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Mössbauer study of red blood cells from patients with erythremia

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Red blood cell samples from several patients with erythremia have been studied by Mössbauer spectroscopy. Quadrupole splitting and isomer shift for oxyhemoglobin from erythremic red blood cells and those of oxyhemoglobin from normal ones differ slightly, while these hyperfine parameters for deoxyhemoglobins are the same. An additional component in Mössbauer spectra of red blood cell samples was observed in some cases of erythremia. It is proposed that this component is related to ferritin-like iron and its content ranged from 13–15% to 5–7%.

Mössbauer spectroscopy; Erythremia; Hemoglobin; Fe^{2+} electronic structure; Ferritin; (Normal adult red blood cell, Erythremic red blood cell)

1. INTRODUCTION

Erythremia is a malignant blood disease in which there is an overproduction of red blood cells like the overproduction of white blood cells in leukemia. Using the Mössbauer effect it was shown earlier that Fe^{2+} electronic and active site in the molecular structure of oxyhemoglobin in red blood cells from patients with leukemia differed slightly from those of normal adult oxyhemoglobin [1–3]. Recently Ortalli et al. [4] had shown similar results. Therefore, it would be of interest to test the Fe^{2+} electronic structure of hemoglobin during erythremia for comparison with data for leukemic hemoglobin. On the other hand, the Mössbauer effect study of model erythroleukemia using the lines of murine erythroleukemia cells and human K-562 cells had shown that iron ions had been incorporated into ferritin during non-differential cell growth only while induction of erythroid differentiation had led to hemoglobin biosynthesis and some iron deposited into ferritin had been used for this process [5–8]. Therefore, it would be of interest also to reveal additional iron-containing components.

2. MATERIALS AND METHODS

Red blood cell samples were prepared from venous blood of patients with erythremia by the methods described in detail in [2,9]. The concentrated erythrocytes were saturated with oxygen. One part of them was immediately frozen with liquid nitrogen while the other part was frozen after 4 days of incubation for oxyhemoglobin deoxygenation at room temperature. Thus, samples with effective thickness of 0.7 mg Fe/cm^2 containing oxy- and deoxyhemoglobin were made (see [9]). A detailed description of the Mössbauer spectra measurements at 87 K and its computer fitting have been given in [2,9].

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3. RESULTS

Mössbauer spectra of oxygenated red blood cells and deoxygenated hemolysates from two patients with erythremia are shown in figs 1 and 2. The main component of these spectra is hemoglobin in oxy- and deoxyforms. As we had pointed out, the Fe^{2+} electronic structure non-equivalence in different subunits should be taken into account during the fitting of Mössbauer spectra of tetrameric hemoglobins [2,9,10]. However, for simplification and comparison with analogous results from [2,3,9] we consider the Fe^{2+} electronic structure equivalence in different subunits in the present work. Therefore, Mössbauer spectra of hemoglobins were fitted using one quadrupole split doublet with Lorentzian line shapes. The estimations of Mössbauer parameters (isomer shifts δ and quadrupole splittings ΔE_Q) are given in table 1.

It is clearly seen that the values of ΔE_Q and δ for oxyhemoglobin in red blood cell samples from patients with erythremia are slightly higher than those of normal adults and coincide with the data of normal fetal and several samples of leukemic oxyhemoglobins (see [2]). This fact indicates that the Fe^{2+} electronic and the active site of the molecular structure in oxyhemoglobin in erythremic red blood cells could be modified in comparison with that of normal adult oxyhemoglobin. In contrast, the values of ΔE_Q and δ for deoxyhemoglobin in the samples of erythremic red blood cell hemolysates and those of normal adult deoxyhemoglobin are the same (see [9]).

It was interesting to observe an additional component in Mössbauer spectra of blood samples from patients Z. and D. (see figs 1 and 2). We assumed that this additional component is related with ferritin-like iron by analogy with model erythroleukemia cells (see [5–8]). Therefore, we used the values of ΔE_Q and δ for ferritin at 82 K from [6] to fit the Mössbauer spectra

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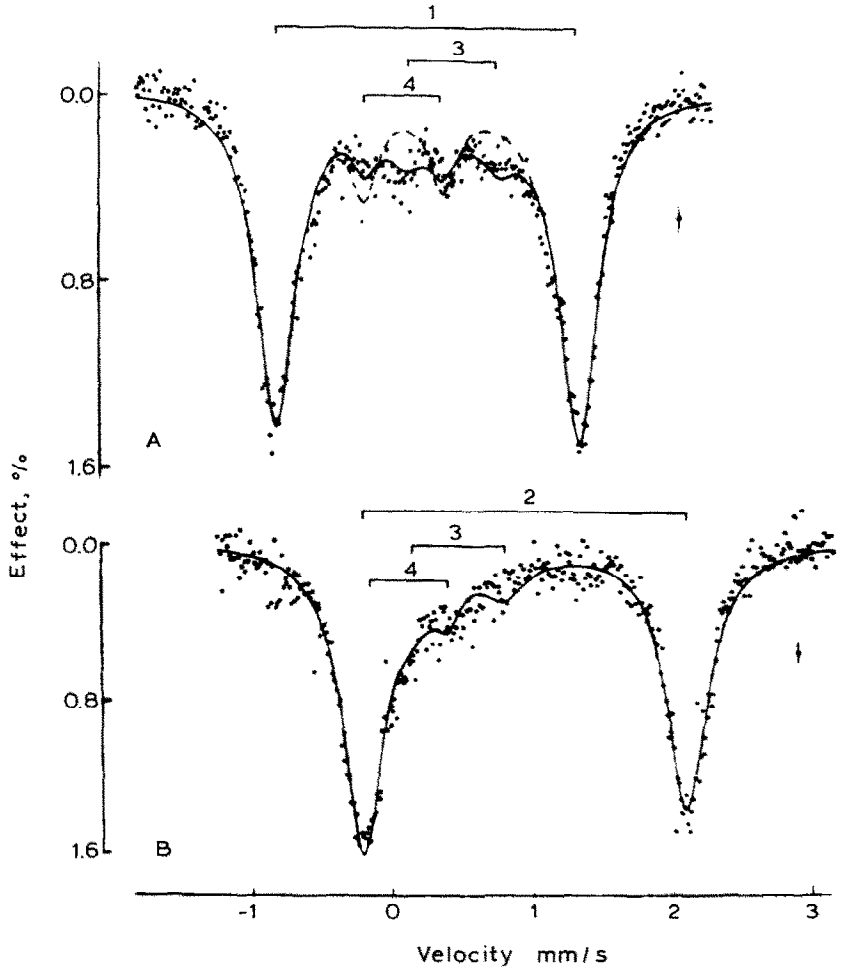

Fig.1. Mössbauer spectra of red blood cell samples from patient Z. with erythremia. (A) Oxygenated red blood cells. (B) Deoxygenated hemolysate. 1, oxyhemoglobin; 2, deoxyhemoglobin; 3, ferritin-like iron; 4, ^{57}Fe nuclei in the scintillator detector beryllium window. The dashed and continuous lines result from least squares fitting excluding and including the ferritin-like component, respectively.

given in figs 1 and 2. The dashed lines show the results of Mössbauer spectra approximation without ferritin-like component while the continuous lines are the result of spectra fitting including the ferritin-like component. The content of ferritin-like iron in erythremic red blood cell samples (relative area S of additional component) is given in table 1. So we could say that red blood cell samples from two patients with erythremia contain an additional component, probably ferritin-like iron. However, liquid helium temperature measurements are needed for evidence.

4. DISCUSSION

The differences between Mössbauer hyperfine parameters of normal adult and erythremic oxyhemoglobins indicate that the Fe^{2+} electronic structure of oxyhemoglobin in red blood cells from patients with erythremia is transformed like that of fetal and leukemic oxyhemoglobins which had been considered in [2,3], i.e. the energies of the low-lying triplet states decrease relative to the ground singlet state (the Fe^{2+} electronic structure was chosen from [11]). These changes in the Fe^{2+} electronic structure in oxyhemoglobin from patients with erythremia could be a result of weakness of the iron-ligand bonds. Similar differences between normal adult and fetal oxyhemoglobins were interpreted as a weakness of the Fe^{2+} bonds with the axial ligands N_ϵ(His F8) and O_2. Recently, XANES studies of adult and fetal hemoglobins [12,13] had evidenced differences in stereochemistry of the active sites in both hemoglobins, in particular it had been shown that the Fe-O-O angle should be lower in fetal oxyhemoglobin [13]. So we could conclude that the Fe-O-O angle is lower in oxyhemoglobin in erythremic red blood cells than that in normal adult oxyhemoglobin which results in a weakness of Fe^{2+}-O_2 bond.

On the other hand, Mössbauer hyperfine parameters of deoxyhemoglobins from normal adult and erythremic red blood cells do not distinguish while slight difference between ΔE_Q values for normal adult and fetal deoxyhemoglobins had been observed [9] (this difference was in agreement with the results of XANES spectroscopy of adult and fetal deoxyhemoglobins

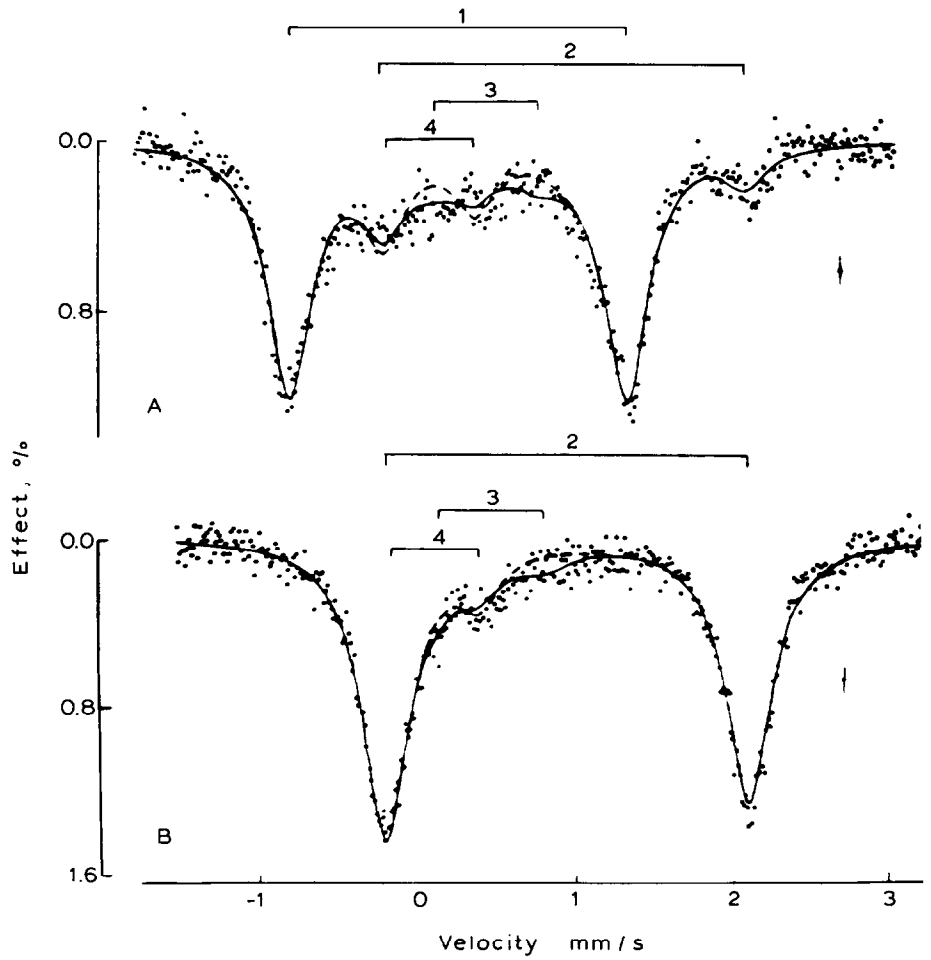

Fig.2. Mössbauer spectra of red blood cell samples from patient D. with erythremia. (A) Oxygenated red blood cells. (B) Deoxygenated hemolysate. 1, oxyhemoglobin; 2, deoxyhemoglobin; 3, ferritin-like iron; 4, ^{57}Fe nuclei in the scintillator detector beryllium window. The dashed and continuous lines result from least squares fitting excluding and including the ferritin-like component, respectively.

which had indicated the difference in Fe^{2+}-N_ϵ(His F8) bond [12]). Therefore, it can be concluded that modifications in erythremic hemoglobin may be possible in proximal heme region which effects Fe^{2+}-O_2 bond. The preliminary results of the measurement of the hemoglobin oxygen saturation curve for one patient with erythremia by the method described in [2] should be mentioned. We have observed an unusual curve with Hill parameter n = 1.83 and P_{50} = 11.9 mmHg (pH = 6.96, SD 0.242). The value of the Hill parameter indicates the decrease of cooperativity in erythremic hemoglobin studied. However, these results should be verified with a number of patients with erythremia.

As to an additional component in Mössbauer spectra of two patients with erythremia, it should be noted that we did not observe any additional component in the samples of adult, fetal and leukemic hemoglobins in red blood cells or hemolysates, although Ortalli et al. [4] had observed an additional component in some leukemic blood samples (Ortalli et al. [4] had associated this component with Hb-OH while the parameters estimated indicate the presence of hemochromes). The results of Mössbauer study of ^{57}Fe incorporation into ferritin and hemoglobin in model erythroleukaemia cells [5–8] indicate that iron is accumulated in ferritin in the absence of hemoglobin synthesis. Therefore, the presence of ferritin-like iron in erythremic red blood cells may reflect some changes in hemoglobin biosynthesis. On the other hand, intracellular hemoglobin denaturation leads to appearance of additional ferritin-like components in Mössbauer spectra of erythroleukemia cells [7,14] and red blood cell samples from patients with thalassemia, sickle-cell anemia and hemoglobin Hammersmith [6,15,16]. It should be noted that additional components with hyperfine parameters which were different from those of ferritin were observed in recent Mössbauer studies of some abnormal hemoglobins [17–20]. Therefore, two questions should be resolved: (i) whether an additional component in Mössbauer spectra of erythremic red blood cells is ferritin-like iron; (ii) whether ferritin content increase is a result of hemoglobin biosynthesis disturbance or intracellular denaturation of hemoglobin, if an additional component in erythremic red blood cells is really ferritin-like iron. We hope further investigations can solve these problems.

Table 1

Mössbauer parameters of hemoglobin samples from patients with erythremia and normal adult and fetal hemoglobins

	Sample	δ[a,b] (mm/s)	ΔE_Q[a] (mm/s)	S (%)
Patient Z.	oxyhemoglobin	0.274	2.144	15
	deoxyhemoglobin	0.929	2.319	13
	deoxyhemoglobin	0.932	2.312	14
Patient D.	oxyhemoglobin[c]	0.282	2.126	5.2
	deoxyhemoglobin	0.947	2.315	7.4
Patient L.	oxyhemoglobin[c]	0.276	2.133	–
	deoxyhemoglobin[d]	0.950	2.303	–
Patient S.	oxyhemoglobin[c]	0.277	2.125	–
Normal adult[e]	oxyhemoglobin	0.258	2.081	–
	deoxyhemoglobin	0.951	2.308	–
Normal adult[e]	oxyhemoglobin	0.260	2.075	–
	deoxyhemoglobin	0.945	2.317	–
Normal fetal[e]	oxyhemoglobin	0.267	2.120	–
	deoxyhemoglobin	0.934	2.276	–
Normal fetal[f]	oxyhemoglobin	0.274	2.140	–
	deoxyhemoglobin	0.941	2.282	–

[a] Experimental error ± 0.012 mm/s
[b] Isomer shifts are given relative to metallic iron at 295 K
[c] Additional content of deoxyhemoglobin (~6–7%)
[d] Additional content of oxyhemoglobin (10%)
[e] Data from [9]
[f] New measurements

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REFERENCES

[1] Oshtrakh, M.I. and Semionkin, V.A. (1985) in: Applications of the Mössbauer Effect (Proc. Int. Conf. Appl. Mössbauer Effect, 1983), vol.5 (Kagan, Yu.M. and Lyubutin, I.S. eds) pp.1639–1642.
[2] Oshtrakh, M.I. and Semionkin, V.A. (1986) FEBS Lett. 208, 331–336.
[3] Oshtrakh, M.I. and Semionkin, V.A. (1987) Biophysika 32, 197–202.
[4] Ortalli, I., Pedrazzi, G. and Varacca, V. (1988) Hyperfine Interact. 42, 913–916.
[5] Ofer, S., Fibach, E., Kessel, M., Bauminger, E.R., Cohen, S.G., Eikelboom, J. and Rachmilewitz, E.A. (1981) Blood 58, 255–262.
[6] Bauminger, E.R. and Ofer, S. (1982) Proc. Ind. Natl. Sci. Academy, Int. Conf. Appl. Mössbauer Effect 1981, pp.61–71.
[7] Fibach, E., Bauminger, E.R., Konijn, A.M., Ofer, S. and Rachmilewitz, E.A. (1983) in: Structure and Function of Iron Storage and Transport Proteins (Urushizaki, I. et al. eds) pp.129–132.
[8] Fibach, E., Konijn, A.M., Bauminger, E.R., Ofer, S. and Rachmilewitz, E.A. (1987) J. Cell. Physiol. 130, 460–465.
[9] Oshtrakh, M.I. and Semionkin, V.A. (1985) Mol. Biol. 19, 1310–1320.
[10] Oshtrakh, M.I., Semionkin, V.A., Burykin, B.N. and Khleskov, V.I. (1989) Mol. Physiol. 66, 531–535.
[11] Bacci, M., Cerdonic, M. and Vitale, S. (1979) Biophys. Chem. 10, 113–117.
[12] Bianconi, A., Congiu-Castellano, A., Dell'Ariccia, M., Giovannelli, A., Burattini, E., Castagnola, M. and Durham, P.J. (1985) Biochim. Biophys. Acta 831, 120–124.
[13] Congiu-Castellano, A., Bianconi, A., Dell'Ariccia, M., Della Longa, S., Giovannelli, A., Burattini, E. and Castagnola, M. (1987) Biochem. Biophys. Res. Commun. 147, 31–38.
[14] Fibach, E., Bauminger, E.R., Konijn, A.M., Ofer, S. and Rachmilewitz, E.A. (1983) Blood 62, 928–930.
[15] Jacobs, A., Peters, S.W., Bauminger, E.R., Eikelboom, J., Ofer, S. and Rachmilewitz, E.A. (1981) Br. J. Haematol. 49, 201–207.
[16] Rachmilewitz, E.A., Bauminger, E.R., Eikelboom, J., Ofer, S., Peters, S.W. and Jacobs, A. (1982) in: The Biochemistry and Physiology of Iron (Saltman, P. and Hegenauer, J. eds) pp.373–383.
[17] Ding, X.Q., Hsia, Y.F., Shi, S.Y., Liu, R.C., Wang, S.X., Wang, H.B., Chen, Y., Chen, P.Z. and Liu, Y.T. (1986) Acta Biochim. Biophys. Sin. 18, 313–319.
[18] Zeng, X.S. and Ren, B.Z. (1987) Jinan Liyi Xuebao N 1, 36–43.
[19] Ding, X.Q., Hsia, Y.F., Shi, S.Y., Liu, R.C., Wang, S.X., Wang, H.B., Chen, Y., Chen, P.Z. and Liu, Y.T. (1988) Hyperfine Interact. 42, 893–896.
[20] Xuanhui, C., Nanming, Z., Xiufang, Z., Naifei, G., Youwen, H. and Rongxin, W. (1988) Hyperfine Interact. 42, 897–900.