error for $\Gamma$ was $\pm$ 0.026 mm/s. * Fixed parameter.

tives may be related with the presence of more than one quadrupole doublet in the spectra, resulting from any structural differences at the active sites. Therefore it is reasonable to take into consideration the small differences of the heme iron stereochemistry in non-equivalent subunits shown by X-ray structure analysis (see [26]).

To take account of the non-equivalent hemoglobin subunits, we fitted Mössbauer spectra of hemoglobins with two quadrupole doublets. The Mössbauer parameters are given in Table 2. The areas of both doublets appeared to be almost the same, in agreement with the equal distribution of the $^{57}Fe$ nuclei in each type of subunits in hemoglobin tetramer. Asymmetric Mössbauer spectra of $HbAO_2$ in RBC and CS, $HbFO_2$ and $HbLO_2$ were better fitted with two quadrupole doublets (see Fig. 1, solid lines), and the values of $\chi^2$ were strongly decreased, while symmetric spectra were fitted without any significant changes of $\chi^2$ values. Therefore, the slight differences of the $\Delta E_Q$ values for both doublets could be related with structural differences in the active sites of $\alpha$- and $\beta$-subunits of normal adult hemoglobin, of $\alpha^L$- and $\beta^L$ -subunits of lyophilized hemoglobin, of $\alpha$- and $\gamma$-subunits of fetal hemoglobin as well as of $\alpha^*$- and $\beta^*$-subunits of patient's hemoglobin. However, in this case it is difficult to explain the different line widths of both doublets for oxyhemoglobins.

The results of the X-ray structural analysis of $HbAO_2$ crystals [27] showed different possibilities of the $O_2$ molecule rotation around the Fe-O axis in the $\alpha$- and $\beta$-subunits. The rotation of $O_2$ in $\alpha$-subunits is sterically limited, and terminal oxygen forms

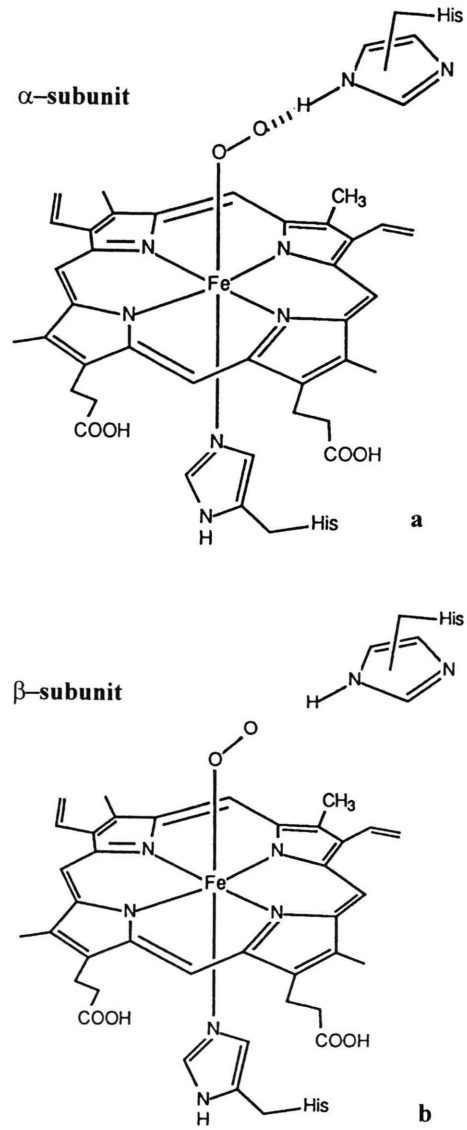


Fig. 5. The porphyrin (heme) structures in $HbAO_2$ tetramer. a: $\alpha$-subunit; b: $\beta$-subunit.

a strong hydrogen bond with $N_\varepsilon$(*His E7*) (see Figure 5a). In contrast, the $O_2$ molecule in $\beta$-subunits is free to rotate over a large range around the Fe-O axis (see Figure 5b). Further studies also showed that in $\alpha$-subunits *His E7* stabilizes the bound $O_2$ molecule, possibly by hydrogen bonding, while in $\beta$-subunits the $O_2$ molecule has unlimited freedom of rotation and a hydrogen bond with $N_\varepsilon$(*His E7*) is apparently lacking [28]. Basing on these results we could suppose that a high value of $\Gamma_2$ at 87 K

reflects the various orientations of the $O_2$ molecule in $\beta$-subunits frozen at this temperature. Theoretical calculations showed small variations of $\Delta E_Q$ within 0.5 mm/s with variations of $O_2$ molecule orientations around the Fe-O axis [29]. Moreover, the $\Delta E_Q$ value is influenced by variations of the Fe-O-O angle [30]. Therefore, the distribution of the $\Delta E_Q$ values related with possible orientations of the $O_2$ molecule about its equilibrium position could lead to the broadening of the second doublet line. The value of $\Gamma_1$ at 87 K could reflect limitations of the $O_2$ molecule rotation in $\alpha$-subunits.

Basing on the stereochemical differences of the heme iron in $\alpha$- and $\beta$-subunits we could also suppose a different Fe(II) spin state in non-equivalent subunits. The heme iron in $\beta$-subunits is in the heme plane [27], and therefore in the low spin state $S = 0$. On the other hand, the heme iron in $\alpha$-subunits is out of the heme plane [27] and may be in the intermediate spin state $S = 1$ (The iron spin state/stereochemical relationship in heme proteins was considered in [31]). In this case, two Fe(II) electronic configurations for the Fe(II)-$O_2$ bond could be used as the ground states. Thus, the Fe(II)-$O_2$ bond may be described by the Pauling model Fe(II)($S = 0$)-$O_2$($S = 0$) for $\beta$-subunits and by the Goddard and Olafson model Fe(II)($S = 1$)-$O_2$($S = 1$) for $\alpha$-subunits (see: [32, 33]). It is possible that the $O_2$ molecule has a different equilibrium position in $\beta^L$-subunits of lyophilized $HbAO_2$ with more possible orientations. This difference may be a result of the of $H_2O$ molecule being influenced by the Fe(II)-$O_2$ bond in the heme pockets of $HbAO_2$ in RBC or CS (see [16]). The same explanation of the relations between Mössbauer parameters and Fe(II)-$O_2$ bonds may be used for $HbFO_2$ and $HbLO_2$, taking into account an additional corresponding stereochemical differences of the heme iron in non-equivalent subunits.

Comparison of the differences of the heme iron stereochemistry in $\alpha$- and $\beta$-subunits in HbA (see [26]) with Mössbauer parameters from Table 2 showed that we could relate the smaller $\Delta E_Q$ value with the smaller distances of Fe-$N_\varepsilon$(*His F8*) and Fe-heme plane in $\beta$-subunits. This supposition correlates with our calculations of the quadrupole splitting temperature dependence by the iterative extended Hückel methods for the heme models in $\alpha$- and $\beta$-subunits of HbA [34]. The difference of the theoretical $\Delta E_Q$ values for $\alpha$- and $\beta$-subunits at 87 K is comparable with the experimental results. It is possible that the heme iron stereochemistry in $\alpha$- and $\gamma$-subunits of HbF differ from those of HbA due to smaller iron-ligand distances or other changes. It should be noted that association of $2\alpha\beta$ and $2\alpha\gamma$ subunits in tetramers could lead to a slightly different structural modification of $\alpha$-subunits in HbA and HbF.

Thus, the features of Mössbauer spectra of some human ferrous hemoglobins are considered as a result of the variations of the heme iron electronic structure and stereochemistry in non-equivalent subunits and in different tetrameric hemoglobins and, therefore, as a result of the presence of superposition of two quadrupole doublets in Mössbauer spectra of tetrameric hemoglobins.

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[1] J. M. Baldwin, Prog. Biophys. Molec. Biol. **29**, 225 (1975).
[2] M. F. Perutz, Ann. Rev. Biochem. **48**, 327 (1979).
[3] U. Gonser, R. W. Grant, and J. Kregzde, Appl. Phys. Lett. **3**, 189 (1963).
[4] U. Gonser and R. W. Grant, Biophys. J. **5**, 823 (1965).
[5] G. Lang and W. Marshall, Proc. Phys. Soc. **87**, 3 (1966).
[6] A. Vertes, L. Korecz, and K. Berger, Mössbauer Spectroscopy, Academia Kiada, Budapest 1979, p. 344.
[7] K. Spartalian and G. Lang, in: Applications of Mössbauer Spectroscopy, Vol. II, Academic Press, Inc., New York 1980, p. 249.
[8] A. Trautwein and E. Bill, in: Transition Met. Chem., Proc. Workshop 1980, Verlag Chemie 1981, p. 239.
[9] D. P. E. Dickson, in: Mössbauer Spectroscopy Applied to Inorganic Chemistry, ed. G. J. Long, Vol. 1, Plenum, New York 1984, p. 339.
[10] M. I. Oshtrakh, Hyperfine Interact. **66**, 127 (1991).
[11] M. I. Oshtrakh, Z. Naturforsch. **51a**, 381 (1996).
[12] M. I. Oshtrakh and V. A. Semionkin, Mol. Biol. Moscow **19**, 1310 (1985).
[13] B. N. Burykin, V. A. Khleskov, M. I. Oshtrakh, and V. A. Semionkin, Mol. Biol. Moscow **21**, 1677 (1987).
[14] M. I. Oshtrakh and V. A. Semionkin, FEBS Lett. **208**, 331 (1986).

[15] M. I. Oshtrakh and V. A. Semionkin, FEBS Lett. **257**, 41 (1989).
[16] M. I. Oshtrakh, J. Inorg. Biochem. **56**, 221 (1994).
[17] E. R. Bauminger and S. Ofer, Proc. Ind. Natl. Sci. Academy, Proc. Int. Conf. Appl. Mössbauer Effect, New Delhi 1982, p. 61.
[18] M. I. Oshtrakh and V. A. Semionkin, Biophysica Moscow **28**, 135 (1983).
[19] L. Cianchi, F. Pieralli, F. Del Giallo, M. Mancini, G. Spina, and L. Fiesoli, Phys. Lett. **100A**, 57 (1984).
[20] B. Boso, P. G. Debrunner, G. C. Wagner, and T. Inubushi, Biochim Biophys Acta **791**, 244 (1984).
[21] G. R. Hoy, D. C. Cook, R. L. Berger, and F. K. Friedman, Biophys. J. **49**, 1009 (1986).
[22] V. E. Prusakov, R. A. Stukan, and V. I. Goldanskii, Biomed. Sci. **2**, 127 (1991).
[23] J. A. Frier and M. F. Perutz, J. Mol. Biol. **112**, 97 (1977).
[24] A. Bianconi, A. Congiu-Castellano, M. Dell'Ariccia, A. Giovannelli, E. Burattini, M. Castagnola, and P. J. Durham, Biochim. Biophys. Acta **831**, 120 (1985).
[25] E. Bill, E. Di Iorio, A. Trautwein, and K. Winterhalter, Proc. Ind. Natl. Sci. Academy, Proc. Int. Conf. Appl. Mössbauer Effect, New Delhi 1982, p. 648.
[26] M. F. Perutz, G. Fermi, B. Luisi, B. Shaanan, and R. C. Liddington, Acc. Chem. Res. **20**, 309 (1987).
[27] B. Shaanan, J. Mol. Biol. **171**, 31 (1983).
[28] J. S. Olson, A. J. Mathews, R. J. Rohlfs, B. A. Springer, K. D. Egelberg, S. G. Sligar, J. Tame, J.-P. Renaud, and K. Nagai, Nature London **336**, 265 (1988).
[29] Z. S. Herman and G. H. Loew, J. Amer. Chem. Soc. **102**, 1815 (1980).
[30] R. F. Kirchner and G. H. Loew, J. Amer. Chem. Soc. **99**, 4639 (1977).
[31] W. R. Scheidt and C. A. Reed, Chem. Rev. **81**, 543 (1981).
[32] L. Pauling, Nature London **203**, 182 (1964).
[33] W. A. Goddard, III and B. D. Olafson, Proc. Natl. Acad. Sci. USA **72**, 2335 (1975).
[34] V. A. Khleskov, B. N. Burykin, A. B. Smirnov, and M. I. Oshtrakh, Biochem. Biophys. Res. Communs. **155**, 1255 (1988).