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Modeling of mammalian ribonuclease inhibitor complex with bacterial ribonuclease binase

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Motivation and Aim: Ribonucleases appear to be a promising replacement for conventional chemotherapy [1] because the side effects of systemic chemotherapy used to treat cancer and directly target DNA are often severe [2]. RNases play a key role in RNA metabolism [3]. One of the promising representatives of therapeutic RNases is bacterial ribonuclease binase, which is not inhibited by the mammalian ribonuclease inhibitor (RI) and selectively eliminates a number of malignant cells. At the same time, pancreatic RNase A is inhibited with high efficiency by RI and does not have a cytotoxic effect on malignant cells [4]. It was postulated that the cytotoxic effect of bacterial RNases is due to the fact that they do not interact with RI. However, this was not verified. The purpose of this work was to test the possibility of interaction between binase and RI.

Methods and Algorithms: molecular modeling of binase complexes with RI was performed using the MOE program, protein-protein docking was performed by the ZDock server [5], and model analysis was performed by the Qasdom server [6]. The interaction of binase with RI in solution was evaluated by microscale thermophoresis (MST). Analysis was performed using the Monolith NT.115 (NanoTemper technology) and data analysis was performed using MO.Affinity Analysis software v.2.3.

Results: An experimental verification of the interaction of binase with RI, carried out in solution by the MST method, showed that they form a stable complex with a dissociation constant in the nanomolar range. A model structure of the complex of RI with binase was constructed for both reduced form of RI and oxidized one. It was found that in both cases the binase active site in such a complex is oriented away from RI and remains available for ribonuclease activity, while the RNase A catalytic site is blocked. Significant amino acids of the binding site of binase with barstar (a natural inhibitor of binase) – Gln28, Arg58, Arg82, Arg86 [7] – as well as the active site of binase – Lys26, Asp53, Val54, Phe55, Ser56, Asn57, Arg58, Glu59, Glu72, Arg82, Arg86, His101, His102 [8] do not interact with RI in our model. Energies of binase complex with the oxidized and reduced forms of the ribonuclease inhibitor were –575 and –617 kcal/mol respectively.

Conclusion: For the first time, it was found that bacterial RNase binase forms a complex with RI. In contrast to RNase A, the active site of binase in complex with RI is not blocked, and binase can degrade the substrate, realizing its cytotoxic effect.

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References

1. Makarov A.A., Ilinskaya O.N. Cytotoxic ribonucleases: molecular weapons and their targets. *FEBS Lett.* 2003;540:15-20. doi: 10.1016/S0014-5793(03)00225-4.
2. Schirrmacher V. From chemotherapy to biological therapy: A review of novel concepts to reduce the side effects of systemic cancer treatment (Review). *Int J Oncol.* 2019;54:407-419. doi: 10.3892/ijo.2018.4661.
3. Deutscher M.P., Li Z. Exoribonucleases and their multiple roles in RNA metabolism. *Prog Nucleic Acid Res Mol Biol.* 2001;66:67-105. doi: 10.1016/S0079-6603(00)66027-0.
4. Blackburn P., Jailkhani B.L. Ribonuclease inhibitor from human placenta: interaction with derivatives of ribonuclease A. *J Biol Chem.* 1979;254:12488-12493.
5. Pierce B.G., Wiehe K., Hwang H., Kim B.-H., Vreven T., Weng Z. ZDOCK server: interactive docking prediction of protein–protein complexes and symmetric multimers. *Bioinformatics.* 2014;30:1771-1773. doi: 10.1093/bioinformatics/btu097.
6. Anashkina A.A., Kravatsky Y., Kuznetsov E., Makarov A.A., Adzhubei A.A. Meta-server for automatic analysis, scoring and ranking of docking models. *Bioinformatics*. 2018;34:297-299. doi: 10.1093/bioinformatics/btx591.
7. Wang L., Pang Y., Holder T., Brender J.R., Kurochkin A.V., Zuiderweg E.R.P. Functional dynamics in the active site of the ribonuclease binase. *Proc Natl Acad Sci.* 2001;98:7684-7689. doi: 10.1073/pnas.121069998.
8. Ermakova E.A. A comparative study of the interaction of Bacillus amyloliquefaciens ribonuclease (barnase) and Bacillus intermedius ribonuclease (binase) with barstar by brownian dynamics simulation. *Biophysics*. 2006;51:202-208. doi: 10.1134/S0006350906020072.