Biochemistry (Moscow), Vol. 68, No. 12, 2003, pp. 1300-1306. Translated from Biokhimiya, Vol. 68, No. 12, 2003, pp. 1616-1623.

Special Features of the DNA-Hydrolyzing Activity of the Antibodies in Systemic Lupus Erythematosus

T. A. Nevzorova*, D. A. Temnikov, and V. G. Vinter

Kazan State University, ul. Kremlevskaya 18, Kazan 420008, Russia; E-mail: Tatyana.Nevzorova@ksu.ru

Received February 12, 2003

Abstract—Two types of IgG anti-DNA antibodies exhibiting DNA-hydrolyzing activity have been isolated from blood serum of patients with systemic lupus erythematosus. This DNase activity of antibodies differs from serum DNases by the non-processive mode, temperature resistance, pH optimum, and the rate of DNA hydrolysis. It is suggested that the anti-DNA antibody molecule possessing DNase activity contains two sites: one site determines specificity of antibody–DNA interaction, whereas the other is responsible for manifestation of the catalytic activity.

Key words: polyclonal catalytic antibodies, abzymes, systemic lupus erythematosus, IgG, DNA hydrolysis

Effective binding to antigens due to formation of specific noncovalent hydrogen bonds and hydrophobic, electrostatic, and van der Waals interactions is a characteristic feature of antibodies. Taking into consideration highly specific interaction of antibodies with corresponding antigens, L. Pauling proposed the existence of common mechanisms underlying antibody–antigen and enzyme–substrate recognition [1]. He also suggested the possibility of the existence of antibodies exhibiting enzymatic activity. Now convincing evidence exists that antibodies can not only bind and eliminate antigens but also exhibit catalytic activity. Such antibodies are called abzymes (antibody enzyme) or catalytic antibodies. Natural abzymes have been found in blood serum of patients with autoimmune diseases (systemic lupus erythematosus, scleroderma [2, 3], autoimmune thyroiditis, polyarthritis [4], rheumatoid arthritis [5]), various forms of viral hepatitis [6], AIDS [7], proliferative diseases of lymphoid tissue [8], asthma [9], and in milk of healthy postpartum women [10]. Among natural catalytic antibodies exhibiting various enzymatic activities, anti-DNA antibodies with DNase activity attract much interest. These anti-DNA abzymes are suggested to be directly involved in pathogenesis of the above-mentioned diseases by participating in elimination of auto-aggressive lymphocytes via apoptosis or being additional factors in the development of certain pathologies. DNA- and RNA-hydrolyzing antibodies can be used for diagnostics of various autoimmune diseases and for evaluation of the course of a pathological processes and efficacy of medical treatment [11].

* To whom correspondence should be addressed.

In the present study, we investigated DNase activity of anti-DNA antibodies isolated from serum of patients with systemic lupus erythematosus, an autoimmune disease of unclear etiology.

MATERIALS AND METHODS

The following chemicals were used in the study: Sephadex G-25, protein A-Sepharose CL-4B, *o*-phenylenediamine, and agarose NA (Pharmacia, Sweden); DEAE-cellulose 23SH, acrylamide, N,N′-methylene-*bis*-acrylamide, SDS, glycine, protein standards of molecular mass, and calf thymus DNA (Serva, Germany); chicken erythrocyte DNA (Reanal, Hungary); Tween-20 (Merck, Germany); and horseradish-conjugated diagnostic antibodies against human IgG (conjugate) (Best, Novosibirsk, Russia). Other chemicals produced by Reakhim (Russia) were of "special purity" grade.

Blood serum of five patients with systemic lupus erythematosus and five healthy subjects was obtained from Kazan hospitals.

Plasmid pBR-322 DNA was isolated and purified by the method of alkaline extraction followed by subsequent gel filtration on Sepharose 4B [12]. The quality of plasmid DNA at each stage of the purification procedure was evaluated spectrophotometrically and electrophoretically.

Isolation of IgG. Blood serum antibodies were sedimented by ammonium sulfate fractionation (50% saturation). The pellet obtained after centrifugation was then dissolved in 20 mM Tris-HCl buffer, pH 8.8, containing

0006-2979/03/6812-1300$25.00 ©2003 MAIK "Nauka/Interperiodica"

300 mM NaCl (buffer A), and desalted on Sephadex G-25 in buffer A. The resulting mixture of proteins was dialyzed at 4°C for 14 h against 500 volumes of 20 mM Tris-HCl buffer, pH 8.8, containing 30 mM NaCl (buffer B) and applied onto a DEAE-cellulose column equilibrated with buffer B. The sorbent-bound fractions were eluted by a linear concentration gradient of NaCl (from 30 to 300 mM) in 20 mM Tris-HCl buffer, pH 8.8. The final step of IgG-antibody purification included affinity chromatography on protein A-Sepharose in 10 mM Tris-HCl buffer, pH 7.5, 125 mM NaCl (buffer C). After dialysis against buffer B the following fractions of IgG-antibodies were applied on to the protein A-Sepharose column (5 ml) by double circulation: the fraction (fraction 1) that did not bind to DEAE-cellulose, and the sorbent-bound IgG (fraction 2). Proteins which did not interact with protein-A-Sepharose (fractions 1a and 2a) during column loading were washed out with buffer B up to total disappearance of the protein absorbance. IgG-fractions (1b and 2b) eluted by 0.1 M glycine-HCl buffer, pH 2.6, were immediately neutralized with 1 M Tris-HCl buffer, pH 8.0. The resulting IgG fractions 1b and 2b were dialyzed at 4°C for 14 h against phosphate buffered saline (PBS) containing 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 2.7 mM KCl, 137 mM NaCl, pH 7.3, normalized by concentration and subjected to thermal treatment at 56°C for 40 min.

Enzyme-linked immunoassay was used to determine antibody interaction with native DNA and antibody DNA-binding activity [13]. Sera were diluted 1 : 100, and antibody preparations were used at the concentration 0.1 mg/ml (in pentaplicates). Results were statistically treated using Student's *t*-test. For objective evaluation, results were expressed in arbitrary units representing a ratio of optical density of an experimental sample referred to the optical density of standard serum from patients with systemic lupus erythematosus characterized by high content of anti-DNA antibody.

SDS-PAGE. Purity of antibody preparation at each purification stage was investigated by SDS-PAGE under non-dissociating and dissociating (in the presence of 2-mercaptoethanol) conditions according to Laemmli [14]. After electrophoresis proteins were stained with $AgNO_3$ [15].

Plasmid DNA hydrolysis. DNA-hydrolyzing activity of anti-DNA antibodies was examined by conversion of plasmid pBR-322 supercoiled DNA into circular and linear forms. The optimal conditions for DNA hydrolysis by autoantibodies were determined by varying pH of the reaction medium and concentrations of bivalent cations and ATP. The reaction medium contained 25 mM Tris-HCl buffer, pH 7.5, 50 mM NaCl, 5 mM $MgCl_2$, 0.5 mM EDTA, and 0.2 mg/ml plasmid pBR-322 supercoiled DNA. The reaction was started by adding: a) intact or heated (at 56°C for 40 min) serum proteins in final concentration 0.07-5.0 mg/ml; b) 0.4 mg/ml of 1b or 2b fractions after affinity chromatography on protein A-Sepharose (before addition they were heated at 56°C for 40 min); c) 0.8 mg/ml of IgG heated at 56°C for 40 min; d) equal quantities of the purified IgG (final concentration 0.8 mg/ml), preheated at 56°C for 40 min. They were sequentially added into the reaction medium after certain time intervals (d). The reaction medium was incubated at 37°C, and aliquots of 12 μl were taken during the incubation period (1-27 h).

Electrophoretic analysis of pBR-322 plasmid DNA in agarose gel. This was carried out in 0.8% agarose gel followed by DNA staining with ethidium bromide (1 μg/ml) for 15 min. Gels were photographed in UV-light, using an orange filter [12]. Photographs were scanned, graphic files were computationally treated using our original method for obtaining densitograms [16]. The relative content (%) of various forms was determined by the area of the corresponding peaks. Absolute error of determination of relative content of various DNA forms did not exceed 2%.

Fluorescent spectroscopy. Fluorescence of ethidium bromide–DNA complex was measured using a Hitachi MPF-4 spectrofluorimeter (fluorescence excitation at 365 nm). The fluorescence was recorded in 1 cm cuvette at 600 nm. Aliquots of the reaction media containing 1.5 μg DNA were added to 2 ml of ethidium bromide solution (1 μg/ml in 0.01 M Tris-HCl buffer, pH 7.8, containing 0.02 M NaCl, 5 mM EDTA) [17].

RESULTS

Figure 1 shows the scheme of purification of IgG preparations containing anti-DNA antibody. The fraction of total IgG obtained by ammonium sulfate fractionation followed by subsequent desalting on Sephadex G-25 was then separated into two fractions during ion-exchange chromatography on DEAE-cellulose. Fraction 1 did not adsorb on the anion exchanger, whereas fraction 2 was eluted from DEAE-cellulose by ~0.175 M NaCl. SDS-PAGE revealed the presence of IgG in both fractions. Subsequent IgG purification of both fractions employed affinity chromatography on protein A-Sepharose; this step subdivided each fraction into two subfractions. Their composition was analyzed by PAGE (Fig. 2). Electrophoresis under non-dissociating conditions revealed that fractions 1a, 1b, and 2b contained only one protein of molecular mass of 150 kD, which corresponded to IgG. Staining of gels after electrophoresis of fractions 1b and 2b under dissociating conditions (with 2-mercaptoethanol) revealed the presence of only two protein bands corresponding to light and heavy chains of IgG. Other protein bands were not found. Enzyme-linked immunosorbent assay revealed a high level of antibody against native DNA in fraction 1b (1.799 ± 0.175). A much smaller level of this antibody was also found in frac-

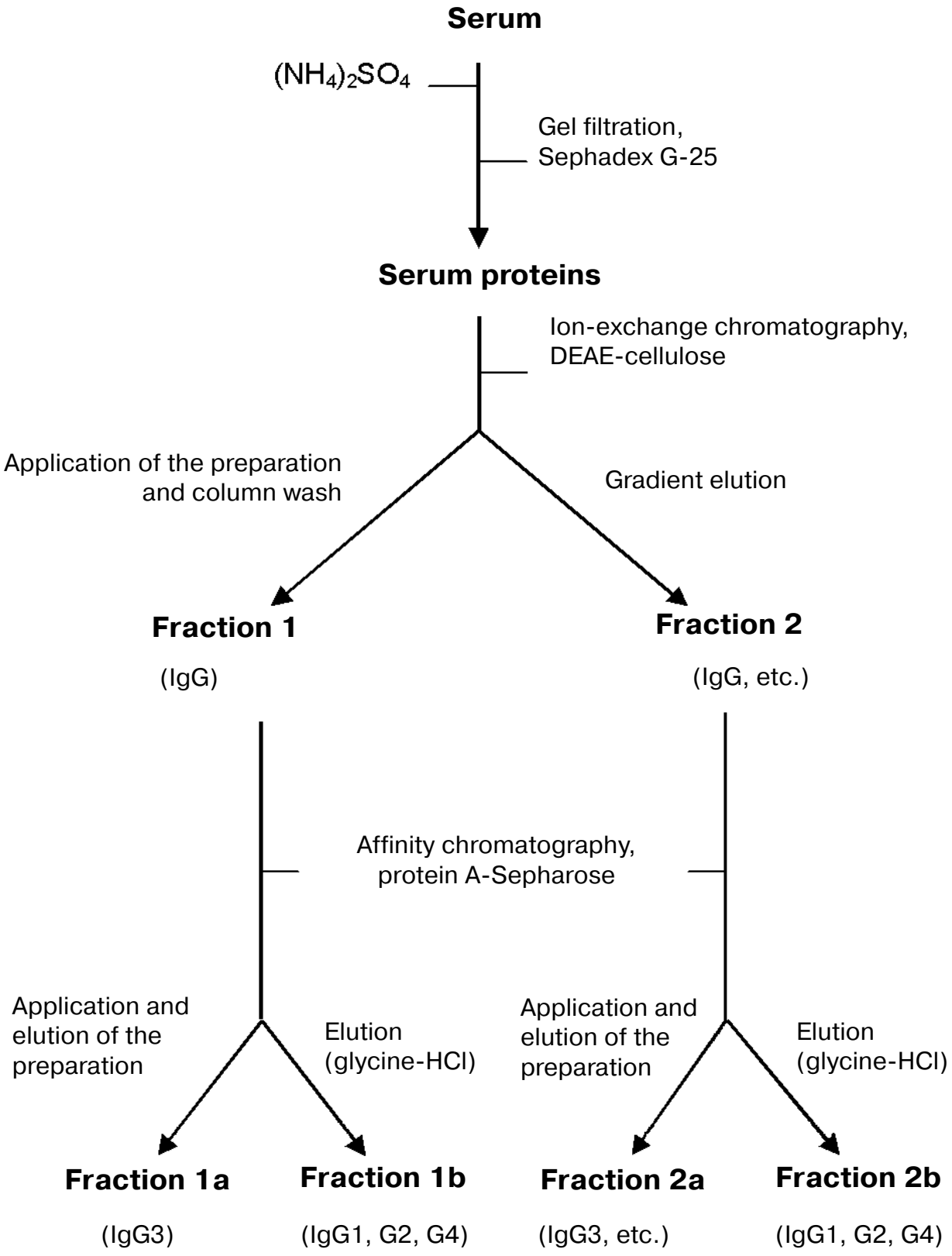

Fig. 1. Scheme of purification of IgG from blood serum of patients with lupus erythematosus.

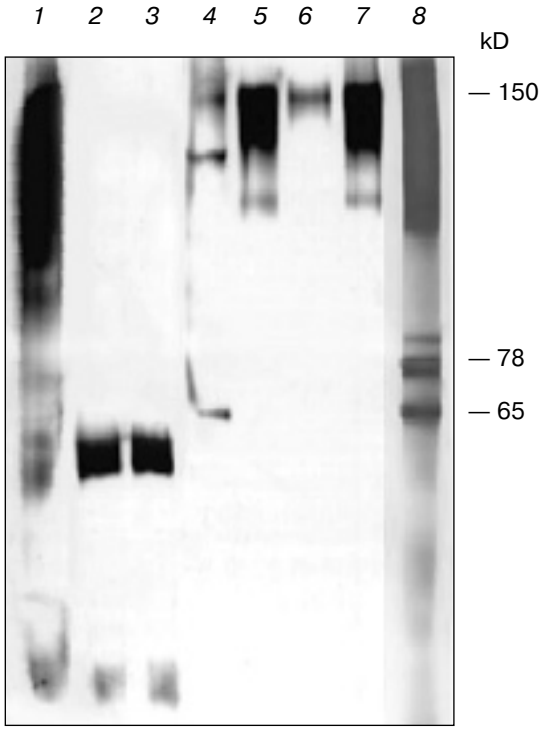

Fig. 2. SDS-PAGE of the IgG antibody after purification on protein A-Sepharose. Lanes: *1*) commercially purchased IgG; *2*, *3*) fractions 2b and 1b under dissociating conditions (with 2-mercaptoethanol); *4*) fraction 2a; *5*) fraction 2b; *6*) fraction 1a; *7*) fraction 2b; *8*) protein markers of known molecular mass.

tions 1a (0.107 ± 0.018) and 1b (0.277 ± 0.008). No antibody against native DNA was found in fraction 2a.

Supercoiled DNA of pBR-322 plasmid was used as substrate for determination of DNA-hydrolyzing activity of the IgG fractions. The formation of single breaks in DNA molecule was determined using fluorescent analysis and/or plasmid DNA electrophoresis in agarose gel.

Human blood serum is known to contain significant amounts of its own DNases, which might contaminate the resulting IgG preparation. So we investigated the effect of heating (at 56°C for 40 min) on inactivation of serum DNases. Data of Table 1 show that heating of blood serum of healthy volunteers caused total inactivation of serum nucleases. Based on this observation, all anti-DNA antibody preparations tested for DNase activity were preheated at 56°C for 40 min. It should be noted that blood sera of patients with systemic lupus erythematosus retained high DNase activity after the heat treatment at 56°C for 40 min. This was especially demonstrative during accentuations of this disease.

Two of four IgG fractions, obtained after affinity chromatography on protein A-Sepharose, namely fractions 1b and 2b, exhibited nuclease activity with respect to pBR-322 supercoiled DNA.

Both preparations (antibody fractions 1b and 2b) exhibited DNA-hydrolyzing activity within the range of pH from 7.4 to 7.8, with pH-optimum at 7.5. Magnesium ions (5 mM) activated this reaction, whereas Mn^{2+}, Ca^{2+}, Zn^{2+}, and ATP did not influence antibody DNA-hydrolyzing activity.

Kinetic studies of plasmid pBR-322 DNA hydrolysis by antibody fractions 1b and 2b revealed (Fig. 3) that IgG of fraction 2b is more active than IgG of fraction 1b. The latter hydrolyzed DNA in 15 h, whereas the former hydrolyzed DNA in 10 h. Prolonged incubation did not cause further quantitative conformational changes in DNA structure. However, the kinetic curve of the plasmid pBR-322 DNA hydrolysis catalyzed by both antibody

Table 1. Effect of preincubation on serum DNA-hydrolyzing activity

Electrophoretic method							Fluorescence method
Substrate	Preparation	Antibody treatment at 56°C for 40 min	Incubation	DNA content, %			Increase in fluorescence* ($I_t - I_0$)
				supercoiled	circular	linear	
pBR-322 DNA	—	—	—	65.0	35.0	0	0
pBR-322 DNA	—	—	10 h at 37°C	65.0	33.4	< 2.0	3.5
pBR-322 DNA	serum from volunteer	—	10 h at 37°C	2.0	79.0	19.0	13.0
pBR-322 DNA	serum from volunteer	+	10 h at 37°C	65.0	34.0	< 2.0	4.0
pBR-322 DNA	serum from patient with systemic lupus erythematosus	+	10 h at 37°C	36.0	48.0	16.0	8.5

* I_t, fluorescence intensity after time interval *t* after antibody addition; I_0, fluorescence of supercoiled DNA.

fractions has the shape of two-stage substrate cleavage which is unusual for catalytic behavior of enzymes. Since in these experiments we used excess of the substrate (plasmid pBR-322 DNA), equal portion of fractions 1b and 2b were added to corresponding reaction media after 15 and 10 h, respectively (Fig. 3). The second addition of antibody preparation caused further reduction of supercoiled DNA accompanied by the increase in opened circular DNA. These opened circular DNA molecules were obviously resistant to the catalytic antibodies, because the amount of linear form of DNA remained unchanged. Similar results were obtained during repeated addition of preheated serum of patients with lupus erythematosus to the reaction medium. Table 2 shows that repeated addition of the preheated serum into the incubation medium caused sharp increase in the fluorescence intensity of ethidium bromide–DNA complex. This suggests the appearance of additional opened circular DNA molecules and increased ethidium bromide binding to DNA.

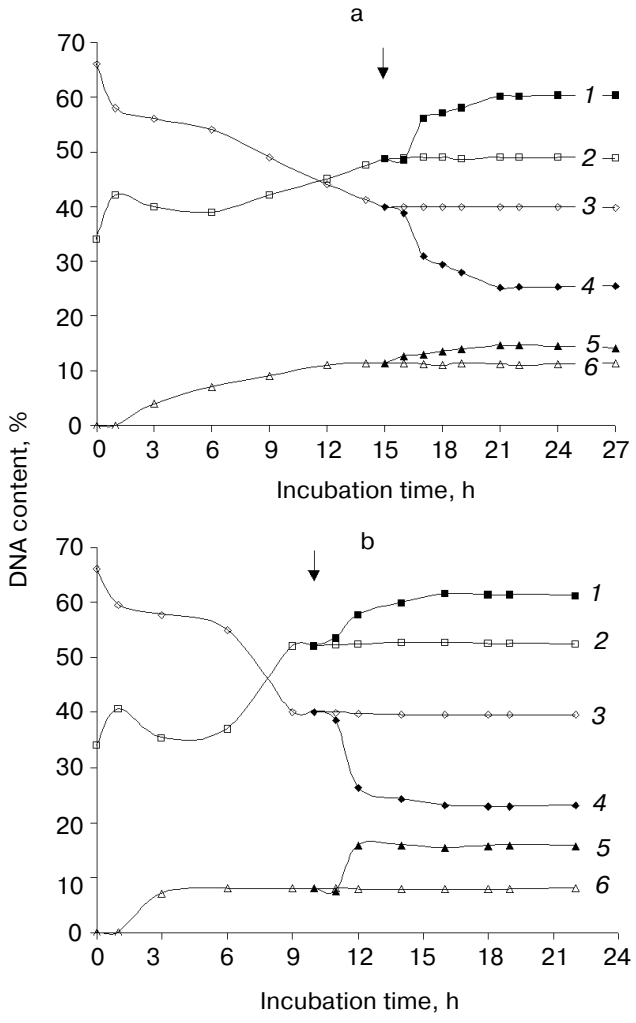

Fig. 3. Kinetics of the pBR-322 plasmid DNA hydrolysis by anti-DNA-antibodies of fractions 1b (a) and 2b (b). Simultaneous addition of one antibody portion: *3*) supercoiled DNA; *2*) circular DNA; *6*) linear DNA. Stepwise addition of two antibody portions (the arrow indicates time of repeated addition of the antibody into the incubation medium): *4*) supercoiled DNA; *1*) circular DNA; *5*) linear DNA.

DISCUSSION

Antibodies to native DNA are known [18, 19] to constitute one of the IgG fractions. So, isolation of catalytic antibodies exhibiting DNA-hydrolyzing activity required maximal purification of serum IgG from other serum proteins. Thus, much attention was paid to isolation and characterization of purified IgG antibodies.

Our study revealed the existence of two types of IgG antibody to native DNA which differed in adsorption on DEAE-cellulose. Resulting catalytic antibodies against native DNA (fractions 1b and 2b) exhibited DNase activity which differed from serum DNases known in the literature. DNase activity of anti-DNA antibody present in serum of patients with systemic lupus erythematosus is thermostable. Heating of serum from patients with erythematosus or resultant antibody preparations at 56°C for 40 min did not influence catalytic activity of antibodies evaluated by their ability to convert supercoiled plasmid DNA into opened forms. The same thermal treatment caused total inactivation of serum DNases. These differences in thermostability of serum DNases and nuclease activity of anti-DNA antibodies in systemic lupus erythematosus may be used for the development of new methods for diagnostics and evaluation of effectiveness of medical treatment.

It is known that serum DNases type I and type II have pH optimum of 7.3-7.6 and 5.2, respectively [20]. Comparative study of catalytic behavior of antibody preparations of fractions 1b and 2b revealed pH optimum value which was close to that of DNase type I.

DNases type I can hydrolyze their substrate only in the presence of 15 mM Mg^{2+} or Mn^{2+}, whereas in the case of catalytic anti-DNA antibody only 5 mM Mg^{2+} activated pBR-322 plasmid DNA hydrolysis. Thus, optimal conditions for manifestation catalytic activity of anti-DNA antibody and DNase type I differ somewhat. It is possible that the catalytic mechanism of DNA hydrolysis by antibody preparations of fractions 1b and 2b involves nucleophilic attack by hydroxyl-ion activated by Mg^{2+}.

Shevelev et al. [21] found that in the presence of salt concentration in the incubation medium (50 mM) with ionic strength not less than 0.15, the endonuclease activity of DNases is almost totally inhibited. This may explain low DNase activity in serum of healthy subjects before heating of serum, which requires much time for conversion of supercoiled DNA into opened form under these conditions. The presence of salts in the reaction medium

Table 2. Fluorescent spectroscopy data on pBR-322 DNA hydrolysis by serum antibodies from patients with systemic lupus erythematosus

Substrate	Protein concentration, mg/ml	Incubation, h	Increase in fluorescence* ($I_t - I_0$)
pBR-322 DNA	2.5 5.0	18 ** 24	7.5 10.0
pBR-322 DNA	2.5 2.5	18 24	7.5 ± 0.3 8.0 ± 0.3
pBR-322 DNA	5.0 5.0	18 24	9.0 9.0
pBR-322 DNA	—	24	1.5 ± 0.5

* I_t, fluorescence intensity after time interval t after antibody addition; I_0, fluorescence of supercoiled DNA.
** Time of the second addition of the same amount of the antibody into the incubation medium.

is additional argument for catalytic DNase activity in antibodies.

Studies of kinetics of supercoiled DNA hydrolysis revealed that the two antibody fractions are characterized by different rates of the hydrolytic reaction. We found that in contrast to serum DNases, the shape of kinetic curve of supercoiled DNA cleavage by antibody preparations is characterized by two-stage DNA cleavage subsequently reaching a plateau, rather than the usual linear or hyperbolic curve.

Good evidence exists that natural antibodies can exhibit their own catalytic activity: phosphatase, protease, DNase, RNase, etc. [3, 22-25]. In contrast to corresponding enzymes, abzymes are characterized by low catalytic activity, which is explained by high affinity of catalytic antibodies to antigens (0.1-10 μM) when high rates of chemical reactions are not possible. It is suggested that antibodies as well as enzymes are conformationally active and their interaction with antigens is characterized by conformational rearrangements of both antibodies and antigens. Such conformational changes may explain prolonged period of hydrolysis of plasmid pBR-322 supercoiled DNA by anti-DNA antibodies in lupus erythematosus.

According to our data, both antibody preparations (fractions 1b and 2b) do not hydrolyze all supercoiled DNA even during prolonged incubation for more than 22 h. It is possible that the IgG of fraction 1b and 2b are endonucleases introducing single strand breaks into supercoiled DNA molecules and converting them into open circular forms which are resistant to subsequent catalytic attack by these antibodies. Such mechanism could well explain lack of accumulation of DNA linear forms.

It is known that enzyme–substrate complex exists for a limited time interval determined by the time required for existence of intermediate reaction complex [26], whereas associative antigen–antibody complex dissociates at a slower rate [27]. Moreover, in contrast to formation of enzyme–substrate complex, which depends on the amount of substrate and enzyme in the incubation medium, effective antibody–antigen interaction requires certain antibody/antigen ratio. Incomplete hydrolysis of supercoiled DNA molecules in the incubation medium containing excess of substrate (DNA) and low concentration of anti-native DNA antibody may be attributed to formation of stable immune antibody–DNA complex. It is possible that anti-DNA-abzyme–DNA interaction initially involves mechanisms typical for formation of antigen–antibody immune complexes and catalytic properties of antibodies are realized later. By analogy with many enzymes [28], active antigen-binding site of abzymes might have two sites: the first one represents "anchor region" responsible for specific interaction between antibody and DNA, whereas the second site determines catalytic activity. However, in contrast to enzymes, catalytic cleavage of phosphodiester bond of DNA is not accompanied by DNA release from the antibody molecule.

Nevinsky and his colleagues [23, 29, 30] demonstrated that oligomeric forms of IgG-antibodies, their Fab-fragments, and isolated light chains exhibit endonuclease activity. In some cases, catalytic activity of isolated light chains was higher than that of oligomeric forms. Studying human milk sIgA, these authors found [31] that light chains of immunoglobulins have lower affinity to DNA, and these light chains were mainly responsible for DNase activity. Heavy chains of sIgA readily bound to DNA-cellulose but they did not exhibit nuclease activity. This is consistent with results by Li et al. [32]; using gene engineering method, these authors demonstrated that heavy chains of IgG play a major role in the antibody binding to DNA in lupus erythematosus. The existence of two sites involved in antibody–DNA interaction would explain the non-processive mode of the antibody effect on DNA when after conversion of supercoiled plasmid pBR-322

DNA molecule into the open circular form DNA remains bound to the antibody.

REFERENCES

1. Pauling, L. (1948) *Am. Scientist*, **36**, 51-59.
2. Gololobov, G. V., Shuster, A. M., Kvashuk, O. A., and Gabibov, A. G. (1991) *Mol. Biol.* (Moscow), **25**, 593-602.
3. Shuster, A. M., Gololobov, G. V., Kvashuk, O. A., Bogomolova, A. E., Smirnov, I. V., and Gabibov, A. G. (1992) *Science*, **256**, 665-667.
4. Vlassov, A. V., Baranovsky, A. G., Kanyshkova, T. G., Prints, A. V., Zabara, V. G., Naumov, V. A., Breusov, A. A., Giege, R., Buneva, V. N., and Nevinsky, G. A. (1998) *Mol. Biol.* (Moscow), **32**, 559-569.
5. Bronstein, I. B., Shuster, A. M., Gololobov, G. V., Gromova, I. I., Kvashuk, O. A., Belostotstaya, K. M., Alekberova, Z. S., Prokaeva, T. V., and Gabibov, A. G. (1992) *FEBS Lett.*, **314**, 259-263.
6. Baranovsky, A. G., Matyushin, V. G., Vlassov, A. V., Zabara, V. G., Naumov, V. A., Giege, R., Buneva, V. N., and Nevinsky, G. A. (1997) *Biochemistry* (Moscow), **62**, 1358-1366.
7. Gololobov, G. V., Mikhalp, S. V., Starov, A. V., Kolesnikov, A. V., and Gabibov, A. G. (1994) *Appl. Biochem. Biotechnol.*, **47**, 305-315.
8. Kozyr, A. V., Kolesnikov, A. V., Yakhnina, E. I., Astsaturov, I. A., Varlamova, E. Yu., Kirillov, E. V., and Gabibov, A. G. (1996) *Byull. Eksp. Biol. Med.*, No. 2, 204-206.
9. Paul, S., Volle, D. J., Beach, C. M., Johnson, D. R., Powell, M. J., and Massey, R. J. (1989) *Science*, **244**, 1158-1161.
10. Kanyshkova, T. G., Semenov, D. V., Vlassov, A. V., Shipitsyn, M. V., Yamkovoi, V. I., Buneva, V. N., and Nevinsky, G. A. (1997) *Mol. Biol.* (Moscow), **31**, 1082-1091.
11. Nevinsky, G. A., Breusov, A. A., Baranovskii, A. G., Prints, A. V., Kanyshkova, T. G., Galvita, A. V., Naumov, V. A., and Buneva, V. N. (2001) *Med. Sci. Monit.*, **7**, 201-211.
12. Kuznetsova, N. N., and Vinter, V. G. (1997) *Methods of Gene Engineering* [in Russian], Bioinformservis, Moscow.
13. Sattarova, L. I., Gafiyatullina, L. A., Agliullina, D. G., and Vinter, V. G. (1994) *Biotekhnologiya*, **11/12**, 38-41.
14. Laemmli, U. K. (1970) *Nature*, **227**, 680-685.
15. Osterman, L. A. (1983) *Study of Biological Macromolecules by Isoelectrofocusing, Immunoelectrophoresis and Radioisotope Methods* [in Russian], Nauka, Moscow.
16. Temnikov, D. A. (1999) *Pribor. Tekh. Eksp.*, **6**, 59-62.
17. Paoletti, C., Le Pekg, J. B., and Lehmman, J. R. (1971) *J. Mol. Biol.*, **55**, 75-99.
18. Okamura, M., Kanayama, Y., Amastu, K., Negoro, N., Kohda, S., Takeda, T., and Inoue, T. (1993) *Ann. Rheum. Dis.*, **52**, 14-20.
19. Winkler, T., Henschel, T., Kalies, I., Baenkler, H., Skvaril, F., and Kalden, J. (1988) *Clin. Exp. Immunol.*, **72**, 434-439.
20. Shapot, V. S. (1968) *Nucleases* [in Russian], Meditsina, Moscow.
21. Shevelev, I. V., Legina, O. K., Tutaev, K. Yu., and Krutyakov, V. M. (1998) *Mol. Biol.* (Moscow), **32**, 741-744.
22. Buneva, V. N., Andrievskaya, O. A., Romannikova, I. V., Gololobov, G. V., Yadav, R. P., Yamkovoi, V. I., and Nevinsky, G. A. (1994) *Mol. Biol.* (Moscow), **28**, 738-741.
23. Kit, Yu. Ya., Semenov, D. V., and Nevinsky, G. A. (1995) *Mol. Biol.* (Moscow), **29**, 893-906.
24. Semenov, D. V., Kanyshkova, T. G., Kit, Yu. A., Khlimankov, D. Yu., Akimzhanov, A. M., Gorbunov, D. A., Buneva, V. N., and Nevinsky, G. A. (1998) *Biochemistry* (Moscow), **63**, 935-943.
25. Savel'ev, A. N., Eneyskaya, E. V., Shabalin, K. A., Filatov, M. V., and Neustroev, K. N. (1999) *Protein Peptide Lett.*, **6**, 179-184.
26. Murray, R., Granner, D., Mayes, P., and Rodwell, V. (1993) *Harper's Biochemistry*, 21st Edn. [Russian translation], Mir, Moscow.
27. Roitt, I., Brostoff, J., and Male, D. (2000) *Immunology* [Russian translation], Mir, Moscow.
28. Blow, D. (2000) *Structure with Folding and Design*, **8**, R77-R81.
29. Baranovsky, A. G., Kanyshkova, T. G., Mogilnitsky, A. S., Naumov, V. A., Buneva, V. N., Gusev, E. I., Boyko, A. N., Zargarova, T. A., Favorova, T. A., and Nevinsky, G. A. (1998) *Biochemistry* (Moscow), **63**, 1239-1248.
30. Vlassov, A. V., Andrievskaya, O. A., Kanyshkova, T. G., Baranovsky, A. G., Naumov, V. A., Breusov, A. A., Giege, R., Buneva, V. N., and Nevinsky, G. A. (1997) *Biochemistry* (Moscow), **62**, 474-479.
31. Nevinsky, G. A., Kanyshkova, T. G., Semenov, D. V., Vlassov, A. V., Gal'vita, A. V., and Buneva, V. N. (2000) *Appl. Biochem. Biotechnol.*, **83**, 115-129.
32. Li, Z., Schettino, E. W., Padlan, E. A., Ikematsu, H., and Casali, P. (2000) *Eur. J. Immunol.*, **30**, 2015-2026.