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Review

Nucleotide binding architecture for secreted cytotoxic endoribonucleases

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ABSTRACT

Vertebrate secreted RNases are small cationic protein endowed with an endoribonuclease activity that belong to the RNase A superfamily and display diverse cytotoxic activities. In an effort to unravel their mechanism of action, we have analysed their nucleotide binding recognition patterns. General shared features with other nucleotide binding proteins were deduced from overall statistics on the available structure complexes at the Protein Data Bank and compared with the particularities of selected representative endoribonuclease families. Results were compared with other endoribonuclease representative families and with the overall protein–nucleotide interaction features. Preferred amino acids and atom types involved in pair bonding interactions were identified, defining the spatial motives for phosphate, base and ribose building blocks. Together with the conserved catalytic triad at the active site, variability was observed for secondary binding subsites that may contribute to the proper substrate alignment and could explain the distinct substrate preference patterns. Highly conserved binding patterns were identified for the pyrimidine and purine subsites at the main and secondary base subsites. Particular substitution could be ascribed to specific adenine or guanine specificities. Distribution of evolutionary conserved residues were compared to search for the structure determinants that underlie their diverse catalytic efficiency and those that may account for putative physiological substrate targets or other non-catalytic biological activities that contribute to the anti-pathogen role of the RNases involved in the host defence system. A side by side comparison with another endoribonuclease superfamily of secreted cytotoxic proteins, the microbial RNases, was carried on to analyse the common features and peculiarities that rule their substrate recognition. The data provides the structural basis for the development of applied therapies targeting cellular nucleotide polymers.

1. Introduction

The vertebrate secreted RNase superfamily comprises small secreted proteins showing very diverse catalytic and biological properties [1]. A variety of biological functions have been attributed to some family members, ranging from angiogenesis to host defence [2–6]. Mammalian homologues are grouped in eight lineages which are referred as the canonical RNases [5] (Fig. 1A). The family members were first gathered together as pancreatic type RNases, in honour to the family reference prototype, the bovine pancreatic RNase A, conforming the so-called "RNase A superfamily". The catalytic mechanism of RNase A was already proposed in the 60s decade prior to the three-dimensional structure knowledge [7]. RNase A, is nowadays one of the best studied enzyme and represents an ideal model to understand the endoribonuclease catalytic mechanism and polymeric substrate binding mode [8–11]. The protein active site architecture reveals several phosphate binding subsites adjacent to the main catalytic site which contributes to align the RNA substrate (Fig. 1B). Together with a primary role in RNA digestion in ruminants for RNase A, a variety of non-catalytic biological properties were described for the other family members [2,12,13]. Other RNases were identified in many organs and tissues, and found not only in mammals, but in reptiles, birds, amphibians and fishes, setting the basis to define a properly vertebrate secreted RNase superfamily [5,14,15]. The antibacterial activity of distant related RNases suggested that the family evolved from an ancestral host–defence function [4]. Another suggestive hypothesis based on the angiogenic properties of low order family members, such as bird and fish RNases, ascribed

Abbreviations: ECP, eosinophil cationic protein; EDN, eosinophil derived neurotoxin; pn, Rn and Bn, protein binding sites for nucleotide phosphate, ribose and bases; RMSD, residual mean standard deviation.

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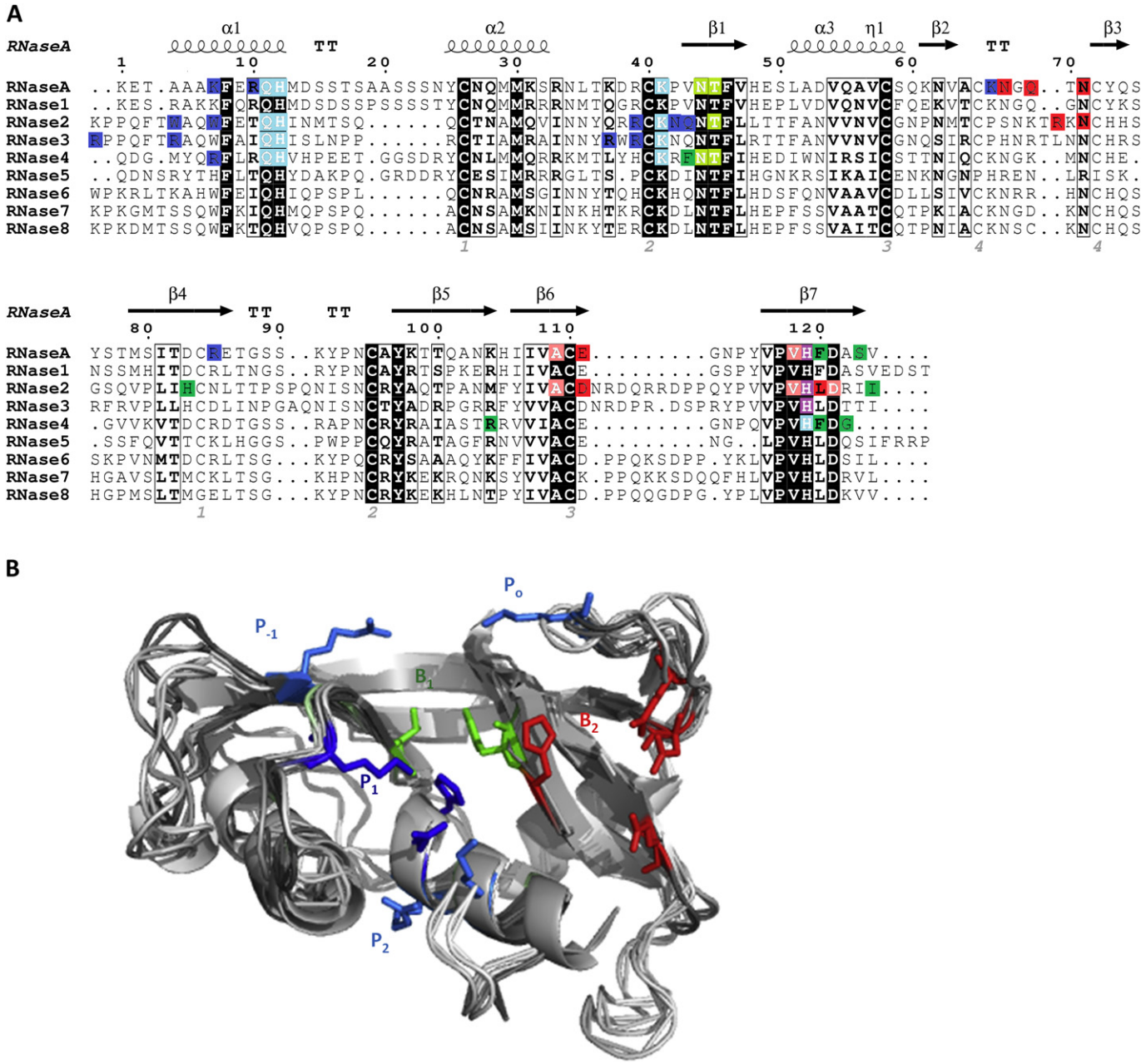

Fig. 1. (A) Sequence alignment of human RNase A superfamily members. Secondary structure elements of RNase A are depicted at the top. Strictly conserved residues are boxed in black and conserved residues, as calculated by a similarity score, are boxed in white. Coloured residues in RNase 1, RNase 2 (EDN), RNase 3 (ECP) and RNase 4 refer to those identified in protein complexes (see Table 2), and ascribed to phosphate/ribose (blue), pyrimidine (green) and purine (red) bases. Cysteine pairings for disulfide bridges are numbered below. The figure was created using the *ESPript* software [100]. (B) Representation of the superimposed three-dimensional structures of the RNases showing the subsites location and corresponding residue side chains for RNase A, coloured according to the same criteria as above.

an ancestral angiogenesis role [15,16]. All members, even with a low primary sequence identity, around 30%, share a common three-dimensional fold. All the so-called canonical RNases conserve the catalytic triad identified in the RNase A reference enzyme by His12, His119 and Lys41, where Lys is located at the family signature CKXXNTF, together with a main base subsite for pyrimidines. On the contrary, variability is found for the residues ascribed to secondary binding sites and a huge amount of work is still pending to accurately understand the enzymatic properties of most of the vertebrate secreted RNases.

The RNase A superfamily is an $\alpha + \beta$ protein type and one of the representative classified RNases superfamilies [17]. According to its catalytic mechanism (EC 3.1.27.5), the enzyme cleaves ester bonds with an endoribonuclease activity. Endoribonuclease families embrace prokaryote and eukaryote members that can participate in cell RNA processing or are secreted proteins with a cytotoxic defence role. In particular, together with the RNase A superfamily, microbial RNases represent another reference superfamily, which includes bacterial and fungal RNases [18]. Some representative members have also been thoroughly characterized, as RNase T1, binase or RNase Sa, and provide a wealth of information on the nucleotide binding mode patterns [19].

In a wider context, nucleotide binding motives have been analysed by the statistical analysis of databases of nucleic acid–protein