ONCOLOGY

Peculiarities of Hemoglobin Interaction with Serum Proteins of Mice with Ehrlich Carcinoma

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In male C57B1/6 mice with transplanted Ehrlich carcinoma, hemoglobin forms a complex with serum proteins characterized by a molecular weight of about 300 kDa. The complex incorporates proteins weighing 100, 68, 65, and 15 kDa identified by MALDI-TOF mass spectrometry as haptoglobin, serum albumin, gi|26341396 nameless protein *Mus musculus*, and α-hemoglobin, respectively. This complex can possess biological activity and contribute to the control of tumor growth.

Key Words: *experimental tumors; blood serum proteins*

Peculiarities of growth and metastasizing of experimental tumors (specifically, Ehrlich carcinoma) attest to an important role of serum proteins in these processes. However, the attempts to isolate and identify specific protein failed [1,3]. It can be hypothesized that not individual protein, but a complex of proteins exhibits biological activity. The possible complex formation is indicated changes in the interaction between blood serum proteins and tumor cells in animals with transplanted tumor [2]. The detected modification of proteins are related to changes in their glycosylation during tumor growth. Probably, protein glycosylation is important for the charge, spatial structure of protein molecules and their interaction during the formation of protein complexes. In comparison with blood serum proteins of intact animals, these complexes have a modified charge and spatial structure, so they can be isolated and characterized by ion-exchange chromatography (by their charge) and gel filtration (by the size and spatial structure). No large molecular complexes can be formed in the serum, otherwise they would disturb blood rheology and physiological functions. Such complexes can be formed between cell proteins (after their release from destroyed cells) and serum proteins. Hemoglobin is the most prevalent protein in blood cells.

Our aim was to study peculiarities of interaction between hemoglobin and serum proteins in mice with Ehrlich carcinoma.

MATERIALS AND METHODS

The experiments were carried out on male C57B1/6 mice obtained from Stolbovaya nursery (Moscow region). Ehrlich carcinoma (aneuploid strain ELD from Bank of Tumor Strains, N. N. Blokhin Cancer Research Center) was transplanted intramuscularly into the right hind leg (1×10^6 cell/mouse in 0.1 ml RPMI 1640 medium).

After the tumor surpassed 1000 mm^3, the complex of hemoglobin with serum protein was isolated from the serum. Moderate spontaneous hemo-

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lysis was observed during serum collection, which was associated with low resistance of mouse erythrocytes to damaging factors. The serum was added to 0.01 M tris buffer (pH 7.4) containing 0.01% sodium azide on a PD-10 column and kept at 4°C for 12 h. The precipitate was separated by centrifugation at 10,000 g for 20 min. The proteins were transferred into a column packed with Sephadex Q FF. Unbound serum proteins were eluted with 0.01 M tris buffer (pH 7.4) with 0.01% sodium azide, while bound proteins were extracted using a linear gradient with increasing concentration of NaCl (0.5 M) in tris buffer (pH 7.4) with 0.01% sodium azide. The gradient was set by a GP-250 (Pharmacia) gradient programmer. The eluted proteins were monitored at 280 nm in a flow cell. Eluted hemoglobin was visually monitored and then assessed by absorption at 414 nm. The isolated complex containing hemoglobin was transferred to a column packed with HW-50, thereafter visually colored aliquots were collected.

Separation of protein fractions was carried out by ion exchange chromatography, gel filtration and SDS electrophoresis [5].

Identification of proteins forming complex with hemoglobin was performed by MALDI-TOF mass spectrometry [4].

Analysis of hemoglobin isolated from erythrocytes of intact and experimental mice was performed after removal of serum proteins in 1077 den-

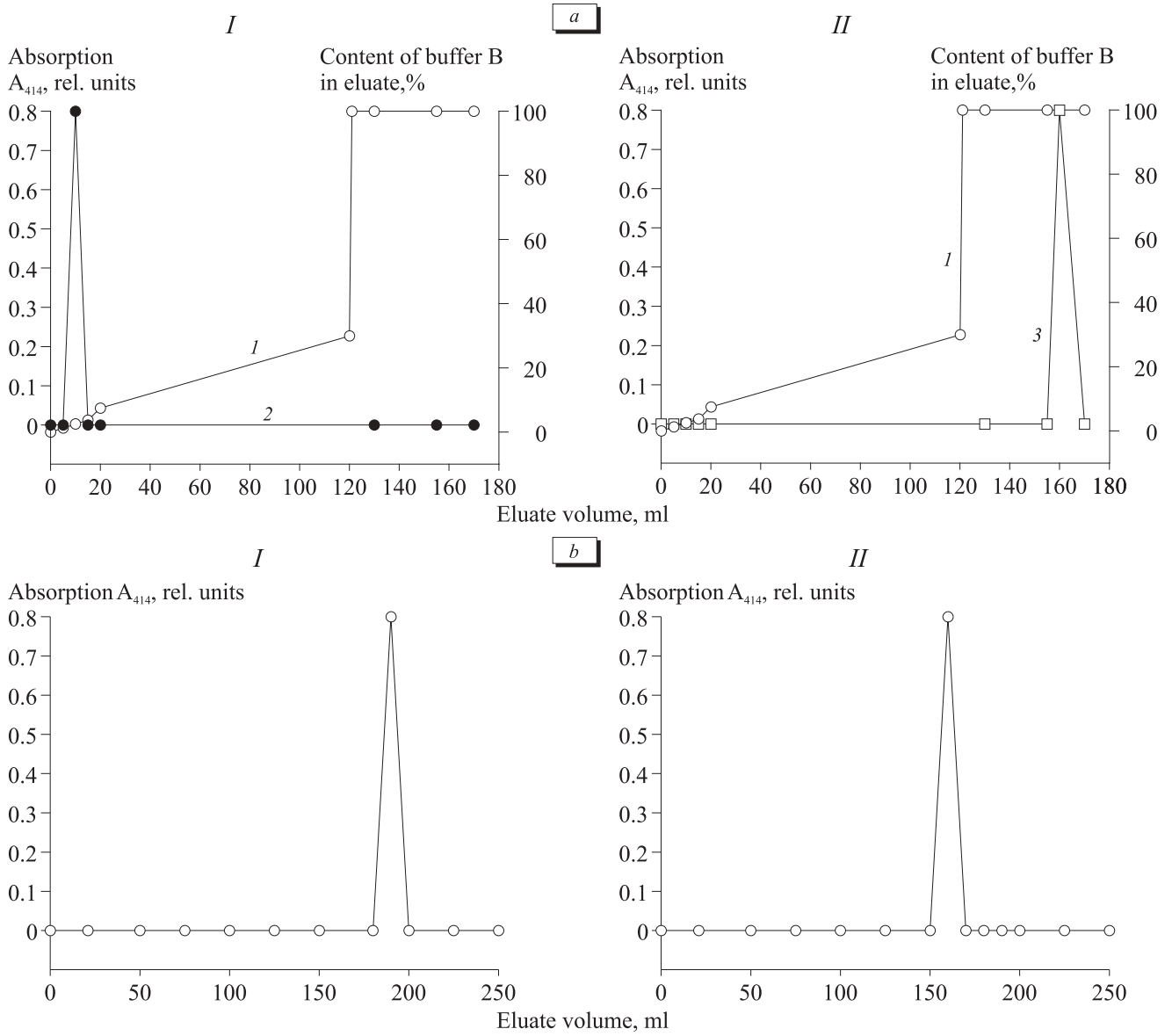

Fig. 1. Elute profile of serum hemoglobin from intact mice (*I*) and experimental mice with transplanted Ehrlich carcinoma (*II*) on a column packed with Q FF sepharose (*a*) and HW-50 (26×100, *b*). *1*) intact mice; *2*) experimental mice; *3*) NaCl concentration gradient.

sity gradient. Hemoglobin was isolated and purified by gel filtration.

RESULTS

Figure 1, *a* shows elution profile of serum hemoglobin of intact mice and experimental mice with Ehrlich carcinoma on a column packed with Q FF Sepharose. Hemoglobin of intact animals was eluted in a volume of 10 ml (Fig. 1, *a*, *I*), while the volume of eluted hemoglobin in experimental mice was 160 ml (Fig. 1, *a*, *II*). Since separation was performed in this case by ion exchange chromatography, it can be hypothesized that hemoglobin either changed its electrical charge or formed a complex with blood proteins in tumor-bearing mice. To test these hypotheses, the hemoglobin-containing fractions were analyzed by gel filtration in a HW-50 column (Fig. 1, *b*). Hemoglobin was eluted in volumes of 190 and 155 ml in intact and experimental mice, respectively. According to column calibration, the effluent volumes corresponded to proteins with molecular weights of 68 and 310 kDa, respectively. The isolated proteins had different optical density at 280 and 414 nm. For hemoglobin complex of experimental mice, absorption A_{280}=1.0 rel. units at λ=280 nm and A_{414}=0.44 rel. units at λ=414 nm (A_{280}/A_{414}=2.3), while the control values were A_{280}=0.18 rel. units and A_{414}=0.47 rel. units (A_{280}/A_{414}=0.38). The change in the ratio of optical density for hemoglobin and its molecular weight can be explained only by the formation of hemoglobin complex with other serum proteins.

Figure 2 shows MALDI-analysis of hemoglobin fractions isolated from erythrocytes of intact and tumor-bearing mice under conditions excluding the interaction of hemoglobin with serum proteins. In both cases, the molecular weight of hemoglobin subunits was equal, demonstrating two peaks corresponding to 15,064 and 15,091 Da. Ion exchange chromatography of hemoglobin fractions performed under conditions excluding interaction of hemoglobin with blood serum proteins showed that the protein was eluted in a volume of 10 ml both in control and experimental mice. Thus, neither charge, nor molecular weight changed in erythrocytes.

TABLE 1. MALDI-TOF Mass Spectrometry of Proteins Forming Complex with Hemoglobin

Electrophoresis value of band molecular weight, kDa	Protein code	Protein molecular weight, Da	Protein name
100	gi|8850219	38 727	Haptoglobin
68	gi|33859506	68 678	Serum albumin
64	gi|26341396	64 961	Nameless protein
15	gi|6680175	15 076	α-hemoglobin

Therefore, spontaneous hemolysis in the serum of experimental mice with Ehrlich carcinoma is accompanied by the formation of hemoglobin complex with serum proteins. Figure 3 shows electrophoregram of fractions containing hemoglobin isolated from the serum of control and experimental mice. The proteins were applied in excessive amounts for evaluation of all constituents of the protein complex. The hemoglobin fraction isolated from control serum contained no admixtures. By contrast, the electrophoregram of experimental mice revealed the presence of proteins with molecular weights of 100, 68, 65, and 15 kDa. The electrophoregram

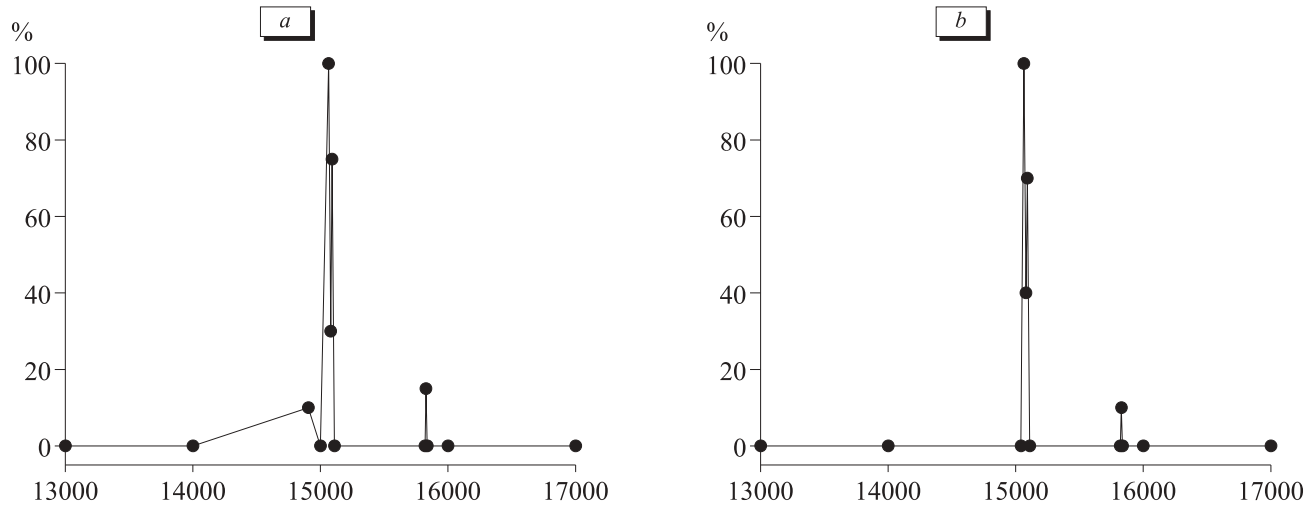

Fig. 2. MALDI spectrum of hemoglobin isolated from erythrocytes of intact mice (*a*) and experimental mice with transplanted Ehrlich carcinoma (*b*). Abscissa: molecular weight of protein; ordinate: intensity (% of maximum).

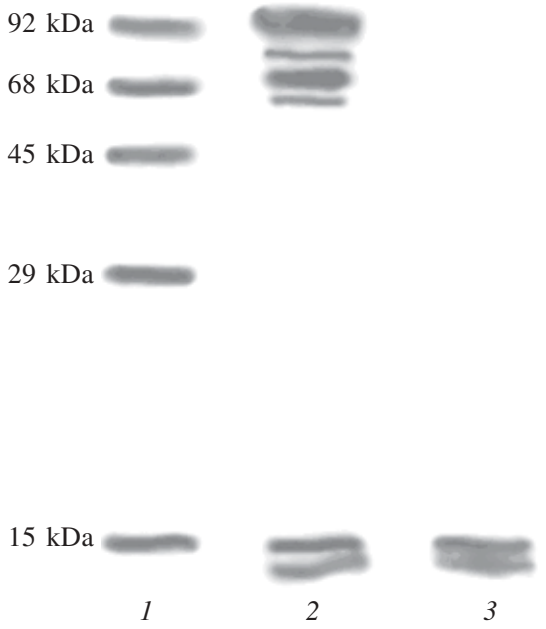

Fig. 3. Electrophoresis of the fractions containing hemoglobin isolated from erythrocytes of intact mice and experimental mice with transplanted Ehrlich carcinoma.

bands were cut and identified by MALDI-analysis (Table 1). The bands of 100, 68, 65, and 15 kDa were identified as haptoglobin, serum albumin, gi|26341396 nameless protein (*Mus musculus*), and α-hemoglobin, respectively.

Thus, hemoglobin can form complexes with serum proteins of tumor-bearing animals with molecular weight of about 300 kDa. The complex contains proteins with lower molecular weights of 15, 65, 68, and 100 kDa. Under normal conditions, hemoglobin also forms a complex with serum protein (haptoglobin). However, we did not observe it in our experimental paradigm. Probably, it is related to the fact that glycosylation of haptoglobin is modified during tumor growth, which enhances the resistance of its complex with serum protein to destruction during the chromatographic processing in comparison with the complexes formed in healthy animals. Probably, this complex possesses biological activity. The role of hemoglobin complexes with serum proteins in the tumoral animals requres further investigation.

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