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Role of Viscosity and Permeability of the Erythrocyte Plasma Membrane in Changes in Oxygen-Binding Properties of Hemoglobin during Diabetes Mellitus

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Changes in viscosity and permeability of the plasma membrane and conformation of erythrocyte hemoglobin hematoporphyrin were found in patients with diabetes mellitus. The decrease in oxygen binding and increase in deoxyhemoglobin concentration during diabetes mellitus were accompanied by changes in viscosity and permeability of the membrane for Na^+, H^+, Ca^{2+}, and K^+. Our results suggest that oxygen-binding properties of hemoglobin depend on viscosity and permeability of the erythrocyte plasma membrane.

Key Words: *erythrocyte; plasma membrane; permeability; viscosity; hematoporphyrin*

Our previous Raman spectroscopy (RS) studies revealed conformational variations in erythrocyte hemoglobin porphyrin (HP) reflecting changes in oxygen binding in patients with various disorders [1,2]. Study of blood cells from patients with hypertension accompanied by disturbed carbohydrate metabolism and hypercholesterolemia revealed changes in plasma membrane permeability for H^+, Na^+, K^+, and Ca^{2+} (Na^+/H^+ exchange, function of Ca^{2+}-dependent K^+ channels, and Ca^{2+}-ATPase activity). Changes in erythrocyte membrane permeability and oxygen-binding properties of hemoglobin is usually related to the effect of various plasma components (glucose, cholesterol, and triglycerides; pH and pCa) on plasma membrane permeability [3,4].

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Here we evaluated whether changes in plasma membrane permeability (Na^+/H^+ exchange, Ca^{2+}-dependent K^+ channel, and Ca^{2+}-ATPase) and viscosity can modulate oxygen-binding properties of HP in erythrocytes from patients with diabetes mellitus (DM).

MATERIALS AND METHODS

Experiments were performed with whole venous blood from healthy donors and DM patients. The diagnosis of DM was made according to the results of clinical examination at the Cardiology Research-and-Production Complex (Russian Ministry of Health) and Department of Internal Diseases of the Pisa University (Italy). Whole blood samples were taken from the cubital vein and stabilized with an anticoagulant (20-50 U heparin per 1 ml blood). The samples were centrifuged at 4000 rpm and 4°C for 10 min [3,4]. The plasma and white blood cells were removed. Erythrocyte pellet was incubated in

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a sodium-phosphate medium. The composition of this medium varied depending on the technique of study. The major components were 75-140 mM NaCl, 75-140 mM KCl, 0.2 mM $MgCl_2$, 10 mM glucose, and 20 mM HEPES-OH (pH 7.4).

The intensity of Na^+/H^+ exchange, activity of Ca^{2+}-ATPase, and function of Ca^{2+}-dependent K^+ channels in erythrocytes were determined by a potentiometric study with ion-selective electrodes [4]. Conformational changes of HP were recorded by the technique of RS [1,2,9,12]. They were estimated by the following parameters of Raman spectra: ratio between the intensity of bands 1375 cm^{-1} and 1580 cm^{-1} (I_{1375}/I_{1580}), which reflects the efficiency of binding of O_2 (and/or other ligands) to hemoglobin; and I_{1375}/I_{1355} ratio, which reflects the ratio between various forms of hemoglobin (oxyhemoglobin and deoxyhemoglobin). Viscosity of the plasma membrane was measured by the method of electron paramagnetic resonance spectroscopy [11]. Spin-labeled analogues of stearic acid (5- and 16-doxyl-stearic acid, 5-DS and 16-DS) served as the probes. The order parameter (S, mobility of acyl chains in fatty acid) was calculated from electron paramagnetic resonance spectra of the probe 5-DS to estimate erythrocyte membrane viscosity. The nitroxyl tumbling correlation time (τ, multiplied by the base of logarithm lne^{-9}) was determined for the probe 19-DS [11]. The contents of cholesterol, glucose, and creatinine in blood plasma and concentration of hemoglobin were measured routinely.

The results were analyzed by nonparametric Wilcoxon test (MedCalc software). The differences were significant at $p<0.05$.

RESULTS

Oxygen-binding properties of HP and intracellular homeostasis of pH and pCa probably depend on viscosity of the erythrocyte plasma membrane [6, 8,9]. We measured viscosity of blood erythrocyte membrane in healthy donors and DM patients (Table 1). Nitroxyl radicals incorporated into the membrane were used as spin labels. The paramagnetic fragment of these radicals is located in different regions of the acyl chain [7]. This method allowed us to evaluate viscosity of the membrane at various distances from its surface. The nitroxyl radicals of 5-DS and 16-DS are located at a distance of 0.6-0.8 and more than 2.2 nm from the outer membrane surface, respectively.

A 2-fold increase in triglyceride and total cholesterol content in blood plasma from patients with carbohydrate metabolism disturbances (DM) was accompanied by a decrease in S and τ parameters of spin labels incorporated into the erythrocyte plasma membrane (Table 1). The decrease in erythrocyte membrane viscosity was observed not only in the outer layer (0.6-0.8 nm from the surface), but also in deep layers of the membrane (2.2 nm from the surface) [5,6,8-10]. These findings attest to a significant decrease in microviscosity during DM.

In the next series permeability of the erythrocyte membrane to univalent and bivalent ions was estimated by the rate of Na^+/H^+ exchange, function of Ca^{2+}-dependent K^+ channels, and Ca^{2+}-ATPase activity. The study was performed with cells from healthy donors and patients with disturbed carbohydrate metabolism and hypertension (Table 2). The rate of Na^+/H^+ exchange and function of Ca^{2+}-dependent K^+ channels increased, while Ca^{2+}-ATPase activity decreased in patients with type 2 DM.

Raman spectra of the blood from healthy donors and DM patients were recorded (Fig. 1). Raman spectra of whole blood and erythrocyte suspension in the range of 1800-1000 cm^{-1} were presented by bands corresponding to the porphyrin spectrum (Fig. 1) [2,6,11]. In both cases the selected family of bands corresponded to stretching of C-N (1375-1355 cm^{-1}) and C=C bonds (1610-1562 cm^{-1}) sensitive to valence and spin of the iron atom, respectively. A comparative study of Raman spectra for erythrocyte porphyrin in healthy donors and DM patients revealed typical changes in the ratio between bands corresponding to C-N and C=C bonds of erythrocyte HP (Table 3).

DM patients were characterized by a significant decrease of I_{1375}/I_{1580} and I_{1375}/I_{1355} in the Raman spectrum for erythrocyte HP and decreased blood hemoglobin concentration. Conformational chan-

TABLE 1. Parameters of Spin Labels in the Erythrocyte Membrane (S and t) and Concentrations of Cholesterol (mmol/liter) and Triglycerides (mmol/liter) in Blood Plasma from Healthy Donors and Patients with DM

Group	S	$\tau \times e^{-9}$	Cholesterol	Triglycerides
Healthy donors	0.275 (100%)	1.56 (100%)	4.8 (100%)	1.1 (100%)
Patients	0.262 (95%)	1.43 (92%)	5.6 (106%)	2.4 (205%)

Note. Here and in Table 3: % of control is shown in brackets.

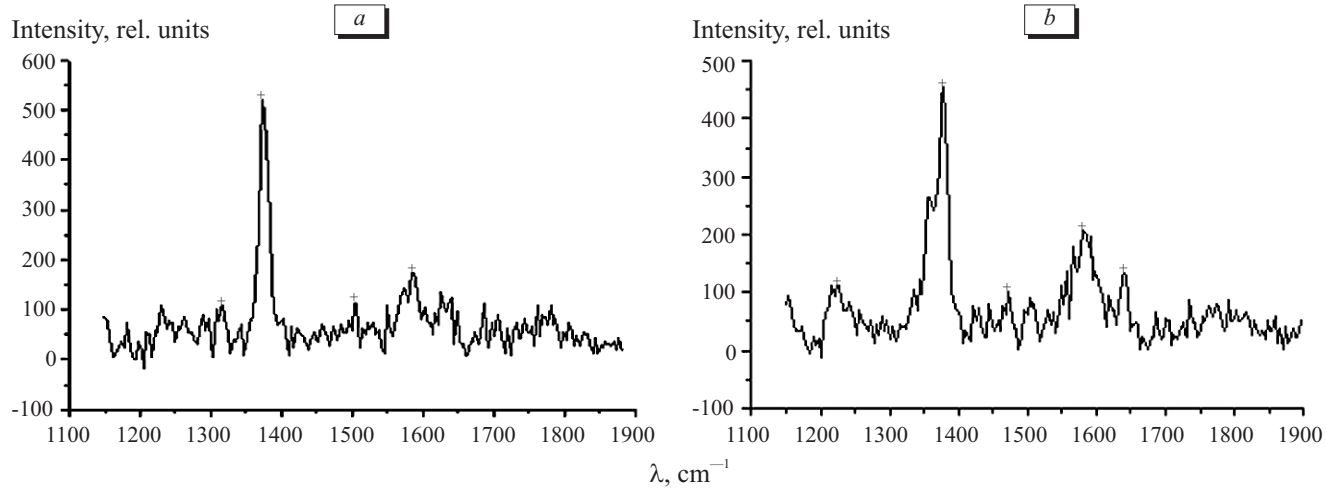

Fig. 1. Raman spectra for erythrocyte hemoglobin hematoporphyrin in the blood from healthy donors (*a*) and patients with diabetes mellitus (*b*).

ges of HP contributed to a decrease in the efficiency of oxygen binding to hemoglobin (of I_{1375}/I_{1580}) and reduction of oxyhemoglobin concentration (I_{1375}/I_{1355}) in DM patients. Moreover, we revealed a decrease in hemoglobin concentration in these patients (HbA1c).

Probably, conformational changes in globin during DM stabilized porphyrin in the form of deoxyhemoglobin, which accelerated its transition into oxyhemoglobin and reduced efficiency of oxygen binding [8,10,12].

The efficiency of oxygen transport by erythrocytes decreases in DM patients. Previous experiments revealed changes in gas diffusion through the plasma membrane, efficiency of O_2 binding to porphyrin, and concentration of oxyhemoglobin in these patients [5,6,8]. We detected the presence of a hemoglobin form with low oxygen-binding activity in DM patients. It determines the development of tissue hypoxia due to abnormalities in binding and transport of O_2 in the blood. Our findings suggest that variations in some parameters of extracellular medium (total cholesterol content and triglyceride concentration), viscosity, and permeability of the erythrocyte plasma membrane for gases and ions can induce conformational changes in HP and decrease the efficiency of oxygen binding and transport during DM.

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TABLE 2. Function of the Ion-Transport System in Erythrocytes from Healthy Donors and Patients with DM

Group	Na^+/H^+ exchange, μmol H^+/liter cells×min	Ca^{2+}-ATPase, μmol Ca^{2+}/liter cells×min	Ca^{2+}-dependent K^+ channels, mol K^+/liter cells×min
Healthy donors	107.4±10.6	175.3±27.1	3.65±0.74
Patients	254.8±11.2*	123.1±15.0**	8.7+2.85**

Note. *$p<0.01$ and **$p<0.05$ compared to the control.

TABLE 3. Ratio between Bands of Raman Spectra for Blood Hematoporphyrin (I_{1375}/I_{1580} and I_{1375}/I_{1355}) and Hemoglobin Concentration (HbA1c) in Healthy Donors and Patients with DM

RS parameters	Efficiency of oxygen binding (I_{1375}/I_{1580})	Amount of Hb-O_2 complexes (I_{1375}/I_{1355})	Hemoglobin concentration, HbA1c (%)
Control	2.75±0.03 (100%)	0.70±0.03 (100%)	8.1±0.6 (100%)
DM	2.30±0.03 (83.6%)	0.39±0.04 (55.7%)	6.8±0.83(84%)

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