Biochemistry of frog ribonucleases

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Abstract. Frogs have unique pyrimidine base-specific RNases, with structures similar to those of turtle, iguana and chicken RNases. Among the four frog RNases discussed here, three from Rana pipiens, R. catesbeiana and R. japonica oocyte cells show antitumour activity, and the latter two show lectin activity towards sialic acid-rich glycoproteins. In this review, (i) we compare their unique primary structures with respect to the locations of their disulphide bridges, three-dimensional structure, base specificity and heat stability as compared with RNase A, and (ii) we summarize current knowledge about the mode of action of lectin and the antitumour activities of the three frog RNases.

Key words. Frog RNase; lectin activity; antitumour activity; pyrimidine base-specific RNase; amphibian RNase.

Introduction

Most of pyrimidine base-specific RNases as typified by bovine pancreatic RNase A have four intramolecular disulphide bridges, and their locations are very well conserved. Since the discovery of turtle pancreatic RNase, which lacks one disulphide bridge [1], the primary structures of several RNases lacking one disulphide bridge of RNase A Cys65–Cys72, have been elucidated. They are angiogenin [2], chicken bone marrow RNase [3], an RNase derived from v-mys-transformed myeloma monocytic cells [4], chicken liver RNase [5], iguana pancreatic RNase [6] and four proteins (RNases) derived from frog liver and oocytes [7–10]. Among them, frog proteins conserved the three disulphide bridges among the four of RNase A, and have another novel one near their C termini. These structurally exceptional RNases lacking a disulphide linkage (Cys65–Cys72) might be temporarily designated as angiogenin/turtle/chicken/frog type RNases. These structurally unique RNases also showed highly characteristic enzymological, physical and physiological properties (angiogenesis, lectin activity and antitumour activity). In this review, we will summarize the unique nature of frog-derived RNases with respect to structure, enzymatic nature and physiological function in comparison with turtle and chicken RNases and angiogenin.

The biochemistry of this group of RNases was historically studied employing three different approaches. Beintema et al. elucidated the amino acid sequence of turtle pancreatic RNase in 1985 [1], and, subsequently, that of the iguana [6], from the viewpoint of comparative and evolutionary biochemistry. Levy et al. described the presence of cytosine base-specific RNase from chicken liver [11]. Miura et al. further purified this RNase [12], and later Hayano et al. elucidated the...
amino acid sequence of this RNase (RNase CL2) and showed it to be a pyrimidine-specific and cytosine-preferential RNase [5]. As for the frog RNases, Nagano et al. purified bullfrog (Rana catesbeiana) liver RNase and showed it to be a pyrimidine base-specific RNase [13, 14]. Roth [15] and Nagano et al. [13, 14] also detected the presence of an RNase inhibitor in frog tissues. The bullfrog RNase inhibitor does not inhibit mammalian RNases and vice versa, as frog RNases are insensitive to the mammalian RNase inhibitor [15]. Malica Blaszkiewicz also purified an RNase from R. esculenta [16]. Nitta et al. further purified the RNase from R. catesbeiana liver and elucidated its primary structure (RC liv.) [9]. Riordan et al. isolated a factor that has angiogenicity from human carcinoma cells and determined its primary structure of cSBL and jSBL were determined by Titani et al. [7] and Kamiya et al. [8], and their RNase activities were demonstrated by these investigators [20].

Quite independently, Shogen and his associates showed that a protein from R. pipiens oocyte cells (onconase; P30) had antitumour activity and was toxic to cells. They also determined its amino acid sequence [10]. The structure indicated that this protein is also a member of the RNase A superfamily [10].

Primary structure of frog RNases

The primary structure of a frog RNase, cSBL, was first recognized by Titani et al. [7] as that of a protein which is homologous with RNase A, and which confers lectin activity to it. Subsequently, the primary structures of three frog RNases, liver RNase from R. catesbeiana [9] (Rc liv.), a lectin from R. japonica (jSBL) [8] and onconase from R. pipiens [10] were elucidated. The structures of these proteins indicated that they are homologous proteins belonging to the RNase A superfamily of enzymes, based on their high homology with RNase A and having most of the amino acid residues involved in the catalysis of RNase A as well as some base recognition functions, particularly components of the B1 base recognition site of RNase A (fig. 1). Frog RNases have six half-cystine residues (thus three disulphide bridges) at the same locations as in RNase A, but they lack one disulphide bridge connecting the 65th and 72nd positions (RNase A numbering). They have a fourth disulphide bridge (Cys107–Cys126), and one of the half-Cys residues (Cys126) is located near the C-terminus of each enzyme. The locations of the three conserved disulphide bridges are the same as those of chicken, turtle and iguana RNases. However, chicken, turtle and iguana RNases do not appear to have the novel disulphide bridge observed in frog RNases. As shown in figure 1, catalytic components of the P1 site (His12, His119 and Lys41, RNase A numbering), are all conserved, while some amino acid residues which are thought to augment activity, though their roles have yet to be clarified, such as Asp121 and Gln11 are replaced.

Among the B1 site components – Thr45, Phe120 and Ser123 – the first two are conserved in frog RNases. In the P2 site, Lys7 is partly conserved. In the B2 site, only His119 and Glu111 are conserved. The effects of some of the replacements on base specificity are discussed below. The disulphide bridge located near the C-terminus with the active site His119 (RNase A numbering) is probably responsible for stability and increased enzymatic activity at higher temperatures such as 60 °C (see below).

The most characteristic feature of the four frog RNases is a γ-pyroglutamyl residue at the N-terminus. Several highly characteristic biological properties, including enzyme activity, appear to be related to this γ-pyroglutamyl residue. Frog lectins and onconase are insensitive to the placental RNase inhibitor, and show cytotoxicity towards several cell lines. They also have lower enzymatic activities than RNase A. However, a recombinant onconase with an N-terminal Met instead of the γ-pyroglutamyl residue showed decreased cytotoxicity and lower enzymic activity. In contrast, a recombinant onconase without this Met and with a cyclized γ-glutamyl residue again showed cytotoxicity and enzymatic activity [21]. Thus, the γ-pyroglutamyl residue appears to be important for these biological roles.

Cloning of cDNA of frog RNases

Complementary DNA (cDNA) encoding complementary sialic acid-binding lectin (cSBL) was cloned from a cDNA library prepared from bullfrog liver. The cDNA consisted of 853 base pairs containing 224 nucleotides of a 5′-flanking region, followed by a leader sequence for the signal peptide (22 amino acid residues) and a 333-nucleotide region coding mature cSBL, a 3′-flanking region and a poly(A) tail [22]. The amino acid sequence deduced from the cDNA was completely in agreement with that of protein sequencing [7]. Because the amino terminal of the cSBL protein was a γ-pyroglutamyl residue, processing of mature cSBL involves