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DNase, RNase, and Phosphatase Activities of Antibodies Formed upon Immunization by DNA, DNase I, and DNase II

M. A. Krasnorutskii, V. N. Buneva, and G. A. Nevinsky*

Institute of Chemical Biology and Fundamental Medicine, Siberian Branch of the Russian Academy of Sciences, pr. Lavrentieva 8, 630090 Novosibirsk, Russia; fax: (383) 363-5153; E-mail: nevinsky@niboch.nsc.ru

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Abstract—Relative DNase, RNase (efficiency of hydrolysis of ribo- and deoxyribooligonucleotides (ON)), and phosphatase (removal of the ON 5′ terminal phosphate) catalytic activities of antibodies (AB) obtained after rabbit immunization by DNA, DNase I, and DNase II were compared. It is shown that electrophoretically homogeneous preparations of polyclonal AB from non-immunized rabbits did not exhibit such activities. Immunization of rabbits by DNA, DNase I, and DNase II results in generation of IgG abzymes that exhibit high activity in the ON hydrolysis reaction and even higher activity in cleavage of 5′ terminal phosphate of ON. In this case K_m values for supercoiled plasmid DNA and ON found in reactions of their AB-dependent nuclease hydrolysis and phosphatase cleavage of 5′ terminal phosphate differ by 2-4 orders of magnitude. This shows that nuclease and phosphatase activities belong to different abzyme fractions within polyclonal AB. Thus, in this work data indicative of the possibility of a formation of antibodies exhibiting phosphatase activity after immunization of animals with DNA, DNase I, and DNase II, were obtained for the first time. Possible reasons for production of AB with phosphatase activity after immunization of rabbits with these immunogens are discussed.

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Key words: immunization of rabbits with DNA, DNase I, and DNase II; abzymes with RNase, DNase, and phosphatase activities

Artificial antibodies (AB) against stable analogs of transition states of chemical reactions with catalytic functions (or abzymes) are already well described [1].

It is known that the blood of healthy donors may contain polyclonal auto-AB to very different antigens [2, 3]. However, the relative amount of auto-AB is significantly higher in the blood of people with very different autoimmune diseases (AID) [2, 3], while the presence in the blood of AB with different catalytic activities is characteristic just of different AIDs [1, 4-8]. It is assumed that there are two main pathways of natural abzyme formation. Like artificial abzymes, natural abzymes can be produced directly against antigen that in certain conditions can imitate a transition state [1]. Among such abzymes are AB specifically hydrolyzing vasoactive intestinal peptide (VIP) (asthma) [9], thyroglobulin (autoimmune thyroiditis) [10], myelin basic protein (multiple sclerosis) [11], and a number of different proteins in many AIDs [5-8]. Abzymes with nuclease activities can be produced directly against free DNA and RNA or against their complexes with different proteins [5-8].

Abzymes can be also of antiidiotypic nature: in this case they are formed in accordance with Erne theory [12-14]. If idiotypic AB are targeted against an enzyme active center, then the corresponding secondary antiidiotypic AB may have structural characteristics of the initial enzyme active center and, as a result, they may exhibit catalytic activity. A number of enzymes were used to obtain monoclonal abzymes with acetylcholine esterase [14, 15], carboxypeptidase [16, 17], and β-lactamase [18] activities. It is supposed that the blood of patients with systemic lupus erythematosus (SLE) can contain DNA-hydrolyzing AB against topoisomerase I [19].

In the case of different AIDs, anti-DNA and anti-RNA antibodies with nuclease activities are the result of autoimmunization by DNA and RNA complexes with various proteins, including histones that appear in blood

Abbreviations: AB, antibodies; AID, autoimmune diseases; mBSA, methylated BSA; ON, oligonucleotide; pAB, polyclonal antibodies; pIgG, polyclonal IgG; RA, relative activity; scDNA, supercoiled DNA; SLE, systemic lupus erythematosus; VIP, vasoactive intestinal peptide.

* To whom correspondence should be addressed.

after cell apoptosis [20]. By now IgG and/or IgM as well as IgA are described which hydrolyze DNA, RNA, proteins, and polysaccharides isolated from blood of patients with different AIDs (SLE, Hashimoto's thyroiditis, polyarthritis, multiple sclerosis) as well as with lymphoproliferative and some viral diseases (viral hepatitis, HIV infection, and tick-borne encephalitis [1, 4-8, 21]).

Canonical DNases are known to hydrolyze only DNA and they do not exhibit anti-RNA activity, while RNases are not able to hydrolyze DNA. Unlike canonical nucleases, IgG-, IgA-, sIgA-, and IgM-abzymes from blood of patients with AIDs and milk of healthy women more efficiently hydrolyze RNA than DNA [4-8, 22-25]. It was shown in work [26] that sIgA from human milk hydrolyze ribosomal RNA. Interestingly, different mouse monoclonal IgG against different context B-DNA hydrolyzed any RNA at rates 30-100 times exceeding those in DNA hydrolysis [27]. It was also shown that immunization of rabbits with DNase I results in production of idiotypic AB1, immunization by which results in production of antiidiotypic AB2 with DNase activity [28]. However, it was unknown until recently whether autoimmunization by DNA (or RNA) results only in formation of polyfunctional abzymes able to hydrolyze both DNA and RNA, or perhaps the simultaneous formation of Ig exhibiting only DNase or only RNase activity, or whether some other activities appear. It was also not clear whether AB with RNase activity, which are of antiidiotypic nature, can be produced as well. To elucidate this question, we recently immunized rabbits with DNase I, DNase II, RNase A, and mBSA (methylated BSA) complexes with DNA and RNA and compared relative activities of the resulting AB in hydrolysis of plasmid scDNA (supercoiled DNA) and different polyribonucleotides [29-33]. It has been shown that in all cases pIgG (polyclonal IgG) efficiently hydrolyzing both RNA and DNA are formed. The ratio of these activities and AB affinity to DNA and RNA depended on the used antigen, but on the whole, the abzymes obtained in the case of all mentioned antigens were 3-4 orders of magnitude more active in RNA hydrolysis than with that of DNA [29-33]. We believe that for different AIDs, DNA and RNA hydrolyzing abzymes comprise a "cocktail" directed against DNA, RNA, as well as against secondary AB against very different enzymes hydrolyzing nucleic acids [8, 34].

The works [29-33] use of polymeric substrates did not allow the authors to determine whether the same or different abzyme fractions within polyclonal AB catalyze hydrolysis of nucleic acids and exhibit phosphatase activity.

The aim of this work was to analyze the enzymological features of AB obtained upon immunization of rabbits with DNA, DNase I, and DNase II in reactions of hydrolysis of different $d(pN)_n$, $(pN)_n$, and 5′-terminal phosphate cleavage.

MATERIALS AND METHODS

Immunization of rabbits. Healthy three-month-old rabbits underwent triple immunization by the same immunogen: DNA complex with mBSA (1.5 mg highly polymeric DNA (free of RNA) from calf thymus in mixture with 1 mg mBSA), DNase I (0.5 mg), DNase II (0.5 mg), and BSA (1 mg) using physiological solutions (0.85% NaCl, 0.02 M NaH_2PO_4, pH 7.2) according to [29-33]. Mixtures of 0.5 volume of Freund's complete adjuvant and 0.5 volume of antigen solution were used. The mixture was stirred until homogeneous gel formation and introduced subcutaneously into pads. Two repetitive immunizations were carried out using a mixture with incomplete Freund's adjuvant after 21 days and then 7 days later. Immunization results were registered two months after the third immunization.

Isolation of antibodies from rabbit blood. Electrophoretically and immunologically homogeneous pAB preparations from rabbit blood were obtained using modified technology developed earlier for abzyme isolation from AID patient blood; affinity chromatography on protein A-Sepharose was used followed by high performance gel filtration on a Superdex 200 HR column as described in the literature [24, 25, 35, 36]. For protection against contamination, AB fractions were filtered through a Millex filter (Millipore, USA), pore size 0.2 μm. Then they were used for analysis after storage in neutral buffer during 1 week at 4°C. The absence of IgA and IgM in IgG preparations was confirmed by immunoblotting [22, 23, 30-33]. AB purity was analyzed by electrophoresis in 4-15% gradient polyacrylamide gel (0.1% SDS with (or without) 10 mM dithiothreitol) according to Laemmli. Proteins were incubated for 1 min at 100°C in 50 mM Tris-HCl buffer, pH 6.8, containing 2% SDS, 10% glycerol, and 0.025% bromphenol blue, and then they were applied on the gel. After electrophoresis the proteins were stained with $AgNO_3$ [29-33]. Electrophoretic homogeneity of AB was shown. AB preparations, all characteristics of which are shown in works [29-33], were used in this work.

Determination of DNase, RNase, and phosphatase activities of AB. In analysis of the IgG RNase and phosphatase activities, the standard reaction mixture (15 μl) contained 50 mM Tris-HCl buffer, pH 7.5, 81-159 nM AB, as well as 18-136 μM oligonucleotide (ON) ($(pU)_9$, $(pC)_{10}$, $(pT)_{10}$, or $d(pC)_{10}$). Reaction mixtures for DNase activity determinations contained additionally 5 mM $MgCl_2$, 1 mM EDTA, as well as 8-60 μM $d(pT)_{10}$ or $d(pC)_{10}$ as substrates. All reaction mixtures for each separate experiment contained an equal amount of 5′-[^{32}P]ON; the necessary concentration of used substrates was provided by addition to reaction mixtures of required amounts of cold $(pN)_n$ or $d(pN)_n$. After incubation of a mixture for 1-3 h at 37°C, the reaction was stopped by addition of 5 μl formamide containing 0.25% bromphe-

nol blue, 0.25% xylene cyanole, and 20 mM EDTA. Reaction products were analyzed by PAGE (89 mM Tris-borate buffer, pH 8.3, 2 mM EDTA, and 8 M urea). For analysis of phosphatase activity, 20% gel was used, and the electrophoresis time corresponded to the dye penetration into the gel by 0.5-1.0 cm. Under these conditions a single radioactivity band corresponded to all original and hydrolyzed 5′-[^{32}P]ON and separation of these ON and free [^{32}P]orthophosphate was observed. A 30% gel and longer electrophoresis time were used for analysis of 5′-[^{32}P]ON hydrolysis extent. For relative activity determination, radioluminescent scanning was used (Molecular Imager FX system; BioRad Laboratories, USA). First, relative phosphatase activity was calculated on the basis of radioactivity in the band corresponding to [^{32}P]orthophosphate as percentage of total radioactivity in bands [^{32}P]ON and [^{32}P]P_i. Relative DNase and RNase activities were also first calculated as percentage of radioactivity in bands of different length radiolabeled products in comparison with total radioactivity corresponding to all radioactivity bands in the case of each reaction mixture. Finally, relative rates of ON nuclease hydrolysis and phosphatase activity (nmol/min) were calculated using data on the content of hydrolysis products (%) in reaction mixtures with regard to total concentration of ON used.

Determination of kinetic parameters of reaction. All measurements were carried out under pseudo-first order reaction conditions using linear regions of reaction rate dependence on the AB concentration and time. The decrease of initial ON and accumulation of [^{32}P]orthophosphate under these conditions was 10-40%. The V_{max} (k_{cat}) and K_m values were estimated by nonlinear regression using the MicroCal Origin v5.0 software and are presented graphically in Lineweaver–Burk double-reciprocal coordinates [37]. Error of determinations of these parameters does not exceed 20%.

Reagents. Reagents of the following firms were used in this work: Sigma (USA), Pharmacia (Sweden), and SDS of Merck (Germany). Synthesis and characterization of all electrophoretically homogeneous d(pN)$_n$ and (pN)$_n$ were carried out as described earlier [24, 25].

RESULTS

Earlier, DNase and RNase activities were studied in electrophoretically and immunologically homogeneous pIgG of antibodies from the blood of rabbits immunized with DNA complex with mBSA, as well as with DNase I and DNase II [29-31]. Three rabbits were used in each published work. The use of a number of methods including analysis of AB activity in gel with copolymerized DNA or RNA (*in situ* technique) showed that IgG preparations are not contaminated by canonical DNases and RNases and the catalytic activities are their intrinsic characteristics rather than that of contaminating enzymes [29-31]. All three AB preparations in the case of each of these three antigens exhibited close characteristics in hydrolysis of scDNA and polyribonucleotides. Taking this into account, for analysis of an average situation, in this work three mixtures were obtained which contained equimolar amounts of each of three IgG preparations (used in works [29-31]) corresponding to AB obtained after immunization of rabbits with DNA, DNase I, and DNase II. Relative DNase and RNase activities of the AB in these works [29-31] were estimated using scDNA and different heterogeneous length poly(N) as substrates. The scDNA hydrolysis in the presence of AB preparations resulted in formation of only relaxed DNA with its subsequent hydrolysis. Like known RNases, rabbit pIgG better hydrolyzed poly(C) than poly(U), but unlike canonical RNases and polyclonal AB of patients with AIDs, they were completely inactive in hydrolysis of poly(A) and poly(G) [29-31]. The use of scDNA and heterogeneous length poly(N) limited the possibilities in determination of kinetic characteristics of catalysis of scDNA and polymeric RNA hydrolysis as well as that of products of their AB-dependent degradation and analysis of their phosphatase activity. Owing to this, in this work for more detailed analysis of physicochemical parameters that characterize AB-dependent catalysis of different length DNA and RNA hydrolysis and removal of their 5′-terminal phosphate, single-stranded 5′-[^{32}P](pN)$_{9\text{-}10}$ and 5′-[^{32}P]d(pN)$_{10}$ were used.

It was shown that AB from blood of non-immunized (equimolar mixture of three preparations; Fig. 1a) and immunized with mBSA (equimolar mixture of three preparations; Fig. 1, b and d) rabbits do not hydrolyze either 5′-[^{32}P](pN)$_n$ or 5′-[^{32}P]d(pN)$_n$, and do not cleave their 5′-terminal phosphates. However, AB from blood of rabbits immunized with DNA, DNase I, and DNase II efficiently cleaved 5′-terminal phosphates from both 5′-[^{32}P](pN)$_n$ and 5′-[^{32}P]d(pN)$_n$. For example, Fig. 1c shows data of electrophoretic analysis of [^{32}P]orthophosphate cleavage from 5′-[^{32}P](pC)$_{10}$, which is catalyzed by IgG from blood of rabbits immunized with DNase II. As mentioned above, the rate of poly(N) hydrolysis by preparations from blood of rabbits immunized with AB preparations corresponding to different antigens was 3-4 orders of magnitude higher than for DNA. A similar situation is observed when hydrolyses of 5′-[^{32}P](pN)$_n$ and 5′-[^{32}P]d(pN)$_n$ are compared. Figure 1e shows as an example of data for 5′-[^{32}P](pC)$_{10}$, hydrolyzed by IgG preparation from blood of rabbits immunized with DNase II. Data of electrophoretic analysis of AB relative activity in hydrolysis of different 5′-[^{32}P]ribooligonucleotides and cleavage of their 5′-terminal phosphate were assessed as described above.

Figures 2 and 3 show dependences of relative rates of 5′-[^{32}P](pC)$_{10}$ and 5′-[^{32}P](pU)$_9$ RNase hydrolysis and cleavage of their 5′-terminal phosphates on concentra-

tion of these ON for preparations corresponding to three different antigens. It is seen that all dependences of 5′-[^{32}P](pC)$_{10}$ and 5′-[^{32}P](pU)$_9$ RNase hydrolysis have the typical shape of Michaelis–Menten hyperbolic curves with saturation at high concentrations. The table shows K_m values for oligoribonucleotide substrates and k_{cat} calculated using the hyperbolic nonlinear regression approximation of such curves and the Lineweaver–Burk inverse coordinates, which gave identical results. It is seen that the affinity of all the AB preparations to (pC)$_{10}$ (in terms of K_m values) is approximately 3.4-6.4 times higher than to (pU)$_9$. This agrees with 3-4-fold higher affinity (K_m per mononucleotide) of these IgG preparations to poly(C) compared to poly (U) [29-31].

Note that for the used oligoribonucleotide concentrations, dependences of reaction rates of phosphatase cleavage of 5′-terminal phosphate correspond to linear regions of hyperbolic curves for all AB preparations (Figs. 2 and 3). To estimate possible K_m and k_{cat} values, we began with computer analysis of these curves using the hyperbolic approximation. In Fig. 2 all three dependences of the rate of 5′-terminal phosphate cleavage by phosphatase from 5′-[^{32}P](pC)$_{10}$ are very similar. Computer analysis of these curves resulted in approximate estimation of possible K_m and k_{cat} values (0.8-3.5 mM and 1.6-1.9 min^{-1}, respectively). In Fig. 3, the rate of 5′-terminal phosphate cleavage from 5′-[^{32}P](pU)$_9$ by phosphatase noticeably exceeds the rate of RNase cleavage at the used substrate concentrations, and there are significant differences in the curves corresponding to AB obtained upon rabbit immunization with DNA, DNase I, and DNase II. Hyperbolic approximation using nonlinear regression of curves for 5′-[^{32}P](pU)$_9$ provided possible estimates of K_m and k_{cat} values (2-5 mM and 0.6-1.0 min^{-1}, respectively). However, there could be a great error in these estimations because K_m values for 5′-[^{32}P](pN)$_{9\text{-}10}$, which characterize their affinity to catalytic centers of AB exhibiting phosphatase activity, in conformity with such estimation it by almost three orders of magnitude exceeds those for centers of abzymes exhibiting RNase activity. Taking this into account as well as regarding large ON amounts spent for analysis of phosphatase activity, we carried out direct analysis of K_m and V_{max} (k_{cat}) using high concentrations of 5′-[^{32}P](pC)$_{10}$ in the case of AB from rabbits immunized

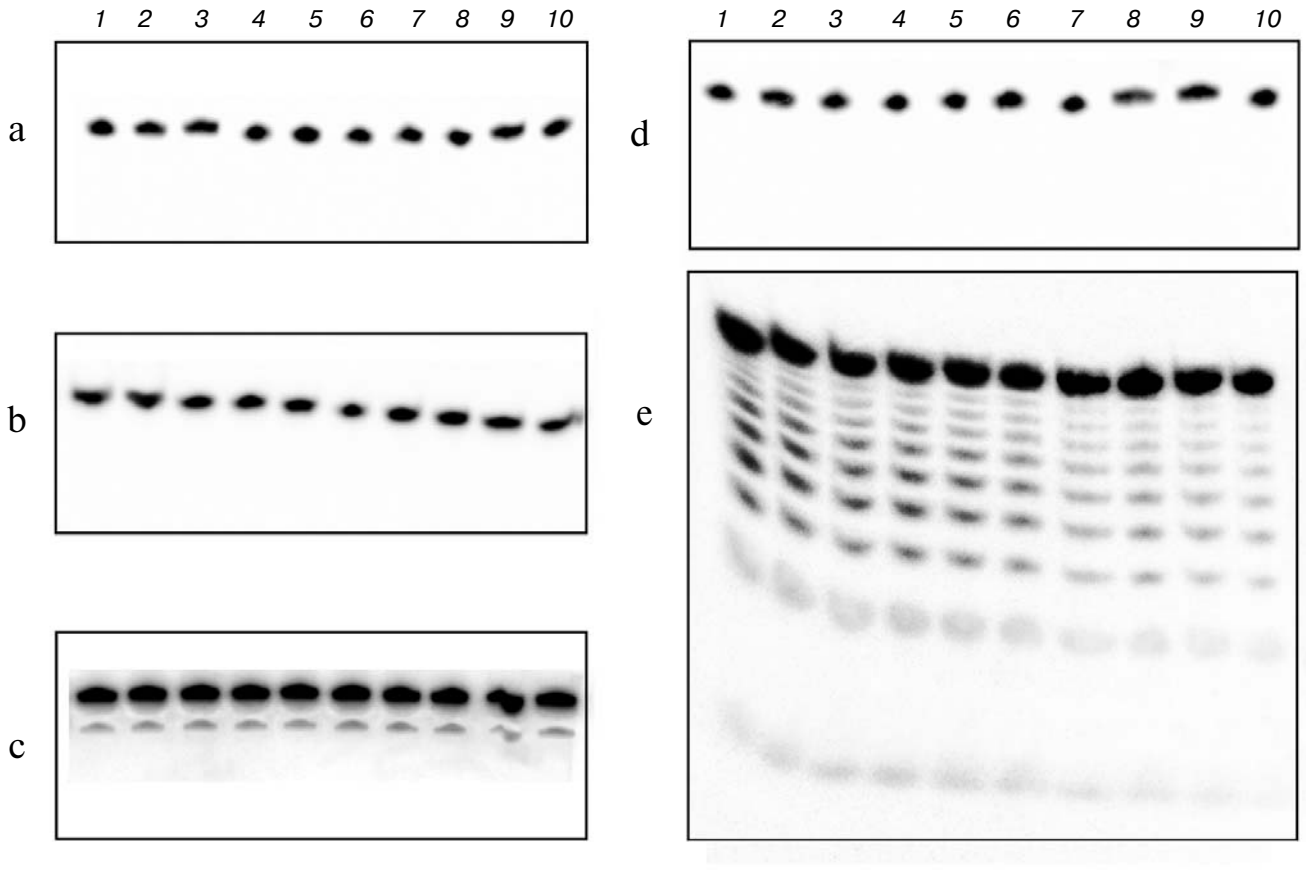

Fig. 1. Electrophoretic analysis of 5′-terminal phosphate removal from 5′-[^{32}P](pC)$_{10}$ (phosphatase activity) by AB from blood of rabbits not immunized (a), immunized by mBSA (b), and by DNase II (c) using 20% polyacrylamide gel. Analysis (30% polyacrylamide gel) of 5′-[^{32}P](pC)$_{10}$ hydrolysis (RNase activity) in the presence of AB from the blood of rabbits immunized by mBSA (d) and DNase II (e). In all cases (a-e) 5′-[^{32}P](pC)$_{10}$ was used at the following concentrations (μM): 7.6 (*1*), 9.5 (*2*), 11.9 (*3*), 14.8 (*4*), 18.5 (*5*), 23.1 (*6*), 28.9 (*7*), 36.2 (*8*), 45.2 (*9*), 56.5 (*10*).

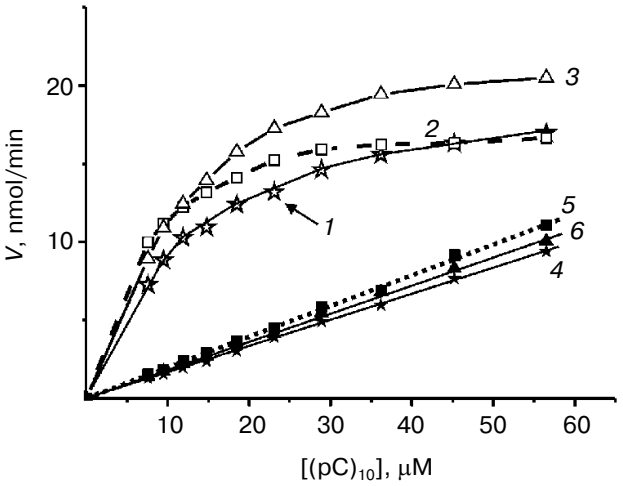

Fig. 2. Rate dependences of 5′-[^{32}P](pC)$_{10}$ cleavage by RNase (curves *1-3*) and 5′-terminal phosphate cleavage by phosphatase (curves *4-6*) by AB (100 nM) from blood of rabbits immunized with DNA (curves *1* and *4*), DNase I (curves *2* and *5*), and DNase II (curves *3* and *6*) on concentration of (pC)$_{10}$.

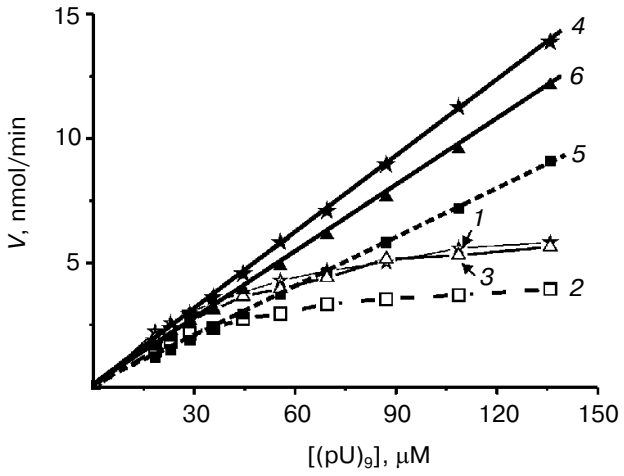

Fig. 3. Dependences of relative rate of 5′-[^{32}P](pU)$_9$ hydrolysis by RNase (curves *1-3*) and 5′-terminal phosphate cleavage by phosphatase (curves *4-6*) in response to AB (100 nM) from blood of rabbits immunized with DNA (curves *1* and *4*), DNase I (curves *2* and *5*), and DNase II (curves *3* and *6*) on (pC)$_{10}$ concentration.

with DNase II and for 5′-[^{32}P](pU)$_9$ only for anti-DNA AB (Fig. 4). The K_m and k_{cat} values were 0.9 mM and 1.7 min^{-1} for (pC)$_{10}$ and 2.9 mM and 1.0 min^{-1} for (pU)$_9$, which corresponds to the above-mentioned range of these values (0.8-3.5 mM) estimated using the hyperbolic approximation. Therefore, the ribo-ON affinity to active centers of AB exhibiting phosphatase activity is approximately 50-450 times lower than to centers with RNase activity. Interestingly, the k_{cat} values characterizing AB with phosphatase activity are 19-20 times higher than those for abzymes with RNase activity. Therefore, simultaneous analysis of RNase and phosphatase activities of studied rabbit AB was possible only because saturation of active centers exhibiting RNase activity takes place at lower concentrations (as compared with centers with phosphatase activity).

Note that 5′-terminal phosphate removal from deoxy-ON (5′-[^{32}P]d(pC)$_{10}$ and 5′-[^{32}P]d(pU)$_{10}$) stimulated by AB isolated from blood of rabbits immunized with DNA, DNase I, and DNase II occurred at approximately at the same rate as a similar cleavage from ribo-ON. However, the rate of 5′-terminal phosphate cleavage from deoxy-ON upon their incubation with AB preparations much exceeded that of their DNase cleavage. This made difficult quantitative estimation of K_m and k_{cat} values characterizing interaction of DNase centers with deoxy-ON. However, the data indicate that the interaction of DNA-hydrolyzing AB from the blood of immunized rabbits with deoxy-ON takes place at micromolar concentrations comparable with those for ribo-ON. This agrees with the similar affinity of ribo- and deoxy-ON to abzymes isolated from the blood of patients with different AIDs, the phosphatase activity of which (unlike analyzed rabbit AB) is usually much lower than the DNase and RNase activities [5-8].

DISCUSSION

The data show that in addition to AB with DNase and RNase activities, rabbit immunization with DNA,

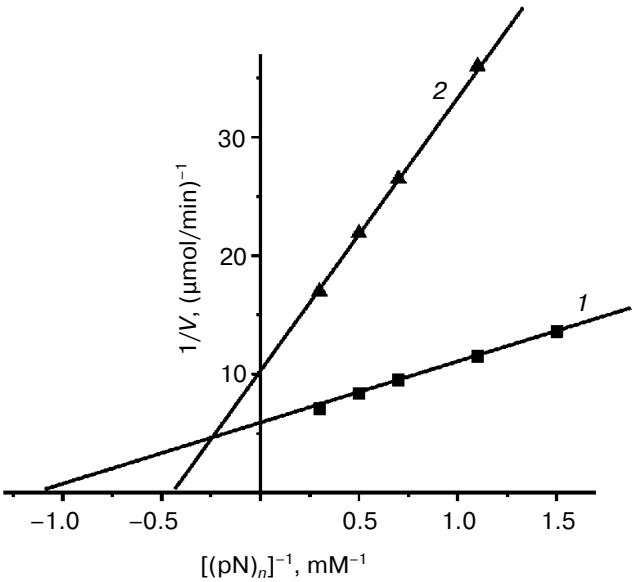

Fig. 4. Dependences of reaction rate of 5′-terminal phosphate cleavage (phosphatase activity) from 5′-[^{32}P](pC)$_{10}$ (*1*) and 5′-[^{32}P](pU)$_9$ (*2*) by 100 nM pIgG from blood of rabbits immunized with DNase II (*1*) and DNA (*2*) in coordinates $1/V$ vs. $1/[S]$.

K_m and k_{cat} characterizing the interaction of oligonucleotides with pIgG preparations from blood of rabbits immunized with DNA, DNase I, and DNase II

Substrate, activity type, parameter	Antibodies obtained upon immunization of rabbits by antigens*		
	DNA	DNase I	DNase II
	RNase activity Substrate $(pC)_{10}$		
K_m, μM	13.5 ± 0.6**	6.5 ± 0.4	12.8 ± 0.9
k_{cat}, min^{-1}	0.21 ± 0.02	0.19 ± 0.02	0.26 ± 0.03
k_{cat}/K_m, $min^{-1}\cdot M^{-1}$	$1.6\cdot10^4$	$2.9\cdot10^4$	$2.0\cdot10^4$
	Substrate $(pU)_9$		
K_m, μM	46.0 ± 1.4	41.7 ± 2.2	56.0 ± 1.5
k_{cat}, min^{-1}	$(7.8 \pm 0.1)\cdot10^{-2}$	$(5.2 \pm 0.5)\cdot10^{-2}$	$(8.0 \pm 0.8)\cdot10^{-2}$
k_{cat}/K_m, $min^{-1}\cdot M^{-1}$	$1.7\cdot10^3$	$1.2\cdot10^3$	$1.4\cdot10^3$
	Phosphatase activity, substrates $(pU)_9$ and $(pC)_{10}$		
K_m, μM	–	2.9 ± 0.3; $(pU)_9$	0.9 ± 0.1; $(pC)_{10}$
k_{cat}, min^{-1}	–	1.0 ± 0.2; $(pU)_9$	1.7 ± 0.2; $(pC)_{10}$
k_{cat}/K_m, $min^{-1}\cdot M^{-1}$	–	$2.9\cdot10^3$	$5.3\cdot10^2$; $(pC)_{10}$

* Each preparation is an equimolar mixture of AB preparations from the blood of three rabbits.
** Standard deviation (three independent determinations).

DNase I, and DNase II results in production of abzymes exhibiting phosphatase activity. The K_m values for scDNA and ON in the case of AB from blood of rabbits immunized with DNA, DNase I, and DNase II are characteristic of the highly efficient interaction of AB with antigens and are for 2-4 orders of magnitude lower than those for DNase I (K_m = 46-58 μM) [38] and RNases [24, 25].

Some published data should be considered in analysis of DNase and phosphatase activities of rabbit AB. The DNA- and RNA-dependent enzymes are known to demonstrate affinity to different DNA and RNA characterized by K_m (K_d) values varying from 10^{-6} to 10^{-10} M [39, 40]. However, the affinity of mononucleotides, extended DNA, and RNA to different phosphatases and ATPases in terms of K_m is comparable and, depending on the particular enzyme, it changes from 10^{-4} to 10^{-3} M [39, 40]. Evidently, a similar situation of significant difference of DNA and RNA affinity (at least 2-3 orders of magnitude) is also observed for abzymes with nuclease and phosphatase activities.

In work [41], relative phosphatase (AMP and ATP substrates) and DNase (scDNA and deoxy-ON substrates) activities of polyclonal AB from blood of MRL-lpr/lpr mice with spontaneous autoimmune SLE and mouse monoclonal IgG obtained using hybridoma technologies were compared. The K_m values for scDNA (1-70 nM [29-31) in the case of polyclonal IgG from rabbits immunized with DNA, DNase I, and DNase II are comparable with those for scDNA (12-58 nM [41]) found earlier for AB from blood of mice with spontaneous SLE as well as for IgG from blood of patients with different AIDs (30-92 nM) [6-8]. Upon transition from scDNA to $d(pN)_{10}$ and $(pN)_{10}$, the affinity of AB from blood of AID patients to these nucleic acids decreases only by 1-2 orders of magnitude (0.1-20 μM) [24, 25]. A similar difference in K_m values for scDNA (1-70 nM [29-31]) and ribo-ON (6.5-56 μM; table) is observed for AB from blood of immunized rabbits.

On the whole, K_m values for scDNA, deoxy-, and ribo-ON in the case of the above-mentioned AB of any origin, including those from blood of immunized rabbits, are 2-5 orders of magnitude below those for AMP and ATP (0.6-2.0 mM), which characterize terminal phosphate cleavage from these mononucleotides by phosphatase that is catalyzed by AB from blood of mice with spontaneous SLE [41].

It was shown that AMP and ATP do not inhibit scDNA hydrolysis catalyzed by mouse AB even upon saturating concentrations of these mononucleotides [41]. In turn, scDNA and deoxy-ON deprived of the 5′-terminal phosphate did not inhibit terminal phosphate cleavage from AMP and ATP. This showed that phosphatase and nuclease activities are exhibited by different abzyme fractions of the total pool of mouse polyclonal AB [41]. Monoclonal mouse IgG with phosphatase activity were efficient in terminal phosphate cleavage from AMP and ATP (K_m = 1-3 mM) but did not hydrolyze scDNA and ON, while in high concentrations (1-3 mM) they cleaved the terminal phosphate from $d(pN)_n$ oligonucleotides [41]. It was shown earlier that monoclonal mouse AB to

DNA better hydrolyze RNA than DNA [27]. This means that in the case of spontaneous development of AID there may be only preferable production of AB exhibiting either phosphatase or DNase and RNase activities belonging to different fractions of the polyclonal abzymes. In principle, one cannot exclude the possibility of formation of abzymes which active centers are able to catalyze both hydrolysis of nucleic acids and terminal phosphate removal. However, no monoclonal AB exhibiting these two enzyme activities were found, while polyclonal abzymes showed pronounced difference in oligonucleotide affinity to AB fractions with nuclease and phosphatase activities [41]. Evidently, in the case of rabbit immunization with DNA complexed with mBSA, DNase I, and DNase II there can be production of abzymes with nuclease and phosphatase activities similar to those in autoimmune mice.

When the same AB molecule (the same AB active center) exhibit nuclease and phosphatase activities, the K_m values for oligonucleotides, detected in reactions of substrate hydrolysis and its terminal phosphate cleavage, should coincide within experimental error. A large difference in affinity (2-3 orders of magnitude) of total polyclonal AB preparations to ON, found during analysis of phosphatase and nuclease activities, show that these reactions are catalyzed by different abzyme fractions of the total pool of rabbit catalytic AB. This conclusion is also supported by the fact that the affinity of ON to pAB with nuclease activity corresponds to that in the case of monoclonal and polyclonal AB with this activity [40], while affinity of substrate RNA, detected using reaction of terminal phosphate cleavage, corresponds to the mononucleotide and oligonucleotide affinity to canonical phosphatases, ATPases, and abzymes with phosphatase activity [39, 40].

Note that in the case of AIDs, potential immunogens stimulating formation of antiidiotypic AB with phosphatase activity can be phosphatases, ATPases, and different blood enzymes removing phosphate from low molecular weight substrates as well as cellular enzymes exhibiting these activities revealed in the blood upon apoptosis. The question arises, what antigens are able to stimulate formation of AB with phosphatase activity upon rabbit immunization by DNA complex with mBSA, DNase I, and DNase II? In the case of immunization with polymeric DNA, rabbit blood may contain DNA fragments, including those 5′-phosphorylated. It might be that production of AB against 5′-terminal nucleotide units like DNA can result in formation of AB with phosphatase activity. Besides, hydrolysis of DNA in complex with mBSA may be associated with formation of mononucleotides, whose complexes with mBSA are evidently also able to stimulate formation of AB with phosphatase activity. In work [41], to obtain monoclonal abzymes with phosphatase activity, the authors used as immunogen the BSA conjugate with ATP, which, in principle, can model mBSA complexes with different mononucleotides.

As mentioned above, in the case of rabbit immunization with DNase I and DNase II, a part of abzymes are of antiidiotypic nature, and these AB bind to primary antibodies to DNase I and DNase II [30, 31]. However, another part of the IgG does not interact with idiotypic AB to DNase I and DNase II, but these AB exhibit DNase and RNase activities. This can be because such abzymes are AB to nucleic acids forming complexes with DNase I and DNase II [30, 31]. Since blood always contains in different concentrations 5′-phosphorylated DNA, RNA, and mononucleotides, able to interact with DNase I and DNase II, used as immunogens, the pathway of formation of abzymes with phosphatase activity upon immunization of animals by these DNases can be the same as that upon immunization with DNA and its complexes with mBSA.

AB against analogs of transition states are usually characterized by 10^2-10^6 times lower reaction rates than those for canonical enzymes [1]. The known k_{cat} values for natural abzymes vary from 0.001 to 15.6 min^{-1} [4-10, 22-25]. Presently abzymes from milk of healthy women [42] and autoimmune mice [41] exhibiting phosphatase activity have been studied. The k_{cat} values that characterize 5′-terminal phosphate removal from ribo- and deoxyribo-ON by antibodies from blood of rabbits immunized with DNase I and DNase II (1.0-1.7 min^{-1}; table) are 1-2 orders of magnitude lower than those for AB with phosphatase activity from the blood of autoimmune mice (k_{cat} = 84-372 min^{-1} [41]) or comparable with those for AB from milk of lactating women (k_{cat} = 0.14-1.0 min^{-1} [42]). However, on the whole these k_{cat} values exceed those for most known abzymes with different activities and are comparable with reaction rate constants for a large number of repair and restriction enzymes [6-8].

This work for the first time deals with analysis of hydrolysis of short single-stranded ribo- and deoxyribooligonucleotides and removal of 5′-terminal phosphate by AB; the data also show that it is possible to use immunization of animals with DNA, DNase I, and DNase II to produce not only antibodies hydrolyzing DNA and RNA, but also abzymes with phosphatase activity as well.

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