

# Identification of 2',3'-cGMP as an intermediate of RNA catalytic cleavage by binase and evaluation of its biological action

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## Abstract

© 2015 Pleiades Publishing, Ltd. Binase, the RNase from *Bacillus pumilus*, is an endonuclease that cleaves the phosphodiester bond between the 3'-guanyl residue and 5'-OH residue of an adjacent nucleotide, with the formation of a corresponding intermediate, 2',3'-cGMP. Subsequent hydrolysis of 2',3'-cGMP into a 3'-phosphate is highly specific and proceeds slowly. Thus, a question arises in respect to the time interval that this positional isomer exists during catalytic cleavage of RNA by binase, and whether it may contribute to antitumor activity of the enzyme. In this study, we found, by implementing an enzyme-linked immunosorbent assay, that during catalytic cleavage of RNA by binase, 2',3'-cGMP is maintained in the reaction mixture for about one hour. Activation of phosphodiesterases did not lead to a complete elimination of 2',3'-cGMP. The highest amount of 2',3'-cGMP was detected at pH 8.5, underat which conditions, it reached nanomolar levels. The linital RNA concentration in the reactions was in the range of 100-1000 µg/mL. We found that exogenous 2',3'-cGMP, as well as its positional isomer, 3',5'-cGMP, do not induce apoptosis of human lung adenocarcinoma A549 cells, which are sensitive to binase apoptogenic activity. Taking into consideration the data on binase internalization, and on the role of 2',3'-cGMP in the activation of opening of mitochondrial pores, we propose that 2',3'-cyclic guanosine phosphates contributes to apoptotic processes, induced by binase, only when generated intracellularly.

<http://dx.doi.org/10.1134/S1068162015010136>

## Keywords

2' 3'-cGMP, A549, binase, cycle-forming guanyl-specific RNases, human lung carcinoma