ISSN 0006-2979, Biochemistry (Moscow), 2008, Vol. 73, No. 8, pp. 950-956. © Pleiades Publishing, Ltd., 2008.
Original Russian Text © Yu. Ya. Kit, M. A. Starykovych, V. A. Richter, R. S. Stoika, 2008, published in Biokhimiya, 2008, Vol. 73, No. 8, pp. 1177-1184.

Detection and Characterization of IgG- and sIgA-Abzymes Capable of Hydrolyzing Histone H1

Yu. Ya. Kit[1]*, M. A. Starykovych[1], V. A. Richter[2], and R. S. Stoika[1]

[1]*Institute of Cell Biology, National Academy of Sciences of Ukraine, Drahomanov Str. 14/16, Lviv 79005, Ukraine; E-mail: kit@cellbiol.lviv.ua*
[2]*Institute of Chemical Biology and Fundamental Medicine, Siberian Division of the Russian Academy of Sciences, pr. Akademika Lavrent'eva 8, 630090 Novosibirsk, Russia*

Received October 1, 2007
Revision received February 1, 2008

Abstract—Immunoglobulins IgG and sIgA actively hydrolyzing histone H1 have been detected on analyzing proteolytic activity of antibodies isolated by chromatography on Protein A-agarose from blood serum of patients with multiple sclerosis and from colostrum of healthy mothers. These antibodies hydrolyze other histones less actively and virtually failed to cleave lysozyme of chicken egg. By gel filtration at acidic pH and subsequent analysis of protease activity of chromatographic fractions, it was shown that IgG and sIgA molecules were responsible for hydrolysis of histone H1. Anti-histone H1 antibodies of IgG and sIgA classes were purified by affinity chromatography on histone H1-Sepharose from catalytically active antibody preparations. The protease activity of anti-histone H1 IgG antibodies was inhibited by serine proteinase inhibitors, whereas anti-histone H1 sIgA antibodies were insensitive to inhibitors of serine, asparagine, and cysteine proteases.

DOI: 10.1134/S0006297908080142

Key words: antibodies, protabzymes, multiple sclerosis, blood serum, colostrum, proteolytic activity

Interaction of antibodies with antigens can lead to degradation of the antigens. Peptides, proteins, nucleic acids, and oligosaccharides, which are present in humans and other mammals, can serve as substrates for catalytically active antibodies [1-3]. Antibodies with proteolytic activity have been called protabzymes [1]. Protabzymes were first found in blood serum of patients with bronchial asthma [4]. They were of the IgG class and could cleave a vasoactive intestinal peptide. Later, protabzymes of the same class were found in blood serum of patients with autoimmune diseases (autoimmune thyroiditis [5], autoimmune myocarditis [6], multiple sclerosis [7], etc.) and also in patients with hemophilia A [8] and some malignancies [9].

The substrate specificity of IgG-protabzymes and their proteolytic activity in blood serum of patients are shown to closely correlate with the type and severity of disease; therefore, they are considered to be promising markers for prediction of the course of autoimmune diseases [1, 10].

Protabzymes of IgM and IgG classes were also found in blood serum of healthy subjects [11]. Normally, protabzymes can function protectively in viral and bacterial infections [11, 12] and also prevent age-related neurodegenerative changes in the brain [13]. Milk of healthy mothers was shown to contain secretory IgA capable of cleaving β-casein of human and cow milk [14]. Casein-hydrolyzing protabzymes were also found in patients with AIDS [15].

These data suggest that protabzymes can play an important physiological role in the human body. Therefore, further studies of them seems be promising for better understanding of humoral immunity functions in norm and disease.

In this work, the presence of histone H1-hydrolyzing protabzymes has been shown in blood serum of patients with multiple sclerosis and in colostrum of healthy mothers.

MATERIALS AND METHODS

Isolation of immunoglobulins. Blood sera of patients with multiple sclerosis and of healthy donors were provid-

Abbreviations: AB) antibody; MBP) myelin basic protein.
* To whom correspondence should be addressed.

ed by T. I. Negrich (L'vov National Medical University). Specimens of colostrum of healthy mothers were obtained in the L'vov Regional Prenatal Center. IgG was isolated from blood serum as described in [16]. Blood serum (2 ml) was centrifuged at 5000*g* for 20 min and placed onto a column which contained 1 ml of Protein G-agarose Fast Flow equilibrated with buffer A (20 mM Tris-HCl (pH 7.5) and 0.15 M NaCl). The column was washed successively in buffer A supplemented with 0.5% NP-40 and in buffer A, and antibodies were eluted with 0.1 M glycine-HCl (pH 2.6). Then the antibody-containing fraction was immediately neutralized with 1.5 M Tris-HCl (pH 8.8) and dialyzed against buffer A. Secretory IgA was isolated from mothers' colostrum as described in [17]. Colostrum (5 ml) was centrifuged at 5000*g* for 30 min, the lipid fraction and cell precipitate were removed, and the plasma was placed onto a column which contained 2 ml of Protein A-agarose Fast Flow. The column was washed successively with buffer A supplemented with 0.5% NP-40 and buffer A. The antibodies were eluted with 0.1 M glycine-HCl (pH 2.6) and neutralized with 1.5 M Tris-HCl (pH 8.8). Antibody-containing fractions were dialyzed against 20 mM Tris-HCl (pH 7.0) for 18 h, and then the antibody preparations were placed onto a column containing Fractogel TSK DEAE-650 and equilibrated with the same buffer. sIgA was eluted with 20 mM Tris-HCl buffer (pH 7.5) supplemented with 0.1 M NaCl. Homogeneity of antibody preparations was analyzed by electrophoresis in a gradient polyacrylamide gel (7-18.5%) in the presence of 0.1% SDS [18].

Affinity chromatography of antibody preparations on histone-H1-Sepharose. To purify antibodies with affinity for histone H1 (anti-histone-H1 AB), IgG and sIgA preparations were subjected to chromatography on a column containing histone H1-Sepharose. This was done by placing 2.5-3 mg antibodies onto a column with histone H1-Sepharose (1 ml) equilibrated with buffer A. The column was washed in buffer A, and the antibodies were eluted with 0.1 M glycine-HCl (pH 2.6). The antibody-containing fractions were immediately neutralized with 1.5 M Tris-HCl (pH 8.8) and dialyzed against 20 mM Tris-HCl (pH 7.4) for 18 h.

Analysis of proteolytic activity. Histone H1, calf thymus total histones, and chicken egg lysozyme were used as substrates for proteolysis. The reaction was performed in buffer containing 20 mM Tris-HCl (pH 7.5) and 0.1 M NaCl in the presence of 0.3 mg/ml protein and 0.05-0.3 mg/ml antibodies for 1 h at 37°C. To analyze proteolytic activity of the chromatographic fractions, aliquots (30 μl) were supplemented with 6 μg histone H1, and the mixtures were incubated for 2 h at 37°C. The protease activity of anti-histone-H1 AB was inhibited with 1.5 mM PMSF and mixture of inhibitors of serine, cysteine, and asparagine proteases containing AEBSF, pepstatin A, E-64, bestatin, leupeptin, and aprotinin diluted 1 : 10. The reaction was stopped by addition of fourfold denaturing buffer (0.26 M Tris-HCl (pH 6.8), 4% SDS, 8% 2-mercaptoethanol, 40% glycerol), and the reaction products were separated by electrophoresis in 12% polyacrylamide gel in the presence of 0.1% SDS. The gels were stained with Coomassie G-250.

Gel filtration of AB under conditions of immune complex dissociation (pH shock). Preparations of AB capable of hydrolyzing histone H1 were separated by gel filtration on a column (180 × 5 mm) with Toyopearl TSK HW-55. The antibodies were precipitated with 50% ammonium sulfate, and the precipitate was dissolved in 0.1 M glycine-HCl (pH 2.6). The protein (96 μg in 40 μl) was placed onto the column and was eluted with the same buffer. Chromatographic fractions (300 μl) were collected, neutralized with 1.5 M Tris-HCl (pH 8.8), dialyzed against buffer A for 18 h, and analyzed by electrophoresis in a gradient polyacrylamide gel (7-18.5%) in the presence of 0.1% SDS.

Reagents. A preparation of calf thymus total histones was kindly provided by M. D. Lutsik, and histone H1 was purified from this preparation as described in [19]. Chicken egg lysozyme was from Fermentas (Lithuania); acrylamide, N,N′-methylene bis-acrylamide, glycine, and Tris were from Merck (Sweden); PMSF and the mixture of protease inhibitors were from Sigma (USA); sorbents Fractogel TSK DEAE-650 (M) and Toyopearl TSK HW-55 were from Merck (Germany) and Toyo Soda (Japan), respectively. Histone H1 was immobilized on Activated CH-Sepharose 4B (Sigma-Aldrich, USA) according to the producer's protocol.

RESULTS AND DISCUSSION

Analysis of proteolytic activity of antibody preparations. Protabzymes of IgG class hydrolyzing myelin basic protein (MBP) were found in cerebrospinal fluid and blood serum of patients with multiple sclerosis [1, 7]. MBP is a member of a family of proteins with molecular weight of 17-21.5 kD that have highly homologous amino acid sequences in various animals [20]. MBP has about 25% of basic acids (arginine, lysine, and histidine) uniformly distributed along the polypeptide chain, and this is responsible for its high isoelectric point (p*I* 12-13) [21]. Therefore, we supposed that blood serum of patients with multiple sclerosis should contain protabzymes capable of hydrolyzing other positively charged proteins in addition MBP. To test this supposition, histone H1 from calf thymus chromatin also carrying a high positive charge was used as a substrate for proteolysis.

Antibody preparations were isolated from blood sera of patients with multiple sclerosis, of healthy donors, and from mothers' colostrum by chromatography on Protein A-agarose. On analyzing proteolytic activity of 16 antibody preparations from blood sera of patients with multi-

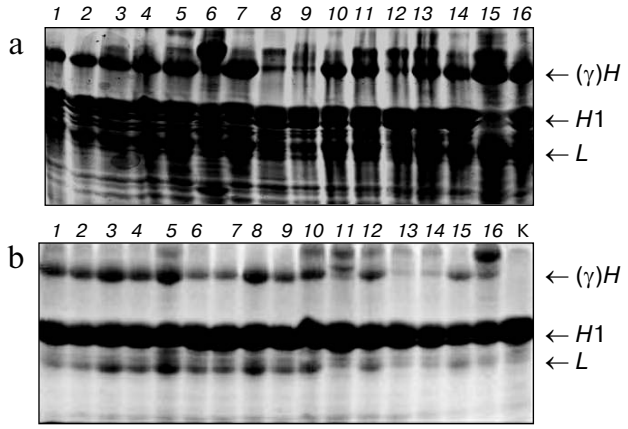

Fig. 1. SDS-PAGE in 12% polyacrylamide gel of products of histone H1 hydrolysis by antibody preparations from blood sera of 16 patients with multiple sclerosis (a) and 16 healthy donors (b) purified by chromatography on a column with Protein A-agarose. Lanes: *1-16*) histone H1 in the presence of antibodies; K) histone H1 in the absence of antibodies. Arrows on the right indicate positions on the gel of IgG *H*- and *L*-chains and of histone H1.

ple sclerosis, the ability to cleave histone H1 was detected in two of them (Fig. 1a). These catalytically active antibody preparations were different in hydrolytic efficiency. The position of immunoglobulin heavy chain polypeptides in the gel indicated that the preparation with lower proteolytic activity was enriched with IgM (Fig. 1a, lane *6*), whereas IgG prevailed in the more active antibody preparation (Fig. 1a, lane *15*). We found another antibody preparation enriched with IgG and capable of hydrolyzing histone H1 upon additional screening of 11 specimens of blood sera of patients with multiple sclerosis (data not presented). Blood sera of these patients were subsequently used for isolation of IgG antibodies and investigation of their proteolytic activity.

None of 16 antibody preparations from blood sera of healthy donors could hydrolyze histone H1 (Fig. 1b). However, this protein was cleaved in the presence of sIgA isolated from pooled colostrum of five healthy mothers by chromatography on Protein A-agarose and purified by ion-exchange chromatography on DEAE-Fractogel (Fig. 2). Thus, protabzymes capable of hydrolyzing histone H1 can be present in colostrum of healthy mothers, as well as in blood serum of some patients with multiple sclerosis.

Protabzymes are characterized by their substrate specificity. We attempted to determine if catalytically active IgG and sIgA preparations could hydrolyze other cationic proteins besides histone H1. Thus, calf thymus total histones, which in addition to the linker histone H1 also contain core histones, and chicken egg lysozyme were used as substrates of protease activity. Histone H1 was effectively hydrolyzed by IgG (Fig. 3a, lane *2*) and sIgA (Fig. 3b, lane *2*), but neither IgG (Fig. 3a, lane *6*) nor sIgA (Fig. 3b, lane *6*) cleaved chicken egg lysozyme. However, IgG and sIgA preparations were different in hydrolyzing core histones of calf thymus. Figure 3a shows that in the presence of IgG from the patient's blood serum the amount of some of histones is considerably decreased along with an increase in the content of low molecular weight products of their hydrolysis (lanes *4* and *5*). In the presence of sIgA from colostrum, these histones remained virtually unchanged (Fig. 3b, lanes *4* and *5*), although the increase in the amount of low molecular weight polypeptides on lane *4* suggested that some of core histones could be hydrolyzed. We cannot yet say unambiguously what histones, in addition to histone H1, can be substrates for proteolytic activity of IgG and sIgA preparations. Considering data on relative electrophoretic mobility of mammalian histones [22], it was supposed that histones H3 and H2B could be substrates for proteolytic activity of IgG antibodies from blood serum of a patient with multiple sclerosis.

Arguments for proteolytic activity of antibodies. The presence of protease activity in affinity-purified antibody preparations does not guarantee their possessing of protabzymes. To prove that the ability to hydrolyze histone H1 is a property of antibody molecules, we used some routine procedures [2]. First, catalytically active IgG preparations from blood serum of patients with multiple sclerosis and those of sIgA from mothers' colostrum were gel filtered under conditions favorable for dissocia-

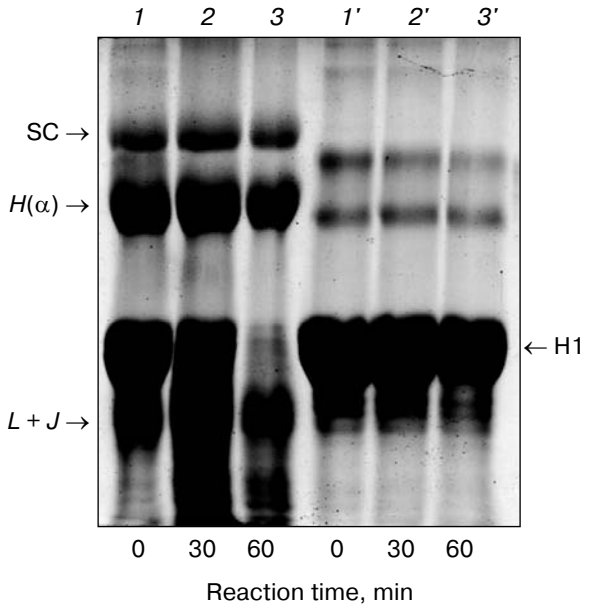

Fig. 2. SDS-PAGE in 12% polyacrylamide gel of products of histone H1 hydrolysis by sIgA preparation from mothers' colostrum purified by successive chromatography on columns with Protein A-agarose and Fractogel TSK DEAE-650. Lanes: *1-3*) histone H1 in the presence of sIgA; *1'-3'*) histone H1 in the absence of sIgA. On the left, positions of sIgA polypeptides are shown (SC, secretory component; *H*(α), heavy chain; *L*, light chain; *J*, joining chain). The position of histone H1 is shown on the right.

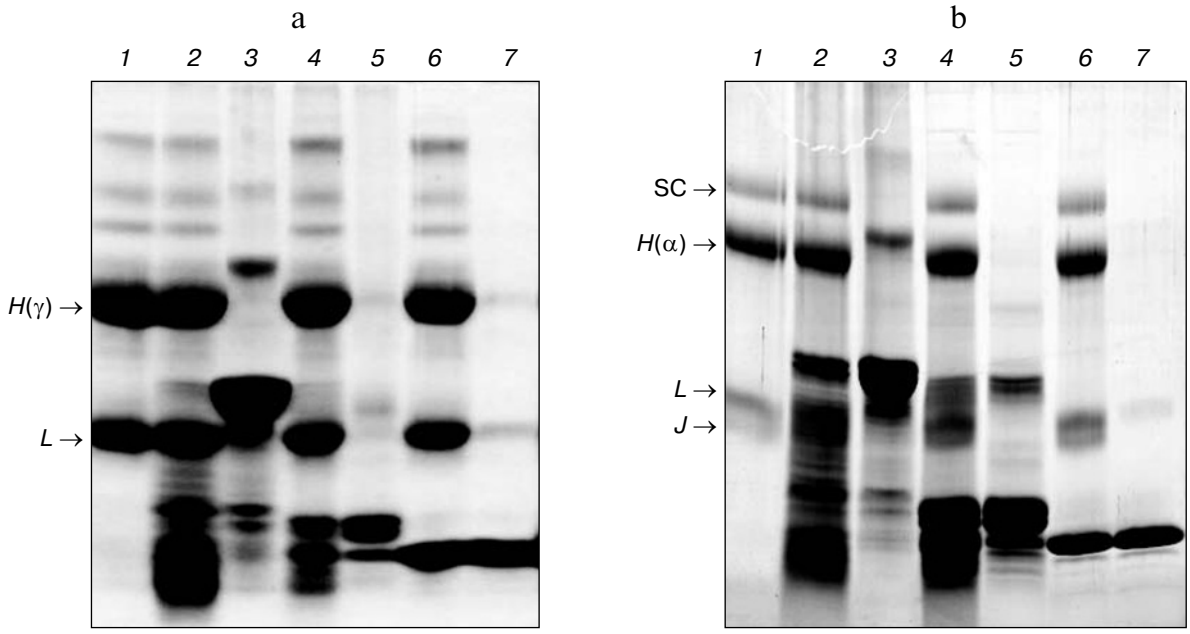

Fig. 3. SDS-PAGE in a gradient polyacrylamide gel (7-16.5%) of products of protein hydrolysis by preparations of IgG (a) and sIgA (b). Lanes: *1*) Ig preparations; *2*) Ig preparations + histone H1; *3*) histone H1; *4*) Ig preparations + total histones; *5*) total histones; *6*) Ig preparations + lysozyme; *7*) lysozyme. The arrows indicate positions of IgG polypeptides (*H*(γ), heavy chain; *L*, light chain) and of sIgA polypeptides (SC, secretory component; *H*(α), heavy chain; *L*, light chain; *J*, joining chain).

tion of immune complexes (Fig. 4a) with subsequent electrophoresis of chromatographic fractions (Fig. 4b) and analysis of their capability for hydrolyzing histone H1 (Fig. 4c). The ability to hydrolyze histone H1 was detected in those chromatographic fractions of catalytically active IgG and sIgA preparations (Fig. 4a) that contained polypeptides of these immunoglobulins (Fig. 4b). The absence of proteolytic activity in the other chromatographic fractions (Fig. 4c) indicated that the histone H1-hydrolyzing activity of IgG and sIgA preparations belonged to immunoglobulins rather than to possible admixtures of proteases from blood serum or colostrum.

Gel filtration of antibodies at acidic pH values promoted a considerably greater decrease in the protease activity of sIgA from mothers' colostrum than in the protease activity of IgG from blood serum of a patient with multiple sclerosis (Fig. 4a). It was supposed that an increased sensitivity of the catalytic activity of sIgA to low pH might be associated with a more complicated structure of this immunoglobulin molecule as compared with the IgG molecule. In addition to light and heavy immunoglobulin chains, sIgA molecule also contains a secretory component and a joining chain [23]; therefore, dialysis in isotonic buffer can be insufficient for complete recovery of the quaternary structure of the molecule.

Determination of the affinity of IgG and sIgA for histone H1. Another and more important condition for the presence of abzymes is the affinity of catalytically active antibodies for the reaction substrates. Because histone H1 was a substrate of the protease activity, protabzymes were isolated from IgG and sIgA preparations by affinity chromatography on a column containing histone-H1-Sepharose. Catalytically active IgG and sIgA preparations contained proteins with affinity for histone H1 (Fig. 5a). By electrophoresis, these proteins were shown to be IgG (Fig. 5b, lane *1*) and sIgA (Fig. 5b, lane *2*) immunoglobulins. Thus, IgG preparations from blood serum of a patient with multiple sclerosis and sIgA from the colostrum of healthy mothers contained antibodies with affinity for histone H1 (anti-histone-H1 AB). Histone H1 was cleaved in the presence of anti-histone-H1 AB of IgG and sIgA classes (Fig. 5c). Consequently, proteolytically active preparations of IgG and sIgA contained anti-histone-H1 AB capable of hydrolyzing histone H1 from calf thymus.

Characterization of the proteolytic activity of the antibodies. We have studied effects of some inhibitors on histone H1 hydrolysis in the presence of IgG and sIgA preparations purified by chromatography on histone H1-Sepharose (anti-histone-H1 AB). This was studied using PMSF (a noncompetitive inhibitor of serine proteases) and a mixture of serine, cysteine, and asparagine protease and aminopeptidase inhibitors. The reaction to the inhibitors of IgG- and sIgA-protabzymes was different. Figure 6 shows that neither the inhibitor of serine proteases (PMSF, lane *2*) nor the mixture of broad spectrum inhibitors (lane *3*) affected the intensity of histone H1

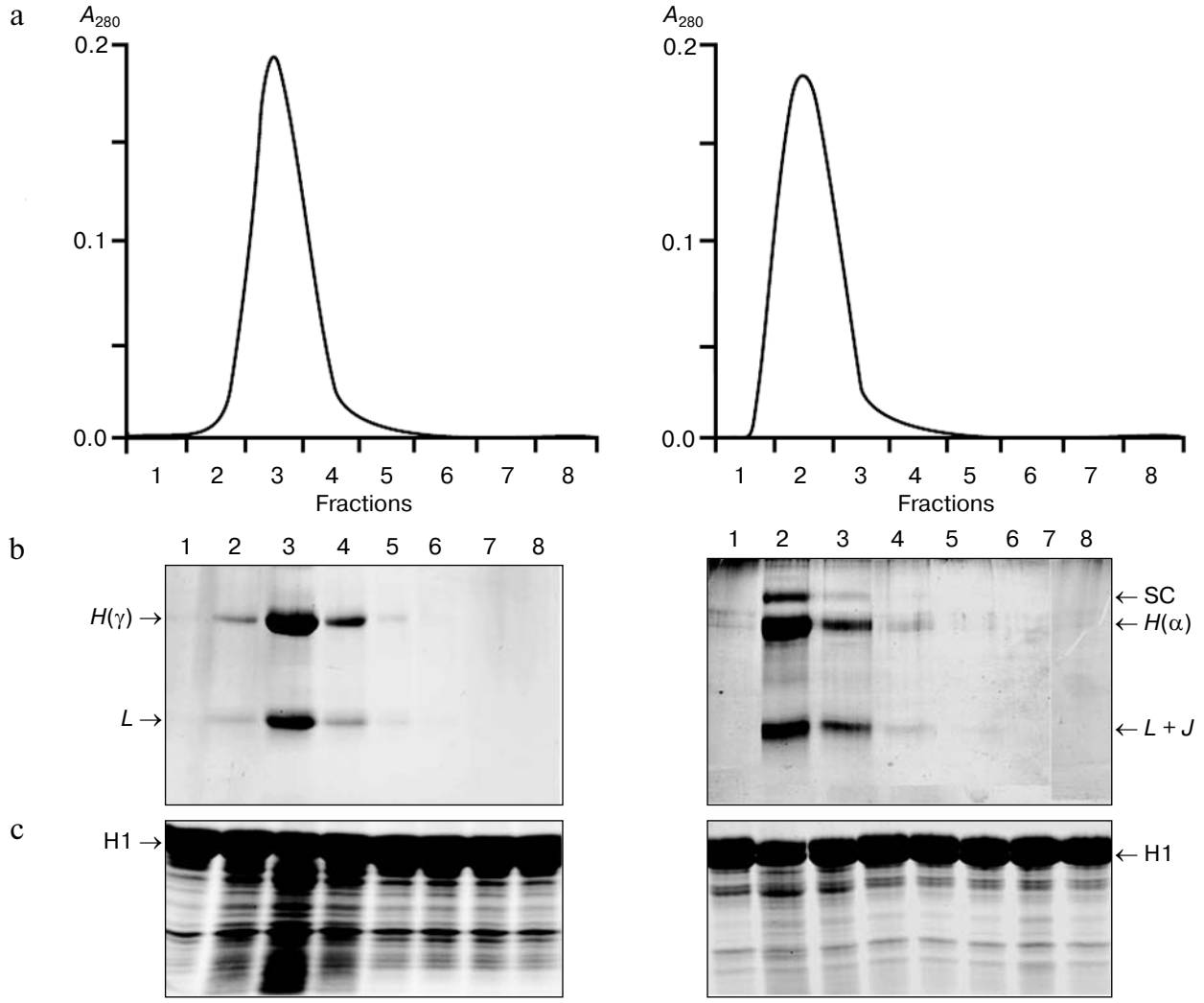

Fig. 4. Analysis of proteolytic activity of preparations of IgG (to the left) and sIgA (to the right). a) Gel filtration of antibodies in 0.1 M glycine-HCl buffer (pH 2.6); b) SDS-PAGE of proteins of chromatographic fractions; c) SDS-PAGE of products of histone H1 hydrolysis in the presence of chromatographic fractions. The arrows indicate positions of IgG polypeptides ($H(\gamma)$, heavy chain; L, light chain) and sIgA polypeptides (SC, secretory component; $H(\alpha)$, heavy chain; L, light chain; $L + J$, light and joining chain) and of histone H1.

hydrolysis in the presence of sIgA-protabzymes isolated from colostrum. However, protease inhibitors significantly suppressed the hydrolysis of histone H1 in the presence of IgG-protabzymes isolated from blood serum of a patient with multiple sclerosis (Fig. 6, lanes *2′* and *3′*). Thus, similarly to other proteases found in human blood serum and milk, IgG-protabzymes from blood serum of a patient with multiple sclerosis capable of hydrolyzing histone H1 were serine type proteases [8, 9, 14, 15]. Because broad spectrum inhibitors failed to affect the histone H1 hydrolysis by sIgA from colostrum, the type of these proteases is still unclear. Note also that sIgA-protabzymes hydrolyzing histone H1 were insensitive to serine protease inhibitors, and this discriminated them from the sIgA-protabzymes found earlier in women's milk and capable of hydrolyzing β-casein [14].

Thus we have shown for the first time that protabzymes hydrolyzing histone H1 are present in blood serum of patients with multiple sclerosis and in colostrum. Note that results of the patients' screening are preliminary, and further studies are required. The too small sample of studied patients makes it uncertain whether the appearance of histone H1-hydrolyzing antibodies in the blood serum of patients with multiple sclerosis is directly associated with this autoimmune disease. Moreover, the catalytic activity of sIgA from colostrum is more likely to indicate the opposite situation. The rather infrequent detection of histone H1-hydrolyzing antibodies in blood serum of patients with multiple sclerosis suggests that these protabzymes can occur in these patients because of some individual features of their immune response. The affinity of catalytically active antibodies

for histone H1 allows us to classify them as anti-histone autoantibodies. Consequently, the histone H1-hydrolyzing activity is more likely to be inherent in autoantibodies of this type. For example, anti-histone-H1 antibodies of the IgG class are known to be present in blood serum of patients with systemic lupus erythematosus [24, 25]. IgG antibodies to cell nucleus antigens were found in blood serum of patients with multiple sclerosis [26, 27]. Autoantibodies with affinity for histone H1 can apparently be produced in some patients with multiple sclerosis, similarly to patients with systemic lupus erythematosus.

On the other hand, antibodies catalyzing hydrolysis of myelin major protein are present in blood serum of patients with multiple sclerosis [1, 7]; thus, protabzymes with a broader substrate specificity capable of cleaving not only MBP protein but also similarly charged histone H1 can be generated in some patients due to their individual features.

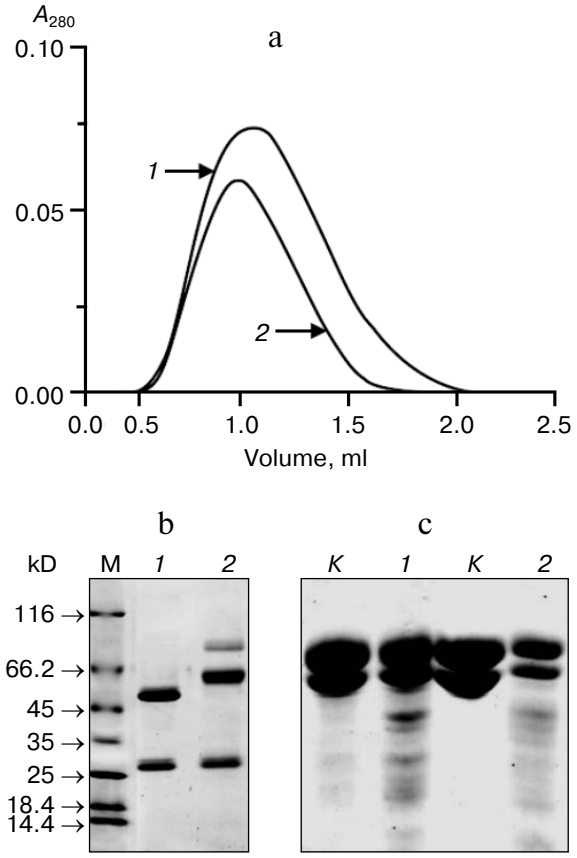

Fig. 5. Purification of antibodies and analysis of their proteolytic activity. a) Affinity chromatography of preparations of IgG (*1*) and sIgA (*2*) on histone H1-Sepharose. b) SDS-PAGE of proteins in eluates: *1*) anti-histone-H1 IgG from blood serum of a patient with multiple sclerosis; *2*) anti-histone-H1 sIgA from colostrum of healthy mothers. c) SDS-PAGE of products of histone H1 hydrolysis by antibody preparations purified by chromatography on histone H1-Sepharose: *K*) histone H1 in the absence of antibodies; *1*) histone H1 in the presence of anti-histone-H1 IgG; *2*) histone H1 in the presence of anti-histone-H1 sIgA.

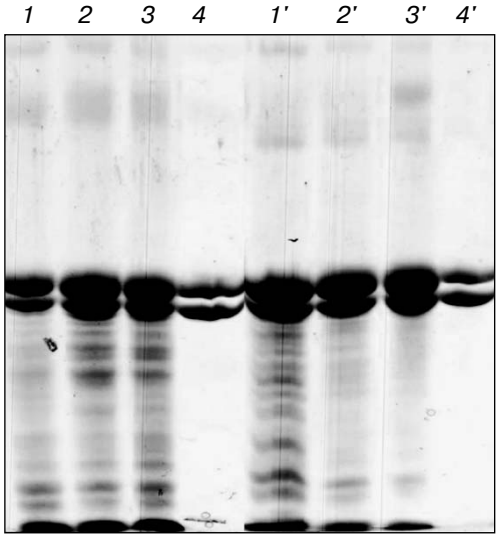

Fig. 6. SDS-PAGE in 12% polyacrylamide gel of products of histone H1 hydrolysis before and after treatment of anti-histone-H1 antibodies (aAB) with protease inhibitors: *1-3*) histone H1 in the presence of anti-histone-H1 sIgA; *1′-3′*) histone H1 in the presence of anti-histone-H1 IgG; *1*, *1′*) aAB + histone H1 in the absence of inhibitors; *2*, *2′*) aAB + histone H1 in the presence of PMSF; *3*, *3′*) aAB + histone H1 in the presence of protease inhibitor mixture; *4*, *4′*) histone H1 in the absence of aAB.

Considering specific features of immune response of the human secretory immune system, sIgA-protabzymes of colostrum are suggested to be products of molecular mimicry of heterologous antigens. Human milk sIgA are known to be produced in response to the influence of microflora on the lymphoid tissue of intestine and bronchi [28]. Possibly, The sIgA affinity for histone H1 from calf thymus and also the proteolytic effect of these antibodies on this protein might be associated with molecular mimicry of antigens from microflora of mothers' mucous membrane.

This work was supported by the Program for Combined Projects of the National Academy of Sciences of Ukraine and the Siberian Division of the Russian Academy of Sciences.

REFERENCES

1. Gabibov, A. G., Ponomarenko, N. A., Tretyak, E. B., Paltsev, M. A., and Suchkov, S. V. (2006) *Autoimmun. Rev.*, **5**, 324-330.
2. Nevinsky, G. A., and Buneva, N. I. (2002) *J. Immunol. Meth.*, **269**, 235-249.
3. Saveliev, A. N., Ivanen, D. R., Kalminskaya, A. A., Ershova, N. A., Kanyshkova, T. G., Buneva, V. N., Mogelnitskii, A. S., Doronin, B. M., Favorova, O. O., Nevinsky, G. A., and Neustroev, K. N. (2003) *Immunol. Lett.*, **86**, 291-297.

4. Paul, S., Volle, D., Beach, C. M., Johnson, D. R., Powell, M. J., and Massey, R. J. (1989) *Science*, **244**, 1158-1162.
5. Li, L., Paul, S., Tyutyulkova, S., Kazatchkine, M. D., and Kaveri, S. (1995) *J. Immunol.*, **154**, 3328-3332.
6. Ponomarenko, N. A., Durova, O. M., Vorobiev, I. I., Aleksandrova, E. S., Telegin, G. B., Chamborant, O. G., Sidorik, L. L., Suchkov, S. V., Aleksandrova, Z. S., Gnuchev, N. V., and Gabibov, A. G. (2002) *J. Immunol. Meth.*, **269**, 197-211.
7. Ponomarenko, N. A., Durova, O. M., Vorobiev, I. I., Belogurov, A. A., Kurkova, I. N., Petrenko, A. G., Telegin, G. B., Suchkov, S. V., Kiselev, S. L., Lagarkova, M. A., Govorun, V. M., Serebryakova, M. V., Avalle, B., Tornatore, P., Karavanov, A., Morse, H. C., Thomas, D., Friboulet, A., and Gabibov, A. G. (2005) *Proc. Natl. Acad. Sci. USA*, **103**, 281-286.
8. Lacroix-Desmazes, S., Moreau, A., Sooryanarayana, S., Bonnemain, C., Stieltjes, N., Pashov, A., Sultan, Y., Hoebeke, J., Kazatchkine, M. D., and Kaveri, S. V. (1995) *Nat. Med.*, **5**, 1044-1047.
9. Paul, S., Li, L., Kalaga, R., Wilkins-Stevens, P., Stevens, F. J., and Solomon, A. (1995) *J. Biol. Chem.*, **270**, 15257-15261.
10. Ponomarenko, N. A., Durova, O. M., Vorobiev, I. I., Belogurov, A. A., Telegin, G. B., Suchkov, S. V., Misikov, V. K., Morse, H. C., 3rd, and Gabibov, A. G. (2006) *Immunol. Lett.*, **103**, 45-50.
11. Paul, S., Karle, S., Planque, S., Taguchi, H., Salas, M., Nishiyama, Y., Handy, H., Hunter, R., Edmundson, A., and Hanson, C. (2004) *J. Biol. Chem.*, **279**, 39611-39619.
12. Lacroix-Desmazes, S., Bayry, J., Kaveri, S. V., Hayon-Sonsino, D., Thorenoor, N., Charpentier, J., Luyt, C. E., Mira, J. P., Nagaraja, V., Kazatchkine, M. D., Dhainaut, J. F., and Mallet, V. O. (2005) *Proc. Natl. Acad. Sci. USA*, **102**, 231-235.
13. Paul, S., Nishiyama, Y., Planque, S., Karle, S., Taguchi, H., Hanson, C., and Weksler, M. E. (2005) *Springer Semin. Immunopathol.*, **26**, 485-503.
14. Odintsova, E. S., Buneva, V. N., and Nevinsky, G. A. (2005) *J. Mol. Recognit.*, **18**, 413-421.
15. Odintsova, E. S., Kharitonova, M. A., Baranovsky, L. P., Sizyakina, L. P., Buneva, V. N., and Nevinsky, G. A. (2006) *Biochemistry (Moscow)*, **71**, 251-261.
16. Brok, J. (1987) in *Immunological Methods* (Frimel, G., ed.) [in Russian], Meditsina, Moscow, pp. 390-413.
17. Kit, Yu. Ya., Semenov, D. V., and Nevinsky, G. A. (1995) *Mol. Biol. (Moscow)*, **29**, 893-906.
18. Laemmli, U. K. (1970) *Nature*, **227**, 2244-2750.
19. Banchev, T., Srebreva, L., and Zlatanova, J. (1991) *Biochim. Biophys. Acta*, **1073**, 230-232.
20. Boggs, J. M. (2006) *Cell Mol. Life Sci.*, **63**, 1945-1961.
21. Chevalier, D., and Allen, B. G. (2000) *Protein Exp. Purif.*, **18**, 229-234.
22. Shechter, D., Dormann, H. L., Allis, C. D., and Hake, S. B. (2007) *Nature Protocols*, **2**, 1445-1457.
23. Royle, L., Roos, A., Harvey, D. J., Wormald, M. R., van Gijlswijk-Janssen, D., Redwan, E.-R. M., Wilson, I. A., Daha, M. R., Dwek, R. A., and Rudd, P. M. (2003) *J. Biol. Chem.*, **278**, 20140-20153.
24. Hardin, J. A., and Thomas, J. O. (1983) *Proc. Natl. Acad. Sci. USA*, **4**, 7410-7414.
25. Wesierska-Gadek, J., Penner, E., Lindner, H., Hitchman, E., and Sauermann, G. (1990) *Arthritis Rheum.*, **33**, 1273-1278.
26. Lu, F., and Kalman, B. (1999) *J. Neuroimmunol.*, **99**, 72-81.
27. Lefranc, D., Almeras, L., Dubucquoi, S., de Seze, J., Vermersch, P., and Prin, L. (2004) *J. Immunol.*, **172**, 669-678.
28. Woof, J. M., and Mastecky, J. (2005) *Immunol. Rev.*, **206**, 64-82.