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Three-dimensional structure of ribonuclease Ms*3′-guanylic acid complex at 2.5 Å resolution

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The crystal structure of ribonuclease Ms•3′-guanylic acid complex has been determined by molecular replacement methods based on the known structure of ribonuclease T_1. The pattern of hydrogen-bonds between the enzyme and the guanine base is similar to that discovered by Arni et al. [(1988) J. Biol. Chem. 263, 15358–15368] in the crystal structure of ribonuclease T_1•2′-guanylic acid complex. As for the possible general base in the trans-phosphorylation step of the catalysis, O ε1 of Glu^{57} is within the hydrogen-bond distance (2.7 Å) of the 2′-O of the nucleotide while N ε2 of His^{39} is significantly more distant (3.4 Å) from the 2′-O.

Ribonuclease Ms; Inhibitor; Crystal structure; *Aspergillus saitoi*

1. INTRODUCTION

Ribonuclease Ms (RNase Ms; EC 3.1.4.23) was isolated and purified from *Aspergillus saitoi* by Ohgi and Irie [1]. RNase Ms hydrolyzes RNA completely into mononucleotides via 2′,3′-cyclic nucleotides without absolute base specificity. The enzyme consists of a single polypeptide chain (M_r 11,401) of 105 amino acid residues, the sequence of which was determined by Watanabe et al. [2]. It has two disulfide bonds (Cys^3-Cys^{11}, Cys^7-Cys^{102}) similar to RNase T_1 (2–10,6–103). There are many similarities between the sequences of RNase Ms and RNase T_1 despite large differences in base specificity. The two enzymes have identical amino acids for 65% of their aligned sequences (Fig. 1). In order to elucidate the origin of its lack of base specificity and to provide further clues for the current controversy over the catalytic residues of this type of microbial ribonucleases (see Results and Discussion), we solved the crystal structure of the RNase Ms*3′-guanylic acid (3′-GMP) complex using molecular replacement methods based on the known structure of RNase T_1.

2. MATERIALS AND METHODS

2.1. *Crystallization and data collection*

The RNase Ms*3′-GMP complex was crystallized as described earlier [3]. The crystals belong to an orthorhombic, space group $P2_12_12_1$, with cell dimensions $a = 47.0$ Å, $b = 62.8$ Å, $c = 37.9$ Å. A total of 4195 independent reflections in the resolution range of 20–2.5 Å were collected in a dynamic limited ω-step scan mode as described before [4] on a Rigaku four-circle diffractometer AFC-5R mounted on an RU-200 rotating anode generator operated at 50 kV, 200 mA. The R_{sym} factor between the symmetry-related structure amplitudes F_{obs} was 2.24%.

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Abbreviations: RNase, ribonuclease; 3′-GMP, 3′-guanylic acid; 2′-GMP, 2′-guanylic acid; r.m.s., root-mean-square

2.2. *Rotation and translation search*

A series of rotation functions were calculated, employing the fast rotation function algorithm [5] with the RNase T_1 molecule (whose coordinates were kindly supplied by Dr. Heinemann) as a model. The search model was constructed as follows. Amino acid residues of RNase T_1 identical with those of RNase Ms were preserved. Homologous and non-homologous residues were replaced with alanine and glycine residues, respectively (Fig. 1). The search model was put in a triclinic P1 cell with dimensions of 80 × 80 × 80 Å. Parameters such as the resolution range and the integration radii were varied. One prominent peak appeared consistently in almost all choices of parameters. When the resolution shell and the integration radius were set to 8–2.5 Å and 15 Å respectively, the highest peak was 5.1 σ higher than the mean' and 1.4 σ higher than the second highest peak, where σ is the standard deviation of the rotation function.

Translation search was carried out using the program BRUTE [6]. The appropriately oriented model was moved in small steps (1 Å or less) over the maximum fraction of the unit cell containing a single permissible origin (one eighth of the unit cell). At each step, the correlation coefficient between the observed and calculated structure factors was computed using only the data within the 5–4 Å resolution range. The highest peak was 0.599 high, 7.6 σ higher than the mean and 1.6 σ higher than the second highest peak, where σ is the standard deviation of the correlation coefficient. The refined Eulerian angles and translation vector were ($\alpha = 124.7°$, $\beta = 34.1°$, $\gamma = 61.4°$) and (15.7° Å, 16.8 Å, 2.1 Å), respectively. The R-factor calculated at this point was 0.477 in the resolution range of 10–2.5 Å. As an independent test of the correctness of this solution, the packing of the models in the unit cell was graphically checked and showed no abnormal contacts.

2.3. *Refinement of the structure*

The starting model was constructed by deleting two residues (Gly^{34}, Ser^{35}) of the model, adding three residues (Glu^1, Ser^{104}, Ser^{105}) of

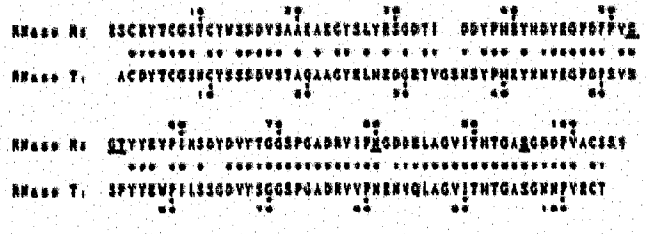

Fig. 1. Aligned amino acid sequences of RNase Ms and RNase T_1. The amino acids are represented by a one-letter symbol. The identical and homologous residues are marked with an asterisk and a plus sign, respectively. The homology relationships used above are N = D = Q = E, V = I and S = T. The original sequence of RNase Ms reported by Watanabe et al. [2] was corrected at three positions. The corrected sequence are underlined (H. Watanabe, K. Ohgi, M. Irie, unpublished data).

RNase Ms to the model and replacing the remaining residues of the model with the corresponding ones of RNase Ms. This procedure and a subsequent structure regularization were carried out using the program package TOM [7,8]. The crystal structure was refined by a stereochemically restrained least-squares program PROLSQ[9] followed by manual refitting of the model to new difference Fourier and fragment-deleted ('omit') difference Fourier maps using TOM. Solvent molecules refined to a temperature factor of $B > 60$ Å^2 were eliminated. The present model of the RNase Ms*3′-GMP complex consists of 803 protein and 24 inhibitor atoms and 142 solvent atoms. The current R-factor is 0.200 for the 3441 reflections in the 7.0–2.5 Å, resolution range with the r.m.s. deviation of bond lengths from ideal values being 0.015 Å.

3. RESULTS AND DISCUSSION

As shown in Fig. 2, the overall three-dimensional structure of RNase Ms molecule is similar to that of RNase T_1 [10,11]. The typical secondary elements are an α-helix with 4.4 turns (Ser^{14}-Glu^{29}) and a five-stranded antiparallel β-sheet (β_1:Pro^{38}-Tyr^{41}, β_2: Tyr^{55}-Met^{61}, β_3:Asp^{75}-Asn^{80}, β_4:Glu^{84}-Thr^{90}, β_5: Phe^{99}-Cys^{102}) as in the cases of RNase St[12] and RNase T_1. When the Cα model of RNase Ms is optimally superimposed on that of RNase T_1, the r.m.s. distances between the corresponding atoms is 0.84 Å. Two significantly large deviations occur in Thr^{10} and Asp^{35}. The latter deviation is clearly due to the deletion of two intervening residues (Glu^{34} and Ser^{35} of RNase T_1, see Fig. 1) while the origin of the former deviation is unclear.

The electron density for 3′-GMP is well defined in the omit map giving rise to the interpretation shown in Fig. 3. The basic conformation of the nucleotide seems to be essentially the same as that of 2′-GMP as found in its complex with RNase T_1 [10] in that the ribose puckering is C2′-endo and the glycosyl torsion angle is syn.

As for the possible catalytic residues, O ϵ 1 of Glu^{57} is within the hydrogen-bond distance (2.7 Å) of the 2′-O of the nucleotide, while N ϵ 2 of His^{39} is significantly more distant (3.4 Å) from the 2′-O. This observation is rather in favour of the classical role of Glu^{57} (as proposed by Takahashi [13]) rather than the new role of His^{39} (as recently proposed by Nishikawa et al. [14]) as the base for the first deprotonation step. Further crystallographic work on the complex of RNase Ms with a dinucleoside monophosphate analogue (rather than a simple mononucleotide) is in progress.

The scheme of enzymatic recognition of the guanine base is almost the same between the case of RNase T_1*2′-GMP complex [10] and the present RNase Ms*3′-GMP complex. Thus there are three hydrogen bonds involving the enzymatic main-chain peptide group ((a) His42NH to N7Gua, (b) Asp43NH to 06Gua and (c) Tyr44NH to 06Gua) and two hydrogen bonds involving the enzymatic sidechain of Glu^{45} ((d) Glu45O ϵ 1 to HN1Gua and (e) O ϵ 2 to HN2Gua). Here it is intriguing to see whether or not the adenine base can be bound to the enzyme in the same topological arrangement as described above, since the reaction catalyzed by RNase Ms is known to be base non-specific (with the preference order of G > A > C > U) rather than G-specific as in RNase T_1 [15]. For the adenine base, the hydrogen-bond interaction (a) defined above (but excluding (b) and (c)) would be possible. Furthermore, since the NH group at position 1 is deprotonated in the

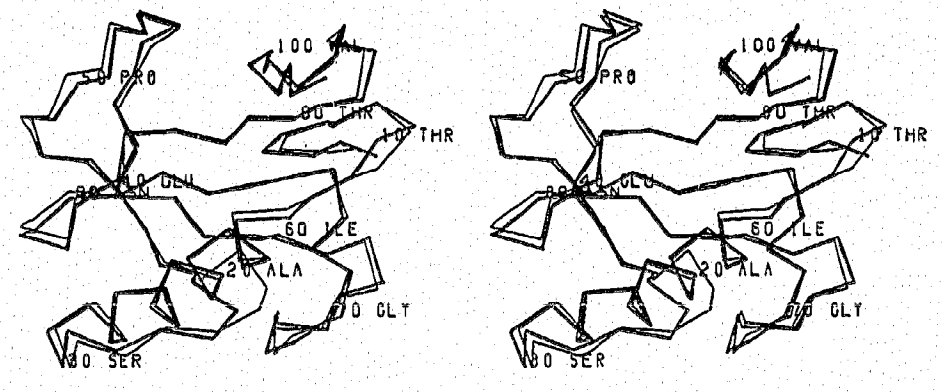

Fig. 2. Superposition of Cα-drawings of the polypeptide chain folding of the RNase Ms*3′-GMP complex (thick line) and of the RNase T_1*2′-GMP complex (thin line). Every 10th amino acid residue is labelled.

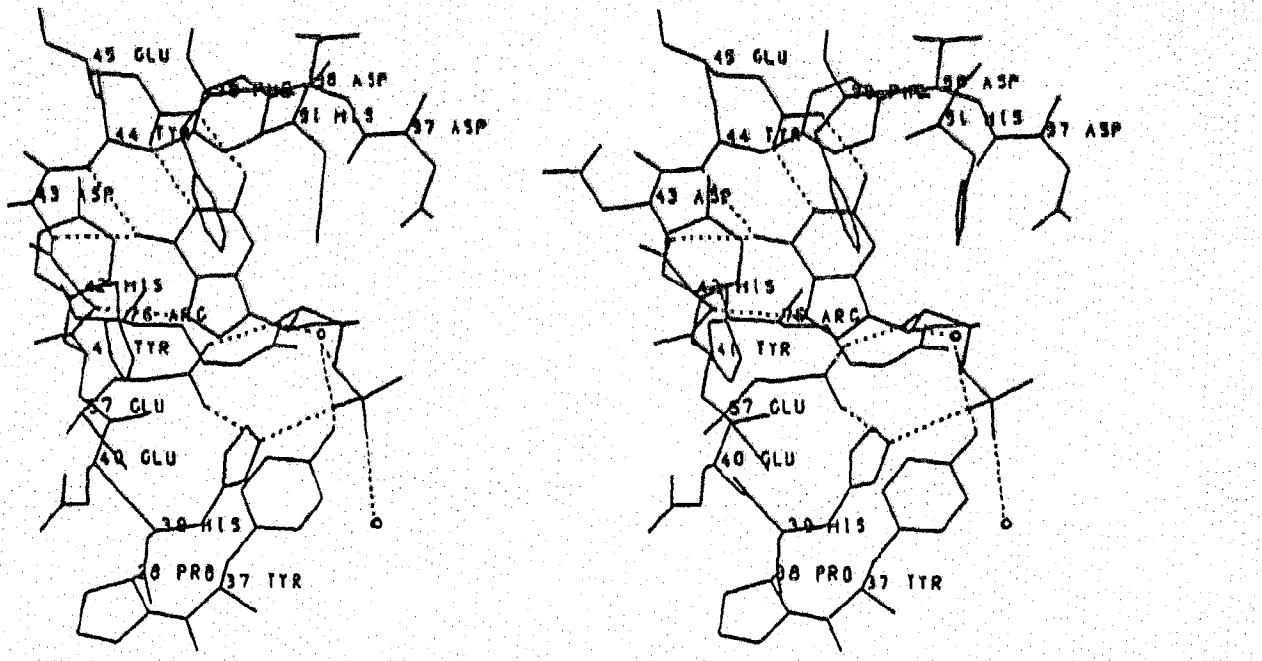

Fig. 3. Stereoscopic drawings of the nucleotide binding site in the RNase Ms*3'-GMP complex. Broken lines and open circles represent hydrogen bonds and water molecules, respectively.

adenine ring, the hydrogen bond (d) would be possible if the carboxylate group of Glu^{45} is protonated. However, even in the pH range around 4.5 where the reaction catalyzed by RNase Ms is optimum, such protonated form will not be dominant. It should be noticed here that the target residue Glu^{45} is conserved in RNase T_1 where, however, the optimum pH is around 7.0 making the interaction with N1 of the adenine base absolutely impossible. In any case, the crystal structures of RNase Ms complexed with certain adenine-containing ligands have to be established to elucidate the origin of the base non-specificity as in a classical work explaining the broad pyrimidine specificity exhibited by RNase S [16]. We have recently succeeded in crystallizing RNase Ms*3'-AMP complex. Attempts at improving the crystal quality are in progress.

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