Modes of binding of guanosine monophosphates to ribonuclease T_1 – A computer-modelling study

P. V. Balaji, W. Saenger† and V. S. R. Rao*

Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560 012, India
†Institute for Crystallography, Free University Berlin, Takustrasse 6, D-1000, Berlin 33, Germany

Computer-modelling studies on the modes of binding of the three guanosine monophosphate inhibitors 2′-GMP, 3′-GMP, and 5′-GMP to ribonuclease (RNase) T_1 have been carried out by energy minimization in Cartesian-coordinate space. The inhibitory power was found to decrease in the order 2′-GMP > 3′-GMP > 5′-GMP in agreement with the experimental observations. The ribose moiety was found to form hydrogen bonds with the protein in all the enzyme–inhibitor complexes, indicating that it contributes to the binding energy and does not merely act as a spacer between the base and the phosphate moieties as suggested earlier. 2′-GMP and 5′-GMP bind to RNase T_1 in either of the two ribose puckered forms (with C3′-*endo* more favoured over the C2′-*endo*) and 3′-GMP binds to RNase T_1 predominantly in C3′-*endo* form. The catalytically important residue His-92 was found to form hydrogen bond with the phosphate moiety in all the enzyme–inhibitor complexes, indicating that this residue may serve as a general acid group during catalysis. Such an interaction was not found in either X-ray or two-dimensional NMR studies.

RIBONUCLEASE (RNase) T_1 (EC 3.1.27.3), secreted by the fungus *Aspergillus oryzae*, is an endonuclease, whose sequence of 104 amino-acid residues is known[1]. It acts only on single-stranded RNA and hydrolyses the P–O5′ phosphodiester bonds on the 3′-side of guanine nucleosides with very high specificity. A variety of physicochemical techniques like NMR and CD spectroscopy, UV difference spectroscopy, chemical modification and kinetic studies have been used [2–4] to elucidate the specific recognition of guanine by RNase T_1. Recent 1.9-Å resolution X-ray crystallographic studies of the Lys25- (ref. 5) and Gln25-RNase T_1–2′-GMP (ref. 6) complexes agree in general with each other regarding the conformation of the bound 2′-GMP molecule (C2′-*endo syn*) but differ in the nature of the hydrogen bonds between 2′-GMP and RNase T_1. The hydrogen-bonding scheme proposed for the RNase T_1–2′-GMP complex based on two-dimensional NMR spectroscopic studies[7] also differs significantly from each of the schemes proposed from X-ray studies (Table 1). A 2.6-Å-resolution X-ray crystallographic study of the RNase T_1–3′-GMP complex[8] showed that the main-chain polypeptide folding is very similar to that seen in the Lys25-RNase T_1–2′-GMP complex[5]. This study could reveal only the hydrogen bonds between the base and the protein (Table 2), which are very similar to those observed in the Lys25-RNase T_1–2′-GMP complex. In contrast, 2D NMR studies[7] predicted that the structure of the RNase T_1–3′-GMP complex is more similar to that of the uncomplexed enzyme rather than to the RNase T_1–2′-GMP complex. The conformation of 3′-GMP in the RNase T_1–3′-GMP complex was not indicated by either the X-ray[8] or the 2D NMR[7] studies. However, ^{1}H NMR investigations[9] on the complexes of RNase T_1 with 2′-GMP, 3′-GMP and 5′-GMP have indicated that 2′-GMP and 3′-GMP adopt C3′-*endo syn* conformation and 5′-GMP adopts C3′-*endo anti* conformation when bound to the enzyme. Thus the puckering of the ribose moiety in the RNase

Table 1. Hydrogen-bonding scheme in the RNase T_1–2′-GMP complex.

	X-ray (Ref. 5)	X-ray (Ref. 6)	2D NMR (Ref. 7)	Present calculations (C2′-*endo*)
Guanine				
N1H	E46 OE1	E46 OE1	E46 OE1 N99 OD1	E46 OE1
N2H	E46 OE2 N98 O	N98 O	N99 OD1	E46 OE2 N98 O
O6	N44 N–H Y45 N–H	N44 N	N44 N–H Y45 N–H	N44 N–H Y45 N–H
N7	N43 N–H	N43 N–H N43 HD2	N44 HD2	N43 N–H N43 HD21
Ribose				
O2′				H40 HE2
O3′			H40 HE2 N98 HD2	
O4′			N43 HD2	
O5′			N98 HD2	
O5′H			Y45 OH	N98 OD1
Phosphate				
O1	Y38 HH H40 HE2 E58 OE2		N98 HD2	N36 HD21
O2			R77 NE	Y38 HH E58 HE2 R77 HE2
O3		Y38 HH E58 HE2 R77 NH2	Y38 HH H40 HE2	H92 HE N98 HD22

Amino-acid residues of RNase T_1 are indicated by the single-letter code for amino acids and the residue number.

*For correspondence.