FEBS Letters 587 (2013) 3601–3608

journal homepage: www.FEBSLetters.org

Review

Structural and functional relationships of natural and artificial dimeric bovine ribonucleases: New scaffolds for potential antitumor drugs

Giovanni Gotte [a], Douglas V. Laurents [b], Antonello Merlino [c], Delia Picone [c,*], Roberta Spadaccini [c,d]

[a] Dipartimento di Scienze della Vita e della Riproduzione, Sezione di Chimica Biologica, Università degli Studi di Verona, Strada Le Grazie 8, I-37134 Verona, Italy
[b] Instituto de Química Física "Rocasolano" (C.S.I.C.), Serrano 119, E-28006 Madrid, Spain
[c] Dipartimento di Scienze Chimiche, Università degli Studi di Napoli, via Cintia, 80126 Napoli, Italy
[d] Dipartimento di Scienze e Tecnologie, Università del Sannio, via Port'Arsa 11, 82100 Benevento, Italy

ARTICLE INFO

Article history:
Received 22 May 2013
Revised 23 August 2013
Accepted 24 September 2013
Available online 7 October 2013

Edited by Christian Griesinger

Keywords:
Ribonuclease A
Dimeric RNase
Antitumor RNase
Bovine seminal ribonuclease
Protein aggregation
3D-domain swapping

ABSTRACT

Protein aggregation via 3D domain swapping is a complex mechanism which can lead to the acquisition of new biological, benign or also malignant functions, such as amyloid deposits. In this context, RNase A represents a fascinating model system, since by dislocating different polypeptide chain regions, it forms many diverse oligomers. No other protein displays such a large number of different quaternary structures. Here we report a comparative structural analysis between natural and artificial RNase A dimers and bovine seminal ribonuclease, a natively dimeric RNase with antitumor activity, with the aim to design RNase A derivatives with improved pharmacological potential.

1. Introduction

Bovine pancreatic ribonuclease (RNase A, EC 3.1.2.27.5) has been the object of many innovative studies in different fields, including protein chemistry, enzymology, evolutionary and structural biology [1]. RNase A represents an extraordinary model system for many biophysical studies, and it is one of the most represented proteins in PDB. It also inspired the hypothesis of protein oligomerization through the mutual exchange of identical structural elements [2], however it took almost thirty years before the first X-ray structures showing this interchange were solved [3,4] and even longer before the first RNase A dimeric N-terminal swapped structure (N_D) was determined [5]. Later on, with the discovery of the C-terminal swapped dimer (C_D), RNase A became the first protein known to be able to oligomerize by swapping different segments of its structure [6,7]. This finding gave a new impulse to the field of 3D domain swapping (3D-DS) because, based on the structure of C_D, the hypothesis of a relationship between 3D-DS and amyloidogenic protein aggregation was put forward [6,8]. To date, more than 300 swapped structures have been deposited in the PDB and in a dedicated database [9], but RNase A is unique in the diversity and number of its quaternary structures, in native or recombinant forms, all of which preserve the catalytic activity.

Beside cleaving RNA, some ribonucleases (RNases) belonging to different sources are endowed with other biological activities of potential pharmacological interest [10], such as tumour treatment, angiogenesis, immunosuppression and antimicrobial actions. However to date, these studies have been essentially limited to the preclinical stage, except for onconase®, a frog RNase which reached phase IIIb in clinical trials for treating certain cancers [11].

To be effective as antitumor drugs, RNases must be efficiently delivered to the specific cells; for RNases this depends on the distribution of charges on the protein surface [12,13]. Strategies to overcome this drawback which limits the potency of RNases have been very recently reviewed [14]. Furthermore, in mammalian cells, most monomeric, pancreatic-like RNases are captured by RNase inhibitor (RI), a very abundant, horse-shoe shaped protein [15], which makes an extremely tight complex (K_d <10^{-15} M) with RNase A and most other mammalian RNases [16,17].

Protein engineering of the RNase contact sites to inhibit the binding with RI [18–20], chemical modifications [21] or the conversion of the protein into a swapped, covalent dimer [22,23] produced RNase A derivatives with higher antitumor activity. However, none of them is as cytotoxic as bovine seminal ribonuclease

* Corresponding author. Address: Department of Chemical Sciences, via Cintia, 80126 Napoli, Italy. Fax: +39 081 674091.
E-mail address: delia.picone@unina.it (D. Picone).

0014-5793/$36.00
http://dx.doi.org/10.1016/j.febslet.2013.09.038

(BS-RNase) [24], a naturally homo-dimeric protein whose subunit shares about 80% of sequence identity with RNase A and very close structural and dynamical features in solution [25]. BS-RNase is characterized by two intersubunit disulfide bonds and the co-existence of two isomers: a swapped (MxM) and an unswapped one (M = M), whose relative populations are about 2:1 at equilibrium [26]. Structural and functional studies on BS-RNase [27,28] and human pancreatic ribonuclease derivatives [29] have indicated that BS-RNase's cytotoxic activity is mainly due to the optimal quaternary assembly of the non-covalent swapped form of the enzyme (see below). In contrast, RNase A dimers are endowed with a limited cytotoxic activity [30] and they are bound by RI [31]. Furthermore, no RNase A dimeric variant displaying a relative subunit orientation that enables the evasion of RI binding [16] under the reducing cytosol conditions [32,33], has been yet produced.

Here we compare the main structural features of known bovine RNase dimers with those of other dimeric antitumor proteins belonging to the RNase A family, with the aim of providing insight for engineering improved variants for potential pharmacological applications [32,34].

2. RNase A artificial aggregates

RNase A (Fig. 1A) is almost unique in its remarkable capacity to self-associate through 3D-DS [4,35], thus, without covalent modification(s) [2,36,37], and only very recently two other proteins, BS-RNase [38] and cyanovirin N [39] were discovered to be able to form different aggregated conformers via 3D-DS.

In 1962, the group lead by the Nobel prize winners Stein and Moore envisaged the exchange (that is, 3D-DS) of S-peptides moieties (i.e., the N-termini) between two RNase A monomers to explain the presence of dimers and larger aggregates after lyophilization of the enzyme from 40% to 50% acetic acid aqueous solutions [2]. All RNase A domain-swapped dimers and multimers characterized to date are meta-stable, soluble and biologically active, as are several oligomers formed by more than 40 3D-DS self-associating proteins [40].

Interestingly, upon oligomerization RNase A partly maintains its enzymatic activity against single strand RNA (ss-RNA) and, moreover, it acquires a depolymerase action on double strand RNA (ds-RNA) [41]. By swapping both the N- and C-termini, which are located outside the disulfide-bond core and contain the catalytically important histidines 12 and 119, respectively, RNase A reconstitutes an active site [2,5,6,42,43] with a composite functional unit (F.U.) [35], in which one histidine belongs to one subunit whilst Lys41 and the second histidine are provided by the partner subunit. This active site represents part of the dimer's "Closed interface", i.e., the interface present in both monomer and 3D-DS oligomers [43], whereas in contrast the "Open interface" is formed only upon swapping [43].

RNase A dimers and oligomers have been extensively studied in the last 10–15 years [23,36,44–60], especially after the proposal

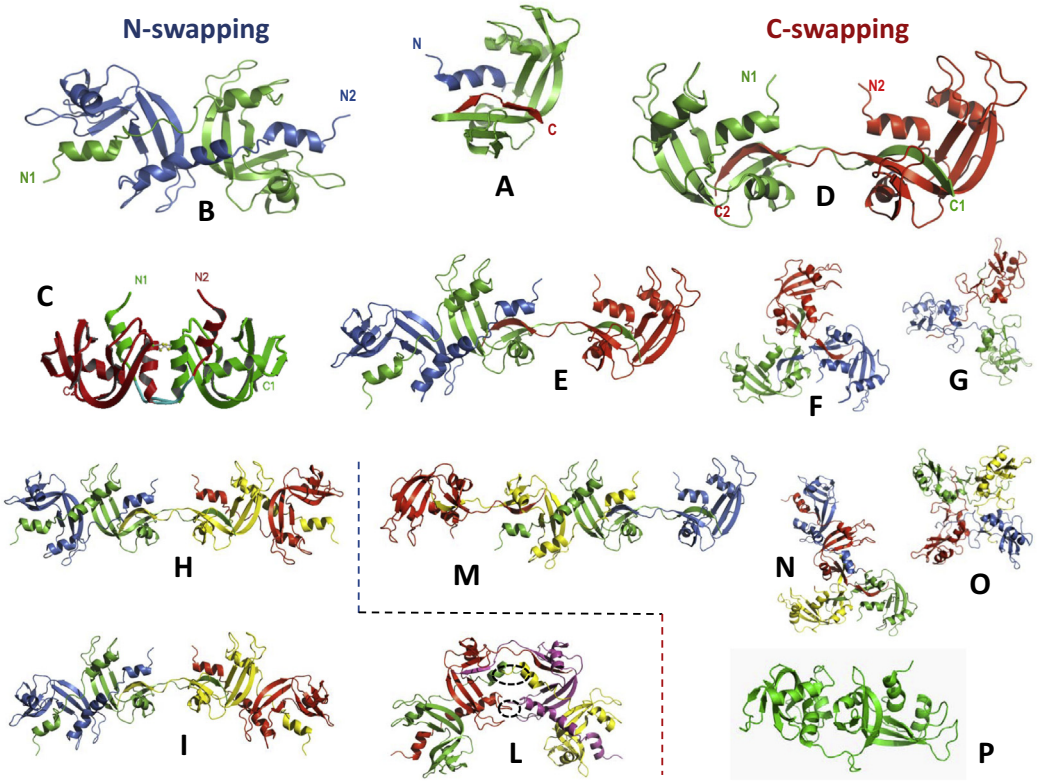

Fig. 1. Structures of RNase A, swapped isomer of BS-RNase, and of the principal RNase A dimers and 3D-DS oligomers. The dashed line divides the tetramers with more N-swapped domains (left, blue segment), from the ones, which have more C-swapped domains shown on the right (red line segment). (A) RNase A: the blue and red N- and C-termini, respectively, are coloured distinctly from the green core of the protein to highlight their role in 3D-DS. (B) RNase A N_D, (PDB entry 1A2W). (C) 3D-DS isomer of BS-RNase covalent native dimer (PDB entry 1BSR); (D) RNase A C_D (PDB entry 1F0V); (E) N+C-doubly-DS trimeric RNase A model: the central green subunit swaps both its N- and C-termini with the blue and red subunits located on its left and right, respectively; this model represents the first seed of a "runaway 3D-DS", which can be close-ended thanks to co-existing double domain swappings. (F) 3D-DS cyclic C-trimer, or C_T (PDB entry 1JS0): the C-termini-only swapping allows a propeller-like structure to form. (G) 3D-DS cyclic trimer model: the same exclusive C-swapping event could permit a "flower-cup-like" C-trimer to be formed. (H) Linear N+C+N-swapped tetrameric model, l-NCN_{TT}. (I) "Quasi-linear" N+C+N-swapped modeled tetramer, ql-NCN_{TT}. (L) Bent N+C+N-swapped tetrameric model, b-NCN_{TT} [68], displaying two additional open interfaces located inside the dotted black circles. (M) Linear C+N+C-swapped tetrameric model, CNC_{TT}. (N) C-swapped-cyclic + Nswapped-linear tetrameric model, N = NC–C–C_{TT}. (O) "Flower-cup-like" C-swapped-only-tetramer, C_{TT}. (P) Tandem, fusion-peptide linked, unswapped RNase A dimer (PDB entry 3MWQ).