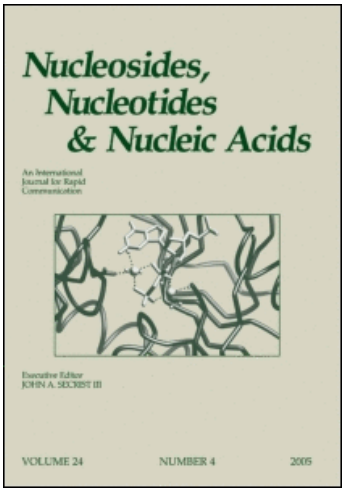

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Solution Structure of Rnase T_1 and Its Complexes with Nucleotides

E. Hoffmann[a]; J. Schmidt[a]; J. Simon[a]; H. Ruterjans[a]
[a] Institut für Biophysikalische Chemie Johann Wolfgang Goethe-Universitat Theodor-Stern, Frankfurt

To cite this Article Hoffmann, E. , Schmidt, J. , Simon, J. and Ruterjans, H.(1988) 'Solution Structure of Rnase T_1 and Its Complexes with Nucleotides', Nucleosides, Nucleotides and Nucleic Acids, 7: 5, 757 — 761
To link to this Article: DOI: 10.1080/07328318808056325
URL: http://dx.doi.org/10.1080/07328318808056325

NUCLEOSIDES & NUCLEOTIDES, 7(5&6), 757-761 (1988)

SOLUTION STRUCTURE OF RNASE T_1 AND ITS COMPLEXES WITH NUCLEOTIDES

E. Hoffmann, J. Schmidt, J. Simon, and H. Rüterjans*

Institut für Biophysikalische Chemie
Johann Wolfgang Goethe-Universität
Theodor-Stern Kai 7, Haus 75A
D-6000 Frankfurt 70

Abstract. The solution structure of RNase T_1 and its complexes with 2'-GMP and 3'-GMP have been investigated by combined use of 2D-NMR spectroscopy and restrained molecular dynamics calculations (MD). An analysis of the nuclear Overhauser effects (NOEs) observed indicates the presence of one α helix as well as of two antiparallel β sheets. Interaction of the nucleotides with the active site leads to changes of the backbone conformation of the amino acids involved. However, the interaction between the protein and 3'-GMP is not as strong as the interaction with 2'-GMP, possibly because of weaker binding.

Introduction. RNase T_1 (MW: 11085; Fig. 1) is a globular protein of 104 amino acids. It generally cleaves single-stranded RNA at the 3' end of guanosine nucleotides via 2',3'-cyclophosphate intermediates. 2'-GMP and 3'-GMP act as competitive inhibitors (1). 2'-GMP forms a tighter complex with RNase T_1 than 3'-GMP. The present study reveals not only the folding manner of the enzyme but also some interesting features of protein-nucleotide interactions.

Materials and Methods. RNase T_1 was isolated from *Aspergillus oryzae* extract (Luitpold Company, Munich), as previously described (2,3). The backbone ^1H resonances of 95 amino acids and most of the side-chain ^1H resonances of RNase T_1 and its complexes with 2'-GMP and 3'-GMP were assigned by using 2D-NMR techniques (4) at 500 MHz.

Results. The secondary structure elements of RNase T_1 were derived from their characteristic medium- and long-range NOEs. For RNase T_1 two antiparallel β sheets and one α helix as well as some β turns were observed. The first β sheet is located near the N-terminus and involves two strands. The second pleated sheet is built up by five strands and forms most of the hydrophobic core of the protein (Fig. 1). This is in contrast to the X-ray structure of RNase T_1, in which only one four-

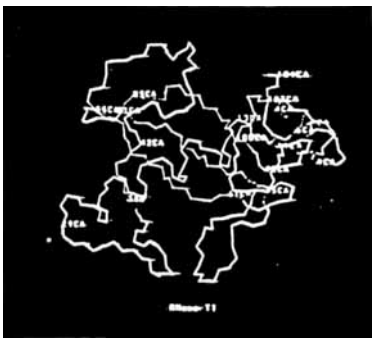

Fig. 1 (left): Backbone conformation of the free RNase T_1 as obtained from a restrained MD calculation in vacuo

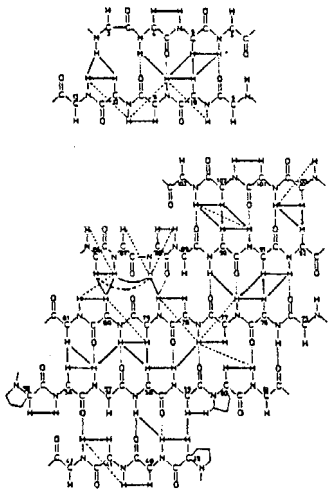

Fig. 2 (right): The β-sheet structure of RNase T_1 as derived from ^{1}H-NMR data.
The smaller sheet involves the amino acid residues Y4 to C6 and N9 to Y11. The larger sheet contains the amino acids residues P39 to Y42 (strand 1), P55 to I61 (strand 2), A75 to N81 (strand 3), L86 to H92 (strand 4), and F100 to C103 (strand 5)
-·-·-·- : short sequential NOEs
——— : $d_{NN}(i,j)$, $d_{\alpha N}(i,j)$ and $d_{\alpha\alpha}(i,j)$ connectivities
····· : backbone hydrogen bonds derived from the respective N_iH - C_i^1H cross peaks in D_2O solution and from NOE data. Note the β bulge at residue A87 in strand 4 of the β sheet

stranded β sheet was found. The β-sheet structures of RNase T_1 are shown in Fig. 2. The α helix of RNase T_1 is located between S13 and D29 and contains 4.5 turns (Fig. 1).

A low-resolution tertiary structure can be derived from the diagonal-plot representation of the NOE data (Fig. 3). The row of NOEs parallel to the diagonal between the amino acids S13 and D29 indicates the α-helix conformation, whereas the row of NOEs, which are arranged

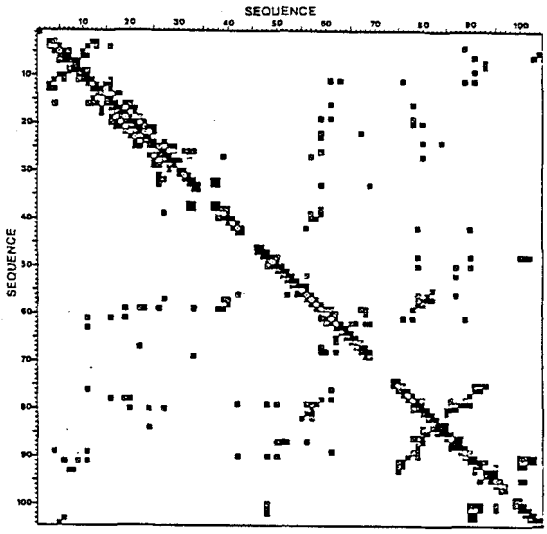

Fig. 3: Diagonal plot of the NOEs used for the determination of the secondary and tertiary structure of RNase T_1-2'-GMP-complex
■ : NOEs between backbone protons
⊠ : NOEs between backbone and side chain protons
▨ : NOEs between side chain protons
When two residues are connected by more than one NOE, only the one which involves the larger number of backbone protons is shown

perpendicularly to the diagonal, indicates the antiparallel β-sheet strands. The two strands of the N-terminal β sheet are connected by a β turn. This β sheet is in contact with the N-terminal end of the α helix. The α helix is near the second strand of the large β sheet. Some contacts of the α helix with side chains of the third strand are observed. The α helix is followed by a loop. Extended loops are formed between strand 1 and 2, as well as between strands 2 and 3, of the large β sheet. Strands 3 and 4 of this sheet are connected by a β turn, and between strands 4 and 5 there is another loop. Finally the NOEs between residues Y4 to C6 and C103 to T104 are proof for the proximity of the N-terminal end of RNase T_1 and its C-terminal end formed by a disulfide bridge between C6 and C103.

The differences in structure of the free RNase T_1 and its 2'-GMP and 3'-GMP complexes are only minimal. Therefore nearly identical NOE values were obtained for the free enzyme and its nucleotide complexes. More sensitive indications of structural changes induced with complex formation can be obtained by comparing the chemical shifts of the resonances of the backbone protons in the free enzyme and those in its nucleotide complexes (Fig. 4).

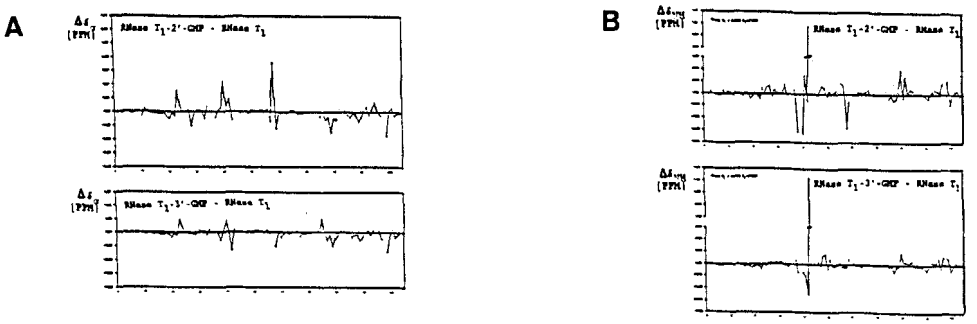

Fig. 4: Differences of the chemical shift values of the $C^{\alpha}H$ (A) and NH (B) resonances of the RNase T_1 complexes and RNase T_1

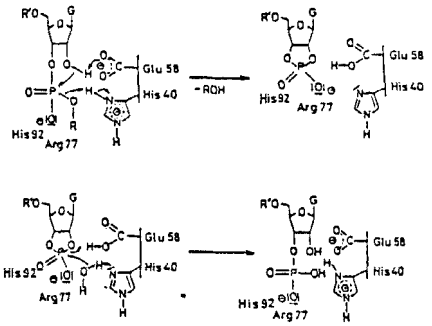

Fig. 5 (left): Possible mechanism of RNase T_1-catalyzed RNA hydrolysis

Table 1 (right): Hydrogen bonds between the inhibitors and RNase T_1

RNase T_1-2'-GMP				RNase T_1-3'-GMP		
	Donor	Acceptor	[Å]	Donor	Acceptor	[Å]
Base	N44-H42N	N7	45	T47-OH	N7	38
	T43-NH	O6	31	T43-OH	O6	44
	E46-NH	O6	31	E46-NH	O6	32
	H18	E46-Oε1	32	F100	O6	23
	H18	N99-Oδ1	44	H18	E46-Oε1	23
	H28	N99-Oδ1	78	H18	E46-Oε2	18
				H18	N99-Oδ1	33
				H28	N99-Oδ1	77
Phosphate	Y38-OH	O3P	100	S35-OH	O3P	90
	N40-H42N	O3P	32	N36-NH	O1P	14
	S77-HrH	O2P	49	N40-H42N	O1P	13
	N98-H62N	O1P	30	HO3P	N36-Oδ1	19
				HO3P	Y38-O(H)	78
Ribose	N40-H62N	O3'	12	Y38-OH	O2'	38
	N43-H62N	O4'	13	N43-H62N	O4'	36
	N98-H62N	O5'	30	N98-H62N	O3'	67
	N98-H62N	O3'	17	O2'H	E58-Oε2	17
	O3'H	N36-Oδ1	68			
	O3'H	Y43-O(H)	42			

The essential results can be summarized as follows:

1. Interaction of the inhibitors with the active site leads to considerable changes of the conformation of the backbone of these amino acids. Correspondingly larger changes are observed for amino acids that are directly involved in binding.
2. Conformational changes are also observed in the loop regions that are directly attached to the active site and in the contact area between the α helix and the five-stranded β sheet.

3. The structure of the RNase T_1-3'-GMP complex is more similar to that of the free enzyme than that of the RNase T_1-2'-GMP complex.
4. Y42, Y45, and N98 and/or N99 are directly involved in binding of the guanine base. N43 and E46 may be also involved.
5. Y38, H40, and E58 are involved in phosphate binding or in the catalysis. This is not sure for R77 and H92.

Restrained MD calculations (8) were performed for the free enzyme as well as for the nucleotide complexes for 20 ps to 30 ps in vacuo. 500 to 600 NOE restraints were used per MD calculation. It is evident that larger conformational differences are found only in the loop regions of RNase T_1. The contacts between the inhibitors and RNase T_1 (Table 1) lead to a possible mechanism of RNase T_1-catalyzed RNA hydrolysis which is depicted in Fig. 5.

REFERENCES

(1) Takahashi, K. and Moore, S. (1982) pp. 435-468 in "The Enzymes", Vol. XV: Nucleic Acids, Part B (3rd ed.; P.D. Boyer, ed.). Academic Press, New York.

(2) Fülling, R. and H. Rüterjans, H. (1978) FEBS Lett. 88, 279-282.

(3) Hoffmann, E.; Dissertation, Frankfurt am Main (1988)

(4) Hoffmann, E., and Rüterjans, H., in preparation

(5) Van Gunsteren, W.F., Kaptein, R., and Zuiderweg, E.R.P. (1984) Proc. NATO/CEGAM Workshop on Nucleic Acid Conformation and Dynamics, Orsay (W.K. Olsen, ed.), 79-92.