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Mechanism of guanosine recognition and RNA hydrolysis by ribonuclease T_1

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ABSTRACT

The fungal ribonuclease RNase T_1 has been co-crystallized with its inhibitor 2'-guanylic acid and was analyzed by X-ray diffraction. The enzyme is folded into an α-helix 4.5 turns long, which is covered by a four-stranded antiparallel ß-sheet. Specific recognition of guanine occurs with two hydrogen bonds between main chain peptide groups of Asn43 and Asn44 and the O(6) and N(1)-H of guanine, as well as by stacking with tyrosines 42 and 45 which sandwich the nucleobase. At the active site, Glu58, His92 are involved in hydrolysis of the phosphodiester in a general acid-base catalysis, and Arg77 is present to neutralize the charge on the phosphate.

INTRODUCTION

Ribonuclease T_1 (RNase T_1; EC 3.1.27.3) from the funges Aspergillus oryzae degrades single-stranded RNA to yield exclusively oligonucleotides with terminal guanosine-3'-phosphate. The mechanism of hydrolysis involves, as also with RNase A, the formation of an intermediate 2',3'-cyclic phosphate, which is hydrolyzed in a second step to yield 3'-phosphate. Due to its high specificity towards guanosine, RNase T_1 is widely used as a tool in nucleic acid chemistry and in sequence studies of RNA. Moreover, it is used as model compound to study protein-nucleic acid interactions (1).

Chemical modifications of RNase T_1, kinetic studies and NMR spectroscopy suggested that residues Glu58, His40, His92 and Arg77 are involved in the hydrolysis mechanism of RNase T_1, see sequence in Fig. 1. Also, it appeared

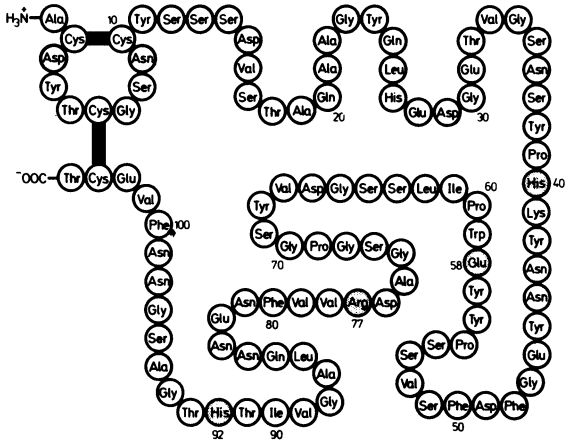

Fig. 1. Covalent structure of RNase T_1 (Ref.1).

that the single tryptophan (Trp59) is involved and it has been established that positions N(1), N(7) and O(6) of the guanine base have to be intact for proper recognition (1). In order to investigate the geometry of the active site of RNase T_1 and to find out the reason for the specific recognition of guanine, we have carried out an X-ray study (2-4). These investigations are summarized in the following.

STRUCTURE DETERMINATION

RNase T_1 (1 % solution) was co-crystallized with 0.25 % 2'-guanylic acid from sodium acetate buffer adjusted to pH 4.0 - 4.4 upon dialysis against 53 % 2-methyl-2,4-peptanediol. Crystals are of the orthorhombic space group $P2_12_12_1$ with unit cell parameters a = 46,8Å, b = 50.2Å, c = 40.4Å. Heavy atom derivatives were prepared by soaking the crystals in 1 - 5 mM solutions of the respective reagent.

Two heavy atom derivatives with lead and platinum and the cross derivative were obtained and the corresponding X-ray intensities including Friedel pairs were measured on a four circle diffractometer. The heavy atoms were located by Patterson and direct methods and the electron density map was computed for the native protein using standard procedures. The figure of merit was $\langle m \rangle = 0.75$ for 3.547 reflections to 2.5Å resolution. A picture of the molecule is displayed in stereo views Fig. 2 and in a schematic drawing in Fig. 3.

THE ARCHITECTURE OF RNase T_1

The overall arrangement of the polypeptide backbone in RNase T_1 is dominated by two significant features. One is the long α-helix which then is followed by a four-stranded antiparallel ß-pleated sheet. The connections between the individual strands of the sheets are composed of rather large loops between the residues 42 and 57, 61 and 76 whereas a short ß-turn is found between residues 81 and 84, see Figs. 2,3.

The structure of the molecule is not only stabilized by hydrogen bonding within the α-helix and the ß-pleated sheet, but also by the orientation of the hydrophyobic residues towards the interior of the globular unit and by the distribution of the negatively charged residues on the periphery of RNase T_1 (see Fig. 1b). It is surprising to find that the pI of RNase T_1, 2.9, is in the acidic range and that this molecule has to recognize a negatively charged RNA. If we look more closely at the distribution of negative and positive charges in RNase T_1, we find that the latter cluster in a narrow zone (Fig. 1c) which is identical with the active site. It appears, that the negatively charged surface of the molecule is constructed such that there is repulsion between the enzyme and the substrate as long as there is not a proper orientation so that the substrate is fitted into the enzyme active site.

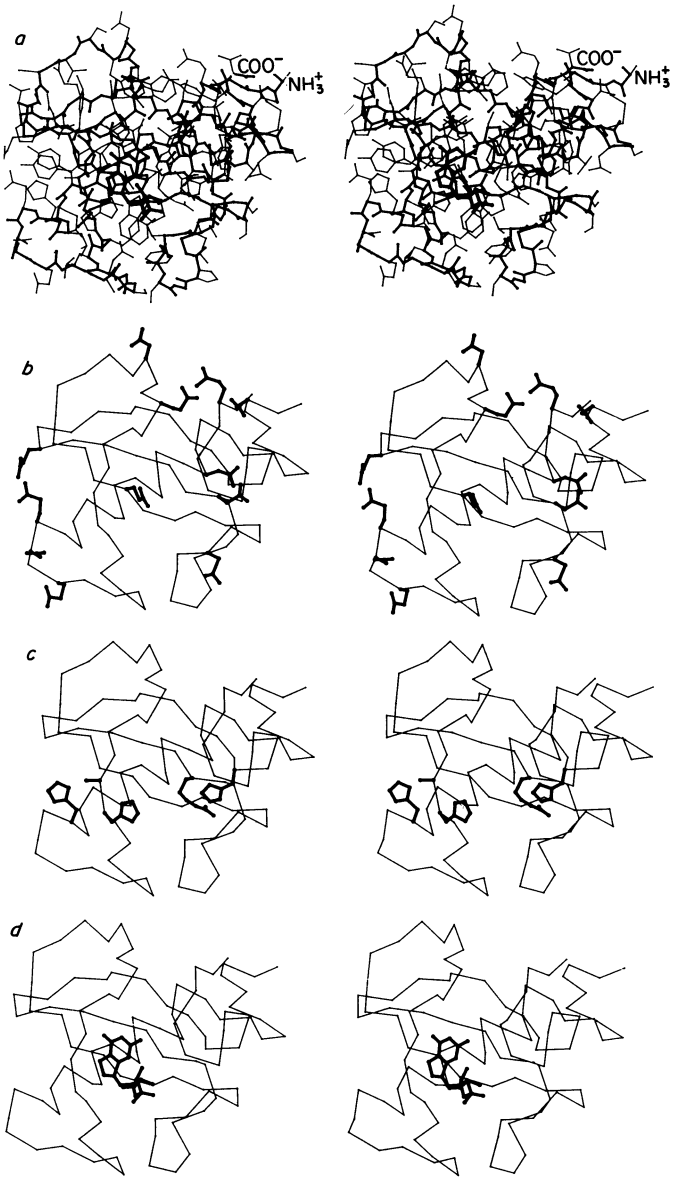

Fig. 2 Stereo drawings of RNase T_1 2'-guanylic acid. *a*, complete structure, *b*, C_α-backbone and acidic residues, *c*, C_α-backbone and basic residues, (Lys41 is not visible in electron density beyond C_β), *d*, C_α-backbone and 2'-guanylic acid. Taken from Ref. 3.

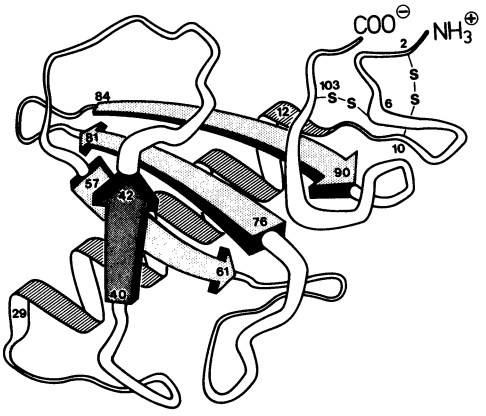

Fig. 3. Schematic drawing of the RNase T_1·2'-guanylic acid complex. Taken from Ref. 3.

SPECIFIC RECOGNITION OF GUANINE

The binding of 2'-GMP to RNase T_1 suggests immediately the geometry of guanine recognition and the geometry of the active site. If we look at guanine alone, it has several hydrogen bond donor and acceptor sites and would be suitable for recognition by a large number of amino acid side chains (Fig. 4). It is therefore very surprising that the hydrogen bonding to guanine in the complex with RNase T_1 is via main chain NH and CO groups. These are (Asn43)NH---O(6) and N(1)H---(Asn44)CO (see Fig. 5). The N2 amino group of guanine is not involved in any obvious hydrogen bond formation. The O(6) carbonyl oxygen of guanine is stacked on the plane of Tyr42 which is located within the enzyme molecule and on the back side of the recognition site. Tyr45 is located over the guanine base so that we observe in fact a sandwiching of guanine.

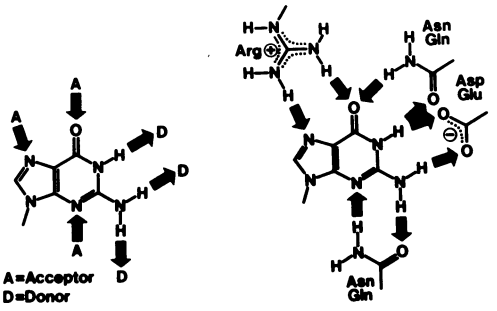

Fig. 4. Guanine can be recognized specifically by a large number of hydrogen bonding interactions with amino acid side chains. Taken from Ref. 4.

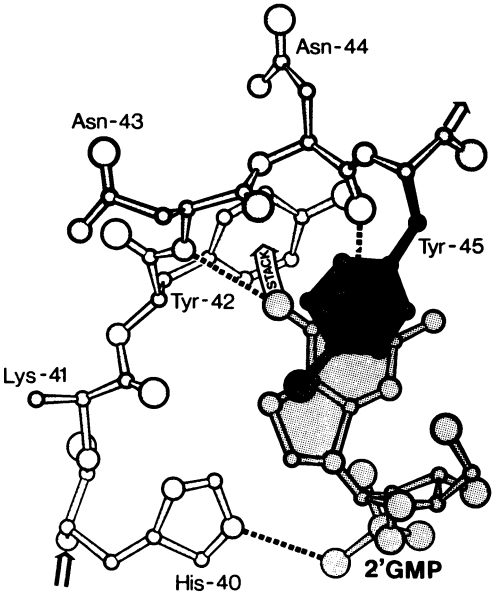

Fig. 5. Illustration of specific RNase T_1• guanylic acid interaction. The arrow points at a stack between guanine O(6) and Tyr42.

The question why nature has not used amino acid side chains to recognize guanine seems to be clear if we look at the possibilities of fine tuning of an interaction if the polypeptide main chain with its many hydrogen bonding donor and acceptor sites is folded into a proper shape. Within the amino acid side chains, groups like carboxyl, guanidinium, amide etc. are rather rigid in their geometry, yet they are flexible about the C-C bonds to which they are attached. In general, it appears that a fold of a polypeptide is a much better candidate for supplying a matrix to which a substrate can be fitted than a number of flexible side chains with a limited and rigid geometry of their functional end groups.

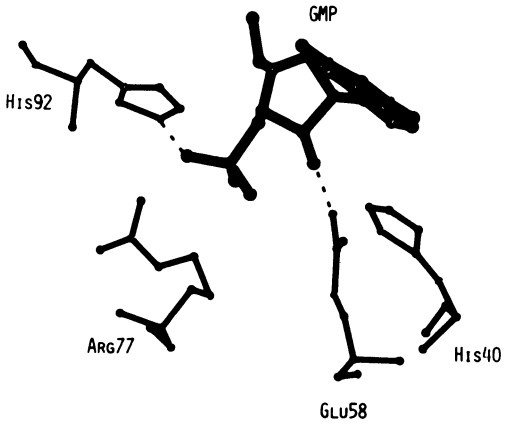

Fig. 6. View of the active site residues of RNase T_1, 3'-GMP has been fitted into the position of 2'-GMP.

GEOMETRY OF THE ACTIVE SITE AND MECHANISM OF RNA HYDROLYSIS

In Fig. 6 the surroundings around the phosphate group of 2'-GMP are displayed. It is clear that Arg77 is in the proper location to neutralize the negative charge of the incoming phosphate group, yet there is no direct salt bridge between these groups. If we had not 2'-GMP but 3'-GMP involved in the binding, as we have in fact with the natural substrate, the $O_{2'}$-hydroxyl of the ribose would be close to Glu58, and the 3'-phosphate would be in close contact with His92. This suggests, as indicated in Fig. 7, that Glu58 acts as a base and subtracts the proton from the $O_{2'}$-hydroxyl. The negatively charged $O_{2'}$ atom then attacks the phosphate group and the leaving oligonucleotide chain obtains a proton from His92 which neutralizes the outgoing $O_{5'}$. The

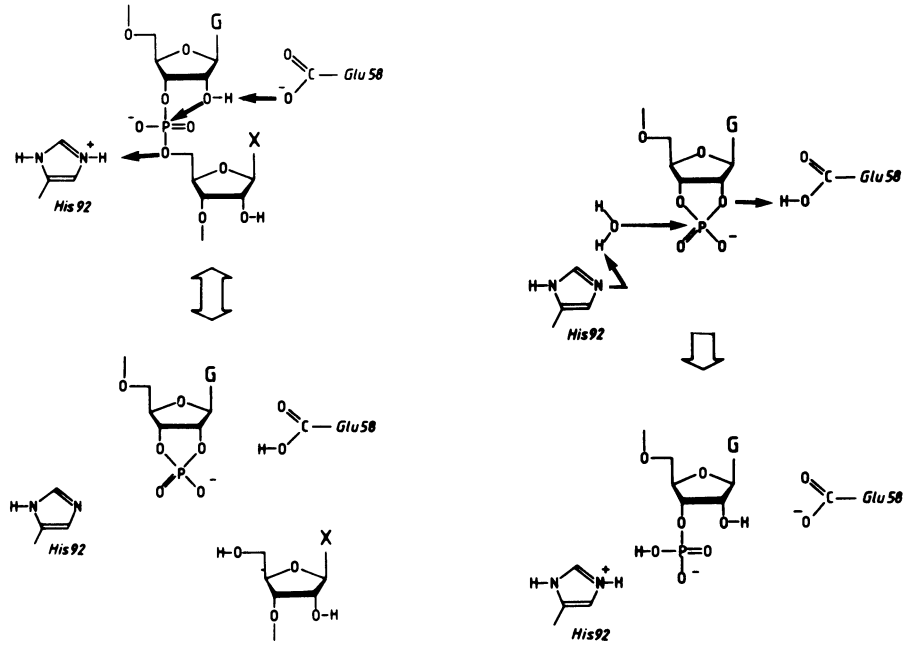

Fig. 7. Schematic mechanism of action of RNase T_1. Taken from Ref. 4.

intermediately formed 2',3' cyclic phosphate is then hydrolized by a water molecule that gives a proton to His92 and the resulting hydroxyl attacks the phosphate to release negatively charged $O_{2'}$ which takes the proton from Glu58. In this mechanism, His40 is not involved in any obvious interaction and it appears, that it only has an activation role for Glu58.

COMPARISON WITH OTHER RIBONUCLEASES

In 1981 and 1982, the crystal structures of a number of ribonucleases belonging to the RNase T_1 family had their structures determined: Ba, St, C2. The topologies of these four ribonucleases all have in common a more or less extended α-helix at the amino terminus and the polypeptide chain is then folded into a four (in some cases five) membered antiparallel ß-pleated sheet. The recognition site seems to be provided by the first strand and the loop between the first and second strand, and the active site residues Glu58, Arg77, His92 are located on the second, third and fourth strands respectively. All these ribonucleases are specific for guanosine or at least for purine and display either close or at least some homology among them. Since these enzymes are obtained both from prokaryots (bacteria) and eukaryots (fungi) there has been a structural conservation in the evolution which is quite remarkable. It appears from the three-dimensional structures of these enzymes, that the conservation of polypeptide folding is crucial because the active site residues are located on this four-folded pleated sheet and an alteration in this topology would have disastrous consequences for the activity of these enzymes.

It is clear that this family of ribonucleases is very different from the mammalian RNase A, which has no homology whatsoever with the RNase T_1 family and cleaves at the 3' site of pyrimidine residues. Also, the topology bears no resemblance because the pleated sheet architecture and arrangement of the helices is very different and RNase A consists of two domains between which the active site is located. The mechanism of cleavage however is reminiscent again because we have two histidines (His12, His119) instead of His92 and Glu58, and Lys41 which replaces Arg77 in RNase T_1. The mechanism is also of the general acid-base type and involves the intermediate formation of a 2',3' cyclic phosphate.

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