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Simulation of the Charge Relay Structure in Ribonuclease A

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Ribonuclease A is well known from X-ray diffraction analysis as an enzyme which changes the conformation, when the substrate binds with the enzyme. Therefore amino acids were rotated in order to simulate the coupling movements of the amino acid side chains, water molecule, and the substrate. From the study of the movements of amino acids and the quantum chemical calculations, a charge relay structure composed of Asp 121, His 119 and a water molecule was simulated in the active site of the ribonuclease A. And the water located in the very position where the hydroxyl group of the water molecule can attack phosphorus of the substrate in the second step of the hydrolysis. Moreover it was reported that Lys 7 as well as Lys 41 may participate in the enzymatic reaction.

Keywords——Charge Relay Structure; enzymatic reaction; molecular orbital; structure; ribonuclease A; complex; enzyme; proton transfer; histidine; aspartic acid

There has been great progress in understanding the reaction mechanism of ribonuclease A (RNase A). The active site of RNase A was clarified from X-ray diffraction analyses.[2,3] The side chains of His 12, His 119 and Lys 41 have been implicated in the active site by chemical studies.[4] A reaction mechanism was presented by Findlay *et al.*[5] The reaction was divided into two steps, which were transphosphorylation from 3′ oxygen to 2′ oxygen of ribose and hydrolysis of ribose 2′, 3′-cyclophosphate following the general base catalysis of His 119 for a water near the cyclicphosphate part. In this paper, the second step is studied.

Recently we noticed that there is aspartate near histidine in the active site of RNase A. If such a charge relay structure as acyl-α-chymotrypsin is simulated in RNase A, the structure among aspartate, histidine and water molecule should be fitted into appropriate positions in order to smoothly transfer a proton with the low potential barriers. However the conformation between the aspartate and the histidine is not similar to that in the charge relay structure of acyl-α-chymotrypsin.[6] On the other hand, the structure obtained from X-ray analyses may not be one which the enzyme actually assume when it is acting on the substrate.[3] Therefore displacements of the amino acid side chains may permit the active site to shape itself for the optimal interaction with the substrate. In those circumstances, we thought that the study of the movements of the amino acid side chains will shed light on the reaction mechanism of RNase A. As the result, we could simulate the charge relay structure composed of water molecule, His 119 and Asp 121.

1) Location: *9-1, Shirokane 5-chome, Minato-ku, Tokyo 108, Japan.*

2) G. Kartha, J. Bello, and D. Harker, *Nature* (London), **213**, 862 (1967).

3) *a*) H.W. Wyckoff, K.D. Hardman, N.M. Allewell, T. Inagami, D. Tsernoglou, L.N. Johnson, and F.M. Richards, *J. Biol. Chem.*, **242**, 3749 (1967); *b*) H.W. Wyckoff, K.D. Hardman, N.M. Allewell, T. Inagami, L.N. Johnson, and F.M. Richards, *J. Biol. Chem.*, **242**, 3984 (1967); *c*) H.W. Wyckoff, D. Tsernoglou, A.W. Hanson, J.R. Knox, B. Lee, and F.M. Richards, *J. Biol. Chem.*, **245**, 305 (1970); *d*) "Atlas of Molecular Structures in Biology," Edited by D.C. Philips, and F.M. Richards, 1973, p. 7

4) *a*) C.H.W. Hirs, *Brookhaven Symp. Biol.*, **15**, 154 (1962); *b*) A.M. Crestfield, W.H. Stein, and S. Moore, *J. Biol. Chem.*, **238**, 2413 (1963); *c*) A.M. Crestfield, W.H. Stein, and S. Moore, *J. Biol. Chem.*, **238**, 2421 (1963).

5) D. Findlay, D.G. Herries, A.P. Mathias, B.R. Rabin, and C.A. Ross, *Biochem. J.*, **85**, 152 (1962).

6) R. Henderson, *J. Mol. Biol.*, **54**, 341 (1970).

Method

All molecular orbital calculations were performed by using the Complete Neglect Differential Overlap method (the CNDO/2 method).[7]

As the coordinates of RNase, the data of Wyckoff *et al.* was used.[3d] Since they did not report the coordinates of hydrogens in RNase A, they were determined as follows; r(CH)=1.09 Å and $<$XCH=109°28′ for $-CH_3$, and $-CH_2-$ group; r(NH)=1.0 Å and $<$XNH=120° for $-NH_2$ and –NH–; r(OH)=0.956 Å and $<$XOH=108.9° for alcoholic group; r(NH)=1.0 Å and r(CH)=1.09 Å for the imidazole group of the histidine.

Dinucleotide analogue UpcA similar to substrate UpA was used in X-ray diffraction analysis of RNase A. The inhibitor UpcA must be replaced by uridine 2′,3′-O,O-cyclophosphate in the second step of the enzymatic reaction. Since Saenger and Eckstein reported crystal and molecular structure of the triethylammonium salt of uridine 2′,3′-O,O-cyclophosphorothioate[8] which has the almost same K_m value for pancreatic RNase as uridine 2′,3′-O,O-cyclophosphate,[9] the thioate compound was positioned in the active site of RNase A. In this replacement, the uracil ring was fixed, since its ring is rigidly bound to the pocket in the active site. Plane O–P–S (named "E" plane in the paper by Saenger and Eckstein) is perpendicular to ribose plane. The sulfur in the plane O–P–S was replaced by an oxygen, and the bond length and bond angle of O–P–O was optimized within the E plane by using the CNDO/2 method; the angle O–P–O was 115.4° and two bond

Table I. Coordinates of Uridine 2′,3′-O,O-Cyclophosphate in RNase A[a]

	Atom	x	y	z
1	P	−11.49518	−2.17267	4.72023
2	S	−12.02975	−0.88234	3.36304
	O (A)[b]	−11.98950	−1.09296	3.53696
	O′ (A)[c]	−11.94634	−1.18725	3.64028
3	O	−11.87908	−1.86930	6.11189
	O (B)[b]	−11.95157	−1.78246	6.28538
	O′ (B)[c]	−11.97099	−1.76587	6.35197
	H′ (B)[c]	−12.46796	−0.91408	6.18620
4	O (1′)	−9.68152	−4.62906	2.34084
5	O (2′)	−11.92741	−3.67517	4.34926
	H′ (2′)[c]	−11.19138	−4.35139	4.38086
6	O (3′)	−9.94122	−2.40452	4.64062
	H′ (3′)[c]	−9.68561	−3.37003	4.59107
7	O (5′)	−6.56139	−4.35784	4.22593
8	C (1′)	−10.81314	−5.23139	2.92992
9	C (2′)	−10.88750	−4.63060	4.39391
	H[d]	−9.04872	−4.15131	5.48378
	H[d]	−8.89615	−4.02016	3.71527
10	C (4′)	−9.55716	−3.85534	4.56617
	H[d]	−11.07618	−5.30062	5.23270
	H[d]	−10.90654	−5.19865	3.46383
11	C (4′)	−8.73656	−4.09474	3.29188
12	C (5′)	−7.58320	−5.02651	3.50795
13	N (1)	−10.60000	−6.70000	3.00000
14	C (2)	−11.59910	−7.47602	3.45953
15	O (2)	−12.60027	−6.94945	4.00013
16	N (3)	−11.47007	−8.80000	3.45449
17	C (4)	−10.39645	−9.44786	2.89823
18	O (4)	−10.34354	−10.68642	2.97614
19	C (5)	−9.40324	−8.66943	2.29123
20	C (6)	−9.56091	−7.33725	2.30266

a) Coordinates (Å) corresponds to the order of atoms in Table I of the paper by Saenger and Eckstein.
b) Coordinates after the energy optimization by using the CNDO/2 method.
c) Coordinates in the interaction between H_3PO_4 and NH_3.
d) Coordinates in the interaction between cyclicphosphate and OH^-.

7) J.A. Pople and G.A. Segal, *J. Chem. Phys.*, **44**, 3289 (1966).
8) M. Saenger and F. Eckstein, *J. Am. Chem. Soc.*, **92**, 4712 (1970).
9) F. Eckstein, *FEBS Lett.*, **2**, 85 (1968).

lengths of P–O were 1.676 Å. Thus the structure of uridine 2′,3′-O,O-cyclophosphate was determined. The coordinates are shown in Table I.

In the charge relay structure of RNase A composed of aspartate, histidine and water molecule, imidazole and formic acid obtained from geometry optimization by using STO-3G basis set[10] were used in place of histidine and aspartate, respectively; positions of O of the water and N^{δ} of the imidazole were fixed. Since the CNDO/2 method is used for these calculations, only r(OH) of the water, r(NH) of the histidine and r(OH) of the aspartate were optimized by the CNDO/2 method. When the calculations of the proton transfer from the histidine to the aspartate or from the water to the histidine are performed, the two hydrogen bonds obtained from the movements of the amino acid side chains and the water molecule are not linear completely between the water molecule and the histidine and between the histidine and the aspartate. First the proton transfer from the imidazole to the formic acid ion is calculated. The hydrogen bond distances between O of the water and N of the imidazole and between N of the imidazole and O of the formic acid ion are 2.93 and 2.58 Å, respectively. Two bond lengths of r(CO) in the formic acid ion are same; this model is called "a^1". Since the geometry change of the formic acid ion from the anion structure to the neutral occurs with the proton transfer from the imidazole to the formic acid ion, the energy of the proton transfer from the imidazole to the geometry changed formic acid ion in which r(C–O) and r(C=O) are obtained from the structure of HCOOH is calculated. Hydrogen bond distances between the water and the imidazole and between the imidazole and the formic acid ion are 2.93 and 2.58 Å, respectively. When the formic acid is positioned in place of the formic acid ion, the angle of N···O–C was maintained. This model is called "a^2". In order to avoid artificial results, only the distance between the imidazole and the formic acid ion from the model a^2 was changed from 2.58 to 2.41 Å. This model is called "a^3". When the energies of the proton transfers are calculated, the directions of the proton movements in the bending hydrogen bonds are along the lines between two hydrogens covalently bonded to N of the histidine and O of the aspartate. After the proton transfer from the imidazole to the formic acid ion, the proton transfer from the water to the imidazole is calculated. Hydrogen bond distances between the water and the imidazole anion and between the imidazole anion and the formic acid are 2.93 and 2.58 Å, respectively. This model is called "b^1". Next, since it is shown from ab initio calculations that the water approached to the imidazole anion, as the proton transferring from the imidazole to the formic acid ion,[10] the energies of the proton transfers from the water to the imidazole anion for the distances 2.58 and 2.23 Å are calculated. The former is called as model "b^2", and the latter "b^3". The distance 2.58 Å between N^{ε} of His 119 and O^{δ} of Asp 121 was obtained from the calculations of the interaction between the imidazole anion and the formic acid.[10]

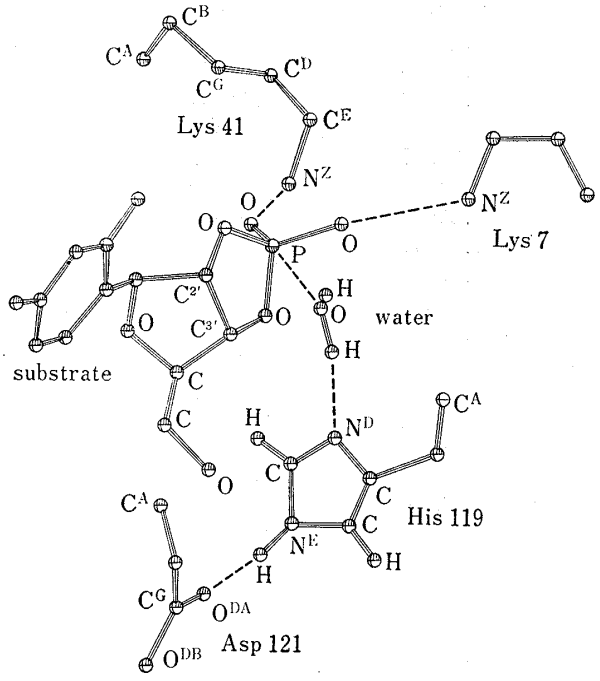

Fig. 1. Structure of the Active Site in RNase A Obtained from the Movements of the Amino Acid Side Chains

Asp 121, His 119 and water molecule construct a charge relay structure. And general base catalysis of N^{δ} is coupling with the attack of the OH group to phosphorus of the substrate. Lys 7 and Lys 41 is fitting to the cyclicphosphate part of the substrate.

Results

Charge Relay Structure from Movements of Amino Acid Side Chains

Potts *et al.* reported about the activity of Asp 121 in RNase A that ribonuclease activity is completely lost with the removal of additional amino acids from the carboxyl terminus of S-protein (Ala 122, Asp 121 and Phe 120).[11] Asp 121 is near His 119 in the active site. Since the general base catalysis of one nitrogen in histidine is increased by the hydrogen bond between aspartate and the other nitrogen of

10) H. Umeyama and S. Nakagawa, *Chem. Pharm. Bull.* (Tokyo) **27**, No. 7 (1979).
11) J.T. Potts Jr., D.M. Young, C.B. Anfinsen, and A. Sandoval, *J. Biol. Chem.*, **239**, 3781 (1964).

histidine,[12] the side chains of Asp 121 and His 119 were moved variously to fit in the conformation keeping the hydrogen bond between both side chains. And the hydrogen bond structure at which we aimed is obtained from the results of *ab initio* calculations.[10] A fitted structure between Asp 121 and His 119 was simulated. The rotation angles for Asp 121 were −22° and 22° to the clockwise direction around the axes of C^{α}–C^{β} and C^{β}–C^{γ} bond, respectively, and for His 119 those were −34° and −144° around the axes of C^{α}–C^{β} and C^{β}–C^{γ} bonds. The structure of the hydrogen bond formed is shown in Fig. 1. The distance between N^{ε} of His 119 and O^{δ} of Asp 121 was 2.58 Å, which is obtained from *ab initio* calculations for the complex between imidazole anion and HCOOH.[10] The angle of N^{ε} of His 119, H covalently bonded to N^{ε} of His 119 and O^{δ} of Asp 121 was 164.6°. The angle of H of

Table II. Coordinates of His 119, Asp 121, H_2O, Lys 7 and Lys 41 Obtained From Movements of Various Amino Acid Residues and Water[a]

Atom		x	y	z
	His 119			
21	C^{α}	−8.00000	−5.70000	11.10000
22	C^{β}	−6.80000	−5.00000	10.50000
23	C^{γ}	−6.29591	−5.37671	9.00201
24	C^{δ}	−5.24311	−5.89916	8.74000
25	N^{δ}	−7.21739	−5.22554	8.01305
26	N^{ε}	−5.32504	−6.18111	7.40816
27	C^{ε}	−6.65688	−5.62195	7.08640
28	$C^{\delta}H$	−4.45928	−6.06528	9.47900
29	$N^{\varepsilon}H$	−4.63594	−6.64484	6.85130
30	$C^{\varepsilon}H$	−7.22631	−5.50180	6.16475
	Asp 121			
31	C^{α}	−5.80000	−9.40000	5.90000
32	C^{β}	−4.50000	−10.00000	6.60000
33	C^{γ}	−3.51437	−9.10323	6.06677
34	$O^{\delta}A$	−3.80392	−7.71667	6.00682
35	$O^{\delta}B$	−2.24329	−9.48989	5.49681
	H_2O			
36	O	−10.10875	−5.04482	7.60443
37	H	−9.10834	−4.88976	7.78782
38	H	−10.37189	−5.84136	8.20007
	Lys 7			
39	C^{α}	−14.00000	−1.10000	13.90000
40	C^{β}	−12.80000	−0.40000	13.50000
41	C^{γ}	−12.48538	−0.82019	12.00854
42	C^{δ}	−13.73692	−0.86246	11.30721
43	C^{ε}	−13.78006	−1.08072	9.80040
44	N^{ζ}	−12.44627	−1.25014	9.26910
	Lys 41			
45	C^{α}	−15.70000	0.80000	−0.30000
46	C^{β}	−16.50000	0.30000	0.70000
47	C^{γ}	−15.50000	0.20000	2.00000
48	C^{δ}	−15.30000	1.50000	2.60000
49	C^{ε}	−14.29621	1.24619	3.76532
50	N^{ζ}	−12.87872	1.01968	3.31879

a) Å.

12) a) H. Umeyama, A. Imamura, C. Nagata, and M. Hanano, *J. Theor. Biol.*, **41**, 485 (1973); b) H. Umeyama, *Chem. Pharm. Bull.* (Tokyo), **22**, 2518 (1974); c) H. Umeyama, A. Imamura, and C. Nagata, *Chem. Pharm. Bull.* (Tokyo), **23**, 3045 (1975); d) S. Nakagawa and H. Umeyama, *Chem. Pharm. Bull.* (Tokyo), **25**, 909 (1977); e) H. Umeyama, A. Imamura, C. Nagata, and S. Nakagawa, *Chem. Pharm. Bull.* (Tokyo), **25**, 1685 (1977).

His 119, O^{δ} of Asp 121 and C^{γ} of Asp 121 forming a hydrogen bond was 132.9°. It may be reasonable since sp^2 hybridization is 120°. The fitted coordinates of His 119 and Asp 121 were shown in Table II.

In relation to the function of general base of N^{ε} of His 119, a water molecule must be placed in a position at which a hydrogen bond between N^{δ} of His 119 and O of the water is possible. The oxygen of the positioned water is at the distance 2.93 Å from N^{δ} of His 119 which is almost the same as the value 2.89 Å obtained from *ab initio* calculations.[10] Thus a charge relay structure composed of water molecule, His 119 and Asp 121 was simulated by moving amino acid side chains in the active site of RNase A. Then the distance between O of the water and phosphorus of the uridine 2′, 3′-ribose cyclophosphate was 4.3 Å, which is longer by about 1 Å than the van der Waals distance obtained from the summation of the values 1.4 Å and 1.9 Å for oxygen and phosphorus, respectively. And the water was placed in the very position at which the attack of the hydroxyl group following the general base catalysis is possible. Accordingly, in the active site of RNase A was obtained a coupled structure between the attack to the phosphorus by the hydroxyl group and the general base catalysis by the complex of His 119 and Asp 121.

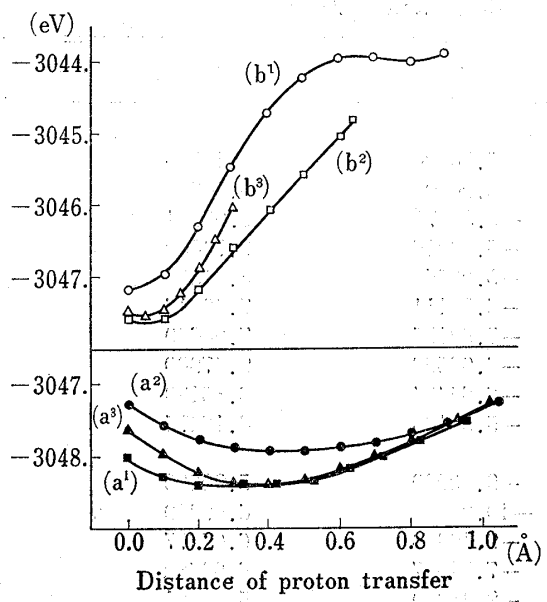

Fig. 2. Potential Curves of Proton Transfer from His 119 to Asp 121 or from Water to His 119

(a) shows the potential curve from His 119 to Asp 121 anion. For (a^1) distances are 2.93 and 2.58 Å between water and His 119, and between His 119 and Asp 121 ion; Asp 121 is obtained from $HCOO^-$. For (a^2) distances are 2.93 and 2.58 Å between water and His 119, and between His 119 and Asp 121 ion; Asp 121 is obtained from HCOOH. For (a^3) distances are 2.93 and 2.41 Å between water and His 119, and between His 119 and Asp 121 ion; Asp 121 is obtained from HCOOH.
(b) shows the potential curve from water to His 119 anion. For (b^1) distances are 2.93 and 2.58 Å between water and His 119 anion, and between His 119 anion and Asp 121; Asp 121 is obtained from HCOOH. For (b^2) distances are 2.58 and 2.58 Å between water and His 119 anion, and between His 119 anion and Asp 121; Asp 121 is obtained from HCOOH. For (b^3) distances are 2.23 and 2.58 Å between water and His 119 anions, and between His 119 anion and Asp 121; Asp 121 is obtained from HCOOH.

Approach of Lys 7 and Lys 41 to the Substrate

The participation of Lys 41 in RNase A has been reported. From X-ray diffraction analysis, however, the amino group of Lys 41 is very far from the phosphorus of the substrate. The distance between N^{ζ} of Lys 41 and the phosphorus of the cyclicphosphate group of the substrate is 8.7 Å. Free rotations for Lys 41 around the bond axes of the side chain were carried out, and the rotations by −120° and 30° to the clockwise direction around the axes of the C^{γ}–C^{δ} and C^{δ}–C^{ε} bonds made N^{ζ} of Lys 41 fit at the distance 2.30 Å to the oxygen of the cyclicphosphate part. The coordinates obtained from the rotations were shown in Table II. There is Lys 7 near Lys 41. When the side chain of Lys 7 rotates by −1, −111, −131 and 174 degrees around the axes of the C^{α}–C^{β}, C^{β}–C^{γ}, C^{γ}–C^{δ} and C^{δ}–C^{ε} bonds, N^{ζ} of Lys 7 comes to the position located at the distance 3.1 Å between N^{ζ} of Lys 7 and the oxygen of the cyclicphosphate part. Two cations of the side chains of Lys 7 and Lys 41 will interact with the mono anion of the cyclicphosphate.

General Base Catalysis Containing Charge Relay Structure

For the charge relay structure obtained above, the calculations of the two proton transfers from His 119 to Asp 121 and from the water molecule to His 119 were carried out at various separations between the water molecule and His 119 by using the CNDO/2 method. Figure 2 shows the results. (a^1) shows the potential curve of the proton transfer from His 119

to Asp 121. It was obtained in the case that the lengths of the two C–O bonds of the aspartic acid ion are same. When the C–O bonds keep the geometry of the aspartic acid and, that is, the bond lengths of r(CO) are different, a similar curve (a^2) or (a^3) was obtained. Although the proton transfer curve from His 119 to Asp 121 shown by (a^1), (a^2) or (a^3) in the figure does not have the potential barrier, it has the potential minimum between N^ε of His 119 and O^δ of Asp 121. When the proton bonded to N^ε of His 119 moves to the potential minimum, His 119 will become from the neutral molecule to the anion. As the result, the water molecule will come near to N^δ of His 119 keeping the hydrogen bond. The decrease of the distance occurring from the ionization of the histidine was described from the *ab initio* calculations.[10] In order to investigate the effects of the proximity of the water molecule, the potential curves (b^1), (b^2) and (b^3) at the various distances 2.93, 2.58 and 2.23 Å, respectively, between O of the water molecule and N^δ of His 119 were calculated. As the separation decreases from 2.93 to 2.23 Å, the potential energy of the proton transfer became smaller. Accordingly, the movement of the proton between N^ε of His 119 and O of Asp 121 lowered the potential of the proton transfer from the water to N^δ of His 119 through the decrease of the distance between the water molecule and His 119. The result obtained above showed the significance of the simulated charge relay structure in RNase A. When the proton transfer curve from the water to His 119 was calculated, His 119 was assumed to be anion; that is, the proton between N^ε of His 119 and Asp 121 was assumed to be covalently bonded to O of Asp 121. Because, from the MO levels and the atomic orbital coefficients of *ab initio* calculations, the N^δ basicity of His 119 of the complex was very similar to that of the structure in which His 119 is anion.

Discussion

The simulated structure composed of aspartate, histidine and water is very significant in lowering the activation energy. Then, why is the fitted structure not found in the data of X-ray diffraction analysis?. As one interpretation, the structure obtained from X-ray analysis may not be truly native. As another interpretation, since the amino acid side chains may always move from the flickering in the active site, the substrate may be decomposed after the formation of a fitted structure among the amino acid side chains and the substrate. In the hydrolysis mechanism by Findlay *et al.*, the nitrogen of His 119 pulls the proton of the water molecule and the hydroxyl group attacks to the phosphorus of the uridine 2′,3′-cyclophosphate.[5] The general base catalysis is very similar to that of acyl-α-chymotrypsin.[6] In the case of acyl-α-chymotrypsin, Asp 102, His 57 and the water molecule composed a charge relay structure.[12b,d] We noticed that there was Asp 121 near His 119 in the active site of RNase A. As the result, the charge relay structure obtained from the movements of the amino acid side chains was very similar to that obtained from *ab initio* calculations. However we could not simulate the completely appropriate structure in the active site. Moreover only the interaction between the histidine and the aspartic acid ion was considered. In future, *ab initio* calculations containing other amino acid side chains as well as Asp 121 and His 119 should be carried out. Nevertheless the simulated structure composed of Asp 121 and His 119 and the water molecule is thought to be valuable.

Usher *et al.*[13] and Roberts *et al.*[14] reported that His 119 and Lys 41 play significant roles in stabilizing a pentacovalent phosphorus intermediate. From the results of X-ray diffraction analyses, however, N^ζ of Lys 41 is placed at the distance 15 Å with the phosphorus bonded to 3′-oxygen of the ribose in the dinucleotide analogue UpcA. Lys 41 must be

13) D.A. Usher, *Proc. Natl. Acad. Sci. U.S.A.*, **62**, 661 (1969).
14) G.C.K. Roberts, E.A. Dennis, D.H. Meadows, J.S. Cohen, and O. Jardetzky, *Proc. Natl. Acad. Sci. U.S.A.*, **63**, 1151 (1969).

displaced by about 12 Å in order to participate in the enzymatic reaction. Therefore the movements of Lys 7 and Lys 41 may explain the enzymatic reaction.

His 12 plays a significant role on the proton release from 2′ oxygen in the transphosphorylation step. When the rotations of 8 and 23 degrees to the clockwise direction were performed around C^{α}–C^{β} and C^{β}–C^{γ} with the rotation of −10 degrees around C^{1}–N^{1} of the inhibitor UpcA, the distance between N^{ε} of His 12 and $O^{2'}$ of UpcA was 3.95 Å. However, when the uridine 2′,3′-O,O-cyclicphosphate was positioned in place of UpcA, the distance between N^{ε} of His 12 and $O^{2'}$ of the substrate intermediate was 6.33 Å. Accordingly, since the intermolecular distance is too large to participate in the enzymatic reaction, His 12 might be excluded in the second hydrolysis step.

Conclusion

In the second step of the hydrolysis by RNase A, Asp 121 may participate in the enzymatic reaction as one of the members constructing the charge relay structure. The charge relay structure is composed of Asp 121, His 119 and the water molecule similar to that in acyl-α-chymotrypsin.

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15) C.K. Jonson: ORNL-3794 (Oak Ridge National Laboratory, Oak Ridge, Tennessee, 1971). ORTEP Program was used to describe Figure 1.