ISSN 0026-8933, Molecular Biology, 2017, Vol. 51, No. 1, pp. 96–101. © Pleiades Publishing, Inc., 2017.
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MOLECULAR CELL BIOLOGY

UDC 577.152.277:578

Exogenous *Bacillus pumilus* RNase (Binase) Suppresses the Reproduction of Reovirus Serotype 1

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Received December 29, 2015; in final form, February 15, 2016

Abstract—The experimental study identified the antiviral activity of *Bacillus pumilus* RNase (binase) against the reovirus of serotype 1/strain Lang. For the first time, it has been found that 50 μg/mL of binase effectively reduced the hemagglutinin and cytocidal activity of reovirus in Vero cell line. The preincubation of the enzyme with reovirus before infection of the cells inhibited the viral replication. To determine the stage-dependent effect of reovirus reproduction upon binase inhibition, the infected cells were treated with binase or RNase A at different phases of the infectious cycle. The treatment of virus-infected cells has revealed that both enzymes have a maximal antiviral effect on the reovirus propagation during early phases of the reovirus reproduction cycle, with binase being more effective than RNase A. It has been hypothesized that the combined action of the oncolytic reovirus and binase is promising for the elimination of tumor cells carrying mutated *RAS* gene.

Keywords: binase, *Bacillus pumilus* RNase, reovirus, antiviral agent

DOI: 10.1134/S0026893316060042

INTRODUCTION

RNA viruses, such as Ebola, AIDS, and influenza, are the most dangerous in the world. They cause local epidemics and global pandemics accompanied by the massive loss of human life. A high degree of variability in the genomes of RNA viruses allows them to withstand the host immune system and to resist the action of drugs. Reoviruses (respiratory enteric viruses) have a unique genome structure that consists of segmented double-stranded RNA. These viruses are widely distributed in nature and affect not only humans, but also animals, birds, and fish. They penetrate into the upper respiratory human path and cause pharyngitis, rhinitis, and diseases that are often accompanied by fever and sometimes pneumonia. When entering the body by the fecal-oral route, reovirus affects the human small intestine that results in enteritis with intestinal spasms and diarrhea. Reoviruses were shown to lead to the development of diabetes and polyendocrinopathy [1]. Infants and children under 6 months of age are particularly sensitive to reovirus infection. The disease occurs in severe form; both pneumonia and encephalitis are possible [2]. According to the WHO, about 2 million people are hospitalized with reovirus infections each year. In 2008, reovirus infections were the cause of death in 453000 children. The main share of severe cases has occurred in countries with a low quality of life [3]. Reoviruses bind to syalated strands of glycoprotein, adhesion protein A (JAM-A), on the host cellular membrane and penetrate into cells using the dynamin- and clathrin-dependent or independent pathways. Intestinal infections can lead to capsid proteolysis either in the cytoplasm or outside the cell [4]. Despite the use of anti-reovirus vaccines in veterinary patients, passive seroprophylaxis does not lead to the desired effect because of the latency of the virus [5].

Currently, reoviruses have been proposed for use as oncolytic agents [6]. However, it is not possible to implement this plan without effective inhibitors of the replication of these viruses. The action of selective inhibitors of reovirus replication allows one to prevent possible side effects. Research in this field is just beginning [7]. Currently, the search for antiviral agents is an urgent task.

In recent years, it has been proved that RNases of mammals, amphibians, and microorganisms can resist infections caused by RNA viruses. Bacterial RNase appeared to have both prophylactic and antiviral effects [8] and to be more efficient than its eukaryotic analogs [9–13]. This is partially due to its lack of interaction with RNase inhibitors, which are present

Abbreviations: HAU, hemagglutinating units; HAR, hemagglutination reaction; $TCID_{50}$, tissue cytopathic dose causing the death of 50% of the cells; CE, cytopathic effect.

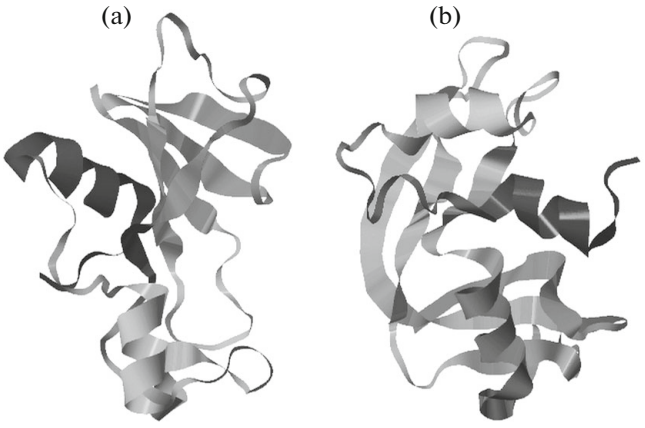

Fig. 1. Structure of (a) binase and (b) RNase A molecules.

in mammalian cells [8, 9, 14, 15]. The antiviral effect of *Bacillus pumilus* RNase (binase) against RNA viruses (viruses of rabies, foot and mouth disease (FMD), and seasonal influenza A and B) has been demonstrated on guinea pigs [12], rabbits [9], mice [8, 9], and chicken embryos [8]. We have recently revealed the antiviral effect of binase against pandemic influenza A (H1N1pdm) virus (2009) [13, 16]. Thus, the binase-based preparation may be considered as a potential antivirus agent. The enzyme degrades virus RNA in the host cell and reduces, thereby, virus reproduction.

In this work, we analyzed the antiviral effect of binase against the reovirus of serotype 1/strain Lang and compared this effect with that of RNase A, which is the antiviral preparation of a broad spectrum of activity.

EXPERIMENTAL

Preparations of RNases. We studied bacterial and eukaryotic enzymes. Binase, guanyl-specific *Bacillus pumilus* RNase 7P (molecular weight, 12.2 kDa; 109 amino acid residues; pI = 9.5), was isolated in homogeneous form from the cultural liquid of *Escherichia coli* BL21 bearing the pGEMGX1/ent/Bi plasmid according to previously described procedure [17]. The binase catalytic activity towards synthetic substrates and high-polymeric east RNA was characterized in [18, 19]. The preparation of pancreatic RNase was also studied (Samson Med, Ltd, Russia) (molecular weight, 13.7 kDa; 124 amino acid residues; pI = 9.6). The molecular structures of the enzymes are presented in Fig. 1.

Viruses. We used reovirus of serotype 1/strain Lang (Reo1-Lang, a collection of FCTRBS Kazan, Russia) with a mean baseline infectious titer of 10^{-4} $TCID_{50}$/mL (tissue cytopathic infectious dose leading to the 50% cell death). The virus was reproduced in the culture of African green monkey kidney embryonic cells (Vero).

Cell culture. We used a culture of the Vero cell line from the collection of Institute of Cytology of RAS (St. Petersburg, Russia). The cells were grown on DMEM nutrient medium containing a single set of amino acids, vitamins (Sigma, United States), and 5–10% fetal calf serum (FCTRBS Kazan, Russia) at 37°C and 5% CO_2. The initial concentration of the cells was (14–15) × 10^5 cells/mL. The same medium without serum was used as a support medium.

Cytotoxicity analysis of RNases. After the formation of a continuous monolayer (60% confluence), the medium in the wells was replaced with a fresh medium (control) or medium containing binase or RNase A at concentrations of 50, 100, or 300 μg/mL. The cells were cultivated in the presence of enzymes for 24 h, followed by the calculation of the total number of cells in wells and the evaluation of the proportion of apoptotic cells using the fluorescent dye merocyanine 540 [20] on a BD FACSCanto II Flow Cytometer (Becton Dickinson, United States). The variant with the addition of classical apoptosis inductor, camptothecin [21] at a concentration of 10 μM was used as a positive control.

Treatment of the virus with RNases. The effect of RNases on the reovirus replication was estimated in the Vero cell culture. The antiviral action of RNases was studied at nontoxic concentrations of 5 and 50 μg/mL. RNase was preincubated with the virus sample (100 or 1000 $TCID_{50}$/mL) for 30 and 60 min, followed by the infection of Vero cells.

To determine the stage at which the reovirus replication cycle is influenced by the studied enzymes, the RNase preparation was introduced in the cell culture after virus adsorption for 2 h (the cells of the infected monolayer adsorb ~90% of the virus for 2 h at 37°C), for 6 h (the latent period of the intracellular reproduction is 6–9 h), and for 24 h (the period of 15–54 h corresponds to the maximal reproduction of the reovirus). After a certain exposure of the virus to cells, the nonadsorbed virus was removed, the cell monolayer was rinsed with the nutrient medium without serum, and the supported medium containing RNase A or binase was added at a concentration of 50 μg/mL. The nutrient medium without enzymes was added to the control sample. The reproduction of the reovirus was evaluated on a monolayer of the two-day culture of Vero cells infected at doses of 100 or 1000 $TCID_{50}$/mL for 72–96 h (control) in the presence or absence of RNases by the time of manifestation of the cytopathic effect (CPE) of the virus and by hemagglutination titer.

Evaluation of reovirus infectivity. The reovirus infectious titer before and after treatment with RNase was evaluated in Vero cell culture. The parent virus-containing material was used to prepare a series of ten-fold dilutions (10^{-1} to 10^{-6}) using DMEM medium. The titer was calculated by the Reed and Muench method in a modification by Ashmarin [22] and expressed in log($TCID_{50}$/mL). The antiviral activity of

the compounds was evaluated by reducing the virus titer in the presence of preparations (60-min incubation of the virus with each preparation at a concentration of 50 μg/mL compared with the corresponding control samples (without preparations). The kinetics of the decrease in the infectious titer was evaluated for 7 days as log($TCID_{50}$/mL).

Inhibition of the cytopathic activity of the virus. As the result of the enzyme action, the decrease in the virus accumulation Δlog($TCID_{50}$/mL) was evaluated by titration and calculated as described earlier [23]:

$$\Delta\log(TCID_{50}/mL) = A_c - A_o,$$

where A_c is the accumulation of the virus when cultivated without the studied enzyme and A_o is the accumulation of the virus with the addition of the studied enzyme in the nutrient medium.

Inhibition coefficient (K_i, %) of the virus accumulation was calculated by the formula [2]

$$K_i = (A_c - A_o)/A_c \times 100.$$

Evaluation of reovirus content. The reovirus titer in cells was evaluated by the standard method, i.e., in the hemagglutination reaction (HR) using 1% suspension of human erythrocytes of group 1 (0) [24]. The virus content was expressed in hemagglutination units (HAU) per 1 mL. Statistical data processing was performed by the method of variation statistics using the Microsoft Excel program. The results were considered significant at $P < 0.05$ according to Student's test.

RESULTS AND DISCUSSION

We previously evaluated the cytotoxic effect of the studied RNases towards Vero cells. Binase and pancreatic RNase were not shown to have significant apoptosis-inducing action in the whole range of studied concentrations (Fig. 2). The proportion of apoptotic cells did not exceed 10% in any of the treatment options, whereas in the control sample (without enzymes), this parameter was evaluated to be 6.4 ± 2.3%. When the apoptosis inductor, camptothecin (10 μM), was added, after 24 h of cultivation, the proportion of apoptotic cells in population was 24.7 ± 3.1%.

Infection by the reovirus led to a change in cell morphology. The cytopathic effect of the virus was expressed in the appearance of rounded granular cells, which were then combined into giant multinucleated cells and symplasts. After 72 h, the reovirus-infected cells were destroyed and detached from the bottom of a glass vial (Fig. 3).

The study of the antiviral efficiency of RNase drugs against the Reo1-Lang virus showed that the virus infectivity was significantly reduced after the treatment with both RNase A and binase. At an enzyme concentration of 5 μg/mL, the inhibition coefficient of the virus infectivity for binase was twice as high as

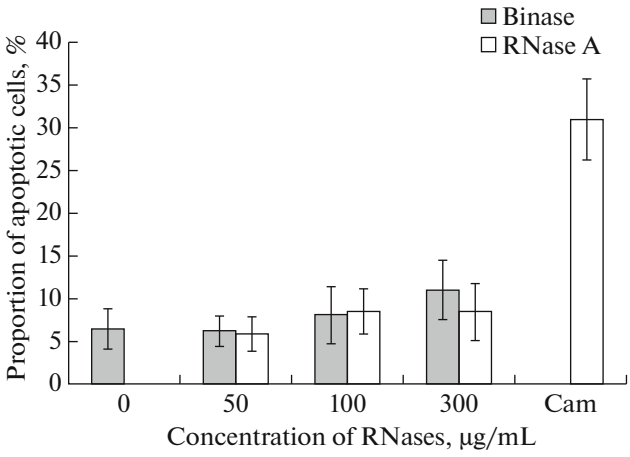

Fig. 2. Cytotoxic effect of binase and RNase A on Vero cells expressed as the relative number of apoptotic cells in the population. Cam, camptothecin (10 μM). Total number of cells was taken as 100%.

for RNase A, and the difference was 4.7 times at a concentration of 50 μg/mL (Table 1).

The inhibitory activity of the enzymes against the reovirus in the Vero cell culture was evaluated by the reduction of the hemagglutination titer. The effect was shown to depend not only on RNases concentration but also on their preincubation time with the virus. It was found that binase inhibited virus reproduction more efficiently at a high concentration (50 μg/mL) and prolonged preincubation time (60 min) than at a concentration of 5 μg/mL and preincubation time of 30 min. Binase completely inhibited hemagglutination at the concentration of 50 μg/mL and prolonged preincubation time with the virus at a dose of 100 $TCID_{50}$/mL, whereas RNase A reduced the virus reproduction by 82.5% under the same conditions (Table 2).

In addition, we studied the stage at which the reovirus replication cycle is predominantly influenced by the studied enzymes. It was found that both RNases act most efficiently at the early stages of virus replication, i.e., 2 h after cell infection. In this case, RNase A and binase retard the CE manifestation in the cell monolayer by 6–8 h and 20–24 h, respectively, compared with the control sample. The introduction of RNases 6 h after the infection led to the less pronounced antiviral effect. RNase A and binase retard the CE manifestation of the reovirus by 4–5 h and 12–15 h, respectively. The treatment of the cells by the enzymes 24 h after the infection did not influence the time of the CE manifestation (Fig. 4).

Thus, the comparative study showed that binase at a dose of 50 μg/mL reduced the reovirus infection titer by more than three orders of magnitude compared with the infected cells untreated by binase. Preincubation of the reovirus with the enzyme before cell infection leads to a decrease in the replication and hemag-

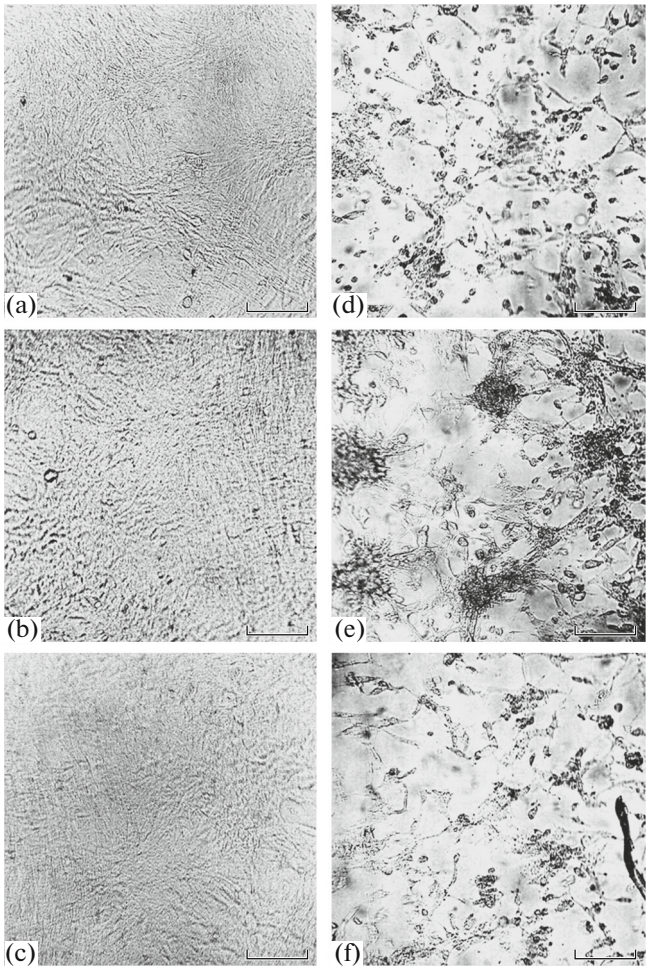

Fig. 3. Changes in morphology of Vero cells. Noninfected cells in the growing process: (a) 24 h; (b) 48 h; (c) 72 h. Cells infected by Reo1-Lang virus: (d) 24 h; (e) 48 h; (f) 72 h. Scale bar is 100 μm.

glutination activities by 77.7% and 67–100%, respectively. RNases have the maximal antiviral effect at the early stages of the reproduction cycle of the Reo1-Lang virus, with the efficiency of binase being significantly higher than that of RNase A.

The structures of these RNases have certain differences that consist of the presence of four disulfide bonds in the RNase A molecule and their absence in binase. Four antiparallel β-strands and three α-helices in RNase A form the secondary structure of the enzyme. Binase also contains three α-helices; five antiparallel and two parallel β-strands form two β-sheets (Fig. 1). RNase A is specific to pyrimidine RNA bases [25], and binase is specific to purine bases, in particular to guanine [14, 15, 26]. It has been found previously that these two enzymes have an almost equal inhibitory effect on the replication of influenza A virus in chicken embryos [8]. Binase appeared to be a more efficient antiviral agent than RNase A on the mice model, which seems to be associated with the suppression of the enzymatic activity of RNase A by mammalian RNase inhibitor [8, 9]. It is possible that the increased antireovirus activity of binase compared to that of RNase A (Tables 1 and 2) is also associated with the inhibition of RNase A by inhibitors, which are present in Vero cells.

Binase has a number of other advantages. At a low concentration that is nontoxic for eukaryotic cells, this enzyme exhibits a low immunogenicity and does not cause a T-cell immune response by the type of superantigen [27]. Moreover, binase is distributed quickly and evenly throughout the body and is slowly eliminated from the organism [8]. The feature of the cytotoxic effect of this RNase is in its selective toxicity towards tumor cells, which express the *RAS*, *KIT,* and *AML1* oncogenes [28]. These properties of binase allow one to consider this enzyme as a potential anticancer agent, including in combination with virotherapy.

As for molecular mechanisms of cell protection from reovirus infection, it is known that the appearance of viral double-stranded RNA in cells activates dsRNA-dependent protein kinase (PKR), which phosphorylates and inactivates eukaryotic translation initiation factor eIF-2. This leads to the blockage of transcription of viral mRNAs [29]. PKR is not activated in cells expressing mutant *RAS*, and viral replication can continue, i.e., in these cells, the virus replicates freely and causes cytolysis. Based on these data, reoviruses have been proposed for use as oncolytic agents against tumors bearing the mutant *RAS* protein [30]. It has been recently shown that oncolytic reovi-

Table 1. Effect of RNases on the reduction of Reo1-Lang virus infectivity in Vero cell culture

Preparation	Concentration, μg/mL	Preincubation time, min	Inhibition of virus infectivity, $\Delta\log(TCID_{50}/mL)$	Inhibition coefficient (K_i, %)
RNase A	5	60	0.5	11.1
	50		0.75	16.6
Binase	5		1.0	22.2
	50		3.5	77.7
Control	0	0	4.5	0

Table 2. Effect of RNases on reproduction of Reo1-Lang virus in Vero cell culture

Preparation	Concentration, μg/mL	Virus dose, $TCID_{50}$/mL	Preincubation time, min	Titer of HAR, HAE[a]	Reduction of titer of HAR, %[b]
RNase A	5	100	30	22.0 ± 6.93	45
		1000		28.0 ± 4.62	30
		100	60	18.0 ± 5.81	55
		1000		20.0 ± 4.62	50
	50	100	30	9.5 ± 4.36	76.2
		1000		12.0 ± 2.67	70
		100	60	7.0 ± 3.94	82.5
		1000		10.0 ± 2.31	75
Binase	5	100	30	3.0 ± 0.67	92.5
		1000		20.0 ± 4.62	50
		100	60	1.0 ± 1.15	97.5
		1000		14.0 ± 2.31	65
	50	100	30	0.50 ± 0.58	98.7
		1000		16.0 ± 6.53	60
		100	60	0.0	100
		1000		13.0 ± 3.46	67.5
Control	0	100	0	40.0 ± 9.24	0
Control	0	1000	0		

[a] Maximal dilution of the virus sample leading to HAR. [b] Complete absence of HAR is taken to be 100%.

ruses primarily stimulate the anti-tumor immune response and cause indirect elimination of tumor cells [31]. The unique relationship between reoviruses and binase is that they are mainly focused on transformed cells carrying the *RAS* oncogene [27, 32]. Thus, not only can the reovirus replication be regulated by binase, but the cells themselves can also be eliminated as the object of selective cytotoxicity of binase. This creates prospects for the development of new methods for combined anticancer therapy.

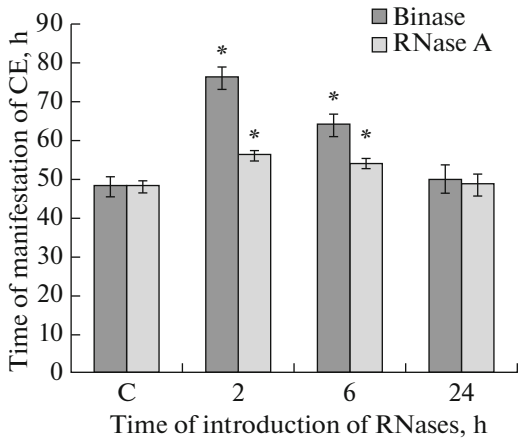

Fig. 4. Time of manifestation of the cytopathic effect of Reo1-Lang virus. RNases were introduced 2, 6, or 24 h after infection of Vero cells. *$P < 0.05$ relative to control (C).

ACKNOWLEDGMENTS

The work was performed within the State program of competitive growth of Kazan (Volga) Federal University among the world's leading scientific and educational centers and by the grant of Russian Science Foundation no. 14-14-00522.

REFERENCES

1. Onodera T., Toniolo A., Ray U.R., Jenson A.B., Knazek R.A., Notkins A.L. 1981. Virus-induced diabetes mellitus. XX. Polyendocrinopathy and autoimmunity. *J. Exp. Med.* **153**, 1457–1473.
2. Bukrinskaya A.G. 1986. *Virusologiya (Virology)*. Moscow: Meditsina.
3. Parashar U.D., Gibson C.J., Bresee J.S., Glass R.I. 2006. Rotavirus and severe childhood diarrhea. *Emerg. Infect. Dis.* **12,** 304–306.
4. Schulz W.L., Haj A.K., Schiff L.A. 2012. Reovirus uses multiple endocytic pathways for cell entry. *J. Virol.* **86**, 12665–12675.

5. Amini M., Ezatkhah M., Bafti M.S. 2014. Effect of vaccination of breeders on avian reovirus antibody titers in broilers. *Onl. J. Vet. Res.* **18**, 772–777.
6. Thirukkumaran C., Morris D.G. 2015. Oncolytic viral therapy using reovirus. *Methods Mol. Biol.* **1317**, 187–123.
7. Kim M., Hansen K.K., Davis L., van Marle G., Gill M.J., Fox J.D., Hollenberg M.D., Rancourt D.E., Lee P.W., Yun C.O., Johnston R.N. 2010. Z-FA-FMK as a novel potent inhibitor of reovirus pathogenesis and oncolysis *in vivo*. *Antivir. Ther.* **15**, 897–805.
8. Shneider M.A., Shtil'bans E.B., Kupriyanov-Ashin E.G., Potselueva L.A., Zaikonnikova I.V., Kurinenko B.M. 1990. Anti-influenza effect of bacterial RNase in experiment and a pharmacokinetic rationale for the method of its application. *Antibiot. Khimioterap.* **35**, 27–33.
9. Alekseeva I.I., Kurinenko B.M., Kleiner G.I., Skuya A.Zh., Penzikova G.A., Oreshina M.G. 1981. Comparative analysis of the antivital activity of pancreatic and micronial RHases. *Antibiot. Med. Tekhnol.* **26**, 527–532.
10. Saxena S.K., Gravell M., Wu Y.N., Mikulski S.M., Shogen K., Ardelt W. Youle R.J. 1996. Inhibition of HIV-1 production and selective degradation of viral RNA by an amphibian ribonuclease. *J. Biol. Chem.* **271**, 20783–20788.
11. Lee Y.H., Wei C.W., Wang J.J., Chiou C.T. 2011. *Rana catesbeiana* ribonuclease inhibits Japanese encephalitis virus (JEV) replication and enhances apoptosis of JEV-infected BHK-21 cells. *Antiviral Res.* **89**, 193–198.
12. Gribencha S.V., Potselueva L.A., Barinskii I.F., Deev S.M., Balandin T.G., Leshchinskaya I.B. 2006. Protective activity of *Bacillus intermedius* RNase in guinea pigs and rabbits with street ravies virus infection. *Vopr. Virusol.* **51**, 41–43.
13. Ilinskaya O.N., Shah Mahmud R. 2014. Ribonucleases as antiviral agents. *Mol. Biol.* (Moscow). **48** (5), 615–623.
14. Ulyanova V., Vershinina V., Ilinskaya O. 2011. Barnase and binase: Twins with distinct fates. *FEBS J.* **278**, 3633–3643.
15. Mitkevich V.A., Schulga A.A., Trofimov A.A., Dorovatovskii P.V., Goncharuk D.A., Tkach E.N., Makarov A.A., Polyakov K.M. 2013. Structure and functional studies of the ribonuclease binase Glu43Ala/Phe81Ala mutant. *Acta Crystallogr. D. Biol. Crystallogr.* **69**, 991–996.
16. Shah Mahmud R., Ilinskaya O.N. 2013. Antiviral activity of binase against pandemic influenza A virus (H1N1). *Acta Naturae.* **5**, 49–56.
17. Schulga A., Kurbanov F., Kirpichnikov M., Protasevich I., Lobachev V., Ranjbar B., Chekhov V., Polyakov K., Engelborghs Y., Makarov A. 1998. Comparative study of binase and barnase: Experience in chimeric ribonucleases. *Protein Eng.* **11**, 775–782.
18. Ilinskaya O.N., Karamova N.S., Ivanchenko O.B., Kipenskaya L.V. 1996. SOS-inducing ability of native and mutant microbial ribonucleases. *Mutat. Res.* **354**, 203–209.
19. Yakovlev G.I., Moiseyev G.P., Struminskaya N.K., Borzykh O.A., Kipenskaya L.V., Znamenskaya L.V., Leschinskaya I.B., Chernokalskaya E.B., Hartley R.W. 1994. Mutational analysis of the active site of RNase of *Bacillus intermedius* (BINASE). *FEBS Lett.* **354**, 305–306.
20. Laakko T., King L., Fraker P. 2002. Versatility of merocyanine 540 for the flow cytometric detection of apoptosis in human and murine cells. *J. Immunol. Meth.* **261**, 129–139.
21. Smolewski P., Grabarek J., Lee B., Johnson G.L., Darzynkiewicz Z. 2002. Kinetics of HL-60 cell entry to apoptosis during treatment with *TNF-alpha* or camptothecin assayed by the stathmo-apoptosis method. *Cytometry*. **47**, 143–149.
22. Starikov V.A., Mikhalishin D.V., Lezova T.N., Borisov A.V. 2014. Analysis of adjuvant activity of ethonium as a component of vaccine against foot-and mouth disease. *Sovrem. Farm. Biopreparaty.* **1**, 30–31.
23. Loginova S.Ya., Borisevich S.V., Semenova I.A., Borisevich G.V., Maksimov V.A., Bondarev V.P., Nebol'sin V.E. 2009. In vitro invstigation of Ingavirin efficacy against influenza B virus. *Antibiot.Khimioterap.* **54**, 13–15.
24. Efimova M.A., Ivanov A.V., Gaffarov Kh.Z., Moskvichev O.V. 2014. Immunobiological properties of reovirus type I. *Dokl. Ross. Akad. S-kh. Nauk*. **1**, 65–68.
25. delCardayre S.B., Raines R.T. 1995. The extent to which ribonucleases cleave ribonucleic acid. *Anal. Biochem*. **225**, 176–178.
26. Karpeiskii, M.Ya., Khandanyan, A.Zh., Chepurnova, N.K., Platonov, A.L., and Yakovlev, G.I., 1981. Analysis of substrate specificity and action mechanism of *Bacillus intermedius* 7P ribonuclase. *Bioorg. Khim.* **7**, 1669–1679.
27. Ilinskaya O.N., Zelenikhin P.V., Petrushanko I.Y., Mitkevich V.A., Prassolov V.S., Makarov A.A. 2007. Binase induces apoptosis of transformed myeloid cells and does not induce T-cell immune response. *Biochem. Biophys. Res. Commun.* **361**, 1000–1005.
28. Mitkevich V.A., Makarov A.A., Ilinskaya O.N. 2014. Cell targets of antitumor ribonucleases. *Mol. Biol.* (Moscow). **48** (2), 181–188.
29. Turnbull S., West E.J., Scott K.J., Appleton E., Melcher A., Ralph C. 2015. Evidence for oncolytic virotherapy: Where have we got to and where are we going? *Viruses*. **7**, 6291–6312.
30. Coffey M.C., Strong J.E., Forsyth P.A., Lee P.W. 1998. Reovirus therapy of tumors with activated Ras pathway. *Science*. **282**, 1332–1334.
31. Errington F., Steele L., Prestwich R., Harrington K.J., Pandha H.S., Vidal L., de Bono J., Selby P., Coffey M. Vile R., Melcher A. 2008. Reovirus activates human dendritic cells to promote innate antitumor immunity. *J. Immunol.* **180**, 6018–6026.
32. Ilinskaya O., Decker K., Koschinski A., Dreyer F., Repp H. 2001. *Bacillus intermedius* ribonuclease as inhibitor of cell proliferation and membrane current. *Toxicology.* **156**, 101–107.

Translated by A. Levina