pH-induced change in nucleotide binding geometry in the ribonuclease T_1–2′-guanylic acid complex

Refinement of X-ray structure at 1.9 Å resolution

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At pH 4.0, the RNase T_1–2′GMP complex (**1**) crystallizes isomorphously with the isoenzyme complex (**2**) (Heinemann, U. and Saenger, W., 1982, Nature 299, 27–31). The X-ray structure of **1** was refined with 1.9 Å data to $R = 0.195$. Polypeptide folding is similar in **1** and **2**. However, the sugar pucker of 2′-GMP is 2′-*endo* (3′*endo* in **2**), and guanine binding involves four hydrogen bonds in **1**, which all differ from the two bonds in **2**. Phosphate contacts Glu58, Arg77, Tyr38 in **1**, but His40 in **2**. These changes are not due to differences in sequence between the mother- and isoenzyme (Gln25-Lys) but are associated with pH changes leading to an inactive enzyme structure.

Ribonuclease T_1 *2′-Guanylic acid* *Enzyme-substrate binding* *pH-induced change* *Conformational change* *Crystallography*

1. INTRODUCTION

Ribonuclease T_1 (RNase T_1, EC 3.1.27.3) from *Aspergillus oryzae* specifically hydrolyzes the 3′-phosphodiester bonds at guanylic acid residues of single-stranded RNA [1–3]. The enzyme consists of 104 amino acid residues of known sequence [4]. Spectroscopic and chemical investigations [1–3] indicated that His40, Glu58, Arg77 and His92 are located at or near the catalytic site and are essential for enzymatic activity. Recently the crystal structure of a complex between an isoenzyme of RNase T_1 (where Gln25 is exchanged with Lys) and the inhibitor 2′-guanylic acid (2′GMP) was determined at 2.5 Å resolution. The molecular structure obtained revealed the polypeptide chain folding of this enzyme and provided an understanding of the base-specific recognition mode and of active site geometry [5,6].

In order to obtain a deeper understanding of the structure of RNase T_1, the mother enzyme was cocrystallized with 2′GMP and the structure refined by stereochemical-restrained least-squares procedures [7] at 1.9 Å resolution. Surprisingly, the nucleotide binding mode is found to be different when compared with the earlier model. We associate the difference with the slightly lower pH of crystallization in the present work.

2. MATERIALS AND METHODS

The mother enzyme of RNase T_1 was purified from Taka-diastase (Sankyo Co.) [8]. Crystallization of the RNase T_1–2′GMP complex was accomplished as in [9] by vapor diffusion techniques with 2-methyl-2,4-pentanediol as precipitant. Conditions were slightly modified, i.e., concentrations of enzyme and 2′-GMP were 18 and 2.5 mg/ml, respectively, and the pH was maintained at 4.0 in the presence of the precipitant whereas it was about 5.0 in the previous study [9]. Crystals of the complex are orthorhombic, space group $P2_12_12_1$

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with a = 46.65(1), b = 50.26(1), c = 40.60(1) Å; i.e., isomorphous to the RNase T_1 isoenzyme–2'GMP complex [9]. Individual reflection intensities, however, are not identical, suggesting some changes in structure and/or packing of the molecules.

Two heavy atom derivatives were obtained by soaking the crystals for two days in mother liquor containing 2.0 mM uranyl acetate or 3.3 mM lanthanum nitrate. Intensity data were collected on a Rigaku-Denki AFC-5 four-circle diffractometer using six crystals for the native data set (1.9 Å resolution) and two crystals for each heavy atom derivative (2.4 Å resolution). After absorption correction [10] and scaling [11,12] of the data, the heavy atoms were located by Patterson methods. The phases of native data were determined by the double isomorphous replacement method taking advantage of the anomalous dispersion effect [12].

An electron density map was calculated based on 3319 data to 2.4 Å resolution using best phases with a figure of merit [13] of 0.784. From this map, a molecular model was constructed in an optical comparator [14] by means of 2 cm·Å^{-1} Kendrew wire models. The atomic coordinates measured from the model were refined by a stereochemical-restrained least-squares technique [7]. The present model of the RNase T_1–2'GMP complex consists of 781 protein and 24 inhibitor atoms and of 46 atoms assigned to water molecules. The conventional R factor is 0.195 for the 6396 reflections [$F_o > 3\sigma(F_o)$] in the 5.0–1.9 Å resolution range.

3. RESULTS AND DISCUSSION

The molecular packing and overall polypeptide chain folding of the mother RNase T_1–2'GMP complex (the low pH form, **1**) is almost identical to that of the isoenzyme complex (the high pH form, **2**) [5,6]. The typical secondary structure elements are an α-helix with 4.5 turns (Ser13-Glu28) and a four-stranded antiparallel β-sheet (β_1: Pro39-Tyr42, β_2: Pro55-Pro60; β_3: Asp76-Phe80 and β_4: Leu86-Thr91). Like the isoenzyme, Ala87-Gly88, forms a β-bulge and bends the β_4 strand significantly.

RNase T_1 has three histidyl residues, His27, His40 and His92, of which the latter two are considered to be important for catalytic activity [3]. NMR studies [15–17] suggested that each imidazole ring of these histidines interacts with an acidic group of the protein or the inhibitor. In the present model, the imidazole nitrogen atoms (NE2) of His27 and His40 are hydrogen bonded to the carboxyl groups of Glu28 and Glu58, respectively, but that of His92 is in contact with the peptide oxygen of Ala75. All these interactions are not seen in **2** because side chains are oriented differently.

Table 1

Interactions between 2'GMP and RNase T_1

Low pH form (**1**)	Distance (Å)	High pH form (**2**)
Guanine base		
N1-Glu46OE1	2.6	N1-Asn44O
N2-Asn98O	2.8	O6-Asn43N
O6-Asn44N	2.6	
N7-Asn43ND2	3.0	
Phosphate		
OP3-Tyr38OH	2.8	OP1-His40NE2
OP3-Glu58OE2	2.3	OP3-Glu58OE2
OP3-Arg77NH2	3.2	

The conformations of 2'GMP in **1** and **2** are similar when torsion angle ranges are considered: *trans* for P-O2'-C2'-C1', *syn* for glycosyl C8-N9-C1'-O4' and *gauche*(−) for O5'-C5'-C4'-O4', the latter leading to an intramolecular N3...O5' hydrogen bonding distance of 2.8 Å. Sugar puckering modes, however, are in the opposite sense, i.e., C2'-*endo* in **1** and C3'-*endo* in **2**. Consequently, the relative spatial dispositions of guanine base and 2'-phosphate groups are different in both models.

If **1** and **2** are compared, the most significant changes are found at the nucleotide binding site where specific guanine-protein recognition takes place and where phosphodiester hydrolysis is catalyzed, see table 1 and figs 1a and 1b. In **2**, the guanine base is bound by only two hydrogen bonds to the protein main chain peptide groups, Asn44CO...HN1Gua and Asn43NH...O6Gua. In contrast, in **1**, four hydrogen bonds are involved, none of them being identical with those of **2**. Two of these four hydrogen bonds are again to main-chain peptide groups, Asn98CO...HN2Gua and Asn44NH...O6Gua, the latter showing that the guanine recognition site is shifted in frame by one peptide unit (from Asn43 to Asn44). N1H is now

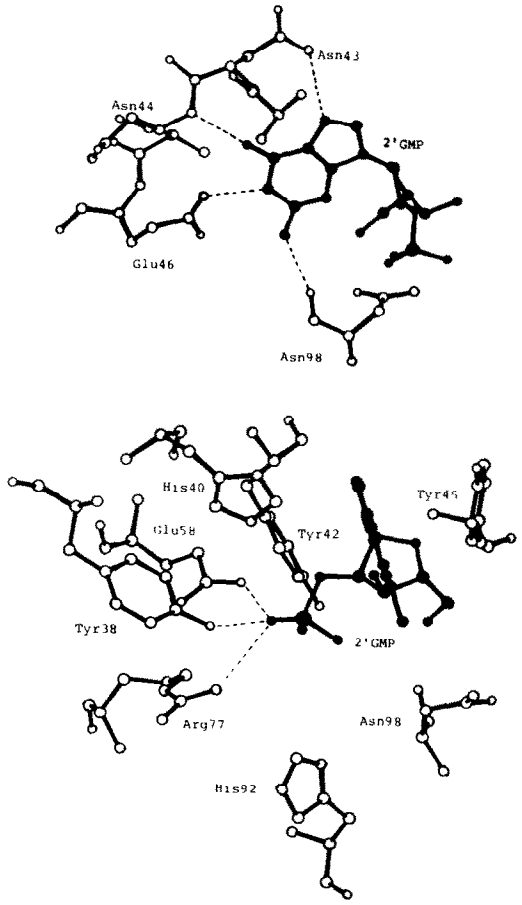

Fig.1. Perspective views of the nucleotide binding site [at the guanine base (a) and at the 2'-phosphate (b)] in the low pH form (1) of the RNase T_1–2'GMP complex. Broken lines represent hydrogen bonds or ionic interactions. Note sandwich formed by Tyr42-Gua-Tyr45 in fig.1b.

hydrogen bonded to the carboxylate of Glu46, and N7 accepts a hydrogen bond from the amide group of the side chain of Asn43. These observations are in line with chemical and spectroscopic data which suggested that N2 and N7 of guanine are not essential for enzymatic catalysis [18,19] but are required for tight binding to the enzyme [20,21].

The different guanine-protein hydrogen bonding schemes in **1** and **2** require some conformational changes of Glu46 and Asn43. These are located at the periphery of the RNase T_1 molecule in **2** but are rotated inward to the hydrogen bond with guanine in **1**. In both cases, the guanine base is sandwiched by the phenolic side chains Tyr42 and Tyr45.

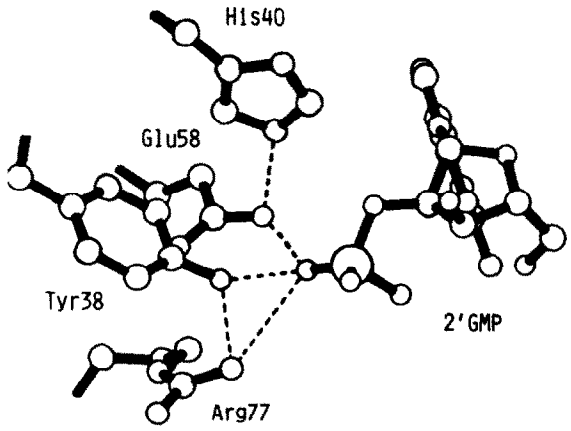

Fig.2. A drawing of the chain of hydrogen bonds observed at the 2'-phosphate portion of the bound inhibitor molecule in the low pH form (1) of the RNase T_1–2'GMP complex.

Consideration of the active site geometry also reveals some major differences between the two crystal forms, especially around the location of the 2'-phosphate group (see fig.2). One of the phosphate oxygen atoms is in hydrogen bonding contact with Arg77NH_2, Tyr38OH and Glu58OE2. The latter interaction requires a proton attached either to phosphate or to Glu58. In **2**, the 2'-phosphate group is in hydrogen bonding contact only with the imidazole ring of His40 whereas the guanidinium group of Arg77 is turned away from the phosphate. In both crystal structures, the imidazole of His92 points toward the phosphate group but is too far to interact directly. However, the model building studies have shown that it would be favourably disposed for hydrogen bonding if a substrate with 3'GMP replaced the inhibitor [5,6].

The differences observed in the RNase T_1–nucleotide interactions cannot be due to the exchange of Gln25 with Lys in isoenzyme because amino acid 25 is located on the α-helix at the surface of the protein molecule and far away from the nucleotide binding site. We have to conclude therefore that at pH 4.0 protonation of some sites on the protein and/or 2'GMP give rise to different interactions and induce conformational changes or vice versa. In this sense, 2'GMP is recognized by RNase T_1 in **2** as a non-productive 'substrate' with weak, yet specific binding whereas in **1**, it is bound

much more tightly (with more hydrogen bonds) as an 'inhibitor'.

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