Hemoglobin as substantial object for biomedical studies and diagnostics

O V Kosmachevskaya¹, E I Nasybullina¹, V G Nikitaev², A N Pronichev², E V Polyakov² and A F Topunov¹*

¹Bach Institute of Biochemistry, Research Center of Biotechnology of the Russian Academy of Sciences, Moscow 19071, Russia
²National Research Nuclear University MEPhI (Moscow Engineering Physics Institute), Moscow 115409, Russia

*aftopunov@yandex.ru

Abstract. Hemoglobin (Hb) is a hemoprotein consisting of the hem prosthetic group and a protein part. Its main function in human organism is oxygen binding and transportation. Different factors can violate native Hb structure. Depending on such factors, hereditary and acquired hemoglobinopathies are distinguished. Acquired ones are associated with the effect of various chemical agents, the modified Hb forms can appear in this case. It is caused by conjunction of different compounds to the molecule (hem and protein ligands). At several conditions Hb binds with the erythrocyte membrane. Changes in Hb structure can disturb its oxygen-carrying function and stability of erythrocytes; furthermore, they hamper the erythrocyte flow through microvessels. These processes take place at many pathological states as well. Because of increasing amount of information, it becomes urgent to use computer medical systems to increase the efficiency of diagnostics, including hemoglobinopathies. In the expert system elaborating by us, the results of the general haematological analysis are used, with addition of the data on modified Hb forms, among them on membrane-bound one.

1. Introduction

For diagnostics of many diseases the data on hemoglobin (Hb) state and Hb-containing erythrocytes (red blood cells - RBC) are very informative. Among hematologic diseases, hemoglobinopathies (hemoglobinoses) are distinguished, to which pathological states caused by violations of normal Hb structure are attributed. Hemoglobin consists of a hem group and a protein part. Depending on the nature of factors violating the Hb structure, hereditary and acquired hemoglobinopathies are distinguished. Hereditary ones are caused by mutations in genes coding Hb protein chains (sickle cell anemia is the best known). Several hundred anomalous hemoglobins are reported; however, not all of them cause clinical implications. Acquired hemoglobinopathies are associated with the effect of various chemical agents on Hb [1, 2]. Changes in Hb structure can violate the oxygen-carrying function and stability of erythrocytes; furthermore, they complicate their flow in microvessels, and this can cause hemolytic anemia and local tissue ischemia.

To detect such diseases, it is necessary to have information of Hb forms synthesizing and functioning in the organism, including modified forms appearing at the oxidative, nitrosative and carbonyl stress conditions and under chronic action of the toxic compounds. Some of such interactions and appropriate modified Hb forms we describe below.
To distinguish molecular Hb forms, the methods of modern biochemistry, molecular biology and genetics are used now. The problems of diagnostics became more labor-consuming due to increasing and more complicated information. It becomes particularly urgent to use computer medical systems and decision support systems to increase the efficiency of diagnostics of various diseases, including hemoglobinopathies and anemias.

2. Hb reduction and methemoglobinemia
Hemoglobin is supporting in physiologically active reduced states (with 2-valent hem iron) thanks to specific NADH- and NADPH-dependent reductases. If enzymes are not enough active, the illness methemoglobinemia takes place, when a considerable portion of Hb is in oxidized (metHb) state. This disease can be either hereditary or caused by the action of various chemical compounds. The second type of disease is often named as “drug” methemoglobinemia, because it can be a result of the use of many medicaments.

The important point in study of enzymatic Hb reduction was the establishing identity of erythrocytic metHb reductase and cytochrome b5 reductase of other tissues [3]. Now they are included in the enzyme nomenclature as one enzyme – NADH: ferricytochrome b5 reductase (EC 1.6.2.2.). Thus there are two types of enzyme deficiency: “erythrocytic”, when it is visible only in red blood cells, and “basic” when it is manifesting also in other tissues. The methemoglobinemia connected with deficiency of cytochrome b5 itself.

The population groups with increased prevalence of hereditary “enzymatic” methemoglobinemia are: Eskimos and Athabasca of Alaska; Navajo (all - USA) and Yakuts of the Viluy river basin (Russia).

3. Hb interaction with different compounds
Hemoglobins can interact with reactive oxygen and nitrogen species – i.e. function at oxidative and nitrosative stress. It is important both at pathogenesis and under action of negative factors.

Such Hb derivate as nitrosoHb is forming when NO is binding by the hem group, and the hypothesis is actively discussing that erythrocytic Hb is one of the most important parts of the metabolic system for NO and its donors. Under hypoxic conditions the reduction of NO$^2$ to NO take place in blood and tissue cells by deoxygenated Hb (deoxyHb) and myoglobin (deoxyMb) respectively. That is, Hb works as nitrite reductase at these conditions [4].

A very important type of posttranslational modification of Hb is its glycosylation (interaction with reactive carbonyl compounds, e.g. with sugars). Hb was the first protein for which this process was reported [5], later its connection with diabetes has been shown [6]. The glycosylated human Hb A is indicated as HbA1c. In general this fraction is not pathological and is about 5-7% for healthy people [7]. The main site for glycosylation is N-terminal amino acid of both Hb beta–chains. The amount of non-enzymatic glycosylated proteins increases at various diseases (diabetes, atherosclerosis, galactosemia etc.), and amount of HbA1c is critical marker of the violation of carbohydrate metabolism [8].

The particular interest have Hb modification products when it is simultaneously treated with reactive nitrogen species and reactive carbonyl compounds. We have shown that in this case various products can be formed, e.g. nitroso- and nitriHb, and the appearance of a form may depend both on the ratio of the concentrations of the substances and on the reaction conditions [9, 10].

Hb can bind sulfides by hem group. It was shown that in case of excess H$_2$S admission to the organism it form complexes with Hb – sulfhemoglobin (sulHb) [11], in pathological conditions its portion can be of 1-10% [12]. Sulfhemoglobinemia may be a consequence of taking medication (e.g. sulfonamides) [13], or as a result of the action of H$_2$S producing bacteria Morganella morganii living in intestine [14]. Sulfides can be also bound to Hb by Cys93 residue of beta-chains.

Strong complexes can be also formed if Hb interacts with rather large molecules, e.g. with heterocyclic and nitrosoaromatic compounds, or with alkyl radicals. They are referred to so-called “unusual” ligands. When Hb reacts with nitrosobenzene the latter can be bound by hem iron and/or by
protein part. Hb complexes with heterocyclic compounds can appear as result of the presence of toxic substances in the environment or after use of several medicaments. Such complexes were found in the blood of chemical plant workers exposed to chronic poisoning [15].

4. Membrane-bound Hb
We should specially highlight the membrane-bound Hb form (MBHb). The fact of Hb binding to erythrocyte membrane was firstly shown in the experiments on obtaining RBC ghosts. Various data on MBHb portion in native erythrocytes were reported: ~3% Hb [16]; 1.1-5.2% [17]; 7-10% [18]. These values could be changed under the influence of different factors.

Hb can interact with the membrane in different ways. The most important is the electrostatic binding of deoxyHb with cytoplasmic domain of anion-transporting Band 3 protein (CDB3) [19, 20]. One can also name covalent binding with membrane components by disulfide bonds and adsorption to membrane lipids with hydrophobic interactions. Hb binding to the membrane can be reversible and irreversible. The portion of irreversible MBHb increases at various diseases: hereditary (sickle-cell anemia and thalassemia) and induced by oxidative agents. We suppose that use of data on MBHb as additional biochemical parameter can sufficiently widen the possibilities for diagnostics of blood system disorders.

We elaborated simple and precise method for spectrophotometric rating of MBHb content in erythrocytes, which is based on the determination of Hb bound with RBC ghosts after full hemolysis. The method has precision less than 0.1% of MBHb in the sample. It was used in experiments with blood of patients from N.N. Blokhin National Medical Research Center of Oncology (in comparison with healthy donors). The diapason of normal MBHb values (3.3-4.9%) correlated with high hemolytic stability was defined. Oncological patients treated with chemotherapy had a shift of MBHb content towards excess. It can be explained both by the development of intoxication caused with medicine preparations, and by the adaptation to anemia accompanying the disease.

Hemolytic stability of erythrocytes depends on MBHb portion: even small fluctuations resulted in level of hemolysis. Forming of MBHb in definite levels can be of physiological significance as a part of adaptive mechanism, but strict Hb binding to the membrane (e.g. as result of oxidative processes) destabilizes membrane, what is resulting in hemolysis and Hb withdrawal to vessels [21].

5. Conclusions
Thus, in the human organism it is possible to find multiple products of different posttranslational Hb modifications (for description of such variations the term “Proteoform” began to be used [22]). We proposed the term “Hemoglobinome” joining all Hb forms presenting in the organism [23]. The most convenient indicators reflecting the functional state of the erythrocyte are the ones connected with Hb. The standard clinical blood analysis includes data on Hb concentration, hematocrit, average Hb concentration and content in erythrocyte, etc. If necessary, the levels of metHb and glycosylated HbA1c are measured, because they correlate with the severity of methemoglobinemia and hyperglycemia, respectively.

The data of various functional and modified (e.g. carboxyHb, sulfoHb, nitrosoHb, nitriHb) Hb forms can be also used in computer diagnostics. We are working on the elaboration of expert system for hemoglobinopathy and anemia diagnostics using all these parameters. The system core is the knowledge base including grouped data on qualitative and quantitative indicators of blood. In addition to standard characteristics, we introduce one more diagnostic one – the membrane-bound hemoglobin.

The expert system represents a tool for supporting the physicians’ decision making in diagnostics of the blood system diseases. The pilot version of the system was proposed [24], and tested on the data of patients of N.N. Blokhin National Medical Research Center of Oncology (Moscow). According to the results of this validation, we can say that the system fully implements all the stated functions and correctly determines the diagnosis. Such system, along with the use of artificial neural networks [25], has been recognized as one of the most promising tendencies of hematological diagnostics [26].
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