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In silico prediction of low molecular weight ricin chain A ribosome inactivating protein based on its interaction with 28S rRNA

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ABSTRACT

Nowadays, ribosome-inactivating proteins (RIPs) are being utilized as potent antiviral strategies and pharmaceutical elements in agricultural and medical industries. They play important role in defense against pathogens in plants and human. To facilitate the molecular handling or improve the pharmakinetics properties, a smaller size of ricin chain A RIP consisting of 137 amino acids (in about half of the original molecule) was designed by using bioinformatics tools including PRIME and VINA molecular interaction and docking analysis based on RIP/28S rRNA interaction. The designed molecule was nominated for the functional and pharmakinetics studies in eukaryotic heterologous expression and drug delivery systems.

Key words: Bioinformatics, Designing, Ribosome inactivating protein, RIP.

INTRODUCTION

Ribosome-inactivating proteins are a group of proteins that enzymatically damage the ribosomes. They exhibit 28S rRNA N-glycosidase activity leading to the irreversible cleavage of an adenine residue at a conserved site of rRNA molecule causing the inhibition of protein synthesis (Barbieri et al. 1993; Stripe 2004). In addition to their N-glycosidase effects, they possess other enzymatic activities such as adenine polynucleotide glycosylase, lipid phosphatase, chitinase, DNase and superoxide dismutase activities (Nielsen and Boston 2001; Stripe and Battelli 2006). RIPs have been identified from a large number of higher plants covering approximately 17 families and 30 species (reviewed by Shu et al. 2009 and Schrot et al. 2015) and Later on they have been isolated from a number of fungi, algae and bacteria, too (reviewed by Schrot et al. 2015). Ribosome-inactivating proteins are generally classified into two categories including type 1 and type 2 proteins. Type 1 is composed of a single chain basic proteins while type 2 RIPs are heterodimers consisting of an A chain having enzymatic N-glycosidase activity and B chain/lectin with sugar binding domain (Stirpe and Battelli 2006). In some plants such as maize and barley, there are peculiar single polypeptide type 1 RIPs without a lectin side chain, but are unusual in being acidic proteins until they are activated by proteolysis. These RIPs are considered as type 3 RIPs (Hey et al. 1995).

Ribosome-inactivating proteins are well known as antiviral elements. Their antiviral mechanism has been suggested to be related to their inhibitory effects on host cell protein biosynthetic apparatus and apoptosis cell death (Barbieri et al. 1993). Ribosome-inactivating proteins have been known to impart a very large level of resistance to plant viruses such as tobacco mosaic virus (TMV),

sunnhemp rosette virus (SRP), potato virus X (PVX), citrus ring spot virus (CRSV) (Peumans et al. 2001; Pandit et al. 2013). They not only exhibit antiviral activities towards different plants viruses but also they show inhibitory activity against numerous animal and human viruses such as human immunodeficiency virus (HIV), human simplex virus (HSV), polio, influenza and hepatitis B viruses (Uckun et al. 2003; He et al. 2008; Pizzo and Antimo 2016). Nowadays, RIPs have attracted a lot of attention in biomedical research towards animal and human cells. They show diverse applications like immunotoxicity (Battelli et al. 1996), abortifacient (Yeung et al. 1988), bioactive properties including antifungal, antibacterial, antioxidant and antitumor activity (Parikh and Tumer 2004; Stirpe and Battelli 2006; Shu et al. 2009; Puri et al. 2009). Their toxic forms also can be utilized as bioweapons and defense elements (Knight 1979; Pizzo and Antimo. 2016). Numbers of RIPs are successfully fused or conjugated to functionally different carriers such as protease inhibitors or hormones in order to create specific bifunctional cytotoxic elements. These chimeric fusions have been exploited to therapeutic purpose in the treatment of cancer cells including hematological or solid cancers. Newly, RIP-based nanoparticles are nominated as excellent candidates for therapeuric applications in biomedical sciences. Thereby, the pharmacology industries are exploring several options, such as specific cellular targets or new drug delivery methods using RIPs (reviewed by Pizzo and Antimo 2016).

Ribosome-inactivating protein commonly play important and vital role in different industries. The genes and the proteins of RIPs are available as strong biotechnological tools (reviewed by Pandit et al. 2013; Sobiya and Jannet 2013). Recently, RIPs DNA are being utilized as suicide tool for gene therapy (reviewed by Vago 2015). In the future, the RIPs are expected to significantly contribute in cancer treatment.

The first identified RIP were of two potent toxins, namely ricin, from the seeds of *Ricinus communis*, and abrin, from the seeds of *Abrus precatorius* (Van Damme et al. 2001). The A chain of ricin is the first RIP based immunotoxin that was generated by its coupling with native antibody (Youle and Neville 1980; Ghetie et al. 1988). In order to facilitate the handling of protein or gene of ricin chain A as biotechnological or pharmaceutical tools, we aimed to design a smallest molecule of chain A by using bioinformatics tools. The designed molecule was nominated for the expression and functional pharmaceutical studies in heterologous eukaryotic systems.

MATERIALS AND METHODS

Designing of ricin chain A with low molecular weight.

As the experimental materials, the protein sequence of ricin chain A (accession number: P02879) from *Ricinus communis* (castor bean) was extracted from the UniProt Knowledge Base webserver41. This sequence was utilized as a target to find out the suitable template sequence and 3D structure within the PDB by SWISS-MODEL online program. The SWISS-MODEL search program revealed *Ricinus communis* RIP (PDB code 1il5) as the best template for target. The template PDB was selected on the basis of query cover (100%), percent identity (99.6%) and resolution of the template PDB structure (2.8 A).The 3D structure of RNA was modeled by SimRNAweb . In order to identify the highest interactive regions between protein and 28S rRNA molecules, PRIME2.0 (a Protein-RNA Interaction ModElling) program was performed. For molecular docking studies, 3DRPC (3D RNA-Protein Complex) structure prediction softwares and Autodock Vina package were utilized. The secondary and the three dimensional structures of test RIP were predicted by online-based PSIPRED and Phyre2.0 softwares.

RESULTS AND DISCUSSION

The aim of the present study was to find out the interactive regions of ricin chain A RIP with those of human 28S rRNA molecule in order to predict and design a smallest RIP molecule with a reduced molecular size. In usual, the utilization and handling of larger molecules are the challenging tasks at experimental, clinical and industrial scales. Thereby, the size of the ricin chain A RIP was selected to be reduced as a pharmaceutically important agent. Ricin is a type 2 ribosome-inactivating protein and consists of an enzymatically active chain A similar to type 1 RIPs, linked to chain B a lectin moiety with specificity for sugars with the galactose structure (Lord et al. 1994). In order to reduce its active size and improve its pharmakinetics properties, the chain A moiety (minus lectin moiety) of ricin toxin similar to type 1 RIPs is utilized using various drug delivery systems. In the present work, for further molecular size decrease, we tried to reduce the size of the ricin chain A, too.

For this purpose, the interactive potential between ricin RIP and 28S rRNA molecules was studied by using PRIME2.0, a Protein-RNA Interaction ModElling program. The output data indicated that the highly interactive region is included between residues 68-119 (consisting of 41 amino acids) in ricin chain A RIP, while the higher interactive site of rRNA molecule included between nucleotides 3334 to 3537 (consisting of 203 nucleotides) (Fig. 1 and Table 1). In both interactive molecules, it was predicted that the highly interactive regions may not included their catalytic sites (including tetra peptide "EAAR" at C-terminal position of RIP and the universally conserved adenine residue at position of 4324).

To further analyze the interactive ability of test RIP molecule with 28S rRNA, docking experiments were carried out using VINA software. For this, the 3D structures of the interactive part of 28S rRNA and test RIP were made by 3DRP and protein blast softwares and then 20 different interactive conformations were docked. The obtained results showed that out of 20 conformations only one has stable interactive structure, having the lowest binding energy. It was predicted to have -5.9 kcal/ mol binding energy (Fig. 2 and Table 2). On the other hand its binding ability was predicted to be 5.9 as compared to the lower abilities of other conformations. Analysis of the interactive structure showed that the alpha-helical regions of RIP molecule could able to interact with rRNA molecule (Fig. 2). Analysis of the secondary structure of test RIP by using PSIPRED software indicated that the region dominated with alpha-helixes contains the catalytic peptide sequence "EAAR" (Fig. 3). Thereby, based on the results obtained from PRIME2.0 and VINA data, the interactive region of protein including the residues between 68-119 plus the catalytic peptide containing region including the residuses between 119-205 was predicted to be the potent reduced acive size of ricin chain A RIP that could be considered and utilized as test experimental molecule (Fig. 4). The residues between 68-205 were predicted to be the highly interactive region of chain A RIP with rRNA molecule. Analysis of the three dimensional structure of the predicted ricin chain A by Phyre2.0 software indicated that it is dominated by alpha-helical structures that was already predicted to be the interactive helixes with 28S rRNA molecule (Fig. 4).

The predicted region is about half of the original ricin chain A molecule that was used as test RIP. The reduced size of the ricin chain A may improve its delivery into heterlogous systems as well as prevent its degradation process inside the target cell. Thereby, its pharmakinetics properties might be further improved by targeting its small protein or gene into heterologous cells.

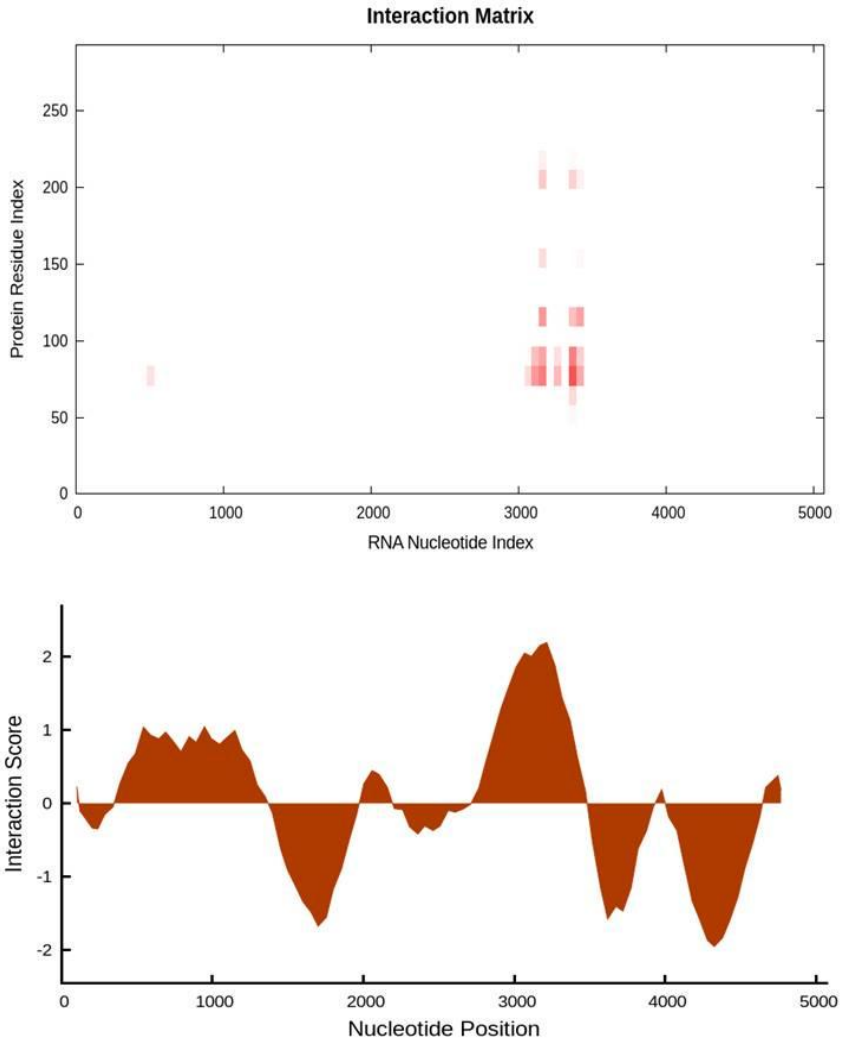

Fig 1: Prediction of interactive regions between RIP and rRNA molecules. The interactive sites between ricin chain A RIP and 28SrRNA molecules were predicted by PRIME 2.0 software. Upper) protein and RNA sequences indexes; Lower) Interaction score versus nucleotide sequence of 28S rRNA molecule

Table 1. Prediction of the interactive regions between RIP and rRNA molecules.

Table1: prediction of the interactive regions between RIP and rRNA molecules

#	Protein region	RNA region	Interaction Propensity	Discriminative Power	Normalized Score
1	68-119	3334-3537	93.14	99	3.03
2	68-119	3132-3335	89.40	99	2.83
3	76-127	3334-3537	89.29	99	2.83
4	101-152	3132-3335	87.23	99	2.72
5	68-119	3050-3253	87.16	99	2.72
6	101-152	3353-3556	86.39	99	2.68
7	76-127	3132-3335	86.34	99	2.67
8	68-119	3353-3556	85.71	99	2.64
9	68-119	3233-3436	84.37	99	2.57
10	101-152	3334-3537	83.98	99	2.55
11	76-127	3050-3253	83.97	99	2.55
12	193-244	3132-3335	83.12	99	2.50
13	76-127	3353-3556	82.62	99	2.48
14	193-244	3334-3537	82.42	99	2.47
15	51-102	3334-3537	81.64	99	2.43
16	68-119	3031-3234	81.56	99	2.42
17	143-194	3132-3335	81.52	99	2.42
18	76-127	3233-3436	81.06	99	2.40
19	68-119	424-627	81.08	99	2.40
20	201-252	3132-3335	79.80	98	2.33

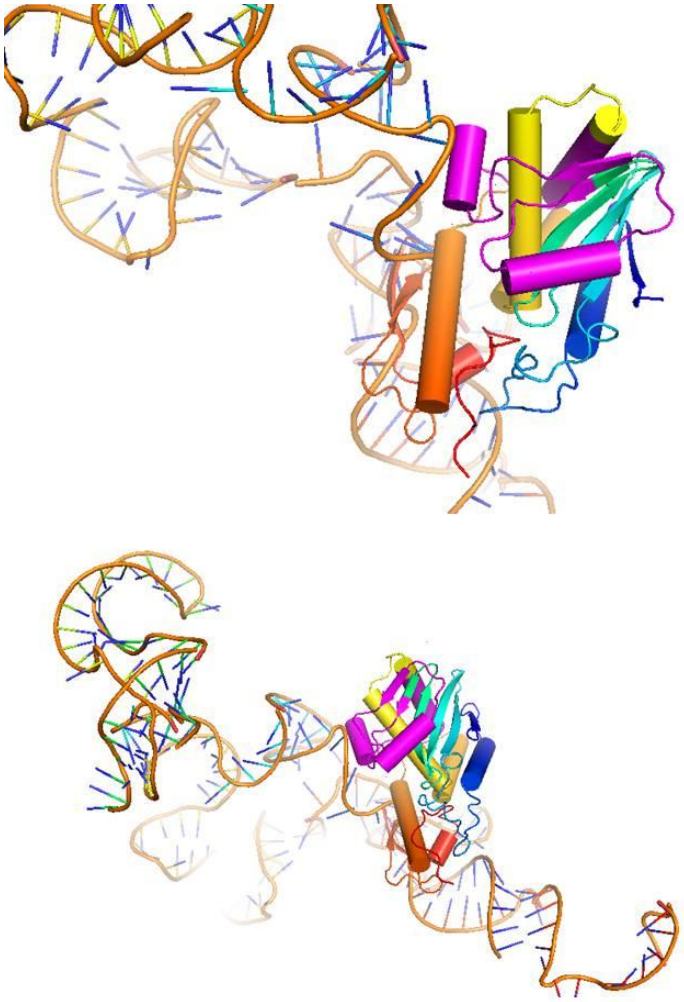

Fig. 2. Molecular docking of RIP molecule with 28S rRNA. The 3D structures of ricin chain A and 28S rRNA were predicted by PROTEIN BLAST and 3DRP softwares and docked together by using VINA molecular docking server.

Table 2. Binding ability of test RIP with 20 different confirmations of 28S rRNA

Table2: Binding ability of test RIP with 20 different conformations of 28S rRNA

mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-5.9	0.000	0.000
2	-5.7	5.053	10.735
3	-5.5	5.796	11.480
4	-5.2	4.098	11.091
5	-5.2	26.367	34.246
6	-5.1	2.475	7.608
7	-4.8	7.712	15.991
8	-4.8	2.806	10.104
9	-4.7	8.742	17.769
10	-4.7	28.290	34.620
11	-4.7	8.361	17.337
12	-4.7	30.815	38.556
13	-4.7	3.491	9.727
14	-4.6	31.082	39.673
15	-4.6	23.322	32.045
16	-4.5	7.144	15.863
17	-4.5	8.249	16.302
18	-4.5	26.625	34.482
19	-4.4	7.974	15.033
20	-4.4	5.986	13.907

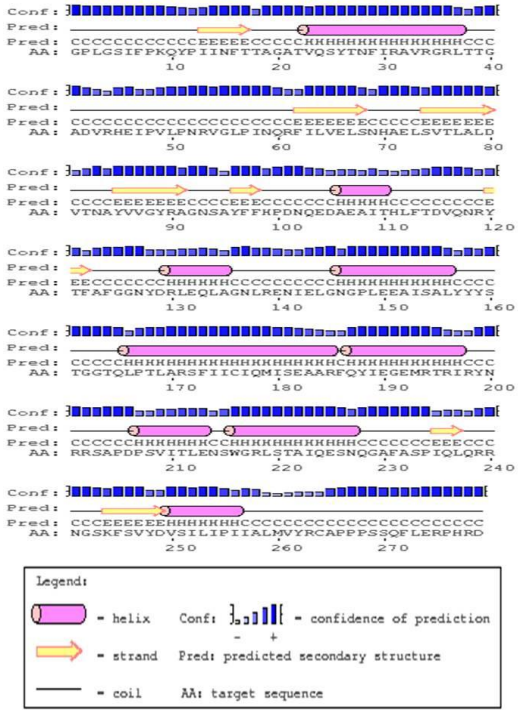

Fig. 3. Prediction of the secondary structure of ricin chain A RIP. The secondary structure of ricin chain A was predicted by PSIPRED software and compared to the interactive structure predicted by VINA.

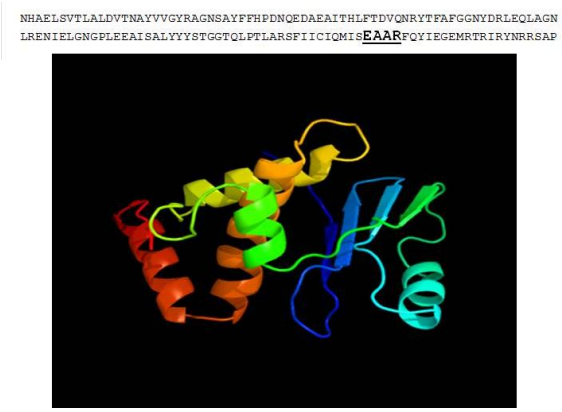

Fig. 4. The amino acid sequence and the 3D structure of the designed RIP. Upper) The amino acid sequence of designed RIP was predicted by PRIME 2.0 and VINA interaction and docking analysis. Lower) The three dimensional structure of designed RIP was predicted by Phyre 2.0 software.

To date, ribosome-inactivating toxins are selectively directed to the target cells as immunotoxins or nanoconjugates to open for huge applications in medicine (reviewed by Pizzo and Antimo 2016). The first RIP based immunotoxins were produced by conjugating ricin chain A with native antibody molecule (Youle and Neville 1980; Ghetie et al. 1988). Later on, the novel immunotoxins were generated by recombination of antibody fragment (with reduced molecular weight) and RIP toxin through recombinant DNA and fusion protein technologies (Maleki et al. 2013; Alewine et al. 2015). The production of such a compact RIP-based immunotoxin not only stabilized its delivery to the target cells but also prevented its degradation process inside cells. To our idea, the size of the fused RIP-based immunotoxin even could be more compact by reducing the active size of the chain A molecule. Thereby, our attempt was made to design and candidate a reduced size of ricin chain A for immunotoxicology studies.

Fusion of chemical Conjugation of RIPs such as ricin chain A to various peptides or proteins eg. Cell-binding ligands have revealed a new selective way of cytotoxicity on various cancerous or malignant cells (reviewed by Pizzo and Antimo 2016). To our idea, the reduction of the molecular sizes of such fused bifunctional products may help the delivery and release of the RIP toxins into the target cells. We herein propose the reduction of the molecular size of the RIP partner to overally decrease the size of the fused or conjugated products.

Besides immunotoxins and peptide-based conjugates, RIP molecules are recently been incorporated into various types of nanoparticles for selective cytotoxity against cancerous target cells (reviewed by Pizzo and Antimo 2016). In this case

also we propose the use of the smaller sizes of RIP molecules to help the possible improvement of pharmakinetic properties of ribosome-inactivating proteins.

In particular, RIP toxin based recombinant therapeutics for the treatment of the cancer and RIP toxin based suicide gene therapy are mainly developed against malignant cells (reviewed by Vago 2015). The cytotoxicity of all these compounds depend on multiple factors such as tumor accessibility and penetration, and the efficient internalization, intracellular sorting and release of catalytically active toxin domain into the cytoplasm. To our idea, the most of the mentioned factors can be improved by reducing the size of the active toxin molecule. Therefore, we herein candidate the reduced size of ricin chain A, as a highly toxic form of RIP, to be used in pharmaceutical studies and drug delivery investigations in eukaryotic systems.

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