Three-dimensional structure of the ribonuclease $T_1 \cdot 3'$-guanylic acid complex at 2.6 Å resolution

Shigetoshi Sugio, Ken-ichi Oka, Hirofumi Ohishi, Ken-ichi Tomita and Wolfram Saenger*

Faculty of Pharmaceutical Sciences, Osaka University, 1–6 Yamada-oka, Suita, Osaka 565, Japan and **Institut für Kristallographie, Freie Universität Berlin, Takustr. 6, 1000 Berlin 33, Germany*

Received 29 January 1985

The mother enzyme of RNase T_1 was co-crystallized with its natural product, 3'-GMP at pH 4.0. The X-ray structure of this complex was refined with 2432 reflections in the 5.4–2.6 Å range using a stereochemical restrained method (conventional $R = 27.4\%$). The overall polypeptide chain folding is very similar in the secondary structure elements to the RNase T_1 in the complex with 2'-GMP crystallized also at pH 4.0, but larger conformational changes occur in the loop regions. The base recognition scheme is identical in both complexes but in RNase $T_1 \cdot 3'$-GMP, the ribose phosphate is not seen in the electron density, probably due to static disorder.

X-ray crystallography *Ribonuclease T_1* *3'-Guanylic acid* *Ribonuclease $T_1 \cdot 3'$guanylic acid complex* *Specific recogniton* *Disordered ribose*

1. INTRODUCTION

In the last 3 years, the crystal structures of several prokaryotic (bacterial) and eukaryotic (fungal) ribonucleases (RNases) have been determined by X-ray diffraction methods. The studies suggest that all these enzymes belong to the same family because the active site is formed by a comparable folding of the polypeptide chain into a 4-stranded, antiparallel β-pleated sheet [1]. In the case of one of these RNases, RNase T_1, the complex with 2'-guanylic acid (2'-GMP) was investigated for an isoenzyme (where Gln is exchanged with Lys25 in the mother enzyme), crystallized at pH 5.3 (**1**) [2,3], and for the mother enzyme, crystallized at pH 4.0 (**2**) [4].

In both crystal structures, 2'-GMP adopts the same *syn* conformation and the base is sandwiched between the phenolic side chains of Tyr42 and Tyr45. Moreover, in **1** two hydrogen bonds are formed between guanine O_6, N_1H and peptide groups of Asn43NH, Asn44CO whereas in **2**, hydrogen bonding involves in addition guanine N_2, N_7 and two other peptide groups (Asn44NH, Asn98CO) as well as the side chains of Glu46, Asn43. The ribose phosphate group is bound to the active site (consisting of His40, Glu58, Arg77 and His92) by one His40...phosphate hydrogen bond in **1**, whereas in **2**, the phosphate interacts with Tyr38, Glu58, Arg77. These differences in 2'-GMP binding are not due to the Lys25-Gln substitution in mother and isoenzyme complexes **2** and **1** but are associated with the change in pH. The pH 5.3 isoenzyme-2'-GMP structure **1** appears to correspond to an active complex whereas the mother enzyme-2'-GMP crystallized at pH 4.0, **2** represents an inactive complex [4].

To elucidate the catalytic mechanism of RNase T_1, the complex with the real reaction product, 3'-GMP, is better suited than the inhibitor 2'-GMP. Therefore, this paper describes the co-crystallization (at pH 4.0) of the mother enzyme of RNase T_1 with 3'-GMP and the structural features of the complex as determined by X-ray diffraction methods at 2.6 Å resolution.

* To whom correspondence should be addressed

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/85/$3.30

2. EXPERIMENTAL

The mother enzyme of RNase T_1 used for this study was purified from Taka-diastase [5]. Crystallization of the RNase T_1–3′-GMP complex (**3**) was carried out by a vapor diffusion method with 2-methyl-2,4-pentanediol under the same conditions (pH 4.0) described for the low pH form of the RNase T_1–2′-GMP complex (**2**). The obtained crystals display orthorhombic space group $P2_12_12_1$ with cell dimensions a = 47.58(1), b = 50.92(1), c = 40.32(1) Å. These parameters differ by no more than 2% (for the a-axis) from those reported for the RNase T_1–2′-GMP complexes **1** and **2** [2–4], suggesting that the crystal structures are near-isomorphous. To determine whether the crystal of **3** contains 3′-GMP, the crystals were redissolved and analyzed by paper electrophoresis (pH 8.0). The molar ratio of 3′-GMP to RNase T_1 in the crystal of **3**, 0.9, was estimated by the UV absorption ratio of 258 to 280 nm.

X-ray intensity data were collected on an automated Rigaku-Denki AFC-5 4-circle diffractometer up to 2.2 Å resolution using 4 crystals. After absorption correction [6], scaling [7,8] and merging of overlapping and symmetry equivalent reflections (R_{merge} = 5.4%), 4063 statistically significant reflections above the $4\sigma(Fo)$ threshold were obtained. Assuming that the crystal structure of **3** is roughly isomorphous to that of **2**, at least at low resolution, an initial phase set in the 10–3 Å range was calculated from a structure containing only the main-chain atoms of **2**, yielding an R factor of 44%. The structure was refined by a stereochemically restrained least-squares procedure [9] followed by manual revision of the peptide main-chain and/or by the addition of newly assigned side-chain and solvent atoms using $2Fo$-Fc and Fo-Fc syntheses. The current structure model consisting of 776 atoms (out of 805 atoms) of the complex and of 24 solvent atoms gives an R factor of 27.4% for 2432 reflections in the 5.4–2.6 Å resolution range.

3. RESULTS AND DISCUSSION

In the structure model of the RNase T_1–3′-GMP complex **3**, 17 atoms could not be located with confidence because they belong to the more flexible N-terminus and to the solvent-exposed side chains of Asp3, Gln25, Glu31, Lys41, Asp49.

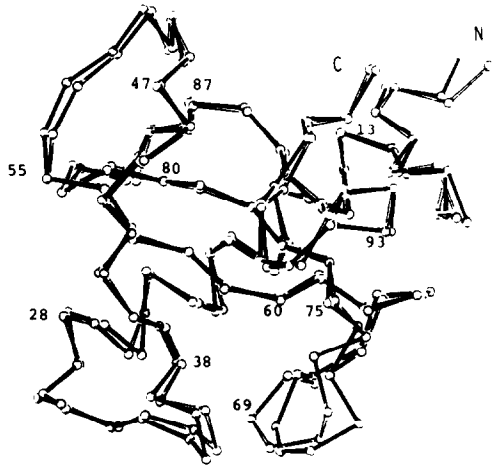

Fig.1. C_α-drawings of the polypeptide chain folding of the RNase T_1–3′-GMP complex **3** (solid lines) and of the RNase T_1–2′-GMP complex **2** (open lines). 'N' and 'C' represent the N- and C-termini, respectively. The two structures can be superimposed with r.m.s. deviation of C_α distances of 0.72 Å. Larger deviations are found at the external loop (residues 69–74) in which the main chain conformations are not well determined in both complexes.

As shown in fig.1, the overall polypeptide folding of **3** is similar to that of **2**. The architecture of the enzyme in the two complexes is retained with r.m.s. deviation of only 0.38 Å for 43 distances between corresponding C_α atoms in the core region including the α-helix (residues 13–28), the 4-stranded anti-parallel β-sheet (residues 38–42, 55–60, 76–80 and 86–91) and the guanine binding site, residues 43–46. Some larger deviations occur in the loop regions, residues 32–38 and 47–55 and, even more pronounced, in the region 69–74. These structural (conformational) changes can be associated with the binding of 3′-GMP instead of 2′-GMP, or they could be due to the differences in crystal packing which are indicated by the change in a-axis dimensions of about 1 Å.

Concerning the 3′-GMP molecule bound to RNase T_1, the electron density for the guanine base and for ribose atom $C_{1'}$ is well defined and can be clearly interpreted, as indicated in fig.2. The interactions between guanine and RNase T_1 are identical to those observed in complex **2**. In contrast to this complex, however, there is no interpretable

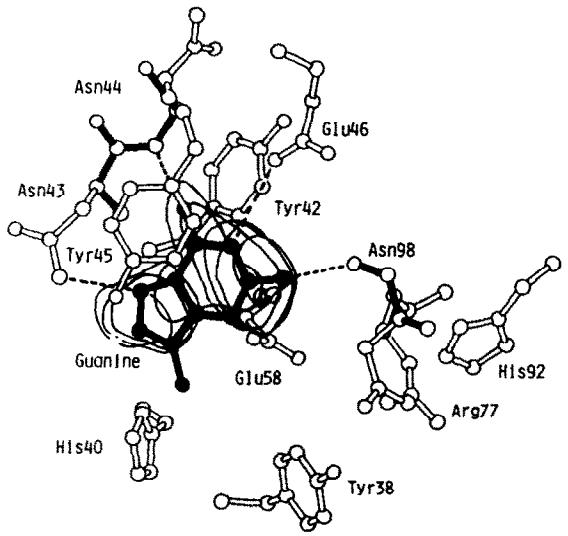

Fig.2. The guanine base recognition site found in the RNase T_1–3′-GMP complex **3**. The guanine base is bound to the enzyme by 4 hydrogen bonds, Asn44N-H...O6Gua, Asn98CO...H-N2Gua, Asn43ND2...N7Gua and Glu460E1...H-N1Gua (dashed lines), and is sandwiched between the phenolic side chains of Tyr42 and Tyr45. The same geometry was observed in **2** [4] but is different in **1** [2,3]. Guanine and peptide main chain bonds are indicated by solid lines, side chains by open lines.

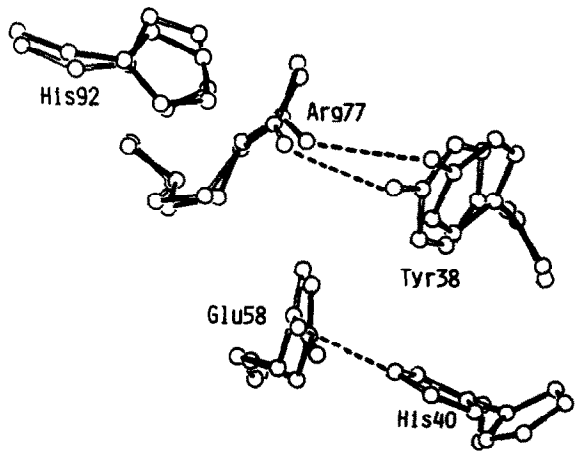

Fig.3. A view of the active site residues in the RNase T_1–3′-GMP complex **3** (solid lines) superimposed on those in the RNase T_1–2′-GMP complex **2** (open lines). Due to a large conformational change of His40 which involves rotations about the C_α–C_β and C_β–C_γ bonds, the imidazole group does not form a hydrogen bond with Glu58 in **3** whereas this interaction occurs in **2** (dashed line).

electron density for the ribose moiety and for the 3′-phosphate group.

Because paper electrophoresis had indicated that the ribose was not hydrolyzed under crystallization conditions (pH 4.0), we conclude that the ribose 3′-phosphate moiety of 3′-GMP is disordered in the complex with RNase T_1. This disorder can be static, if the ribose 3′-phosphate is bound in different RNase T_1 molecules in different, yet closely related sites (due to rotations about the glycosyl $C_{1'}$–N_9 bond), and the overall electron density is smeared out so much that ribose and phosphate groups are not clearly seen; it is also possible that at pH 4.0 some sites are only half protonated and give rise to different hydrogen-bonding schemes. On the other hand, it could well be that the disorder is dynamic and that the RNase T_1 reaction which involves continuous formation of guanosine-2′,3′-cyclic phosphate and its hydrolysis to 3′-GMP, etc., takes place. This process should, however, be associated with movements of the active site residues His40, Glu58, Arg77, His92, which are all clearly seen in the electron density map (fig.3). Moreover, the pH 4.0 is close to an inactive enzyme so that static disorder is more probable.

This finding is surprising because model building studies where 3′-GMP was fitted manually in the active site of the structure of RNase T_1 (determined at pH 5.3) suggested that 3′-GMP would fit ideally, with only minor rotation about the glycosyl link relative to 2′-GMP [3]. In that case, ionized Glu58 would interact with the ribose $O_{2'}H$ (and activate it), and His92 would be in a position to hydrogen bond to the 3′-phosphate group. It appears that, as described for **2** [4], the protonation scheme at the active site is changed drastically at pH 4.0, and therefore the situation for the binding of 3′-GMP may be different compared with pH 5.3.

ACKNOWLEDGEMENTS

The authors wish to thank Professor T. Oshima, Tokyo Institute of Technology, and Dr T. Uchida, Mitsubishi-Kasei Institute of Life Sciences, for technical support and advice on purification of RNase T_1. This work was partly supported by a grant-in-aid for specially distinguished research

(no.59065006) from the Ministry of Education, Science and Culture of Japan, and in Berlin by Sonderforschungsbereich 9, and by Fonds der Chemischen Industrie.

REFERENCES

[1] Hill, C., Dodson, G., Heinemann, U., Saenger, W., Mitsui, Y., Nakamura, K., Borisov, S., Tischenko, G., Polyakov, K. and Pavlovsky, S. (1983) Trends Biochem. Sci. 94, 364–369.

[2] Heinemann, U. and Saenger, W. (1982) Nature 299, 27–31.

[3] Heinemann, U. and Saenger, W. (1983) J. Biomol. Struct. Dyn. 1, 523–538.

[4] Sugio, S., Ohishi, H., Amisaki, T., Tomita, K., Heinemann, U. and Saenger, W. (1985) FEBS Lett. 181, 129–132.

[5] Kanaya, S. and Uchida, T. (1981) J. Biochem. (Tokyo) 89, 591–597.

[6] North, A.C.T., Phillips, D.C. and Matthews, F.S. (1968) Acta Crystallogr. A24, 351–359.

[7] Matthews, F.S. (1975) Acta Crystallogr. A31, 480–487.

[8] Blundell, T.L. and Johnson, L.N. (1976) in: Protein Crystallography, pp.321–380, Academic Press, London.

[9] Hendrickson, W.A. and Konnert, J.H. (1980) in: Biomolecular Structure, Function, Conformation and Evolution (Srinivasan, R. ed.) vol.1, pp.43–57, Pergamon, Oxford.