THE STRUCTURE OF THE COMPLEX OF RIBONUCLEASE S WITH FLUORIDE ANALOGUE OF UpA AT 2.5 Å RESOLUTION

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Received 12 June 1978

1. Introduction

Different physico-chemical methods are used to estimate the mutual position of substrate and enzyme in the course of the catalytic act. A widely exploited approach involves the study of complexes with substrate-like molecules, taking into account their individual inhibitory properties.

Such information for pancreatic ribonuclease, obtained mainly by X-ray and NMR methods, is most thoroughly surveyed in [1,2]. Much of that information was deduced from studies of enzyme–product complexes with nucleoside-3′-phosphates. Atomic coordinates have been published both for the enzyme (RNase S) [3,4] and the enzyme-bound UpA analogue (UpcA) in which the 5′-oxygen of adenosine is replaced by a CH_2 group [3].

The present report describes the results of an X-ray study of RNase S in complex with a non-hydrolysed analogue of UpA, 2′-deoxy-2′-fluoro uridilyl-3′,5′-adenosine, abbreviated further as 2′-F-dUpA. This analogue is assumed to take up, on the enzyme, a position very close to that of unmodified UpA because replacement of 2′-OH by 2′-F produces no significant changes in the conformational state of the ribose, as follows from the practically identical physico-chemical properties and conformation of 2′-F-dUpA and UpA [3].

From the difference maps of electron density at 3–2.5 Å resolution it has been possible to establish the position of the analogue in the active site of the enzyme, to deduce the conformation of 2′-F-dUpA bound to RNase S, and to reveal specific conformational changes of the protein caused by ligand binding.

2. Materials and methods

Crystals of RNase S were grown as described in [5], 2′-F-dUpA was synthesized according to [6], the crystals of the enzyme analogue complex were obtained by soaking of crystals of RNase S in 10 mM 2′-F-dUpA saturated to 75% with ammonium sulphate in 0.1 M sodium acetate, pH 5.5 and 7.2.

Three-dimensional sets of X-ray intensities were collected for crystals of RNase S and its complexes with 2′-F-dUpA (pH 5.5 and 7.2) on a Syntex $P2_1$ diffractometer with a modified collimation system and software. It has been possible to collect a complete set of intensities up to 2.5 Å resolution from one crystal. Primary data reduction included usual Lp and absorption corrections [7], in subsequent scaling a factor $k \cdot \exp(-B \cdot \sin^2\theta/\lambda^2)$ has been applied with least-square selection of k and B.

The difference electron density maps were calculated using, as Fourier terms, the differences in experimental structure amplitudes phased by calculated protein phases obtained by T. Powers [4] in the course of refinement of the structural data in [8]. In some cases, electron density maps of the whole complex proved to be useful.

Atomic coordinates of 2′-F-dUpA were refined by searching for the best fit of the atomic model to electron density using the algorithm described in [9]. For this purpose, a region of the difference map corresponding to the position of the analogue was used. The refinement included optimization of orientational and positional parameters for 2′-F-dUpA as a whole as well as the conformational angles of rotation around ordinary bonds. Changes in the conformation were

constrained by fixed standard values of interatomic distances and bond angles [10,11]. The conformational state of ribose rings was determined by a sequential trial of different possible standard conformations. The real conformation was estimated as that corresponding to the maximal value of overlapping function.

3. Results

Table 1 shows the relative values of electron density in some regions of the difference maps for several complexes. On the map for the dinucleoside phosphate, two extended regions of positive density were observed which correspond to nucleoside portions of the ligand (in sites B_1, R_1, B_2 and R_2, nomenclature as in [1]). In site p_1, which should be the place of phosphate binding, the density is certainly insufficient to account for phosphate. It may be explained by the presence, at p_1 in free RNase S, of a sulfate anion [8] displaced by the phosphate moiety of the ligand when the complex was formed. Nevertheless, on the difference maps at p_1, a pair of positive and negative peaks was observed which indicates a difference in the position of the ligand phosphate group and a sulphate anion at the enzyme site. A similar observation was made in the work on the complex of RNase S with 2′-CMP [12].

From the maps obtained it proved to be possible to establish the conformation of dinucleoside phosphate in the complex studied and to determine its atomic coordinates (table 2). Uridine fragment of 2′-F-dUpA in the complex was found to be in an *anti* conformation with χ_{CN} being of about 220°. A close value of χ_{CN} was observed for 2′-CMP bound to RNase S [12].

For the adenosine fragment of 2′-F-dUpA, the best fit to the map was found to be in close correspondence to *syn* conformation with a value of χ_{CN} of about 125°. This conclusion may be considered as a direct consequence of the applied criterion of choice. It should be noted however that stereochemical estimates derived from the difference maps provide only the most probable basic conformation, not excluding the possible presence of some other conformations, with a preference for the conformation mentioned. The mixture of *syn* and *anti* conformers of purine nucleotides observable in solution [13] implies a small difference in energy between them. So minor variations in the character of the bound nucleotide may provide the preference for an *anti* conformation as described for UpcA [3] or the preference for a *syn* conformation observed for 2′-F-dUpA. These differences support a conclusion about the absence of conformational specificity for B_2 site of the enzyme. (Recently an *anti* conformation was observed also in the purine portion of the RNase S complex with cytidilyl-2′,5′-adenosine, certainly a more distorted version of the natural substrate [14].)

The position of 2′-F-dUpA relative to the functional

Table 1
Maxima of electron density (in e/A^3) in specific regions of the difference maps for several complexes of RNase S

	2′-F-dUpA (10 mM)			3′-CMP [12] (3 mM)	2′-CMP [12] (2 mM)
	pH 5.5		pH 7.2	pH 5.5	pH 5.5
	2.5 A	3 A	3 A	3 A	3 A
Pyrimidine	0.46	0.53	0.60	0.68	0.62
Pyrimidine Ribose	0.63	0.51	0.55	0.53	0.38
Adenosine Ribose	0.64	0.56	0.56		
Adenine	0.57	0.51	0.63		

Table 2
Atomic coordinates of 2′-F-dUpA bound to RNase S (for definition of the coordinate system see [3])

	Atoms	X	Y	Z
Uridine	N1	−10.8	− 6.6	3.8
	C2	−11.8	− 7.5	4.0
	O2	−12.8	− 7.2	4.6
	N3	−11.6	− 8.8	3.6
	C4	−10.4	− 9.1	2.9
	O4	−10.3	−10.3	2.5
	C5	− 9.5	− 8.2	2.7
	C6	− 9.7	− 6.9	3.2
	C1′	−11.1	− 5.2	4.4
	O1′	−10.6	− 4.3	3.4
	C2′	−10.4	− 4.9	5.7
	F2′	−11.3	− 4.3	6.3
	C3′	− 9.0	− 4.4	5.3
	O3′	− 8.5	− 3.5	6.3
	C4′	− 9.5	− 3.6	4.0
	C5′	− 8.4	− 3.4	2.9
	P	− 9.3	− 3.0	7.6
	O	− 9.6	− 4.1	8.4
	O	−10.7	− 2.5	7.1
Adenosine	N1	− 2.4	− 4.6	7.6
	C2	− 3.3	− 3.7	7.4
	N3	− 3.9	− 2.9	8.2
	C4	− 3.4	− 3.1	9.5
	C5	− 2.5	− 4.0	9.9
	C6	− 2.0	− 4.8	8.9
	N6	− 1.0	− 5.7	9.1
	N7	− 2.3	− 3.9	11.2
	C8	− 3.1	− 2.9	11.6
	N9	− 3.8	− 2.4	10.6
	C1′	− 4.8	− 1.3	10.7
	O1′	− 6.0	− 1.8	10.1
	C2′	− 4.5	− 0.1	10.0
	O2′	− 5.0	1.0	10.9
	C3′	− 5.2	− 0.2	8.7
	O3′	− 5.5	1.1	8.2
	C4′	− 6.5	− 0.9	9.1
	C5′	− 7.1	− 1.7	7.9
	O5′	− 8.5	− 2.0	8.3

groups of the protein has been analyzed using the atomic coordinates of the analogue along with the atomic coordinates of RNase S derived from refinement [4]. Certain possibilities have been found for hydrogen bonds stabilizing the position of 2′-F-dUpA in the active site of the protein: $> C2 = O2_{uridine}$ $HN <_{Thr\ 45\ (main\ chain)}$, the bond length 2.8 Å, and $> NH_{uridine}$ $O\gamma_{Thr\ 45}$, the bond length 2.8 Å. The same hydrogen bonds have been reported also for UpcA [1,3] as well as the hydrogen bond between $> C4 = O4_{uridine}$ and −OH of Ser 123. In the case of 2′-F-dUpA, the latter hydrogen bond should not exist as the distance $O4_{uridine}$ $O_{\gamma_{Ser\ 123}}$ is equal to 4.4 Å.

The distances from the phosphorus atom to N_δ of the imidazole rings of His 12 and His 119 were found to be 4.3 Å and 4.4 Å, respectively. It should be noted, however, that the atomic coordinates of His 119 derived for free RNase S may not be satisfactory since the complex formation produces a shift of the imidazole ring of His 119 in the direction of the phosphate group.

Another important feature is the distance between the fluorine atom and N_δ of His 12 which is equal to 3.0 Å. Assuming the position of uridine ribose in an enzyme–substrate complex as close to that in the complex with 2′-F-dUpA, the conclusion may be drawn that a hydrogen bond between the 2′-OH group of the ribose and N_δ of His 12 exists in the productive complex. This hydrogen bond is considered to be important in all mechanisms of enzyme action suggested for RNase [1]. The distance between the fluorine atom and the nitrogen of the carboxyamide group of Asn 44 has been estimated as 3.8 Å. The hydrogen bond between the 2′-OH group of the ribose and the side chain of Asn 44 was found to exist in all RNase–nucleotide complexes studied so far [1,2].

The mutual orientation of the phosphodiester bond and the fluorine atom in 2′-F-dUpA bound to the enzyme appears to be close to that of the productive complex for the formation of the pentacovalent intermediate: the fluorine atom (replaced 2′-OH group of the enzyme–substrate complex) lies opposite O5′ and on the extension of the line P-O5′. The distance between F2′ and P is equal to about 2.8 Å.

Since the catalytic activity of the enzyme has a maximum at pH 7, we compared the structure of the complex at pH 5.5 to that at pH 7.2. The position and conformation of dinucleoside phosphate in the complex at pH 5.5 and pH 7.2 are practically identical. The main difference is that at pH 7.2 the adenosine moiety of 2′-F-dUpA seems to be less flexible whereas the phosphate group is more loosened as follows from the values of electron density in the region mentioned.

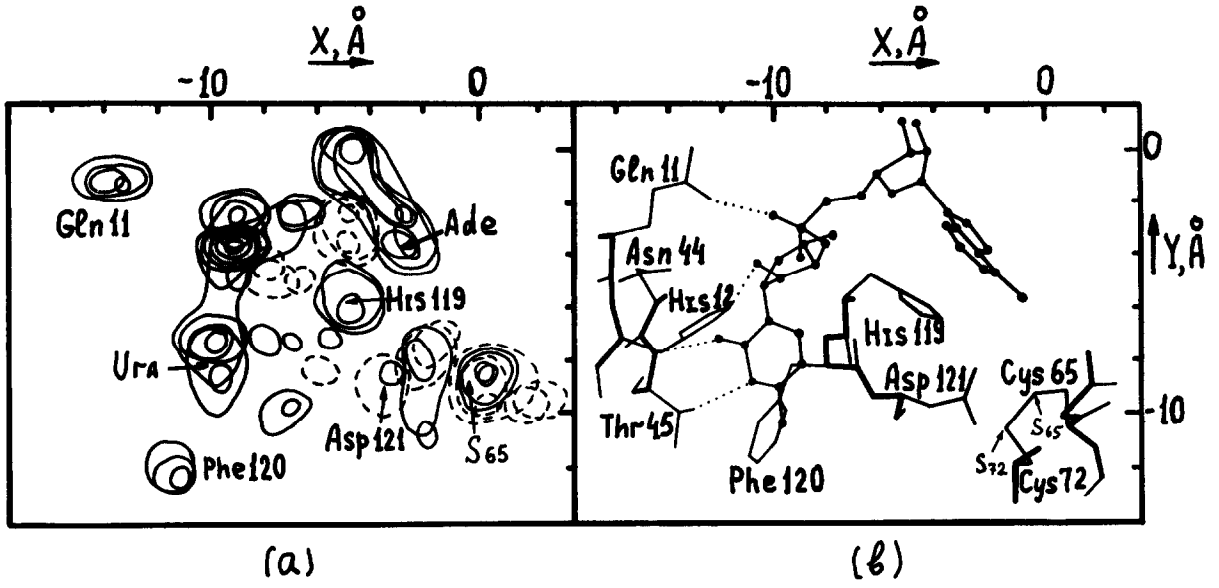

Fig.1. (a) Part of the difference electron density map for the complex of RNase S with 2′-F-dUpA (superposition of several sections), solid lines indicate positive, broken lines negative electron density. (b) The position of 2′-F-dUpA relative to the enzyme as inferred from the difference map. Probable hydrogen bonds are shown by dotted lines.

The complex formation induces noticeable conformational changes of RNase S. A relatively small shift and tighter fixation of the side chain of Phe 120 seems to cause movement of the main chain in the region Phe 120–Asp 121 in the direction from C^{α}_{121} to C^{α}_{120} (fig.1). A pair of negative and positive peaks also indicated a shift of the side chain of Asp 121. There is a positive density on the difference map near the side group of Gln 11 which may be explained by its stabilization due to interaction of the carboxyamide group with the free oxygen of the phosphate group. Most prominent peaks are placed in the region of the loop formed by residues 65–72 and stabilized by an S–S bond. A movement in this region has a direction in common with that for residues 120 and 121. It may be noted that a change in the position of the sulfur atom of Cys 65 (clearly seen on the difference maps) provides room for binding the adenosine part of 2′-F-dUpA. It seems to be of some importance that similar conformational changes, including well observable shifts in the region of the loop 65–72, were found also in the study of complexes of RNase S with 3′-CMP and 2′-CMP [12].

A most prominent conformational change resulting from complex formation involves the shift of the imidazole ring of His 119 in the direction of p_1 which should provide a closer contact of this residue with the phosphate group of the 2′-F-dUpA (fig.1). This shift was not observed in the study of the complex of RNase S with mononucleotides [12].

The results obtained suggest that the structure of the complex of the RNase S with 2′-F-dUpA corresponds well to that of the enzyme–substrate productive complex. In addition, the conformational changes resulting from complex formation are produced by the binding of the uridine fragment of 2′-F-dUpA and give rise to the formation of an optimally positioned productive enzyme–substrate complex.

Acknowledgements

The authors are grateful to Professor B. K. Vainstein for constant interest in this work, Yu. V. Nekrasov for great help in the X-ray experiment, and T. Powers who kindly provided the refined atomic coordinates of RNase S and calculated phases of structure factors.

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