Biochemistry (Moscow), Vol. 66, No. 3, 2001, pp. 295-299. Translated from Biokhimiya, Vol. 66, No. 3, 2001, pp. 365-370.

Study on Conformational Changes in Hemoglobin Protoporphyrin in Essential Hypertension

G. V. Maksimov*, N. V. Maksimova, A. A. Churin, S. N. Orlov, and A. B. Rubin

Department of Biology, Lomonosov Moscow State University, Moscow, 119899 Russia; fax: (095) 939-1115; E-mail: maxim@biophys.msu.ru

Received February 8, 2000
Revision received August 29, 2000

Abstract—Changes in protoporphyrin conformation, partial pressures of O_2 and CO_2, and the mechanisms responsible for regulation of pCa and pH in erythrocytes were studied in essential hypertension (EH). Changes in protoporphyrin conformation in EH were accompanied by a decrease in the partial pressure of O_2 and an increase in the partial pressure of CO_2. This was associated with increased activities of Na^+/H^+-exchange and Ca^{2+}-dependent K^+-channels and with a decreased activity of Ca^{2+}-ATPase. The changes in protoporphyrin conformation in EH are suggested to decrease the efficiency of O_2 metabolism in hemoglobin and increase the values of intracellular pCa and pH of erythrocytes.

Key words: protoporphyrin conformation, Raman-spectroscopy, Na^+/H^+-exchange, Ca^{2+}-dependent K^+-channel, Ca^{2+}-ATPase, essential hypertension

It is now well known that primary (essential) hypertension (EH) is manifested in disorders in intracellular homeostasis of pCa and pH [1]. Some authors think that these disorders are initially caused by the genome and are accompanied by changes in the structure or in the conformation of cell proteins. Using combinational scattering spectroscopy, even slight conformational changes can be studied in separate regions of a protein molecule during the functioning of a cell. Thus, the use of laser radiation with absorption in the range of the protoporphyrin β-band allows the recording of resonance combinational scattering (RCS) spectra of hemoglobin and to study conformational changes in the heme protoporphyrin during the binding of O_2. The lines caused by planar oscillations of C–C and C–N bonds and deformation oscillations of C–H bonds in protoporphyrin, which are sensitive to changes in the oxidation and spin of the iron atom, are dominant in these spectra. Thus, the binding of O_2 by deoxyhemoglobin with the high-spin Fe(II) atom is associated with a displacement of the protoporphyrin-characteristic RCS bands to frequencies near those of the low-spin Fe(III) [2]. To detect the valence state of the iron atom, the polarized bands at 1373 and at 1565 cm^{-1} are usually used because their location in the RCS spectrum shows the electron population of π-orbitals in protoporphyrin. Any effect including changes in the iron atom valence resulting in changes in the electron distribution on protoporphyrin π-orbitals affects the frequency of the corresponding bands in the RCS spectrum. Thus, the frequency is significantly changed in the case of interaction between the axial ligand with a π-orbital and protoporphyrin π-orbitals through dπ-electrons of the iron atom. During complexing of the protein with O_2, the electron density is redistributed from the iron atom to the oxygen molecule with generation of O_2^-; therefore, the effect of the Fe(II) electron shell on the protoporphyrin π-orbital becomes equivalent to the effect of Fe(III) [3]. Thus, fluctuations in the region of 1373 cm^{-1} are sensitive to the bond type in the iron–ligand complex and not only to the third valence in the iron atom.

It is known that formation of the Fe(II)–O_2^- complex fails to change the valence of the iron atom but converts it from the high-spin into the low-spin state, and this is accompanied by a 0.07 nm displacement of the iron atom into the macrocycle plane. This displacement is passed through histidine F-8, and helix F together with histidine are pulled towards the heme, pushing a tyrosine residue out from the cavity. Then salt bridges between α-subunits are broken one-by-one, a proton is released (Bohr's effect), and the pH is therefore decreased. This is probably one of the causes of disorders observed in EH in ionic homeostasis of erythrocytes and in the activation of various systems of ion transport [1].

Therefore, the purpose of the present work was to investigate in EH changes in the hemoglobin protoporphyrin conformation, in activities of various erythrocyte

* To whom correspondence should be addressed.

0006-2979/01/6603-0295$25.00 ©2001 MAIK "Nauka/Interperiodica"

systems of ion transport (Na^+/H^+-exchange, Ca^{2+}-dependent K^+-channel, and Ca^{2+}-ATPase), and in partial pressures of O_2 and CO_2.

MATERIALS AND METHODS

Specimens of whole venous blood from healthy donors (n = 10) and from patients with essential hypertension (n = 10) were used. The diagnosis of EH was established based on clinical examination at the Cardiological Research Complex, Ministry of Public Health of the Russian Federation (Moscow). During the study, both samples of whole blood taken from the ulnar vein and isolated erythrocytes were used. In the latter case, the whole blood samples were taken into tubes containing heparin (20-50 units per ml of blood), which were centrifuged at 4000*g* for 10 min at 4°C. As a partial depolarization of the erythrocyte plasma membrane is specific for EH [1], the precipitated erythrocytes from the patients' blood, after elimination of the blood plasma and other blood cells, were resuspended in a medium containing 75 mM NaCl, 75 mM KCl, 0.2 mM $MgCl_2$, 10 mM glucose, 20 mM Hepes-OH (pH 7.4). The resuspension and precipitation procedures were repeated thrice under the same conditions of centrifugation. Ion contents and partial gas pressures in the donor blood plasma were determined with a Chiron Diagnostics analyzer (USA). Activities of Na^+/H^+-exchange, Ca^{2+}-ATPase, and Ca^{2+}-dependent K^+-channels of erythrocytes were determined using ion-selective electrodes [4].

To study RCS spectra, a capillary containing a sample of whole blood or of erythrocyte suspension was placed into a special holder to provide for focusing of the exciting light beam on the sample. The source of the exciting light during studies of the RCS spectra was a continuous gas laser generating radiation with wavelength of 441.6 nm. Using light filters, the radiation power was maintained at the level of 100-120 mW, and this did not significantly affect the state of the sample. The radiation scattered by the specimen was collected with a lens system onto the entrance aperture of a monochromator. The RCS-spectrum was scanned with a DFS-24 double monochromator (LOMO, Russia). The device has spectral range of 400-850 nm, relative aperture 1 : 5.3, inverse dispersion 0.45 nm/mm, half-width less than 1 cm^{-1}, scanning rate 0.18 nm/sec, and measuring accuracy 4 cm^{-1}. RCS signals were recorded with a FEU-79 photoelectron multiplier (Russia) operating in photon counting regime. The spectra were accumulated with an NTA-1024 multichannel pulse analyzer (EMG, Hungary) compatible with a personal computer.

RESULTS AND DISCUSSION

The characteristic RCS spectrum of whole blood and of erythrocyte suspension in the range from 1800 to 1000 cm^{-1} is a set of bands corresponding to the spectrum of protoporphyrin (Fig. 1, *1* and *2*) [5, 6]. In both cases the band families chosen in the ranges of 1375-1355 and 1610-1562 cm^{-1} correspond, respectively, to oscillations

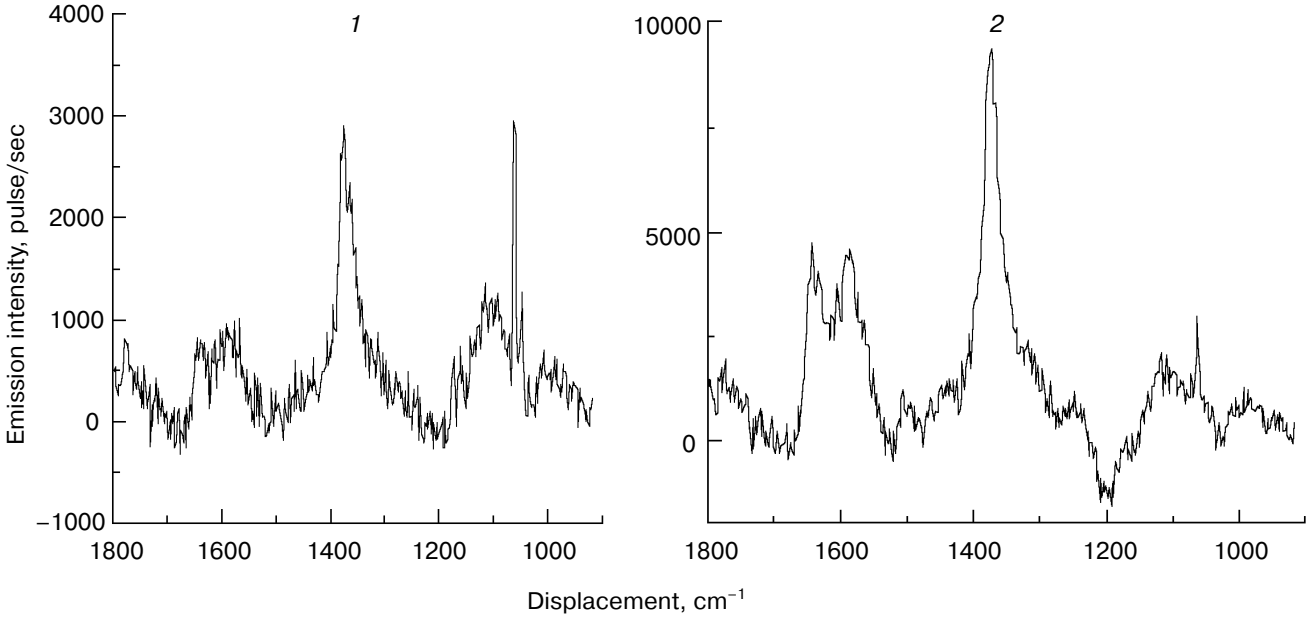

Fig. 1. Characteristic RCS spectra of blood (*1*) and of erythrocytes (*2*).

of C–N bonds sensitive to the valence state of the iron atom and to oscillations of C=C bonds sensitive to the spin of the iron atom. The RCS spectra of both whole blood and erythrocyte suspension from healthy donors and from patients with EH were compared, and specific changes were found in the relative intensities of the bands corresponding to C–N and C=C bonds of the erythrocyte hemoglobin protoporphyrin (Tables 1 and 2). The ratios I_{1373}/I_{1565} in the RCS spectra of the whole blood and of the isolated erythrocytes from patients with EH were significantly increased by 40 and 90%, respectively, suggesting irreversible changes in the protoporphyrin conformation in EH. These differences in the effect and in the parameter ratio in the RCS spectra of whole blood and erythrocyte protoporphyrin in EH seem to be associated with the ionic composition of the blood plasma or with the state of the erythrocytes [7-9]. Changes in the RCS spectra in EH seem to be associated with a redistribution of the electron density in the protoporphyrin molecule and with a displacement of the iron atom relatively to the macrocycle plane. Note that under similar conditions no significant changes have been found in the absorption spectra of protoporphyrin of erythrocytes isolated from the blood of healthy donors and patients with hypertension (Fig. 2).

The changes observed in the protoporphyrin structure suggest an additional enlargement of its "nucleus" that fixes the iron atom in the low-spin state and prolongs its ability for ligand binding (Fig. 3). In the low-spin state of Fe(II), high-energy orbitals are vacant and the iron atom dimensions are smaller than in the case of its high-spin state. Because the "nucleus" diameter in the macrocycle center is 2.02 Å and the diameter of the low-spin Fe(II) atom is 1.91 Å, the latter is incorporated into the macrocycle "nucleus". On the contrary, the high-spin Fe(II) goes out of the plane by a distance of 0.7 Å.

Thus, we have found an increased ratio of the 1373 and 1565 cm^{-1} bands only when changes in the structure of protoporphyrin from the whole blood hemoglobin and from the isolated erythrocytes of patients with EH were studied using RCS spectroscopy. This indicates that the electron density is redistributed between the C=C and C–N bonds of the protoporphyrin molecule, the molecule "nucleus" is enlarged, and that the oxygen–iron complex residence in the macrocycle plane seems to be prolonged. Such conformational changes in protoporphyrin are suggested to be caused by structural rearrangements in the hemoglobin protein that results in the stronger binding of oxygen molecules and in the decreased efficiency of its exchange. In the venous blood from patients with EH the partial oxygen pressure was significantly decreased (Table 1). In experiments on blood samples from healthy donors the conformation of protoporphyrin specific for patients with EH was, as a rule, found in subjects with low partial pressure of O_2 and with high partial pressure of CO_2 in venous blood (Figs. 4 and 5).

Table 1. Parameters of RCS spectrum of hemoglobin protoporphyrin and O_2 and CO_2 blood contents in healthy donors (HD) and in patients with essential hypertension (EH)

Donor	RCS, I_{1373}/I_{1565}	O_2, %	CO_2, mm Hg
HD (n = 10)	2.05 ± 0.2	70.0 ± 5.0	50.6 ± 5.3
EH (n = 10)	2.85 ± 0.2	53.8 ± 5.0	61.2 ± 6.0

It is suggested that changes in the globin conformation in EH should "fix" protoporphyrin in a state that is specific for oxyhemoglobin when the conversion time into deoxyhemoglobin is increased and the binding efficiency of a new oxygen molecule is correspondingly decreased. This hypothesis is based on experimental data. It is known that a short time (some psec) after detachment of the oxygen molecule the iron atom is converted from the low-spin into the high-spin state; however, it remains in the macrocycle plane [10]. It seems that in EH the heme conversion into a stable pyramidal conformation takes a longer time either due to changes in the tertiary structure (with time constant about 800 nsec) or in quaternary structure (with time constant of 60 μsec). This possibility was found by studies on RCS spectra of hemoglobin that revealed a correlation between displacements of the bands 216 and 1357 cm^{-1} caused by oscillations of the Fe-imidazole and pyrrole rings of protoporphyrin. This finding indicates that interactions of protoporphyrin and protein are manifested in the redistribution of the

Table 2. Parameters of RCS spectrum of hemoglobin protoporphyrin and activities of ion-transport systems in erythrocytes isolated from blood of healthy donors (HD) and of patients with essential hypertension (EH)

Donor	RCS, I_{1373}/I_{1565}	HCO_3^-, mmole/liter	Na^+/H^+-exchange, μmole H^+/liter cells per min	Ca^{2+}-ATPase, μmole Ca^{2+}/liter cells per min	K^+-channel, μmole K^+/liter cells per min
HD (n = 10)	1.4 ± 0.16	28.85 ± 2.5	111.0 ± 10.2	167.0 ± 7.3	4.9 ± 0.6
EH (n = 10)	2.65 ± 0.22	38.5 ± 3.6	218.0 ± 19.4	136.0 ± 8.50	7.1 ± 0.8

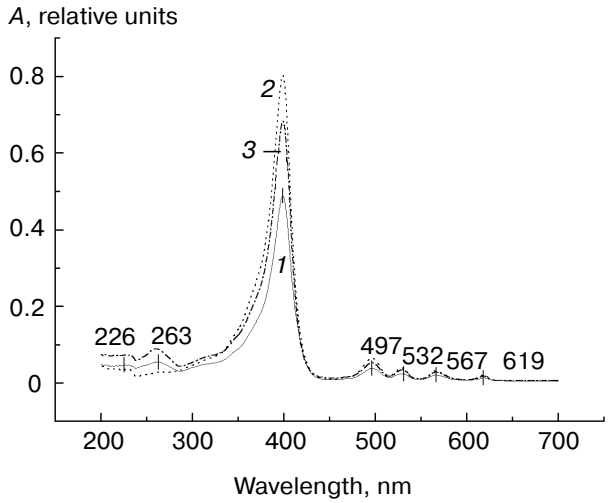

Fig. 2. Characteristic absorption spectra of erythrocytes from a healthy donor (*1*) and from patients with hypertension (*2*, *3*).

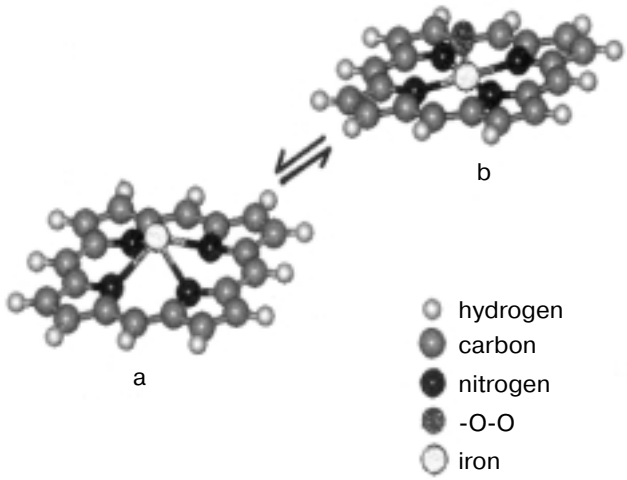

Fig. 3. Scheme of the protoporphyrin macrocycle in the high-spin (a) and low-spin (b) states of the iron atom.

macrocycle electron density and also in the Fe-imidazole bond [11].

Under physiological conditions, the intracellular pH of erythrocytes is recurrently changed synchronously with the functioning of hemoglobin and with CO_2 content. In particular, the conformational changes in protoporphyrin and hemoglobin on the binding of oxygen result in an additional breaking of salt bridges of the α- and β-helices and in decreased intracellular pH of the erythrocyte. On the contrary, with hemoglobin deoxygenation pH increases, and this results in an increased level of 2,3-diphosphoglycerate (2,3-DPG) and in decreased hemoglobin affinity for oxygen [12]. In this regard, we have shown earlier that increased extracellular pH was associated with a decreased ratio of the 1373 and 1565 cm^{-1} bands in the RCS spectrum of erythrocyte protoporphyrin and, consequently, with a decreased lifetime of the oxygen–iron complex [9]. Therefore, in the present work we have studied if changes in the RCS spectrum of protoporphyrin correlate with changes in the activities of various pH-regulating systems of erythrocytes in EH. We have found in EH increased activities of Na^+/H^+-exchange and of Ca^{2+}-dependent K^+-channels and also a decreased activity of Ca^{2+}-ATPase (Table 2). The increased activity of Na^+/H^+-exchange in EH is suggested to be a compensatory mechanism for changes in the

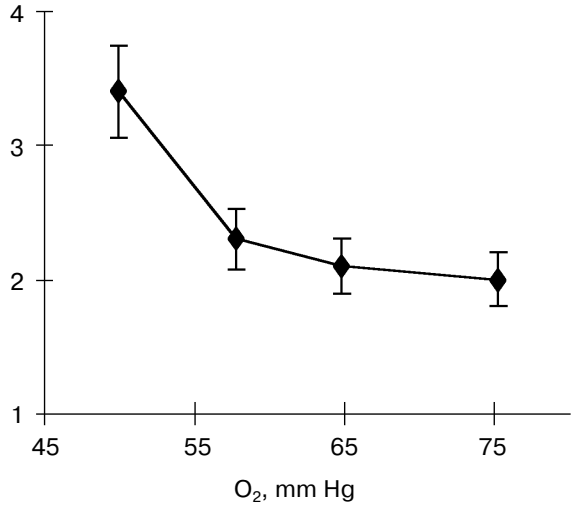

Fig. 4. Changes in the RCS spectrum of protoporphyrin depending on oxygen content in the venous blood of healthy donors. Abscissa axis, content of O_2 (mm Hg); ordinate axis, ratio of 1373 and 1565 cm^{-1} bands (relative units).

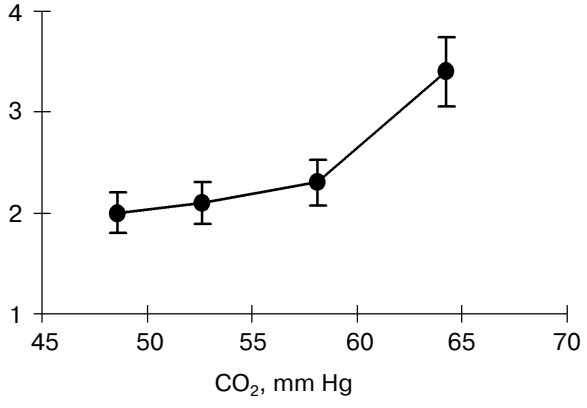

Fig. 5. Changes in the RCS spectrum of protoporphyrin depending on CO_2 content in the venous blood of healthy donors. Abscissa axis, content of CO_2 (mm Hg); ordinate axis, ratio of 1373 and 1565 cm^{-1} bands (relative units).

intracellular pH of erythrocytes in response to the prolonged existence of hemoglobin as oxyhemoglobin. And this in turn initiates a decrease in pCa at the cost of inhibition of Ca^{2+}-ATPase and activates Ca^{2+}-dependent K^+-channels of the erythrocyte plasma membrane [4, 13, 14]. It seems that accumulation of intracellular Ca^{2+} in EH promotes the addition of the cation to the negatively-charged 2,3-DPG molecule. And this addition decreases the affinity of 2,3-DPG for hemoglobin and thus increases the affinity of hemoglobin for oxygen [12].

The present study has shown that changes in activities of the systems regulating the intracellular pH and pCa in erythrocyte should be associated with conformational disorders in protoporphyrin that results in the prolongation of hemoglobin existence as oxyhemoglobin and in decreased efficiency of oxygen binding.

REFERENCES

1. Postnov, Yu. V. (1995) *Kardiologiya*, **10**, 4-13.
2. Anderson, J., Bradbrook, E. F., Cook, A. H., and Lindstead, R. P. (1938) *J. Chem. Soc.*, **23**, 1151-1156.
3. Kitagawa, T., Kyogoku, Y., and Iizuka, T. (1976) *J. Am. Chem. Soc.*, **98**, 5169-5173.
4. Maksimova, N. V., Chizhevskaya, S. Yu., Karpov, Yu. A., and Postnov, Yu. V. (1999) *Kardiologiya*, **5**, 45-49.
5. Plus, R., and Lutz, M. (1974) *Spectrosc. Lett.*, **7**, 133-154.
6. Kaul, R. K., and Kohler, H. (1983) *Klin. Wochenschr.*, **61**, 831.
7. Maksimov, G. V., and Orlov, S. N. (1993) *Biofizika*, **38**, 80-88.
8. Maksimov, G. V., Churin, A. A., Sokolova, T. N., and Pashchenko, V. Z. (1992) *Biol. Nauki*, **6**, 22-26.
9. Maksimov, G. V., Kondratov, V. E., Churin, A. A., Sokolova, T. N., Pashchenko, V. Z., and Orlov, S. N. (1993) in *Nuclear Weapon Test, Environment, and Health of Altai Region Residents* [in Russian], Vol. 7, Barnaul Medical Institute Publishers, Barnaul, pp. 41-61.
10. Dalinger, R. F., Nestor, J. R., and Spiro, T. G. (1978) *J. Am. Chem. Soc.*, **100**, 6251-6252.
11. Stein, P., Terner, J., and Spiro, T. G. (1982) *J. Phys. Chem.*, **86**, 168-170.
12. Dudarev, V. P. (1979) *Hemoglobin Role in Mechanisms of Adaptation to Hypoxia and Hyperoxia* [in Russian], Naukova Dumka, Kiev, p. 138.
13. Orlov, S. N., Pokudin, N. I., Ryazhskii, G. G., and Kotelevtsev, J. V. (1987) *Biol. Membr. (Moscow)*, **4**, 1036-1046.
14. Orlov, S. N., Pokudin, N. I., Kotelevtsev, J. V., and Gulak, P. V. (1989) *J. Membr. Biol.*, **107**, 105-117.