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The therapeutic potential of fungal ribotoxins

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Abbreviations: ABPA, allergic bronchopulmonary aspergillosis; *AOX1*, alcohol oxidase 1 gene; CDR, complementary determining regions; dsFV, disulfide bridged FV; FR2, framework region 2; FV, antibody variable domains; HtA, hirsutellin A; RIP, ribosome-inactivating protein; RNase, ribonuclease, scFV, single chain FV; SRL, sarcin-ricin loop.

Running title: Therapeutic uses of ribotoxins

Abstract

Ribotoxins constitute a family of toxic extracellular fungal RNases that exert a highly specific activity on a conserved region of the larger molecule of rRNA, known as the sarcin–ricin loop. This cleavage of a single phosphodiester bond inactivates the ribosome and leads to protein synthesis inhibition and cell death. In addition to this ribonucleolytic activity, ribotoxins can cross lipid membranes in the absence of any known protein receptor. This ability is due to their capacity to interact with acid phospholipid-containing membranes. Both activities together explain their cytotoxic character, being rather specific when assayed against some transformed cell lines. The determination of high-resolution structures of some ribotoxins, the characterization of a large number of mutants, and the use of lipid model vesicles and transformed cell lines have been the tools used for the study of their mechanism of action at the molecular level. The present knowledge suggests that wild-type ribotoxins or some modified variants might be used in human therapies. Production of hypoallergenic mutants and immunotoxins designed against specific tumors stand out as feasible alternatives to treat some human pathology in the mid-term future.

INTRODUCTION

Ribotoxins are a family of toxic extracellular fungal RNases that exert a highly specific ribonucleolytic activity on the larger molecule of rRNA of the ribosome leading to protein synthesis inhibition and cell death by apoptosis [1-4]. They were discovered during a screening program of the Michigan Department of Health searching for antibiotics and antitumoral agents. The culture filtrates of a mould identified as *Aspergillus giganteus* MDH18894 contained a substance inhibitory to sarcoma and carcinoma induced in mice [5]. The molecule responsible for these effects was proved to be a protein, named α-sarcin, that still today is the best known ribotoxin (Fig. **1A**) [6]. Unfortunately, the rather unspecific cytotoxicity of these proteins when administered to animals harboring different tumors limited their potential clinical use [7].

α-Sarcin, restrictocin, and Asp f 1 are the most exhaustively characterized ribotoxins [1-4,8-10], but many others have been identified and partially characterized in different fungal species [11-16], including the insecticidal protein hirsutellin A (HtA) produced by the invertebrate fungal pathogen *Hirsutella thompsonii* [15,17,18]. All ribotoxins show a high degree of amino acid sequence identity (above 85%) with precisely the only exception of HtA that displays only about 25% sequence identity with the previously known family members [15,18].

Ribotoxins are not the only extracellular RNases produced by fungi. For example, a mould so common as *Aspergillus oryzae*, that is used in the production of various comestible foods, is also the producer of RNase T1 [19],

probably one of the most exhaustively characterized proteins. RNase T1 is therefore the best known member of the family of fungal extracellular RNases [20,21], a group that obviously includes ribotoxins. All of them also show a high degree of sequence [15,16,22,23] and structural similarity [24-29] but, apart from ribotoxins, none of these other fungal RNases has been reported to show cytotoxic activity.

In addition to RNase T1, RNase U2 from *Ustilago sphaerogena* also deserves to be mentioned because it is the non-toxic fungal extracellular RNase most closely related to ribotoxins of from a phylogenetic point of view [3,15,16,23,30]. RNase U2 is a small and highly acidic protein [31] that shows a strong preference for 3'-linked purine nucleotide phosphodiester bonds [32,33], specificity that is rather unusual within the group of microbial RNases. The observation that ribotoxins are larger basic proteins, containing longer and charged loops that are not present in the non-cytotoxic fungal RNases, suggested that these loops are directly related to their cytotoxic properties [30]. It would appear that an RNase T1-like protein would have acquired ribosome specificity by the insertion of short recognition domains that would target it to cleave more specific substrates. This hypothesis has become even more appealing after the recent biochemical characterization of HtA [18], a much smaller ribotoxin presumably with shorter loops.

In summary, all the features outlined above suggest that the study of the evolution and the mechanism of action of ribotoxins is of particular interest, as they appear to be naturally engineered targeted toxins evolved from the other microbial nontoxic RNases to enter cells and specifically inactivate the

ribosomes [34,35]. Identification of the structural features that have allowed these proteins to become such efficient natural killers would be a major step towards their utilization, native or modified, as weapons against different human pathologies.

RIBONUCLEOLYTIC ACTIVITY

Ribotoxins inhibit protein biosynthesis in ribosomal preparations by specifically cleaving a single phosphodiester bond of the large rRNA fragment [36,37]. This bond is also the specific target of the plant family of N-glycosidases known as ribosome-inactivating proteins (RIP) [38,39] best represented by ricin, the toxin found in the seeds from the castor bean plant, *Ricinus communis*. This region, known as the sarcin–ricin loop or SRL (Fig. **2**) is unique and it is located at an evolutionarily conserved site with important roles in ribosome function [40].

Ribotoxins behave as highly specific RNases when assayed against cell-free intact ribosomes, retaining this specificity when assayed against naked rRNA containing the SRL sequence. However, they can also produce extensive non-specific digestion of almost any RNA when used at higher concentrations [9,41]. Ribotoxins follow the same general acid–-base type endonucleolytic reaction scheme as the other members of the RNase T1 family [2,3,42,43]. First, there is a transphosphorylation reaction to form a 2',3'-cyclic phosphate intermediate. Second, this intermediate is hydrolyzed to the corresponding 3'-phosphate. The appearance of this cyclic intermediate, common to all proteins

of the RNase T1 family so far studied is implicit in the denomination of all these enzymes as cyclizing RNases [43]. However, the catalytic efficiency of RNases T1 and U2 against naked RNA, homopolynucleotides, or dinucleotides is several orders of magnitude higher. On the other hand, ribotoxins cleave and consequently inactivate the ribosome with a second order rate constant that matches the catalytic efficiency of the fastest known enzymes [44].

In the case of α-sarcin, during the first step of the reaction, Glu-96 acts as a general base and His-137 as a general acid. The hydrolysis of the cyclic derivative is then catalyzed by the same groups, but their roles are reversed [43,45]. In fact, this Glu/His combination is the most common pair of catalytic residues found in microbial RNases [20]. Another conserved residue, His-50, is required to assist the electrostatic stabilization of the transition state [45]. Mutational studies have also revealed that three other active site residues, Tyr48, Arg121, and Leu145, although not essential, appear to be determinants of the ribotoxin activity of α-sarcin [46-48]. Studies on the crystal structures of complexes of restrictocin with inhibitors led to the proposal that these ribotoxins may use base flipping to enable cleavage at the correct site of the SRL substrates [49]. All studies so far suggest that these three residues would enable the base flipping performed by His50/Glu96/His137 that permits cleavage of the unique phosphodiester bond at the SRL [49].

INTERACTION WITH PHOSPHOLIPIDS

In addition to their ribonucleolytic activity, ribotoxins cross lipid membranes in the absence of any known protein receptor [1,3,50]. Thus, although any ribosome could be potentially inactivated by these proteins, due to the universal conservativeness of the SRL, they are especially active on transformed or virus-infected cells [5,51,52]. This observation has been explained in terms of an altered permeability of their target cells combined with the ability of ribotoxins to interact with acid phospholipid-containing membranes [3,4,52-54]. Indeed, this ability seems to be related to the antitumoral character that led to their discovery [5,55].

In this regard, the use of lipid model systems proved that α-sarcin specifically interacts with negatively charged phospholipid vesicles at neutral or slightly acidic pH, resulting in protein–lipid complexes that can be isolated by centrifugation in a sucrose gradient [53]. Binding experiments revealed a strong ribotoxin–lipid vesicle interaction that caused vesicle aggregation, followed by their fusion into much larger lipidic structures [53]. Indeed, the peptide bonds of the protein were protected against trypsin hydrolysis upon binding to these vesicles [53,55], despite the high number of basic residues present along its sequence [23]. Altogether, all these results proved how α-sarcin, a water-soluble and hydrophilic protein, can interact with phospholipid bilayers through a combination of electrostatic and hydrophobic forces [56]. In good agreement with this scenario, the innate ability of α-sarcin to translocate across a phospholipid membrane in the absence of any other protein has also been demonstrated [50].

The current working hypothesis sustains that a higher content of negatively charged phospholipids in transformed cell membranes [53,54,57-60] would explain the favored toxicity of these proteins against tumors. Unfortunately, there is no direct evidence yet that this abundance of acidic phospholipids is the main explanation for the antitumoral activity of α-sarcin.

STRUCTURAL FEATURES

As mentioned above, ribotoxins show a high degree of identity in their approximately 150 amino acid sequence [14-16,23,61-64], including the conservation of their two disulfide bridges [3,30]. This observation includes HtA [15,18], although this protein is 20 residues shorter than most of the other known ribotoxins. Sequence differences are mainly concentrated at the loops of the ribotoxins suggesting that these differences are responsible for the minor differences observed among their activities [15].

This similarity is also evident between the two ribotoxins whose three-dimensional structure has been solved, restrictocin [26,49] and α-sarcin [29,65-67]. Both proteins folds into a common α + β structure with a central five-stranded antiparallel β-sheet and an α-helix of almost three turns. Residues 1–26 are especially interesting because they form a long β-hairpin that can be considered as two consecutive minor β-hairpins connected by a hinge region (Fig. **1A**). The distal region of this β-hairpin has been deleted, obtaining the Δ(7–22) variant of α-sarcin [68], a mutant retaining the same conformation as the wild-type protein (Fig. **1B**) [69]. Further support for the importance of this N-

terminal extension was obtained by docking and enzymatic studies which revealed that this β-hairpin most probably establishes interactions with specific ribosomal proteins in order to direct the ribotoxin to the SRL [67, 68]. This prediction would also explain the highly diminished cytotoxic properties observed for the deleted variant [68].

Even though α-sarcin and restrictocin show almost identical structures, some small but nevertheless significant functional and structural differences are evident. These differences concern the long non-structured loops and especially the N-terminal β-hairpin, a region of high mobility [26,65]. Interestingly, these aperiodic loops show a well defined conformation despite their exposed character and their lack of repetitive secondary structure [65].

Ribotoxins share their structural core with the non-toxic RNases of the RNase T1 family, in good agreement with their sequence similarities. Both RNase T1 and RNase U2 display identical regular secondary structure elements to ribotoxins despite their different amino acid sequence lengths. The geometric arrangement of the residues involved in the active site is also shared by both families of RNases. In fact, all fungal extracellular RNases whose three-dimensional structure is known exhibit quite different enzymatic specificities, but all of them share this common structural fold concerning the architecture and connectivity of the secondary structure elements [3,26,28,29]. The most significant structural differences among them are, again, related to both the presence of the longer N-terminal β-hairpin in ribotoxins and the different length and charge of their aperiodic loops.

In addition to the deletion mutant mentioned above, many more α-sarcin mutants have been isolated and characterized. These mutational studies have revealed the involvement of several residues, which are conserved among the different microbial extracellular RNases, in catalysis. Thus, it is well known that His137 and Glu96 are the only residues that are essential for the cleavage reaction performed by α-sarcin [35,45,70-75], whereas His-50, Tyr-48, Arg-121, and Leu-145 mostly contribute to the stabilization of the transition state [45-48], as stated above. Most of these residues are located in the central β-sheet and their side-chains point towards the concave face of the protein structure where the substrate is supposed to dock [29].

With regard to the protein regions involved in the interaction with membranes, the use of water-soluble synthetic peptides and mutant variants of α-sarcin within the main β-sheet of this protein suggested that this region (residues 116–139) would be directly involved in this interaction [76-78]. Interestingly, this sheet has also been predicted to be one of the few apolar regions of the protein [30,76]. The study of the mentioned mutants suggested that it would be located within the hydrophobic core of the phospholipid bilayer once the protein-lipid complexes were formed [46,78]. Within this same idea, mutants affecting α-sarcin active site residue Arg121 (R121K and R121Q), showed that the loss of the positive charge at that position produced a dramatic impairment of the protein's ability to interact with phospholipid membranes [46]. Other than this hydrophobic core, mutations affecting single residues located at the N-terminal β-hairpin of α-sarcin and the Δ(7–22) variant suggested that this protein portion would also be another region involved in the interaction with cell

membranes, as they display a different pattern of interaction with lipid vesicles [68,79]. Finally, loop 2 has been proposed by several authors [26,29,30,72] to be also one of the protein regions involved in the interaction with lipids but this possibility has not been directly studied yet.

CYTOXICITY AGAINST INTACT CELLS

α-Sarcin is able to inactivate the ribosomes of a great variety of organisms in cell-free systems [4,35], but it displays marked selectivity when intact cells are used as targets. This specificity seems to be determined by its ability to penetrate cells, as explained above. Thus, α-sarcin is especially active against transformed or virus-infected mammalian cells in the absence of any other permeabilizing agent [51,52,80-83]. This effect is saturable and consistent with passage across the cell membrane as the rate-limiting step. However, no membrane damage or mitochondrial activity alterations are detected [82]. These experiments confirmed that α-sarcin exhibits an intrinsic and rather specific cytotoxic character when assayed against some transformed cell lines, most probably due to the presence of acidic phospholipids on the outer leaflet of the membrane [53,54,57,58]. Consequently, all α-sarcin mutants studied that displayed an altered phospholipid interaction ability, such as that one affecting the positive charge of the active site residues Arg-121 (R121Q) [46] or, again, the Δ(7–22) deletion mutant [68], showed diminished cytotoxic effects on human rhabdomyosarcoma cells [68,79]. Obviously, mutation of the catalytically essential His-137 (H137Q) rendered a non-cytotoxic variant too [52].

THE *A. FUMIGATUS* ALLERGEN Asp f 1

Fungi represent one of the principal sources of allergens. Invasive infection is usually fatal unless treated early, and even then, antifungal therapy is often unsuccessful. The incidence of fungal infections has indeed risen lately, owing to the increase in the number of immunocompromised patients [84]. Furthermore, a link between fungal allergy and severe asthma is accepted, although still poorly understood [85,86]. *Aspergillus fumigatus* is an opportunistic human pathogen and one of the most common sources of allergy and asthma in humans [87,88]. This fungus is also the producer of Asp f 1, a ribotoxin and one of its most important allergens [8,64]. Asp f 1, for example, has been found in the urine of patients with disseminated aspergillosis [8,64,89], although it has also been proven that it is not a major virulence factor in *A. fumigatus* infections [90-92]. However, this protein is clearly involved in the pathogenicity of allergic bronchopulmonary aspergillosis (ABPA), the most severe form of allergic inhalant diseases, as these patients show high levels of Asp f 1-specific IgE [10,93].

Extracts of *A. fumigatus* are frequently used to diagnose allergic reactions, but they are highly complex mixtures very difficult to standardize [94]. Attempts to improve diagnosis are focusing on the use of homogeneous standard preparations of recombinantly produced allergens [95,96]. Unfortunately, recombinant native Asp f 1 is not devoid of cytotoxic activity, and indeed it can trigger anaphylaxis. Taking into account that the ribotoxin-

characteristic N-terminal β-hairpin shows high amino acid sequence variability [3,15,16] and that it is highly flexible and exposed [29,69], it was considered as a major determinant of the immunoreactivity of these proteins [10]. This hypothesis was confirmed through the production and characterization of Asp f 1, α-sarcin, and their corresponding Δ(7–22) variants [10,68]. The two deleted proteins studied showed marked decreases in their reactivity to Asp f 1-IgE antibodies, suggesting that this N-terminal β-hairpin was involved in at least one allergenic epitope [10]. In addition, these experiments reinforced the significant prevalence of Asp f 1-specific IgE antibodies in sera from ABPA patients sensitized to *A. fumigatus* [2,10,96,97]; a prevalence that was also observed for the Asp f 1 variants studied that, even more importantly, also retained most of the IgG epitopes [10].

The amount of data accumulated with these noncytotoxic deletion variants of Asp f 1 seems to favor their use in immunomodulating therapies for *Aspergillus* hypersensitivity and diagnosis, although *in vivo* assays are still required to assess this possibility. Within this idea, it must be remarked how these potentially hypoallergenic ribotoxin molecules have been recently cloned and produced in *Lactococcus lactis* [98], a microorganism ‘generally regarded as safe’ (GRAS). This GRAS status of *L. lactis*, altogether with the fact that the hypoallergenic variants of Asp f 1 are secreted by the engineered bacteria, confers this system with the features required to try immunotherapeutic protocols for Asp f 1-related allergic diseases. The feasibility of this approach will be next tested in animal models in our laboratory after the recent optimization of an allergic murine model sensitized against this allergen

[Álvarez-García, E.; Batanero, E.; García-Fernández, R.; Villalba, M.; Rodríguez, R.; Gavilanes, J.G. and Martínez del Pozo, A., unpublished data].

RIBOTOXINS AS PART OF IMMUNOTOXINS

Immunotoxins are therapeutic agents with a high degree of specificity, composed of targeting moieties, such as antibodies or physiologically important ligands, linked to toxic proteins [99-101]. The design of this type of molecules is helping to close the gap required to achieve one of the oldest goals of antitumoral therapy, proposed by Ehrlich as early as 1906, of targeting cancer cells with a 'magic bullet' consisting of a tissue-specific carrier that would deliver toxic agents to neoplastic tissue [102]. Initially, immunotoxins were prepared by conjugating toxins to monoclonal antibodies, using the whole antibody molecule as targeting moiety [103]. However, it was soon realized that smaller sizes would allow higher index of penetration within solid tumors, easier clearance from blood vessels, and easier manipulation procedures. Within this idea, new approaches have been optimized with the advent of the late extraordinary biotechnological development, including the production of recombinant immunotoxins in different model organisms such as bacteria, yeast, or even insect cells [author(s), unpublished data]. This type of molecules is considered as a second generation of immunotoxins that contain only the antibody variable domains stabilized by a flexible peptide (scFv) or a disulfide bridge (dsFv) (Fig. **3**) [104,105].

Regarding the toxic moiety, the most representative toxins employed have been ricin from plants [106-110] and *Pseudomonas* exotoxin A or diphtheria toxin from bacteria [111-116]. However, although not so frequently employed, ribotoxins have several advantages for their use in the design of immunotoxins; namely, their small size, high thermostability, poor immunogenicity, resistance to proteases, and their highly efficient ability to inactivate ribosomes [3,4,117]. In fact, different ribotoxins have been used as components of immunotoxins [117-125]. The first ribotoxin-based immunotoxins were constructed by chemical conjugation with mitogillin [122], restrictocin [117-119], or α-sarcin [121]. Some years later second-generation versions were also produced by fusing restrictocin cDNA with that encoding the scFv region of a monoclonal antibody directed against the human transferrin receptor, joined by a linear flexible peptide to promote the independent folding of the two immunotoxin moieties. These constructs were further engineered to enhance the intracellular processing and delivery of the ribotoxin [126]. Unfortunately, none of these ribotoxin-based immunotoxins has been yet studied beyond a preliminary characterization, most probably due to their large size, which could hinder their correct internalization into solid tumors, or to the low structural stability of the immunoconjugates prepared.

Much more recently, a new ribotoxin-based immunotoxin approach aimed at solving these problems has been developed. Within this idea, a single-chain immunotoxin composed of the variable domains of the B5 monoclonal antibody bound to α-sarcin through a peptide containing a furin cleavage site (scFv-IMTXαS) (Fig. **3**) has been efficiently produced in the methylotrophic

yeast *Pichia pastoris* [Carreras-Sangrà, N.; Martínez del Pozo, A.; Oñaderra, M.; Gavilanes, J.G. and Lacadena, J., unpublished data]. The B5 Lewis^Y monoclonal antibody, a member of the Nemod antibody family, is specific against Lewis^Y carbohydrates. These tumor-associated antigens are overexpressed on the surface of many carcinomas, including breast and colon solid tumors [127]. Various members of this family of antibodies have already been used as targeting moieties in many different immunotoxins [127-134] and at least three of them have been tested in phase I trials in patients with cancer yielding promising favorable results [130,135].

P. pastoris has emerged as a convenient robust heterologous expression host for immunotoxin production due to its efficient secretory system and its ability to express complex recombinant proteins with correct intra and intermolecular disulfide bonds. The constructs expressed in this system usually produce proteins that do not require additional *in vitro* unfolding and refolding steps, unlike most of the immunotoxins heterologously expressed in bacteria [136-138]. Indeed, *P. pastoris* possesses tightly regulated promoters such as that of the alcohol oxidase 1 gene (*AOX1*), which is perfectly suited for the controlled expression of foreign genes [139]. This explains why *P. pastoris* was the microorganism chosen to produce recombinant scFv-IMTXαS. The immunotoxin produced with this system displays the characteristic ribonucleolytic activity of α-sarcin as well as specific cytotoxicity against cell lines containing the Lewis^Y antigen [Carreras-Sangrà, N.; Martínez del Pozo, A.; Oñaderra, M.; Gavilanes, J.G. and Lacadena, J., unpublished data]. Binding assays performed with different cellular extracts, with a commercial version of

the Lewis^{Y} antigen, or with a synthetic peptide with such a structure that mimics the antigen behavior, have confirmed the high specific affinity conferred to the immunotoxin by its targeting moiety. With the aim to obtain a more cytotoxic immunotoxin, different approaches and designs have been assayed in order to improve toxin delivery into the cytosol of the targeted cells or to increase the stability and/or affinity of the immunotoxin [Carreras-Sangrà, N.; Martínez del Pozo, A.; Oñaderra, M.; Gavilanes, J.G. and Lacadena, J., unpublished data]. The immunoribotoxins constructed by these means appear to be potential good candidates to be studied in the field of cancer therapies.

CONCLUSIONS AND FUTURE PROSPECTS

Ribotoxins are unique RNases displaying an exquisite specific ribonucleolytic action as well as an innate ability to cross membranes. Both activities altogether explain the antitumoral properties that led to their discovery. The initial deception produced by the abandonment of their use as potential anticancerous agents in human therapies has been slowly overcome through the detailed study of their mechanism of action at the molecular level for many years [1-4,9,34]. In this regard, the determination of several high-resolution ribosomal structures, the characterization of a great variety of mutants, and the use of different lipid model vesicles and transformed cell lines have been of great help. Thus, the current knowledge about their mechanism of action suggests that these ribotoxins, or probably some of their modified variants, might be used soon with therapeutic aims. Within this idea, the production of

hypoallergenic variants to treat fungal allergies and different immunotoxins designed against specific tumors stand out as the most feasible alternatives in the mid-term future.

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References

[1] Gasset, M.; Mancheño, J.M.; Lacadena, J.; Turnay, J.; Olmo, N.; Lizarbe, M.A.; Martínez del Pozo, A.; Oñaderra, M. and Gavilanes, J.G. (1994) *Curr. Topics. Pept. Protein Res.*, **1**, 99–104.

[2] Kao, R.; Martínez-Ruiz, A.; Martínez del Pozo, A.; Crameri, R. and Davies, J. (2001) *Methods Enzymol*., **341**, 324–335.

[3] Martínez-Ruiz, A.; García-Ortega, L.; Kao, R.; Lacadena, J.; Oñaderra, M.; Mancheño, J.M.; Davies, J.; Martínez del Pozo, A. and Gavilanes, J.G. (2001) *Methods Enzymol.*, **341**, 335–351.

[4] Lacadena, J.; Álvarez-García, E.; Carreras-Sangrà, N.; Herrero-Galán, E.; Alegre-Cebollada, J.; García-Ortega, L.; Oñaderra, M.; Gavilanes, J.G. and Martínez del Pozo, A. (2007) *FEMS Microbiol. Rev.*, **31**, 212-237.

[5] Olson, B.H.; Jennings, J.C.; Roga, V.; Junek, A.J. and Schuurmans, D.M. (1965) *Appl. Microbiol.*, **13**, 322–326.

[6] Olson, B.H. and Goerner, G.L. (1965) *Appl. Microbiol.*, **13**, 314–321.

[7] Roga, V.; Hedeman, L.P. and Olson, B.H. (1971) *Cancer Chemother. Rep.*, **55**, 101–113.

[8] Arruda, L.K.; Mann, B.J. and Chapman, M.D. (1992) *J. Immunol.*, **149**, 3354–3359.

[9] Wool, I.G. (1997) in *Ribonucleases. Structures and Functions,* eds. D'Alessio, G. & Riordan, J.F. (Academic Press, New York), pp. 131–162.

[10] García-Ortega, L.; Lacadena, J.; Villalba, M.; Rodríguez, R.; Crespo, J.F.; Rodríguez, J.; Pascual, C.; Olmo, N.; Oñaderra, M.; Martínez del Pozo, A. and Gavilanes, J.G. (2005) *FEBS J.*, **272**, 2536–2544.

[11] Lin, A.; Huang, K.C.; Hwu, L. and Tzean, S.S. (1995) *Toxicon*, **33**, 105–110.

[12] Parente, D.; Raucci, G.; Celano, B.; Pacilli, A.; Zanoni, L.; Canevari, S.; Adobati, E.; Colnaghi, M.I.; Dosio, F.; Arpicco, S.; Cattel, L.; Mele, A. and De Santis, R. (1996) *Eur. J. Biochem.*, **239**, 272–280.

[13] Huang, K.-C.; Hwang, Y.Y.; Hwu, L. and Lin, A. (1997) *Toxicon*, **35**, 383–392.

[14] Wirth, J.; Martínez del Pozo, A.; Mancheño, J.M.; Martínez-Ruiz, A.; Lacadena, J.; Oñaderra, M. and Gavilanes, J.G. (1997) *Arch. Biochem. Biophys.*, **343**, 188–193.

[15] Martínez-Ruiz, A.; Martínez del Pozo, A.; Lacadena, J.; Oñaderra, M. and Gavilanes, J.G. (1999) *J. Invertebr. Pathol.*, **74**, 96–97.

[16] Martínez-Ruiz, A.; Kao, R.; Davies, J. and Martínez del Pozo, A. (1999) *Toxicon*, **37**, 1549–1563.

[17] Boucias, D.G.; Farmerie, W.G. and Pendland, J.C. (1998) *J. Invertebr. Pathol.*, **72**, 258–261.

[18] Herrero-Galán, E.; Lacadena, J.; Martínez del Pozo, A.; Olmo, N.; Boucias, D.G.; Oñaderra, M. and Gavilanes, J.G. (2008) *Proteins*, in press, doi10.1002/prot.21910.

[19] Sato, K. and Egami, F. (1957) *J. Biochem.*, **44**, 753–767.

[20] Yoshida, H. (2001) *Methods Enzymol.*, **341**, 28–41.

[21] Loverix, S. and Steyaert, J. (2001) *Methods Enzymol.*, **341**, 305–323.

[22] Sato, S. and Uchida, T. (1975) *Biochem. J.*, **145**, 353–360.

[23] Sacco, G.; Drickamer, K. and Wool, I.G. (1983) *J. Biol. Chem.*, **258**, 5811–5818.

[24] Pace, C.N.; Heinemann, U.; Hahn, U. and Saenger, W. (1991) *Angew. Chem. Int. Ed. Engl.*, **30**, 343–360.

[25] Noguchi, S.; Satow, Y.; Uchida, T.; Sasaki, C. and Matsuzaki, T. (1995) *Biochemistry*, **34**, 15583–15591.

[26] Yang, X. and Moffat, K. (1996) *Structure*, **4**, 837–852.

[27] Campos-Olivas, R.; Bruix, M.; Santoro, J.; Martínez del Pozo, A.; Lacadena, J.; Gavilanes, J.G. and Rico, M. (1996) *Protein Sci.*, **5**, 969–972.

[28] Campos-Olivas, R.; Bruix, M.; Santoro, J.; Martínez del Pozo, A.; Lacadena, J.; Gavilanes, J.G. and Rico, M. (1996) *FEBS Lett.*, **399**, 163–165.

[29] Pérez-Cañadillas, J.M.; Santoro, J.; Campos-Olivas, R.; Lacadena, J.; Martínez del Pozo, A.; Gavilanes, J.G.; Rico, M. and Bruix, M. (2000) *J. Mol. Biol.*, **299**, 1061–1073.

[30] Martínez del Pozo, A.; Gasset, M.; Oñaderra, M. and Gavilanes, J.G. (1988) *Biochim. Biophys. Acta*, **953**, 280-288.

[31] García-Ortega, L.; De los Ríos, V.; Martínez-Ruiz, A.; Oñaderra, M.; Lacadena, J.; Martínez del Pozo, A. and Gavilanes, J.G. (2005) *Electrophoresis*, **26**, 3407-3413.

[32] Rushizky, G.W.; Mozejko, J.H.; Rogerson, D.L. Jr. and Sober, H.A. (1970) *Biochemistry*, **9**, 4966–4971.

[33] Uchida, T.; Arima, T. and Egami, F. (1970) *J. Biochem.*, **67**, 91–102.

[34] Lamy, B.; Davies, J. and Schindler, D. (1992) in *Genetically Engineered Toxins,* ed. Frankel, A.E., (Marcel Dekker Inc., New York), pp. 237–258.

[35] Kao, R. and Davies, J. (1995) *Biochem. Cell. Biol.*, **73**, 1151–1159.

[36] Schindler, D.G. and Davies, J.E. (1977) *Nucleic Acids Res.*, **4**, 1097–1110.

[37] Endo, Y. and Wool, I.G. (1982) *J. Biol. Chem.*, **257**, 9054–9060.

[38] Endo, Y. and Tsurugi, K. (1987) *J. Biol. Chem.*, **262**, 8128–8130.

[39] Endo, Y.; Mitsui, K.; Motizuki, M. and Tsurugi, K. (1987) *J. Biol. Chem.*, **262**, 5908–5912.

[40] Wool, I.G.; Glück, A. and Endo, Y. (1992) *Trends Biochem. Sci.*, **17**, 266–269.

[41] Wool, I.G. (1996) *Trends Biochem. Sci.*, **21**, 164–165.

[42] Lacadena, J.; Martínez del Pozo, A.; Barbero, J.L.; Mancheño, J.M.; Gasset, M.; Oñaderra, M.; López-Otín, C.; Ortega, S.; García, J. and Gavilanes, J.G. (1994) *Gene*, **142**, 147–151.

[43] Lacadena, J.; Martínez del Pozo, A.; Lacadena, V.; Martínez-Ruiz, A.; Mancheño, J.M.; Oñaderra, M. and Gavilanes, J.G. (1998) *FEBS Lett.*, **424**, 46–48.

[44] Korennykh, A.V.; Piccirilli, J.A. and Correll, C.C. (2006) *Nat. Struct. Mol. Biol.*, **13**, 436–443.

[45] Lacadena, J.; Martínez del Pozo, A.; Martínez-Ruiz, A.; Pérez-Cañadillas, J.M.; Bruix, M.; Mancheño, J.M.; Oñaderra, M. and Gavilanes, J.G. (1999) *Proteins*, **37**, 474–484.

[46] Masip, M.; Lacadena, J.; Mancheño, J.M.; Oñaderra, M.; Martínez-Ruiz, A.; Martínez del Pozo, A. and Gavilanes, J.G. (2001) *Eur. J. Biochem.*, **268**, 6190–6196.

[47] Masip, M.; García-Ortega, L.; Olmo, N.; García-Mayoral, M.F.; Pérez-Cañadillas, J.M.; Bruix, M.; Oñaderra, M.; Martínez del Pozo, A. and Gavilanes, J.G. (2003) *Protein Sci.*, **12**, 161–169.

[48] Álvarez-García, E.; García-Ortega, L.; Verdún, Y.; Bruix, M.; Martínez del Pozo, A. and Gavilanes, J.G. (2006) *Biol. Chem.*, **387**, 535–541.

[49] Yang, X.; Gerczei, T.; Glover, L.T. and Correll, C.C. (2001) *Nat. Struct. Mol. Biol.*, **8**, 968–973.

[50] Oñaderra, M.; Mancheño, J.M.; Gasset, M.; Lacadena, J.; Schiavo, G.; Martínez del Pozo, A. and Gavilanes, J.G. (1993) *Biochem. J.*, **295**, 221–225.

[51] Fernández-Puentes, C. and Carrasco, L. (1980) *Cell*, **20**, 769–775.

[52] Olmo, N.; Turnay, J.; González de Buitrago, G.; López de Silanes, I.; Gavilanes, J.G. and Lizarbe, M.A. (2001) *Eur. J. Biochem.*, **268**, 2113–2123.

[53] Gasset, M.; Martínez del Pozo, A.; Oñaderra, M. and Gavilanes, J.G. (1989) *Biochem. J.*, **258**, 569–575.

[54] Gasset, M.; Oñaderra, M.; Thomas, P.G. and Gavilanes, J.G. (1990) *Biochem. J.*, **265**, 815–822.

[55] Oñaderra, M.; Gasset, M.; Martínez del Pozo, A. and Gavilanes, J.G.(1989) *Biochem. Soc. Trans.* **17**, 999–1000.

[56] Gasset, M.; Oñaderra, M.; Martínez del Pozo, A.; Schiavo, G.P.; Laynez, J.; Usobiaga, P. and Gavilanes, J.G. (1991) *Biochim. Biophys. Acta*, **1068**, 9–16.

[57] Connor, J.; Bucana, C.; Fidler, I.J. and Schroit, A.J. (1989) *Proc. Natl. Acad. Sci. U.S.A.*, **86**, 3184–3188.

[58] Zachowski, A. (1993) *Biochem. J.*, **294**, 1–14.

[59] Slivinsky, G.G.; Hymer, W. C.; Bauer, J. and Morrison, D. R. (1997) *Electrophoresis* **18**, 1109-1119.

[60] Orntoft, T.F. and Vestergaard, E.M. (1999) *Electrophoresis* **20**, 362-371.

[61] Rodríguez, R.; López-Otín, C.; Barber, D.; Fernández-Luna, J.L.; González, G. and Méndez, E. (1982) *Biochem. Biophys. Res. Commun.*, **108**, 315–321.

[62] López-Otín, C.; Barber, D.; Fernández-Luna, J.L.; Soriano, F. and Méndez, E. (1984) *Eur. J. Biochem.*, **143**, 621–634.

[63] Fernández-Luna, J.L.; López-Otín, C.; Soriano, F. and Méndez, E. (1985) *Biochemistry*, **24**, 861–867.

[64] Arruda, L.K.; Platts-Mills, T.A.; Fox, J.W. and Chapman, M.D. (1990) *J. Exp. Med.*, **172**, 1529–1532.

[65] Pérez-Cañadillas, J.M.; Guenneugues, M.; Campos-Olivas, R.; Santoro, J.; Martínez del Pozo, A.; Gavilanes, J.G.; Rico, M. and Bruix, M. (2002) *J. Biomol. NMR*, **24**, 301–316.

[66] García-Mayoral, M.F.; Pantoja-Uceda, D.; Santoro, J.; Martínez del Pozo, A.; Gavilanes, J.G.; Rico, M. and Bruix, M. (2005) *Eur. Biophys. J.*, **34**, 1057–1065.

[67] García-Mayoral, M.F.; García-Ortega, L.; Álvarez-García, E.; Bruix, M.; Gavilanes, J.G. and Martínez del Pozo, A. (2005) *FEBS Lett.*, **579**, 6859–6864.

[68] García-Ortega, L.; Masip, M.; Mancheño, J.M.; Oñaderra, M.; Lizarbe, M.A.; García-Mayoral, M.F.; Bruix, M.; Martínez del Pozo, A. and Gavilanes, J.G. (2002) *J. Biol. Chem.*, **277**, 18632–18639.

[69] García-Mayoral, M.F.; García-Ortega, L.; Lillo, M.P.; Santoro, J.; Martínez del Pozo, A.; Gavilanes, J.G.; Rico, M. and Bruix, M. (2004) *Protein Sci.*, **13**, 1000–1011.

[70] Yang, R. and Kenealy, W.R. (1992) *J. Biol. Chem.*, **267**, 16801–16805.

[71] Brandhorst, T.; Yang, R. and Kenealy, W.R. (1994) *Protein Expr. Purif.*, **5**, 486–497.

[72] Kao, R. and Davies, J. (1999) *J. Biol. Chem.*, **274**, 12576–12582.

[73] Lacadena, J.; Mancheño, J.M.; Martínez-Ruiz, A.; Martínez del Pozo, A.; Gasset, M.; Oñaderra, M. and Gavilanes, J.G. (1995) *Biochem. J.*, **309**, 581–586.

[74] Sylvester, I.D.; Roberts, L.M. and Lord, J.M. (1997) *Biochim. Biophys. Acta*, **1358**, 53–60.

[75] Kao, R.; Shea, J.E.; Davies, J. and Holden, D.W. (1998) *Mol. Microbiol.*, **29**, 1019–1027.

[76] Mancheño, J.M.; Gasset, M.; Albar, J.P.; Lacadena, J.; Martínez del Pozo, A.; Oñaderra, M. and Gavilanes, J.G. (1995) *Biophys. J.*, **68**, 2387–2395.

[77] Mancheño, J.M.; Martínez del Pozo, A.; Albar, J.P.; Oñaderra, M. and Gavilanes, J.G. (1998) *J. Pept. Res.*, **51**, 142–148.

[78] De Antonio, C.; Martínez del Pozo, A.; Mancheño, J.M.; Oñaderra, M.; Lacadena, J.; Martínez-Ruiz, A.; Pérez-Cañadillas, J.M.; Bruix, M. and Gavilanes, J.G. (2000) *Proteins*, **41**, 350–361.

[79] García-Ortega, L.; Lacadena, J.; Mancheño, J.M.; Oñaderra, M.; Kao, R.; Davies, J.; Olmo, N.; Martínez del Pozo, A. and Gavilanes, J.G. (2001) *Protein Sci.*, **10**, 1658–1668.

[80] Carrasco, L. and Esteban, M. (1982) *Virology*, **117**, 62–69.

[81] Olmo, N.; Turnay, J.; Lizarbe; M.A. and Gavilanes; J.G. (1993) *STP Pharma. Sciences;* **3**; 93–96.

[82] Turnay; J.; Olmo, N.; Jiménez, A.; Lizarbe, M.A. and Gavilanes, J.G. (1993) *Mol. Cell. Biochem.*, **122**, 39–47.

[83] Stuart, A.D. and Brown, T.D. (2006) *J. Virol.*, **80**, 7500–7509.

[84] Pasqualotto, A. (2006) *Clin. Microbiol. Infect.*, **12**, S25.

[85] Ronning, C.M.; Fedorova, N.D.; Bowyer, P.; Coulson, R.; Goldman, G.; Kim, H.S.; Turner, G.; Wortman, J.R.; Yu, J.; Anderson, M.J.; Denning, D.W. and Nierman, W.C. (2005) *Rev. Iberoam. Micol.*, **22**, 223–228.

[86] Denning, D.W.; O'Driscoll, B.R.; Hogaboam, C.M.; Bowyer, P. and Niven, R.M. (2006) *Eur. Respir. J.*, **27**, 615–626.

[87] Walsh, T.J. and Pizzo, A. (1988) *Eur. J. Clin. Microbiol. Infect. Dis.*, **7**, 460–475.

[88] Bodey, G.P. and Vartivarian, S. (1989) *Eur. J. Clin. Microbiol. Infect. Dis.*, **8**, 413–437.

[89] Lamy, B.; Moutaouakil, M.; Latge, J.P. and Davies, J. (1991) *Mol. Microbiol.*, **5**, 1811–1815.

[90] Paris, S.; Monod, M.; Diaquin, M.; Lamy, B.; Arruda, L.K.; Punt, P.J. and Latge, J.P. (1993) *FEMS Microbiol. Lett.*, **111**, 31–36.

[91] Smith, J.M.; Davies, J.E. and Holden, D.W. (1993) *Mol. Microbiol.*, **9**, 1071–1077.

[92] Smith, J.M.; Tang, C.M.; Van Noorden, S. and Holden, D.W. (1994) *Infect. Immun.*, **62**, 5247–5254.

[93] Kurup, V.P.; Kumar, A.; Kenealy, W.R. and Greenberger, P.A. (1994) *J. Lab. Clin. Med.*, **123**, 749–756.

[94] Piechura, J.E.; Huang, C.J.; Cohen, S.H.; Kidd, J.M.; Kurup, V.P.; Calvanico, N.J. (1983) *Immunology*, 49, 657–665.

[95] Crameri, R.; Hemmann, S.; Ismail, C.; Menz, G. and Blaser, K. (1998) *Int. Immunol.*, **10**, 1211–1216.

[96] Kurup, V.P.; Knutsen, A.P.; Moss, R.B. and Bansal, N.K. (2006) *Clin. Mol. Allergy*, **4**, 11–17.

[97] Greenberger, P.A. (2002) *J. Allergy Clin. Immunol.*, **110**, 685–692.

[98] Álvarez-García, E.; Alegre-Cebollada, J.; Batanero, E.; Monedero, V.; Pérez-Martínez, G.; García-Fernández, R.; Gavilanes, J.G. and Martínez del Pozo, A. (2008) *J. Biotechnol.*, in press, doi:10.1016/j.jbiotec.2007.12.011.

[99] Brinkmann, U. and Pastan, I. (1994) *Biochim. Biophys. Acta*, **1198**, 27–45.

[100] Reiter, Y. and Pastan, I. (1998) *Trends Biotechnol.*, **16**, 513–520.

[101] Kreitman, R.J. (2001) *Curr. Pharm. Biotechnol.*, **2**, 313–325.

[102] Ehrlich, P. (1956) in *The Collected Papers of Paul Ehrlich*, Vol. 1, eds. Himmelweit, F.; Marquardt, M. & Dale, H., (Pergamon Press, New York), pp. 596.

[103] Kreitman, R.J. (2000) *Expert. Opin. Pharmacother.*, **1**, 1117–1129.

[104] Kreitman, R.J. (2003) *Curr. Opin. Mol. Ther.*, **5**, 44–51.

[105] Li, Q.; Verschraegen, C.F.; Mendoza, J. and Hassan, R. (2004) *Anticancer Res.*, **24**, 1327–1335.

[106] Pastan, I.; Chaudhary, V. and FitzGerald, D.J. (1992) *Annu. Rev. Biochem.*, **61**, 331–354.

[107] Ghetie, V.; Swindell, E.; Uhr, J.W. and Vitetta, E.S. (1993) *J. Immunol. Methods*, **166**, 117–122.

[108] Engert, A.; Diehl, V.; Schnell, R.; Radszuhn, A.; Hatwig, M.T.; Drillich, S.; Schön, G.; Bohlen, H.; Tesch, H.; Hansmann, M.L.; Barth, S.; Schindler, J.; Ghetie, V.; Uhr, J. and Vitetta, E. (1997) *Blood*, **89**, 403–410.

[109] O'Toole, J.E.; Esseltine, D.; Lynch, T.J.; Lambert, J.M. and Grossbard, M.L. (1998) *Curr. Top. Microbiol. Immunol.*, **234**, 35–56.

[110] Schnell, R.; Vitetta, E.; Schindler, J.; Barth, S.; Winkler, U.; Borchmann, P.; Hansmann, M.L.; Diehl, V.; Ghetie, V. and Engert, A. (1998) *Leuk. Lymphoma*, **30**, 525–537.

[111] Carroll, S.F. and Collier, R.J. (1987) *J. Biol. Chem.*, **262**, 8707–8711.

[112] Kondo, T.; FitzGerald, D.; Chaudhary, V.K.; Adhya, S. and Pastan, I. (1988) *J. Biol. Chem.*, **263**, 9470–9475.

[113] Kreitman, R.J.; Chaudhary, V.K.; Waldmann, T.; Willingham, M.C.; FitzGerald, D.J. and Pastan, I. (1990) *Proc. Natl. Acad. Sci. U.S.A.*, **87**, 8291–8295.

[114] Kreitman, R.J.; Batra, J.K.; Seetharam, S.; Chaudhary, V.K.; FitzGerald, D.J. and Pastan, I. (1993) *Bioconjug. Chem.*, **4**, 112–120.

[115] Foss, F.M.; Saleh, M.N.; Krueger, J.G.; Nichols, J.C. and Murphy, J.R. (1998) *Curr. Top. Microbiol. Immunol.*, **234**, 63–81.

[116] LeMaistre, C.F.; Saleh, M.N.; Kuzel, T.M.; Foss, F.; Platanias, L.C.; Schwartz, G.; Ratain, M.; Rook, A.; Freytes, C.O.; Craig, F.; Reuben, J. and Nichols, J.C. (1998) *Blood,* **91**, 399–405.

[117] Rathore, D. and Batra, J.K. (1996) *Biochem. Biophys. Res. Commun.*, **222**, 58–63.

[118] Orlandi, R.; Canevari, S.; Conde, F.P.; Leoni, F.; Mezzanzanica, D.; Ripamonti, M. and Colnaghi, M.I. (1988) *Cancer Immunol. Immunother.*, **26**, 114–120.

[119] Conde, F.P.; Orlandi, R.; Canevari, S.; Mezzanzanica, D.; Ripamonti, M.; Muñoz, S.M.; Jorge, P. and Colnaghi, M.I. (1989) *Eur. J. Biochem.*, **178**, 795–802.

[120] Hertler, A.A. and Frankel, A.E. (1989) *J. Clin. Oncol.*, **7**, 1932–1942.

[121] Wawrzynczak, E.J.; Henry, R.V.; Cumber, A.J.; Parnell, G.D.; Derbyshire, E.J. and Ulbrich, N. (1991) *Eur. J. Biochem.*, **196**, 203–209.

[122] Better, M.; Bernhard, S.L.; Lei, S.P.; Fishwild, D.M. and Carroll, S.F. (1992) *J. Biol. Chem.*, **267**, 16712–16718.

[123] Rathore, D. and Batra, J.K. (1997) *Biochem. J.*, **324**, 815–822.

[124] Rathore, D. and Batra, J.K. (1997) *FEBS Lett.*, **407**, 275–279.

[125] Rathore, D.; Nayak, S.K. and Batra, J.K. (1997) *Gene*, **190**, 31–35.

[126] Goyal, A. and Batra, J.K. (2000) *Biochem. J.*, **345**, 247–254.

[127] Pastan, I. and FitzGerald, D. (1991) *Science*, **254**, 1173–1177.

[128] Brinkmann, U.; Pai, L.H.; FitzGerald, D.J.; Willingham, M. and Pastan, I. (1991) *Proc. Natl. Acad. Sci. U.S.A.*, **88**, 8616–8620.

[129] Pai, L.H.; Batra, J.K.; FitzGerald, D.J.; Willingham, M.C. and Pastan, I. (1991) *Proc. Natl. Acad. Sci. U.S.A.*, **88**, 3358–3362.

[130] Pai, L.H.; Wittes, R.; Setser, A.; Willingham, M.C. and Pastan, I. (1996) *Nat. Med.*, **2**, 350–353.

[131] Benhar, I. and Pastan, I. (1995) *Clin. Cancer Res.*, **1**, 1023–1029.

[132] Benhar, I. and Pastan, I. (1995) *J. Biol. Chem.*, **270**, 23373–23380.

[133] Kuan, C.T. and Pastan, I. (1996) *Proc. Natl. Acad. Sci. U.S.A.*, **93**, 974–978.

[134] Bera, T.K. and Pastan, I. (1998) *Bioconjug. Chem.*, **9**, 736–743.

[135] Brinkmann, U. (2000) *In Vivo*, **14**, 21–27.

[136] Cregg, J.M.; Vedvick, T.S. and Raschke, W.C. (1993) *Biotechnology* **11**, 905-910.

[137] Gurkan, C. and Ellar, D.J. (2003) *Protein Expr. Purif.* **29**, 103–116.

[138] Gurkan, C. and Ellar, D.J. (2005) *Microb. Cell. Fact.* 4, 33–40.

[139] Cregg, J.M.; Madden, K.R.; Barringer, K.J.; Thill, G.P. and Stillman, C.A. (1989) *Mol. Cell. Biol.* **9**, 1316-1323.

[140] Humphrey, W.; Dalke, A. and Schulten, K. (1996) *J. Mol. Graph.* **14**, 33-38, 27-28.

[141] Correll, C.C.; Munishkin, A.; Chan, Y.L.; Ren, Z.; Wool, I.G. and Steitz, T.A. (1998) *Proc. Natl. Acad. Sci. U.S.A.* **95**, 13436–13441.

Fig. (1) Three-dimensional structures of (A) wild-type (PDB ID 1DE3) and (B) Δ(7-22) (PDB ID 1R4Y) α-sarcins [29,69]. The diagrams were generated with the VMD program [140].

Fig. (2) Diagram showing the structure of the SRL (PDB ID 430d) [141]. Numbers correspond to rat or *Escherichia coli* (in brackets) nucleotide positions within the 28S (23S) rRNA gene. The bond cleaved by ribotoxins is that on the 3'-side of G4325 (2661). Ricin depurinates A4324 (2660). Both nucleotides have been represented in a darker gray tone. The bulged G is G4319 (2655). The diagram was generated with the VMD program [140].

Fig. (3) Scheme showing the different immunotoxin designs constructed using modified versions of the B5 Lewis^Y monoclonal antibody and the ribotoxin α-sarcin. CDR, complementary determining regions; V_H, variable domain of high chain; V_L, variable domain of light chain; S-S, disulfide bridge; FR2, framework region 2.

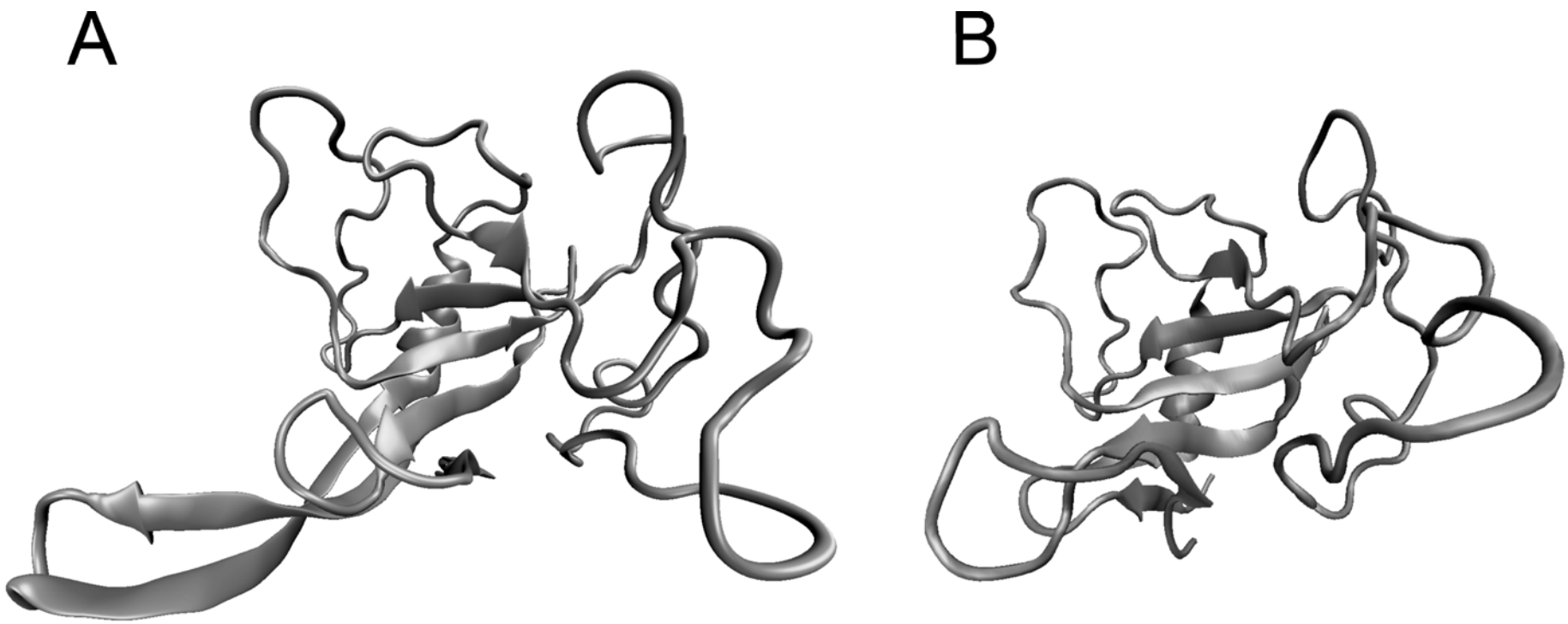

Figure 1

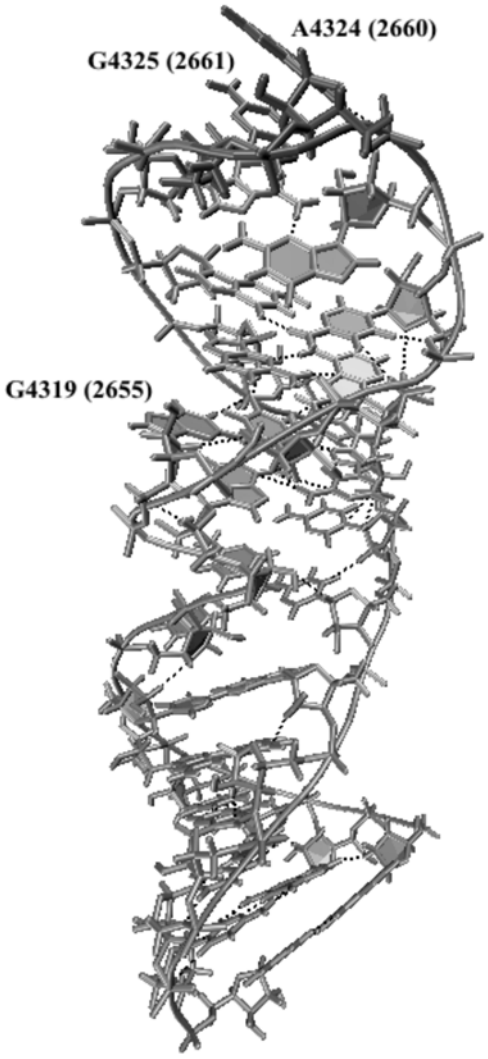

Figure 2

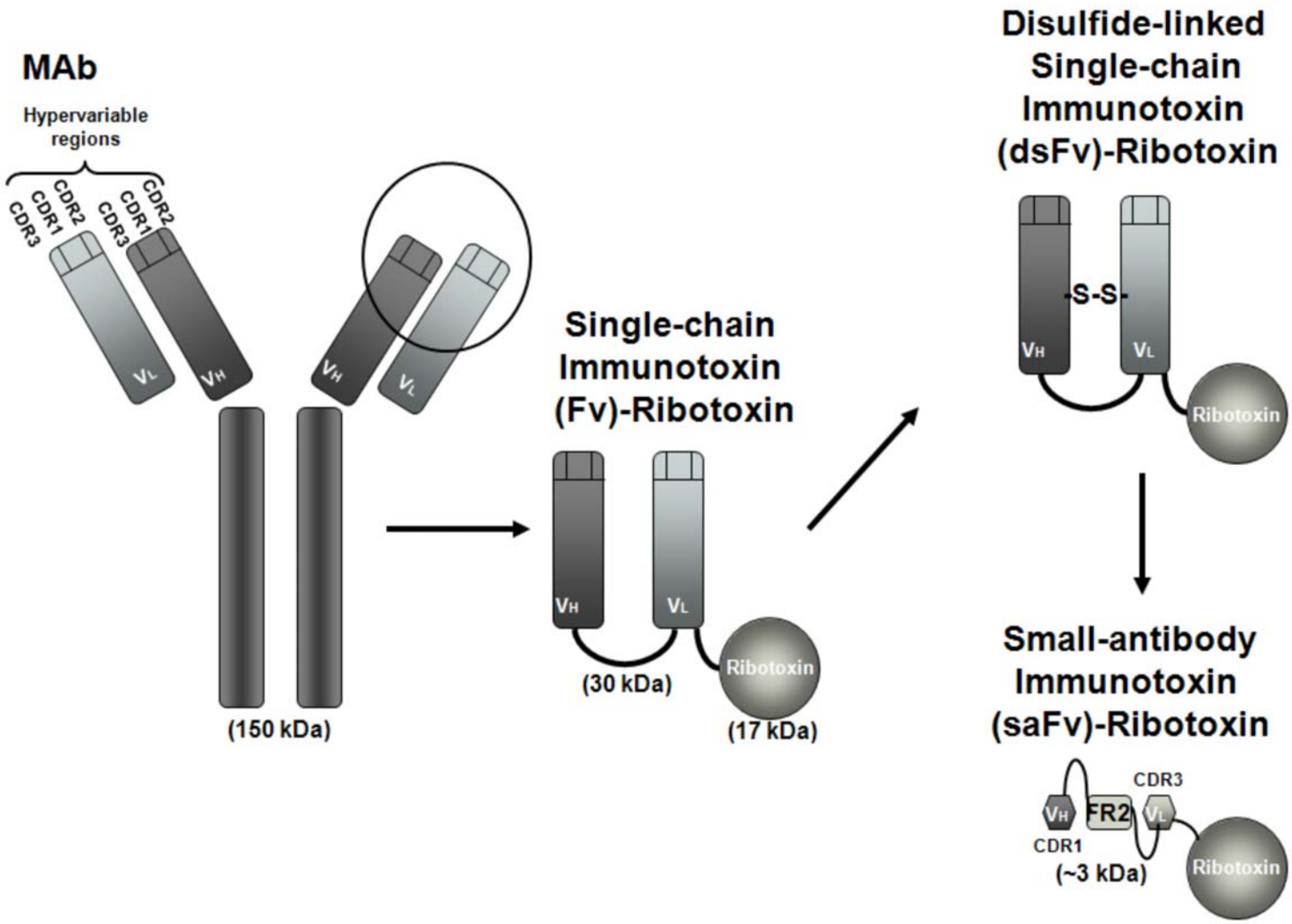
MAb
Hypervariable regions
CDR1
CDR2
CDR3
CDR1
CDR2
CDR3
VL
VH
VH
VL
(150 kDa)
Single-chain Immunotoxin (Fv)-Ribotoxin
VH
VL
Ribotoxin
(30 kDa)
(17 kDa)
Disulfide-linked Single-chain Immunotoxin (dsFv)-Ribotoxin
-S-S-
VH
VL
Ribotoxin
Small-antibody Immunotoxin (saFv)-Ribotoxin
CDR3
VH
FR2
VL
CDR1
(~3 kDa)
Ribotoxin

Figure 3