Binding of a substrate analog to a domain swapping protein: X-ray structure of the complex of bovine seminal ribonuclease with uridylyl(2',5')adenosine

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Abstract

Bovine seminal ribonuclease (BS-RNase) is a unique member of the pancreatic-like ribonuclease superfamily. The native enzyme is a mixture of two dimeric forms with distinct structural features. The most abundant form is characterized by the swapping of N-terminal fragments. In this paper, the crystal structure of the complex between the swapping dimer and uridylyl(2',5')adenosine is reported at 2.06 Å resolution. The refined model has a crystallographic R-factor of 0.184 and good stereochemistry. The quality of the electron density maps enables the structure of both the inhibitor and active site residues to be unambiguously determined. The overall architecture of the active site is similar to that of RNase A. The dinucleotide adopts an extended conformation with the pyrimidine and purine base interacting with Thr45 and Asn71, respectively. Several residues (Gln11, His12, Lys41, His119, and Phe120) bind the oxygens of the phosphate group. The structural similarity of the active sites of BS-RNase and RNase A includes some specific water molecules believed to be relevant to catalytic activity. Upon binding of the dinucleotide, small but significant modifications of the tertiary and quaternary structure of the protein are observed. The ensuing correlation of these modifications with the catalytic activity of the enzyme is discussed.

Keywords: domain swapping; protein-inhibitor complex; protein structure-function; ribonuclease; X-ray diffraction

Bovine seminal ribonuclease (BS-RNase) is a unique member of the pancreatic-like ribonuclease superfamily (D'Alessio et al., 1991, 1996). This homodimeric enzyme, wherein the two subunits are linked by two consecutive disulfide bridges, presents several distinctive properties involving both its structure and catalytic activity. The main structural feature of BS-RNase is the interchange (swapping) of the N-terminal fragments (residues 1–15); these do not interact with the main body (residues 23–124) of their own subunits but, instead, fold against the body of the other subunit (Mazzarella et al., 1993). As active site residues belong to both the main body and N-terminal fragment of the protein, the dimer has two composite active sites, i.e., each active site is made up of

residues from both subunits (Mazzarella et al., 1994). Domain swapping, a phenomenon that was first revealed for this enzyme (Capasso et al., 1983), is not a rare process. Recently, several examples have been reported in the literature (Bennett et al., 1995) and it has been proposed that this mechanism may be involved in the evolutionary pathway of proteins from monomeric to oligomeric states (Bennett et al., 1994, 1995).

Biochemical studies have shown that BS-RNase presents a variety of active forms. The protein purified from bull seminal plasma is a 2/1 mixture of a swapping and a nonswapping dimer (Piccoli et al., 1992). Furthermore, swapping tetramers (Adinolfi et al., 1996) and a fully active monomer, obtained by reducing the inter-chain disulfide bridges (D'Alessio et al., 1975), have been prepared and characterized. Thus, BS-RNase provides a unique example of more than one stable three-dimensional state occurring with a single sequence. On the other hand, the two dimers reveal distinct functional properties. In particular, only the swapping dimer shows mixed cooperativity in the rate-limiting reaction step and special biological functions (D'Alessio et al., 1991), which include an antitumor action (Vescia et al., 1980; Laccetti et al., 1994).

Despite these unusual properties, the sequence of BS-RNase (D'Alessio et al., 1996, and references therein) has only 23 resi-

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Abbreviations: BS-RNase, bovine seminal ribonuclease; MxM, swapping dimer of BS-RNase; M=M, nonswapping dimer of BS-RNase; RNase A, bovine pancreatic ribonuclease; 2',5'-UpA, uridylyl(2',5')adenosine; BS-RNase·UpA, complex of BS-RNase with 2',5'-UpA; HP, hinge peptide; RMSD, root-mean-square deviation.

dues mutated compared to the most well-known member of the family, the monomeric bovine pancreatic ribonuclease (RNase A). Four mutations are located in the peptide 16–22 that connects the N-terminal fragment with the main body of the protein. In particular, it has been suggested that the mutation of Ala19 in RNase A for Pro in BS-RNase plays a major role in the swapping phenomenon (Di Donato et al., 1995; Mazzarella et al., 1995; Vitagliano et al., 1996).

The high sequence similarity between the two proteins includes all residues that are directly involved in the catalytic mechanism of the enzyme. Hence, structural data obtained for RNase A can be transferred to BS-RNase and vice versa. For a long time, structural studies on the interaction of RNase A with substrate analogs did not provide accurate information either because the final models were not well refined (Richards & Wyckoff, 1973; Wodak et al., 1977; Pavlovsky et al., 1978) or because the inhibitor did not bind productively (Aguilar et al., 1992). More recently, several examples of high resolution crystallographic structures as well as NMR studies of these complexes have been reported, providing a more detailed picture of the active site of RNase A (Wlodawer et al., 1983; Lisgarten et al., 1995; Fontecilla-Camps et al., 1994; Zegers et al., 1994; Toiron et al., 1996).

In order to add structural information to the interactions of pancreatic-like ribonucleases with inhibitors and to analyze the effects of binding on the peculiar quaternary structure of BS-RNase, we have undertaken crystallographic studies of complexes of this enzyme with substrate analogs. In the present paper, we report the X-ray structure of the complex between the swapping dimer of BS-RNase and uridylyl(2',5')adenosine (2',5'-UpA), which is the first crystallographic contribution in this field.

Results

Structure of the complex

The complex of BS-RNase with 2',5'-UpA (BS-RNase·UpA) crystallizes in a monoclinic unit cell, space group $P2_1$. As for the native

enzyme, which crystallizes in the orthorhombic space group $P22_12_1$, a whole dimer constitutes the asymmetric unit. The structure, refined at 2.06 Å resolution, gives an *R*-factor of 0.184. The average *B*-factor is 21.1 Å² computed for all the nonhydrogen atoms of the complex, 20.4 Å² for the main-chain atoms (N, C α , C, O) and 21.9 Å² for the side-chain atoms. The dinucleotide atoms present an average *B*-factor of 16.1 Å². An overall view of the complex is shown in Figure 1.

The two subunits, hereafter referred to as SA and SB, are related by a twofold rotation about an axis that is parallel to the crystallographic *c* axis, coupled with a 1.0 Å translation parallel to the rotation axis. This translational component produces a different body-to-tail relationship in the two subunits. A similar structural arrangement has been found for the native dimer, which crystallizes in a different space group (Mazzarella et al., 1993). This finding suggests that this feature is an intrinsic property of the molecule, and is not determined by the crystal packing or by the binding state of the protein.

The electron density map of the complex is well defined for all main-chain atoms and side chains, except for few exposed lysine residues and for the hinge peptide in SB. The overall structure is similar to that of the native enzyme. The RMSD between the two structures is 1.9 Å for the nonhydrogen protein atoms and 1.2 Å for main-chain atoms. The only major deviations are associated to the exposed loop 65–72 in SB and to the hinge peptide (HP). In both subunits of the complex, the loop 65–72 adopts the conformation found in RNase A and in SA of native BS-RNase. This observation strengthens the hypothesis (Mazzarella et al., 1993) that the unusual conformation in SB of native BS-RNase is due to packing contacts.

A key feature of the protein regards the HP whose structure distinguishes the swapping from the nonswapping dimer. Although the complex was crystallized from a protein sample containing both MxM and M=M dimers (Piccoli et al., 1992), the electron density of the HP's region shows that only the swapping dimer is detectable in our crystals. The electron density for the HP in SB is well defined (Fig. 2B), whereas in SA it indicates that the peptide

Fig. 1. Ribbon drawing of the MxM BS-RNase complex with 2',5'-UpA. The molecular twofold axis is vertical. The two subunits of the enzyme are colored differently and the bound dinucleotides and interchain disulfide bridges are drawn in ball-and-stick. Drawing generated by MOLSCRIPT (Kraulis, 1991) and RASTER3D (Merrit & Murphy, 1994).

A

Fig. 2. $3F_o - 2F_c$ omit maps of the region 16–22. (A) Subunit SA showing the two alternative conformations of the HP and (B) subunit SB. The maps were contoured at 1σ . (Figure continues on next page.)

adopts two alternative conformations (Fig. 2A), one of which is similar to that of SB. Both conformations were included in the model and refined with half occupancy.

Inhibitor structure

The structure of 2',5'-UpA is well defined in both active sites of the enzyme (Fig. 3). The two molecules share a very similar structure as shown by the main dihedral angles reported in Table 1. The dinucleotide adopts an extended conformation with the uridine moiety located in the specific pocket for pyrimidine—usually referred to as the B1 binding site for RNase A—the phosphate group in p1 and the adenine base in the less specific B2 site.

The conformation about the uridine glycosidic linkage is anti, whereas that about the adenine glycosidic bond is high-anti. The

torsion angles α' , β' , ϵ' , and ζ' , which define the conformation of the phosphodiester group (see footnote in Table 1), fall into the *-ap*, *ap*, *-ac*, and *ac* regions, respectively, according to the Klyne–Prelog classification. The uridine ribose adopts a C2'-endo conformation (Altona & Sundaralingam, 1972), whereas the adenosine sugar assumes a less common twisted conformation, which is intermediate between the O1'-endo and C1'-exo states. This conformation is probably influenced by the short intermolecular hydrogen bonds that link the hydroxyl oxygens O2' and O3' of the ribose to O3' and O2', respectively, of a symmetry related adenosine ribose (symmetry operation $-x - 1, y + 3/2, -z - 1$).

The protein-inhibitor interactions (Fig. 4), listed in Table 2, closely resemble those found for complexes of RNase A. Strong hydrogen bonds are formed at the pyrimidine-specific binding site, where N and O γ 1 of Thr45 interact with O2 and N3, respectively,

B

Fig. 2. Continued.

of the uridine base. O^{71} of Thr45 makes an additional bond, acting as a hydrogen donor, with O^{81} of Asp83. The phosphate group is hydrogen bonded to the side chains of the catalytically important His12, His119, and Lys41 and to the peptide nitrogen of Phe120. Finally, the downstream adenine, which is located in the less specific purine site, is linked by two hydrogen bonds to the side chain of Asn71 and has an additional weak interaction with Asn67. The side chain of Gln69 is not properly oriented to form hydrogen bonds with the purine base. It is worth noting that the adenine five-membered ring stacks against the side chain of His119. Additional protein-inhibitor interactions at B1 binding site are mediated by two water molecules (see below).

Water molecules and sulfate anions

The final model of the complex includes 108 water molecules with an average thermal factor of $34.1 \text{ \AA}^2$ ranging from $13.8 \text{ \AA}^2$ up to $58.4 \text{ \AA}^2$. Each water molecule is within hydrogen-bonding distance either from a donor or acceptor atom of the complex. Seventy water molecules are related in pairs by the noncrystallographic twofold axis with a deviation of less than $1.4 \text{ \AA}$. Furthermore, 29 water sites of the complex are within $1.0 \text{ \AA}$ from water sites observed in native BS-RNase.

Two water molecules mediate interaction between the inhibitor and the protein residues. One water molecule, which is very well

Fig. 3. $F_o - F_c$ omit map contoured at 2σ of the bound dinucleotide in one of the two active sites. The electron density for the second site is equally well defined.

defined in the electron density map, forms four hydrogen bonds with O4 atom of the uridine base, O^{δ1} of Asp83, O^γ of Ser123, and Thr45 of the protein. The second water molecule, with a weaker electron density, also interacts with O4 of the uridine base and with the peptide nitrogen of Ser123. All these interactions are virtually identical in the two active sites.

The electron density maps show the presence of three sulfate anions. This finding is not surprising given the high concentration of ammonium sulfate in the crystallization medium and the high isoelectric point of the seminal enzyme, which is significantly more basic than RNase A. Two sulfates, placed in equivalent positions of the two subunits, interact with N^{δ2} of Asn67 and are located at close distances from the uridine sugar (Table 2). This position is less than 4.0 Å from the phosphate at 5' end of the pyrimidine nucleotide in the complex of RNase A with

d(ApTpApApG) (Fontecilla-Camps et al., 1994). An additional sulfate anion is located at the interface region between Lys113 of one molecule and N^{δ2} atom of Asn17 of a symmetry-related molecule.

Structural comparison of native and complexed BS-RNase

All the secondary structure elements as well as the main features of the tertiary structure of native MxM BS-RNase are conserved in the structure of the complex. However, a detailed analysis of the two structures reveals subtle, but significant, variations of the tertiary and quaternary structure.

As in RNase A (Wlodawer, 1985), the β -structure of each subunit of BS-RNase comprises two antiparallel β -sheets, V1 and V2, which form a characteristic V-shaped motif with the active site

Table 1. Conformation of the 2',5'-UpA bound to BS-RNase

	Aa		Ba	
	Uridine adenine		Uridine adenine	
Backbone torsion angles (deg)				
O5'-C5'-C4'-C3'	72	45	71	53
C5'-C4'-C3'-O3'	82	118	82	111
C5'-C4'-C3'-C2'	-163	-137	-164	-139
C4'-C3'-C2'-O2'	-79	-119	-70	-124
C1'-C2'-O2'-P	138		140	
Glycosyl torsion angles (deg)				
O1'-C1'-N9-C4		-84		-84
O1'-C1'-N1-C2	-145		-143	
Pseudorotation angles (deg)				
C4'-O1'-C1'-C2' (ν_0)	-4	-46	-7	-54
O1'-C1'-C2'-C3' (ν_1)	-22	35	-24	40
C1'-C2'-C3'-C4' (ν_2)	39	-10	46	-13
C2'-C3'-C4'-O1' (ν_3)	-40	-16	-48	-19
C3'-C4'-O1'-C1' (ν_4)	29	39	36	45
Phase (deg)	23	103	25	104
Phosphodiester torsion angles ^b (deg)				
O2'-P-O5'-C5' (α')	165		161	
P-O5'-C5'-C4' (β')	153		159	
C3'-C2'-O2'-P (ϵ')	-105		-108	
C2'-O2'-P-O5' (ζ')	108		105	

^aThe column headings A and B refer to 2',5'-UpA bound to the body of SA and to the N-terminus of SB and vice versa, respectively (see text).

^bThe angles have been named α' , β' , ϵ' , ζ' by analogy to the nomenclature of the corresponding angles in the 3'-5' oligonucleotides.

located in between. V1 and V2 arms are formed by residues 61–63, 71–75, 105–111, and 116–124 and residues 42–46, 82–87, and 96–101, respectively. In a low-humidity form of RNase A, Radha Kishan et al. (1995) noted a small re-orientation of the two β -sheets, which leads to a slightly more compact structure of the enzyme, and they hypothesized that this modification could be important for the catalytic activity of the enzyme. A similar effect is clearly visible when the structures of the native and liganded form of BS-RNase are compared (Fig. 5A). Separate superimposition of V1 and V2, for SA of the two forms gives an RMSD of 0.20 and 0.23 Å, respectively, indicating that each β -sheet is perfectly well preserved upon binding of the ligand. However, after superimposition of V1, the best fit for V2 requires a further rotation of 3.3°, which reduces the RMSD from 1.00 to 0.23 Å. A similar rotation of 2.8° with comparable values of RMSD was also found for the crystallographically not equivalent SB. Alternatively, the reorientation of the two β -sheets can be estimated by calculating the angle formed by the lines joining the center of mass of V1 and V2 to the center of mass of the elbow residues connecting V1 and V2 (Radha Kishan et al., 1995). This angle reduces from 92.3° and 92.1° for the two subunits of the native protein to 89.2° and 90.0°, respectively for the complex; these figures are comparable to those quoted for RNase A.

Larger variations are observed for the quaternary structure of the liganded enzyme. After superimposition of the V1 and V2 fragments of SA, with an RMSD of 0.40 Å, the best fit for the same residues of SB requires a rotation of 7.6°, which reduces the RMSD

from 2.1 to 0.40 Å. Upon binding of the substrate analog, the quaternary structure of the complex opens up (Fig. 5B). The two chains pivot upon the 16-membered cycle formed by the two interchain disulfide bridges that link Cys31 and Cys32 of SA to Cys32 and Cys31 of SB, respectively. The swapped N-terminal fragments largely follow the body of the chain with which they interact.

Discussion

The structure of the complex of MxM BS-RNase with 2',5'-UpA provides detailed information on the structure of the inhibitor and its interactions with the protein. The inhibitor assumes an extended conformation that is different from those usually found for free nucleotides. However, the conformation is similar to those observed for complexes of RNase A, except for the puckering of the ribose rings, which may, in part, be due to packing contacts. All the main dihedral angles agree well with the values reported in a recent survey of structural parameters of nucleotides bound to proteins (Moodie & Thornton, 1993).

The conformation and the interactions of the residues involved in the binding of the ligand are also similar to those reported for RNase A. Thr45 contributes substantially to the binding of the pyrimidine base in the highly specific B1 site, whereas Asn71 binds the purine base. The side chain of His119, which in native BS-RNase and in several structures of RNase A presents two alternative conformations usually referred to as A and B, adopts the sole state A in BS-RNase·UpA, since the B rotamer is hampered by the presence of the purine base at the B2 binding site. The same rotamer of His119 has been found in the structure of the complexes of RNase A with dinucleotides (Zegers et al., 1994).

It is worth noting that Thr45 interacts not only with the substrate but also makes a strong hydrogen bond with the side chain of Asp83. In RNase A this hydrogen bond is formed when Thr45 binds uridine (Wlodawer et al., 1983), but not when Thr45 binds cytidine (Zegers et al., 1994). In their site mutagenesis studies on RNase A, delCardayre' and Raines (1995) have shown that the replacement of Asp83 by Ala lowers the affinity of the enzyme for uridine but not for cytidine nucleotides. Furthermore, they suggest that the hydrogen bond between Asp83 and Thr45 could anchor the Thr side chain in the proper orientation for the binding of the uridine base. The presence of this bond in the complex of BS-RNase with 2',5'-UpA confirms this hypothesis and shows, at the same time, that the modulation of the specificity of BS-RNase toward pyrimidine bases is similar to that observed for RNase A.

In the present structure, the enzyme-substrate interactions are mediated by two water molecules as in RNase A, thereby confirming the importance of water molecules in stabilizing and modulating protein-substrate interactions in ribonucleases (Gilliland et al., 1994). The presence of a sulfate anion in the proximity of p_0 binding site (Fontecilla-Camps et al., 1994) indicates that the subsite recognition of BS-RNase is also likely to be similar to that of RNase A, even though BS-RNase is active toward double-stranded RNA whereas RNase A is not (D'Alessio et al., 1996).

In addition to these features that characterize BS-RNase as a member of pancreatic-like ribonuclease superfamily, the structure of the complex offers an interesting opportunity to investigate the effects of the binding of a substrate analog on the peculiar structure of the enzyme. Comparison of the native and complexed form of BS-RNase reveals significant variations of the tertiary and quaternary structure of the enzyme. The former becomes more compact

Fig. 4. Stereo diagram of the catalytic site of the MxM BS-RNase-UpA complex showing the hydrogen bonding network (dashed lines), between the dinucleotide and the protein moiety. The two water molecules (W) are present also in RNase A complexes. Due to the swapping of N-termini, His12 belongs to a different chain.

in the complex. This modification is similar to that observed for a low humidity form of RNase A and, to a lesser extent, for the complex of the same protein with uridine vanadate (Radha Kishan et al., 1995). In BS-RNase this effect is highly significant and can

be ascribed to the numerous stabilizing contacts and hydrogen bonds between the dinucleotide and residues belonging to the two arms V1 and V2 of the β -sheet that move upon binding.

At the level of the quaternary structure, the variation is more pronounced. This result is somewhat surprising, as the double interchain disulfide bridge and the swapping of the N-termini severely restrain the relative positions of the two chains. The change of the quaternary structure alters the spatial relationship of the bodies in the dimer, and increases by about 1 Å, the distance between the end points (residues 15 and 23) of the two HPs in the complex. Nevertheless, the HP conformation in SB is similar to SA in the native structure. Furthermore, the two alternative conformations for HP in SA closely resemble those found for the two chains of the native protein. The five HP conformations observed in the two structures can be grouped in two classes with distinct structural features. In one class that includes SA of the native enzyme, SB, and one of the SA alternative conformations of the complex, Gly16 adopts an extended conformation and residues 16–21 form a nested double β -turn, which includes a type IV (residues 16–19) and a type I β -turn (residues 18–21). In the second class, Gly16 is in the α_L state, and residues 18–21 form a type I β -turn. The latter, which is present in all the observed conformations of the hinge peptide, is stabilized by a hydrogen bond between the carbonyl oxygen of Ser18 and the peptide nitrogen of Ser21.

These data suggest that, within the framework of the swapped quaternary structure, HP has a restricted conformational freedom, characterized by two key points: the invariant structural motif represented by the β -turn 18–21, which includes Pro19, and a flexible joint formed by Gly16, which can switch from an extended to an α_L state. A limited flexibility of HP has already been predicted by theoretical studies of the allowed conformations of HP (Mazzarella et al., 1995). The present study shows that even a small change in the distance between the end points of HP, such as that resulting from the change in the quaternary structure of the liganded versus native enzyme, can force the peptide to switch from one conformation to the other.

Table 2. Hydrogen bonds in the active sites of the complex of BS-RNase^a

BS-RNase	2',5'-UpA	Distance (Å)	
		A ^b	B ^b
Thr45 N	O2	2.99	2.99
Thr45 O ^{γ1}	N3	2.91	2.72
Wat	O4	2.47	2.55
Wat	O4	3.29	3.36
Gln11 N ^{ε2}	O1P	3.17	2.90
His12 N ^{ε2}	O1P	2.72	2.63
Lys41 N ^δ	O1P	2.76	2.91
Wat	O1P	3.17	3.09
His119 N ^{δ1}	O2P	2.69	3.25
Phe120 N	O2P	2.81	2.59
Wat	O4'	3.24	3.25
Sul	O3'	2.40	2.40
Asn71 O ^{δ1}	N6A	2.89	2.77
Asn71 N ^{δ2}	N1A	3.37	3.35
Asn67 N ^{δ2}	N7A	2.98	3.26
Wat	N3A	2.94	2.80
2',5'-UpA O3'A ^c	O2'A	2.52	2.34
2',5'-UpA O2'A ^c	O3'A	2.34	2.52

^aAll the interactions with $X \dots A < 3.5$ Å are reported.

^bThe column headings A and B refer to 2',5'-UpA bound to the body of SA and to the N-terminus of SB and vice versa, respectively (see text).

^cAtoms of a symmetry-related molecule (symmetry operation: $-x - 1, y + 3/2, z - 1$).

Fig. 5. $C\alpha$ trace of MxM BS-RNase in the free (thin line) and liganded (thick line) form. **A:** Subunits A of the two forms have been superimposed using residues of V1 arm (see text) to emphasize the closure of the tertiary structure that occurs upon binding of the inhibitor. **B:** Diagram showing the superposition of the quaternary structure. The two subunits rotate pivoting on the interchain disulfide bridges (S-S).

This interplay between the conformational properties of HP and the quaternary structure is a basic feature of the seminal enzyme, which can be correlated to its catalytic properties. It is known that the MxM dimer shows cooperativity, whereas M=M does not (Piccoli et al., 1992). This implies that cooperativity is associated to the interchange of the N-termini that produces two composite active sites, rather than to the presence of the interchain disulfides. The restricted conformational freedom of HP is well-suited for transmitting structural events from one composite active site to the other, thereby providing a structural link for the observed cooperativity in MxM. Accordingly, the interchain disulfides, which are present in both dimers, play the major role of fixing a quaternary structure appropriate for the interchange of N-termini.

The presence of Pro in position 19 favors the swapping of N-terminal helices in dimeric ribonucleases and also favors the formation of type I β -turn for residues 18–21 (Di Donato et al., 1995; Mazzarella et al., 1995). Thus, this single mutation has a double effect on the properties of BS-RNase; it enhances the tendency of the enzyme to form intercommunicating composite active sites and decreases the flexibility of the hinge peptide.

Conclusions

In this paper the first three-dimensional structure of BS-RNase with a substrate analog is presented. The results show that the interactions of BS-RNase with the dinucleotide closely resemble those observed in RNase A. The binding of the ligand induces a small but significant variation at the level of tertiary structure. This modification affects the quaternary structure as well, suggesting that it may act as a switching mechanism for site-site interaction. In principle, the structural effects presented here may in part be due to the different packing contacts between the native and the complexed forms of the protein. In all cases, they demonstrate a somewhat unexpected flexibility of the MxM dimer and provide a deeper insight into the preferred distortion modes of the enzyme.

Materials and methods

Crystallization and data collection

BS-RNase extracted from bovine seminal plasma was purified according to the procedure previously described (D'Alessio et al.,

1996). The uridylyl(2',5')adenosine dinucleotide was purchased from Sigma Chemical Co. Crystals suitable for X-ray crystallography were obtained in unusual conditions involving liquid-liquid phase separation. The best crystals for BS-RNase·UpA complex were obtained using ammonium sulfate 62–64% saturation, 2-methyl-2,4,-pentanediol, 8–10% (v/v), protein concentration 10 mg/mL and a nucleotide/protein molar ratio of 8:1 at 20 °C. The full procedure has been reported elsewhere (Sica et al., 1996).

The crystals are monoclinic (space group $P2_1$) with cell dimensions, $a = 49.5$ Å, $b = 60.6$ Å, $c = 50.7$ Å, and $\beta = 117.4^\circ$. On the basis of a molecular mass of 27.2 kDa and one molecule in the asymmetric unit, the volume per unit mass V_M is 2.50 Å³/Da.

Diffraction data were collected on an imaging plate R-AXIS II. Cu K α radiation was provided by a Rigaku RU-200 rotating anode operating at 50 kV and 180 mA. The data were processed using BIOTEX yielding 15,798 unique reflections from 48,936 total observations collected, with an R_{symm} ($R_{\text{symm}} = \sum |I - \langle I \rangle| / \sum |I|$) of 0.059 for all the data to 2.06 Å resolution. The overall completeness of the data is 92% (91% in the shell 2.25–2.06 Å).

Structure determination and refinement

The structure was solved by molecular replacement using the rotation and translation function implemented in X-PLOR (Brünger, 1992). The structure of native BS-RNase (PDB reference code 1BSR) was used as a starting model. The initial R -factor was 0.47 in the resolution range 10–4.0 Å. The model was refined with PROLSQ (Hendrickson, 1985), using all reflections up to 2.06 Å. Each refinement run was followed by manual intervention using the molecular graphic programs FRODO (Jones, 1978) and O (Jones et al., 1991) to correct minor errors in the position of the side chains. After few refinement runs, the difference Fourier map showed up an extra density in the two active sites, wherein the dinucleotide was easily fitted. Furthermore, the electron density of the region 16–22 of SA indicated the presence of two alternative conformations, which were refined with a fixed occupancy of 0.50. A home-made automated procedure to locate water molecules sites was used.

The geometry of the refined structure was monitored using PROCHECK (Laskowski et al., 1993). The final model has an R -factor of 0.184 using reflections with $F_o > 2\sigma(F_o)$ between 10 and 2.06 Å. Statistics and refinement parameters are presented in

Table 3. Refinement statistics

Resolution limits (Å)	10–2.06
Number of reflections ($F_o > 2\sigma(F_o)$)	14,764
R-factor ^a	0.184
Protein nonhydrogen atoms	1,925
Inhibitor atoms	78
Water sites	108
Sulfate anions	3
Deviation of geometry from ideal values	
1–2 distances (Å)	0.134
1–3 distances (Å)	0.265
1–4 distances (Å)	0.320
Planar group distances (Å)	0.008
Chiral volumes (Å ³)	0.117

^aR-factor = $\sum |F_o - F_c| / \sum |F_o|$ where F_o is the observed structure-factor amplitude and F_c is the calculated structure-factor amplitude.

Table 3. Throughout the refinement noncrystallographic symmetry restraints were not applied. The coordinates for the complex will be deposited in the Protein Data Bank and are available from the authors.

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