

## Antiviral activity of bacterial extracellular ribonuclease against single-, double stranded RNA and DNA containing viruses in cell cultures

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**Abstract** Binase, a small guanyl-preferring extracellular ribonuclease of Gram-positive non-pathogenic soil bacteria *Bacillus pumilus*. Binase is a well-known bacterial ribonuclease and the most essential properties of the enzyme were characterized. Binase has demonstrated antiviral activity in various virus-infected animal models. Most experiments associated with binase treatment of virus-infected animals were performed using single stranded RNA viruses. It is still unclear, whether binase is able to inactivate the double stranded RNA virus. Moreover, the phenomenon of the antiviral activity of binase against the DNA containing virus in animal model is not explained. Here, we presented the experimental results reflecting binase effect towards eukaryotic cells infected with viruses containing different types of nucleic acids. The obtained data revealed the bacterial ribonuclease binase mode of action against single stranded RNA influenza A virus, double stranded RNA reovirus and DNA containing herpes virus to prove future application of new antiviral tool with a broad range of activity.

**Keywords** Binase - ribonuclease – influenza A virus – reovirus – herpes virus- antiviral agent

### 1. Introduction

Influenza is one of the most contagious respiratory diseases caused by seasonal and pandemic influenza virus IV type A (IAV). It kills up to half a million people in a year worldwide. Due to its high mutation rate, vaccine or antiviral drugs are not able to stop the evolution and subsequent emergence of new IAV types or variants during the last 100 years since the incidence of the 1<sup>st</sup> dramatic influenza pandemic in 1918. Rotavirus infection is another common disease caused by reovirus (*Reoviridae* family). According to WHO it kills about 200,000 children during a year. To prevent children death worldwide, development of an efficient antiviral agent is required.

Binase is a well-known enzyme with different biological activities [1] among which antiviral activity is one of most intriguing [2]. It was shown that binase affects both RNA and DNA viruses in infected animals [3-5],

however the molecular basis of antiviral action of binase is still unclear. After internalization, binase can interact with naked virus particles in the cell cytoplasm and nucleus [6-7]. To reveal the specific targets of binase action against the viruses, cell culture models can be very helpful.

Here, we studied the antiviral effect and mode of actions of binase on two RNA containing viruses, influenza and reovirus, as well as DNA-containing herpes virus. Binase is supposed to be a promising tool affecting viral particles intracellularly at post transcription level and inducing the reduction of RNA and DNA containing viruses irrespective to their mutations which could be occurred during epidemic and pandemic periods.

## 2. Material and Methods

Experiments involving viruses were performed using biosafety level 2 containment laboratory approved for such use by the local authorities (RP, Giessen, Germany and Federal Service for Supervision of Consumer Rights Protection and Human Welfare, Russian Federation). Experiments using infectious virus were performed in accordance with German and Russian regulations applicable to the propagation of indicated viruses. The pandemic influenza A/Hamburg/4/2009 (H1N1), reovirus subtype 1 Lang and herpes virus type I (pseudorabies virus) were used. Binase was incubated with infected Madin-Darby canine kidney epithelial (MDCK), African green monkey kidney (Vero) and Madin-Darby bovine kidney epithelial (MDBK) cell lines in Dulbecco's modified Eagle medium supplemented with fetal calf serum and antibiotics at 37°C and 5% CO<sub>2</sub>. The cytotoxicity of binase at different concentrations on the predefined cell monolayers was estimated using MTT assay. The infection and binase treatment were performed as follows. Binase at different nontoxic concentrations (5 and 50 µg/ml or 10 and 100 µg/ml) was pre-incubated with each virus for 30 min, meanwhile the cell monolayers were infected for 60 min at MOI (multiplicity of infection) of 0.01, 0.001 and 0.0001 for IAV, reo- and herpes viruses, respectively. After washing, IAV, reovirus and herpes virus infected cell monolayers were incubated for 24 h, 48 h and 72 h, respectively. The antiviral effect of binase was determined by the reduction of virus titer in supernatants using focus assay for IAV, and plaque assay for reo- and herpes virus. Binase effect on influenza A (H1N1) RNA was detailed by comparing the binase treated and non-treated viral RNA using RT-PCR method. The effect of binase (10 µg/ml) on the IAV genome was confirmed by a plasmid-based reverse genetic approach using a mini-genome system of IAV which includes 4 plasmids containing influenza A (H7N9) viral RNA encoding PB1, PB2, PA, and NP protein and a reporter plasmid encoding GFP (green fluorescent protein) open reading frame in negative-sense of the influenza A NS gene.

## 3. Results and Discussion

Based on cytotoxic effect, the binase concentrations were used to study the antiviral effects on MDCK, Vero and MDBK cell lines at 100 µg/ml and lower. Binase at 10 and 100 µg/ml reduced the pandemic H1N1 viral titer in the MDCK cells after 24 h post infection by 10- and 15-fold, respectively (Fig. a, diagram 1). Binase at 10 µg/ml completely degraded the capsid unprotected influenza A viral RNA segment encoding NS protein (Fig. b). Using viral reverse genetic system, we have shown that binase at 10 µg/ml decreased by 13-fold the activity of influenza A/Anhui/1/2013 (H7N9) viral polymerase complex which express GFP in the cells (Fig. c). Binase at 5 and 50 µg/ml reduced reovirus titer by 50% and 60%, respectively (Fig. a, diagram 2). The effect of binase at 10 and 100 µg/ml against herpes virus was similar, virus titer was reduced by 28-29% (Fig. a, diagram

3). It was previously shown that bacterial ribonuclease binase and eukaryotic ribonuclease RNase A possessed anti-IAV effect in chicken embryo. RNase A was less effective than binase because of the presence of RNase inhibitor in eukaryotic cells [3]. In our experiments, antiviral activity of binase against reovirus and herpes virus was also higher than RNase A activity (two times and four times higher, respectively). Here, we found that binase is more effective against RNA viruses, especially against single stranded virus if compared to double stranded virus. The binase effect on DNA containing viruses was also detected; it was less than on RNA containing viruses but several times higher than this one of eukaryotic RNase A.

### **Conclusions**

Thus, binase at the nontoxic concentrations is effective as an antiviral agents against single- and double stranded RNA viruses as well as against DNA containing viruses. Antiviral activity of binase is higher than the activity of eukaryotic ribonuclease. Binase is able to influence on the viral genome expression. Binase is an attractive tool against broad spectra of viruses regardless of their genome nature and could be applied in human and veterinary medicine.

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## Figure legends

**Fig. (a)** The binase antiviral activity against pandemic influenza A/Hamburg/4/2009 (H1N1) (1), reo 1 Lang (2) and herpes 1 viruses (3); **(b)** RT-PCR analysis of binase treated viral RNA segment encoding NS protein after capsid elimination; **(c)** Decrease of production level of influenza A (H7N9) viral RNP complex in binase treated cells as detected using GFP reporter.

