Biomedical application of Mössbauer spectroscopy with a high velocity resolution: Revealing of small variations

M.I. Oshtrakh^{a,*}, V.A. Semionkin^{a,b}, O.B. Milder^{a,c}, I.V. Alenkina^{a,b} and E.G. Novikov^{a,b}

^a *Faculty of Physical Techniques and Devices for Quality Control, Physical–Technical Department, Ural Federal University, Ekaterinburg, Russian Federation*

^b *Faculty of Experimental Physics, Physical–Technical Department, Ural Federal University, Ekaterinburg, Russian Federation*

^c *Radio–Technical Department, Ural Federal University, Ekaterinburg, Russian Federation*

Abstract. Application of Mössbauer spectroscopy with a high velocity resolution for study different hemoglobins, ferritin, its models and chicken liver and spleen as well as normal and lymphoid chicken spleen demonstrated revealing of small variations of hyperfine parameters related to small variations of iron stereochemistry in biomolecules. These data demonstrate that Mössbauer spectroscopy with a high velocity resolution may be useful in biomedical research to distinguish small variations of iron-containing proteins in normal and pathological cases.

Keywords: Mössbauer spectroscopy with a high velocity resolution, biomedical applications, small variations

1. Introduction

Mössbauer spectroscopy is a powerful tool which allows us to observe the hyperfine splitting of the nuclear energy levels as well as changes of energies of the ground and excited states of Mössbauer nuclei (for instance, ^{57}Fe , ^{119}Sn , ^{197}Au and some other) in the absorption or emission spectrum of γ -rays (detailed description of the Mössbauer effect see, for instance, in [1–3,16]). Iron is one of the most vitally important metals. A number of proteins contain iron as an active site. Some proteins are responsible for the iron transport and storage. The functional properties of these proteins are determined in part by the iron electronic structure and stereochemistry. Therefore, some functional variations of proteins related to the structural heterogeneity may be reflected by the small changes of the iron electronic structure. It is well known that several diseases, so-called molecular diseases, are caused or accompanied by the synthesis of anomalous biomolecules or any other protein biosynthesis disturbance. Some pathological states of the body are caused by environmental factors that may affect biological molecules, cells and tissues. Therefore, Mössbauer spectroscopy was successfully applied for studying biomolecules containing iron in normal and pathological cases [4,7,10,11].

*Corresponding author: M.I. Oshtrakh, Faculty of Physical Techniques and Devices for Quality Control, Physical–Technical Department, Ural Federal University, Ekaterinburg, Russian Federation. E-mail: oshtrakh@mail.utnet.ru.

Mössbauer spectroscopy appeared to be useful for studying so-called “drastic” and “small” changes of the iron electronic structure [6,8,9]. “Drastic” changes imply a change of the valence and/or spin state of iron resulting from the transformation or destruction of proteins. In this case Mössbauer spectra or subspectra may be well defined. “Small” changes imply a change of the iron electronic structure without any change of the valence and spin state resulting from structural modifications of protein. The study of “small” changes is more complicated and requires a high precision, sensitive and stable Mössbauer spectrometer. These structural modifications may be related to protein heterogeneity and functional variety in different organs, in human and various animals as well as in normal and pathological subjects due to their specific structure–function relationship. Small stereochemical variations of iron neighboring atoms may change energies of the ground and low-lying iron electronic terms and, therefore, change electric field gradient (EFG) on the ^{57}Fe . Quadrupole splitting is Mössbauer parameter which depends on the EFG. Therefore, small variations of iron stereochemistry can be revealed by detecting small variations of quadrupole splitting in Mössbauer spectra. However, all previous Mössbauer studies were performed with a low velocity resolution (in 256 or 512 channels) that limited possibilities to reveal small variations of parameters outside the experimental error (real experimental error for Mössbauer hyperfine parameters is ± 1 channel in mm/s if calculated error did not exceed experimental one). Recently new possibilities of Mössbauer spectroscopy with a high velocity resolution (1024–4096 channels) were shown [12–14]. Therefore, in this work we demonstrate these possibilities in revealing small variations in various iron-containing species in recent biomedical research.

2. Experimental

Human adult hemoglobin and rabbit hemoglobin in concentrated solutions were obtained from the Hematological Research Center (Moscow, Russian Federation). Hemoglobin samples were oxygenated and placed into a special holder with about 2.5 ml of oxyhemoglobin solution. Then samples were immediately frozen with liquid nitrogen and stored in liquid nitrogen. Human liver ferritin in lyophilized form was obtained from the Russian State Medical University (Moscow, Russian Federation). We used 100 mg of protein for one sample. Samples of washed and lyophilized chicken liver and spleen in normal case and chicken spleen during lymphoid leukemia were obtained from the Ural State Agricultural Academy (Ekaterinburg, Russian Federation). It is well known that liver and spleen tissues contain a large amount of ferritin molecules. We used 1200–1600 mg of lyophilized tissues for one sample. We also studied industrial samples of ferritin models used for treatment of iron deficiency such as Imferon (Fisons, UK) in lyophilized form and Maltofer[®] (Vifor, Inc., Switzerland) tablet. These samples were prepared with effective thickness about 5 and 10 mg Fe/cm², respectively.

Mössbauer spectra were measured using new Mössbauer spectrometric system on the basis of high precision, sensitive and stable spectrometer SM-2201 with a high velocity resolution (measurements in 4096 channels) and temperature variable liquid nitrogen cryostat with moving absorber. Detailed description and characteristics of the system were given in [15]. The $\sim 1.85 \times 10^9$ Bq $^{57}\text{Co}(\text{Cr})$ source was used at room temperature. Mössbauer spectra of hemoglobin samples were measured at 90 K, while spectra of other samples were measured at 295 K. Spectra of samples with high iron content were measured with a good statistics for presentation in 2048 channels while spectra of samples with low iron content were measured with statistics for presentation in 1024 channels. Additionally, all measured spectra were presented with a low velocity resolution in 512 channels for comparison. Spectra were computer fitted with the least square procedure using UNIVEM-MS program with Lorentzian line shape.

Mössbauer parameters isomer shift δ , quadrupole splitting ΔE_Q , line width Γ , subspectrum area S and statistical criterion χ^2 were determined. Experimental error for determination of the each spectra point was ± 0.5 channel, experimental errors for hyperfine parameters were ± 1 channel while that for Γ was ± 2 channels. It should be noted that spectrometer characteristics determined an integral velocity error which was several times lower than a half of channel value in mm/s during spectra measurements using 4096 channels. The values of isomer shift are given relative to α -Fe at 295 K.

3. Results and discussion

The simplest way to demonstrate possibilities of Mössbauer spectroscopy in revealing of small variations of the hyperfine parameters may be performed using the simplest fitting of measured spectra. In this case we used one quadrupole doublet fit for all measured spectra. Mössbauer spectra of human adult and rabbit oxyhemoglobins measured at 90 K and presented in 1024 channels are shown in Fig. 1(a) and (b). These spectra are similar quadrupole doublets like previous spectra of oxyhemoglobins [5]. Mössbauer hyperfine parameters for the spectra of both oxyhemoglobins presented in 512 channels and in 1024 channels are shown in the plot of quadrupole splitting and isomer shift (Fig. 1(c)). It is clearly seen that ΔE_Q values for human adult and rabbit oxyhemoglobins distinguish better for the high velocity resolution spectra. An increase of ΔE_Q for rabbit oxyhemoglobin indicates that EFG on the ^{57}Fe in this protein also increases. This may be a result of small changes of the energies of low-lying iron electron terms due to small stereochemical variations. For instance, it is possible some changes in the $\text{Fe(II)}-\text{O}_2$ bond in rabbit oxyhemoglobin in comparison with that for human adult oxyhemoglobin.

Mössbauer spectra of Imferon, Maltofer[®] and human liver ferritin measured at room temperature and presented in 2048 channels are shown in Fig. 2(a)–(c). These spectra demonstrated similar quadrupole doublets. Differences in absorption effect are related to differences in the effective thickness of these samples. Mössbauer hyperfine parameters are shown in Fig. 2(d) in the plot of quadrupole splitting and isomer shift. In this case improvement in velocity resolution leads to revealing small differences of both ΔE_Q and δ . It is well known that the iron cores in ferritins are in the form of ferrihydrite while those in Imferon and Maltofer[®] are in the form of β - FeOOH (both are different modifications of hydrous ferric oxide). An increase of ΔE_Q values is related to increase of EFG on the ^{57}Fe in Imferon and Maltofer[®]. A decrease of δ values is related to decrease of the electron density on the ^{57}Fe . This may be a result of stereochemical variations of Fe–O bonds such as changes of angles and distances. In fact, these results demonstrated as rough approximation revealed small structural differences in the iron cores of human liver ferritin, Imferon and Maltofer[®] which are related to small variations of Mössbauer hyperfine parameters.

Mössbauer spectra of chicken tissues demonstrated similar quadrupole doublets; however, absorption effect was significantly less than for extracted human liver ferritin. Therefore, these spectra were presented in 1024 channels. Comparison of Mössbauer hyperfine parameters for human liver ferritin, normal chicken liver and spleen is shown in Fig. 3(a). It is clearly seen that ΔE_Q values differ for all samples while δ value for chicken spleen is slightly higher than that for other samples in the case of a higher velocity resolution. These data demonstrate structural differences in the iron cores of different ferritins in addition to different amino-acid and protein subunits compositions. Comparison of Mössbauer hyperfine parameters for normal and lymphoid leukemia chicken spleens is shown in Fig. 3(b). It was interesting to observe small variations of ΔE_Q and δ values in case of a high velocity resolution. An increase of ΔE_Q means an increase of EFG on the ^{57}Fe as a result of symmetry distortion and a weaker

Fig. 1. Mössbauer spectra of human adult (a) and rabbit (b) oxyhemoglobins presented in 1024 channels and small variations of their hyperfine parameters revealed with a high velocity resolution in comparison with a low velocity resolution (c): human adult oxyhemoglobin – Δ (high velocity resolution), $\blacktriangle$ (low velocity resolution) and rabbit oxyhemoglobin – $\diamond$ (high velocity resolution), $\blacklozenge$ (low velocity resolution). Experimental errors decreased twice for 1024-channel spectra in comparison with 512-channel ones. $T = 90$ K.

Fe–O bond in the iron core of iron storage proteins in lymphoid leukemia chicken spleen in comparison with that in normal chicken spleen. A weaker Fe–O bond may be a result of small increase of Fe–O distance which leads to decrease of the electron density on the ^{57}Fe and slightly less δ value.

4. Conclusion

The results of Mössbauer study of some iron-containing biomolecules and model compounds demonstrated new possibilities to reveal small variations of hyperfine parameters in the case of improving velocity resolution. Small differences of Mössbauer hyperfine parameters for different oxyhemoglobins,

Fig. 2. Mössbauer spectra of Imferon (a), Maltofer[®] (b) and human liver ferritin (c) presented in 2048 channels and small variations of their hyperfine parameters revealed with a high velocity resolution in comparison with a low velocity resolution (d): Imferon – □ (high velocity resolution), ■ (low velocity resolution); Maltofer[®] – △ (high velocity resolution), ▲ (low velocity resolution); human liver ferritin – ◇ (high velocity resolution), ◆ (low velocity resolution). Experimental errors decreased four times for 2048-channel spectra in comparison with 512-channel ones. $T = 295$ K.

for human liver ferritin, its model compounds and chicken liver and spleen as well as for normal and lymphoid leukemia chicken spleen indicated that these differences were sensitive for structural variations in the iron stereochemistry. Therefore, biomedical applications of Mössbauer spectroscopy with a high velocity resolution may be useful for revealing small variations in iron-containing biomolecules in

Fig. 3. Variations of Mössbauer hyperfine parameters for human liver ferritin (○ – high velocity resolution, ● – low velocity resolution), chicken liver (△ – high velocity resolution, ▲ – low velocity resolution) and chicken spleen (◇ – high velocity resolution, ◆ – low velocity resolution) (a) and for normal chicken spleen (◇ – high velocity resolution, ◆ – low velocity resolution) and for lymphoid leukemia chicken spleen (□ – high velocity resolution, ■ – low velocity resolution) (b). Experimental errors decreased twice for 1024-channel spectra in comparison with 512-channel ones. $T = 295$ K.

case of molecular pathology. In this case it would be possible to develop diagnostic tests on the basis of Mössbauer hyperfine parameters.

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References

- [1] H. Frauenfelder, *The Mössbauer Effect*, W.A. Benjamin, New York, 1963.
- [2] V.I. Goldanskii and R.H. Herber (eds), *Chemical Applications of Mössbauer Spectroscopy*, Academic Press, New York and London, 1968.
- [3] P. Gülich, R. Link and A. Trautwein, *Mössbauer Spectroscopy and Transition Metal Chemistry*, Springer-Verlag, Berlin–Heidelberg–New York, 1978.
- [4] M.I. Oshtrakh, Biomedical applications of the Mössbauer effect, *Hyperfine Interact.* **66** (1991), 127–140.
- [5] M.I. Oshtrakh, Comparison of human oxyhemoglobin in lyophilized form, red blood cells, and concentrated solution: the features of Mössbauer spectra and heme iron stereochemistry, *J. Inorg. Biochem.* **56** (1994), 221–231.
- [6] M.I. Oshtrakh, Mössbauer effect in biomedical research: variations of quadrupole splitting in relation to the qualitative changes of biomolecules, *Z. Naturforsch.* **51a** (1996), 381–388.
- [7] M.I. Oshtrakh, Mössbauer spectroscopy of iron containing biomolecules and model compounds in biomedical research, *J. Mol. Struct.* **480,481** (1999), 109–120.

- [8] M.I. Oshtrakh, Study of the relationship of small variations of the molecular structure and the iron state in iron containing proteins by Mössbauer spectroscopy: biomedical approach, *Spectrochim. Acta – Part A Mol. Biomol. Spectrosc.* **60** (2004), 217–234.
- [9] M.I. Oshtrakh, The relationship of Mössbauer hyperfine parameters and structural variations of iron containing proteins and model compounds in biomedical research, *Hyperfine Interact.* **159** (2004), 337–343.
- [10] M.I. Oshtrakh, Mössbauer spectroscopy: application in biomedical research, *Hyperfine Interact.* **165** (2005), 313–320.
- [11] M.I. Oshtrakh, Biomedical applications of Mössbauer spectroscopy, *J. Radioanal. Nucl. Chem.* **269** (2006), 407–415.
- [12] M.I. Oshtrakh, V.A. Semionkin, V.I. Grokhovsky, O.B. Milder and E.G. Novikov, Mössbauer spectroscopy with high velocity resolution: new possibilities of chemical analysis in material science and biomedical research, *J. Radioanal. Nucl. Chem.* **279** (2009), 833–846.
- [13] M.I. Oshtrakh, V.A. Semionkin, O.B. Milder and E.G. Novikov, Biomedical application of Mössbauer spectroscopy with high velocity resolution: preliminary results, in: *Proceedings of the International Conference “Mössbauer Spectroscopy in Materials Science 2008”*, Vol. 1070, M. Mashlan and R. Zboril, eds, AIP Conference Proceedings, Melville, NY, 2008, pp. 122–130.
- [14] M.I. Oshtrakh, V.A. Semionkin, O.B. Milder and E.G. Novikov, Mössbauer spectroscopy with high velocity resolution: new possibilities in biomedical research, *J. Mol. Struct.* **924–926** (2009), 20–26.
- [15] M.I. Oshtrakh, V.A. Semionkin, O.B. Milder and E.G. Novikov, Mössbauer spectroscopy with high velocity resolution: an increase of analytical possibilities in biomedical research, *J. Radioanal. Nucl. Chem.* **281** (2009), 63–67.
- [16] A. Vertes, L. Korecz and K. Burger, *Mössbauer Spectroscopy*, Akadémiai Kiadó, Budapest, 1979.