

Simulation Analysis of Structures on the Reaction Pathway of RNase A[†]

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Abstract: Molecular dynamics simulations of the active site of RNase A have been used to supplement structural and chemical data. A simulation of the CpA substrate complex with a neutral His 12, based on the X-ray structure with deoxy-CpA and protonated His 12, demonstrates a repositioning of important residues that permits them to fulfill their catalytic role; i.e., His 12 is oriented with NE2 in place to accept the proton from the O2' hydroxyl (instead of interacting with the phosphate oxygen, as in the X-ray structure), and Lys 41 is sufficiently close to aid in the proton transfer required for transphosphorylation (instead of being far from the substrate, as in the X-ray structure). Protonated His 119 interacts with a phosphate oxygen, in position to polarize the phosphorus or transfer a proton, as in the Breslow mechanism. Simulations are also reported for complexes with an intermediate (cyclic CMP) and a product (3'-UMP). An analysis is presented of the residues, other than His 12 and 119 and Lys 41, that may play a significant role in catalysis; they include Thr 45, Gln 11, Phe 120, Lys 66, and Asp 121. Lys 7 does not hydrogen bond to the substrate and appears not to participate directly in the reaction.

1. Introduction

Ribonuclease A (RNase A) is one of the most widely studied enzymes.^{1–3} The enzyme cleaves RNA substrates in two steps: the first step is transphosphorylation, in which the P–O2' bond is formed and the P–O5' bond is broken, making a 2', 3'-cyclic phosphate intermediate; the second step is hydrolysis of the cyclic intermediate, in which the P–O2' bond formed in the first step is cleaved, resulting in a normal 3'-terminal phosphate group. The enzyme is thought to function by general acid–base catalysis; i.e., an acid protonates the leaving group, and a base deprotonates the attacking oxygen in each step. According to the established mechanism, when the substrate first binds to the active site, His 12 is in the neutral, deprotonated form. It accepts the proton from the O2' hydroxyl in the first step and then returns it to O2' in the second step. His 119 protonates the O5' leaving group in the first step and deprotonates the water molecule in the second step. This brings the protonation state of each histidine back to its original form, so that the enzyme is ready to repeat the cycle. Recently Breslow et al.⁴ have proposed a mechanism with more complex roles for His 12 and 119.

There are several additional active site residues that may be involved in the reaction. The importance of Lys 41 is indicated by chemical modifications specific to Lys 41 and also by its unusually low pK_a value, 8.6–9.1, compared to pK_a of 10.6–11.2 for all other lysine residues in the enzyme.⁵ Other residues of interest are Lys 66,⁶ Asp 121,⁷ Gln 11, and Phe 120.^{8–10}

Crystal structures of the native enzyme and the enzyme complexed with a number of substrates and substrate analogues have provided important data to supplement the mechanistic studies.^{3,8–11} In particular, Gilbert et al.¹⁰ have determined the crystal structures of RNase A complexed with a substrate analogue (deoxy-CpA), the cyclic phosphate (cyclic CMP) at low temperature (–70 °C), a transition-state analogue (uridine vanadate), and a product (3'-UMP). On the basis of these structures and other data, Gilbert et al. have considered specific mechanistic roles for certain residues in catalysis; their conclusions are generally in accord with the standard mechanism. The structural data support the possibility of general acid–base catalysis by His 12 and His 119 by an in-line mechanism^{12,13} and stabilization of the transition state by ionic interactions with Lys 41. Gilbert et al. also concluded that Thr 45 anchors His 12 and helps to determine the specificity of RNase A, Asp 121 anchors His 119 and assists it in deprotonating the water molecule that hydrolyzes the cyclic phosphate intermediate, and Lys 7 does not participate in catalysis.

Although the crystallographic studies of RNase A are very important, they do not describe the systems that are actually involved in catalysis. Other than the general difficulty that observed structures correspond to trapped species, there are more specific problems in the case of RNase A. A substrate analogue (e.g., deoxy-CpA) rather than a true substrate was studied to prevent the reaction from occurring. The crystal structures have in most cases been obtained at low pH (pH ~5.5) rather than in the range where the enzyme is most active (pH 6–7); this alters the protonation state of the histidines and may affect the active-site hydrogen-bonding network. Since the reaction involves proton transfers, the resulting differences are likely to be significant. Finally, certain essential residues (e.g., Lys 41) are disordered in most of the structures.

In the present paper, we utilize molecular dynamics simulations to supplement the X-ray results. Of particular importance in such simulations is the fact that starting with a given X-ray structure, which involves an inactive species, we can modify the potential function representing the ligand and protein residues so that they correspond to the active species. The X-ray structure for the substrate complex made use of the substrate analogue deoxy-CpA, which lacks the O2' hydroxyl group and is, therefore, unreactive. In addition, His 12 is positively charged in the crystal, and Lys 41 is disordered. Two molecular dynamics simulations of the active-site region of RNase A binding to CpA were made. The first simulation was performed with protonated His 12, as in the crystal structure.¹⁴ Since the neutral form of His 12 is required

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for its participation as a generalized base in catalysis, we have carried out a second simulation of the RNase A/CpA complex with His 12 deprotonated at NE2; this simulation started with the same crystal coordinates as the first so that it provides information on the structural rearrangements expected when His 12 is deprotonated. Comparison of the two simulations shows that, for the real substrate and the correct protonation state, His 12 and Lys 41 occupy positions that permit them to perform their proposed catalytic roles. In addition, we report briefly on the analysis of simulations of RNase A complexed with a cyclic phosphate intermediate (cyclic CMP) and with a product (3'-UMP).

Section 2 outlines the stochastic boundary method used in the simulations. Section 3 contains the results. It focuses on His 12, His 119, and Lys 41 but also comments on the roles of other residues in the active site. Section 4 summarizes the conclusions from the simulations.

2. Stochastic Boundary Methods

Using the stochastic boundary method for solvated proteins,¹⁴⁻¹⁶ we have performed molecular dynamics simulations of RNase A with the substrate CpA and His 12 in its neutral or positively charged form. In addition, we report results for a simulation of RNase A complexed with a cyclic phosphate intermediate (cyclic CMP) and a product (3'-UMP). A transition-state analogue (uridine vanadate) simulation has been discussed in an earlier paper, which also give some results for the positively charged His 12 simulation. X-ray structures provided by G. Petsko and co-workers¹⁰ were used as the starting point for the simulations. The CpA structure was built from the crystal results for deoxy-CpA by introducing the O2'H hydroxyl group; for the deprotonated His 12 simulation, the protonated His potential was replaced by that for the neutral form deprotonated at NE2.

The active site of RNase A is defined by constructing a sphere of 12-Å radius around the phosphorus atom. Space within the sphere not occupied by crystallographically determined atoms is filled by water molecules, introduced from an equilibrated sample of water. The 12-Å sphere is further subdivided into a reaction region (10-Å radius) treated by full molecular dynamics and a buffer region (the volume between 10 and 12 Å) treated by Langevin dynamics. Water molecules diffuse freely between the reaction and buffer regions but are prevented from escaping by an average boundary force. The protein atoms in the buffer region are constrained by harmonic forces derived from crystallographic temperature factors. The forces on the atoms and their dynamics were calculated with the CHARMM program;¹⁷ water molecules are represented by the ST2 model.¹⁸ In each simulation the waters were first equilibrated for 4 ps with the protein fixed, and then the entire system was equilibrated for 8 ps. This involved thermalization of the component atoms and adjustment of the number of waters in the active-site region to obtain the normal liquid density in regions distant from the protein. After equilibration, 20 ps of dynamics was performed for RNase A/CpA with His 12 deprotonated and 50 ps with His 12 protonated; 60 ps of dynamics was performed for the RNase A/cyclic CMP system and 50 ps for the RNase A/3'-UMP system.

Table I lists some characteristics of the simulations (time, average temperature); the number of protein atoms and solvent molecules included in the simulation are given.

3. Results and Analysis

In this section, we report the results of the various simulations, compare them to the related X-ray structures, and discuss their mechanistic consequences. Schematic representations of crystal and molecular dynamics simulation structures (Figures 1-4) are shown for the cases considered. Emphasis in the illustrations is

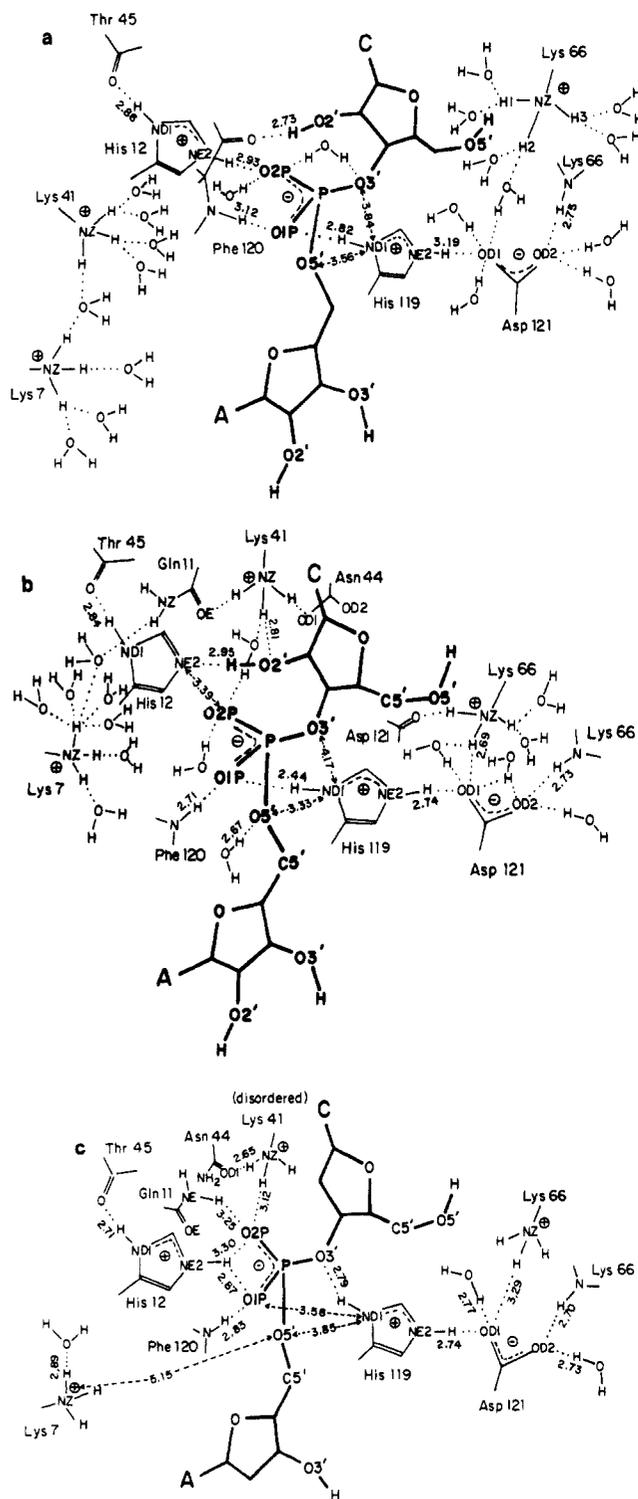


Figure 1. (a) Schematic drawing based on the average structure of RNase A/CpA with His 12 doubly protonated obtained from 50-ps dynamics. Only residues and water molecules of interest are shown; additional protein residues and waters were included in the simulation (see text). (b) Schematic drawing based on the average structure of RNase A/CpA with His 12 deprotonated at NE2 obtained from 20-ps dynamics (see Figure 1a). (c) Schematic drawing based on the crystal structure of the RNase A/deoxy-CpA complex. The residues are positioned to permit the important distances to be included in the diagram. Only the active-site residues that are in the vicinity of the substrate and selected crystal waters are shown. (All crystal structure drawings are based on coordinates supplied by G. Petsko.)

placed on the relative positions of the possible catalytic residues and on the distances involved in the important interactions; water molecules are included if they make hydrogen bonds of interest.

Molecular Dynamics of RNase A/CpA. We consider the simulations with CpA substrate in the presence of a protonated and

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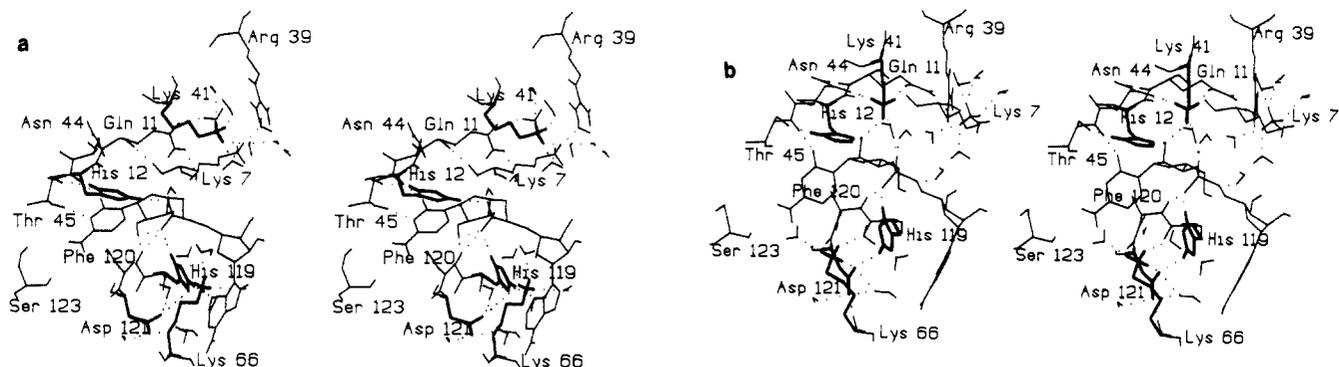


Figure 2. Stereoplots of the average structure of RNase/CpA with His 12 (a) doubly protonated obtained for 50-ps dynamics (see Figure 1a) and (b) deprotonated at NE2 obtained for 20-ps dynamics (see Figure 1a).

Table I. Summary of RNase A Simulations

substrate/inhibitor His 12	CpA protonated	CpA neutral	cyclic CMP protonated	3'-UMP protonated
productn time (ps)	50	20	60	50
temp (K)	298	299	300	294
no. of atoms	735	915	753	791
protein complex	486	485	462	464
ST2 waters	249	430	291	327

unprotonated His 12 (parts a and b of Figure 1, respectively); His 119 is protonated in both simulations. The corresponding X-ray structure used the inhibitor deoxy-CpA and had both His 12 and 119 protonated. In the RNase A/deoxy-CpA crystal structure (Figure 1c), where there is no O2' hydroxyl, NE2 of His 12 makes a bifurcated hydrogen bond to O1P and O2P and ND1 is hydrogen bonded to the C=O of Thr 45. His 119 hydrogen bonds to O3' and Asp 121, and Lys 41 is disorderd. In the average dynamics structure (Figure 1a), NE2 of His 12 hydrogen bonds to O2P but not to O1P. It is repelled by the O2' hydroxyl whose hydrogen is bonded to the backbone oxygen of Phe 120 and O1P. As in the crystal structure of RNase A/deoxy-CpA, ND1 of His 119 does not bond to O5' but hydrogen bonds to O1P; it retains its hydrogen bond to OD1 of Asp 121. The NH terminus of Lys 66 has moved away from Asp 121, hydrogen bonding instead to water molecules (one of which also hydrogen bonds to OD1 of Asp 121). Lys 41 is not near the substrate; the NZ group is 8.64 Å from O2'. This appears to be due to its interaction with Arg 39 and Lys 7 through intermediate water molecules. The side chains of both Lys 66 and 41, as well as Lys 7, hydrogen bond only to water molecules.

The interactions of His 12 and Lys 41 change significantly when the former is deprotonated at NE2, and a dynamics simulation is performed that provides a more realistic model for the initial structure of the catalytic process (Figure 1b); parts a and b of Figure 2 show stereoviews of the protonated and deprotonated His 12 simulations. His 12 has moved such that NE2 is hydrogen bonding to the O2' hydroxyl group of CpA, in accord with its role in deprotonating O2' during transphosphorylation by the generally accepted mechanism as well as in the Breslow proposal.^{4,19} Lys 41 has also moved significantly and is now positioned with its ammonium group making a strong hydrogen bond with O2'. In addition, Lys 41 interacts with OE1 of Gln 11 and indirectly with O2P through a water molecule. Support for the reorientation of Lys 41 comes from the work of Jentoft et al.⁵ who have used NMR to measure titration curves of RNase A in which the lysines were ¹³C-methylated. They found that the ionization of Lys 41 is coupled to the ionization of a histidine residue (perhaps His 12), both of which are perturbed upon substrate binding. The position found in the simulation (Figures 1b and 2b) would account for such a perturbation.

Atom ND1 of His 119, which is protonated in both simulations, hydrogen bonds to O1P; analysis of the dynamics trajectories shows no contribution of ND1-O5' bonding. Thus, its role at the start

of the transphosphorylation reaction may be to make phosphorus more electropositive and facilitate attack by O2'. The position of His 119 is also consistent with protonation of one of the phosphoryl oxygens in the formation of a phosphorane intermediate, as proposed by Breslow et al.⁴ However, it is not certain that their solution mechanism can be extended to the enzyme system, where other catalytic groups are present. Semiempirical²⁰ and ab initio²¹ calculations indicate protonation of a phosphoryl oxygen strengthens the P-O5' bond. Further discussion of this question will be given in subsequent work.

The conformation of the substrate in the region of the hydrolyzed bond is somewhat different from that in the crystal structure. The torsion angles C3'-O3'-P-O5' and O3'-P-O5'-C5' are -132° and -1.6°, respectively, in the crystal structure. Throughout the 20-ps simulation, with His 12 deprotonated the torsion angles, C3'-O3'-P-O5' and O3'-P-O5'-C5' remain roughly constant at 120° and -60°, respectively. The same values are obtained in the simulation of RNase A/CpA with protonated His 12 during 50 ps of dynamics. According to stereoelectronic arguments,²²⁻²⁴ a conformation in which C3'-O3'-P-O5' and O3'-P-O5'-C5' are 60° and 180°, respectively, favors for cleavage of the P-O5' bond. There is a lone pair on O3' that is antiperiplanar to the P-O5' bond; no lone pairs on O5' are antiperiplanar to the P-O3' bond. This is expected to strengthen the P-O3' bond and weaken the P-O5' bond that is to be cleaved. Neither the conformation in the crystal structure nor the molecular dynamics structure after 20 ps of dynamics conform to these stereoelectronic rules. Instead the rather unusual 120°, -60° configuration obtained in the molecular dynamics simulation has a lone pair antiperiplanar to the P-O3' bond and a lone pair in-plane cis to the P-O5' bond. The P-O3' bond would be weakened (via standard stereoelectronic arguments) in addition to the attenuation of the P-O5' bond, although the 120° geometry does not appear to have been considered previously. A complete analysis of the effects on the reaction also requires considerations of the trigonal-bipyramidal transition state, since stereoelectronic effects can be considerably larger for the transition state than for the ground state.^{21,24}

Although the simulation with His 12 protonated is not a good model for the initial stage of the reaction, it may serve as a model of the active-site structure immediately after proton transfer from O2' to NE2 of His 12. Both His 12 and His 119 are now interacting directly with the phosphoryl oxygens. Thus, when His 12 is protonated, its position is such that it can help His 119 polarize O1P and/or O2P. This would make the phosphorus more positive and facilitate attack by O2'. Davis et al.²⁵ have estimated the charge of the leaving oxygen in the RNase A catalyzed cy-

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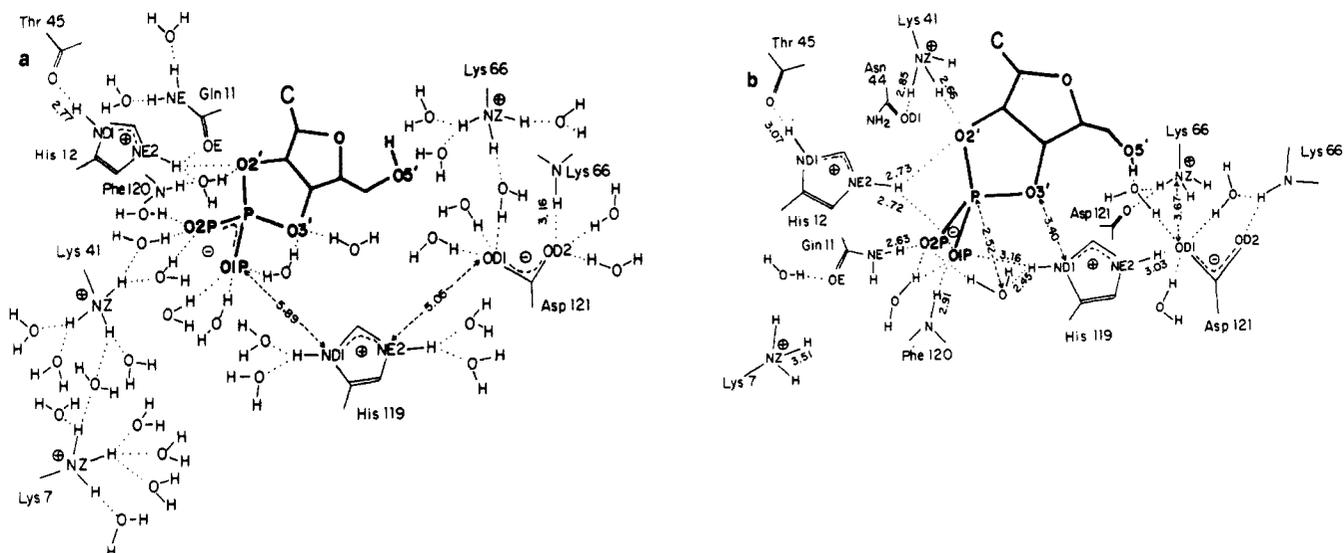


Figure 3. Schematic drawings based on the average structure of RNase A/cyclic CMP obtained (a) for 60-ps dynamics (see Figure 1a) and (b) at low temperature (see Figure 1c).

clization of uridine 3'-phosphate aryl esters. They found a Brønsted $\beta_{18} = -0.2$, which shows an increase in positive effective charge on the leaving oxygen atom compared with that in the nonenzymatic imidazole-catalyzed cyclization, where $\beta_{18} = -0.59$. They attributed the observed increase to electrophilic interactions between the oxygen directly (through hydrogen-bonding interactions) or indirectly via protonation of the monoanions of aryl phosphate esters in water.

Molecular Dynamics of RNase A Complexed with a Cyclic Phosphate Intermediate and a Product. In the cyclic phosphate intermediate the leaving group nucleotide is no longer present so that, in comparison with the CpA complex, additional water molecules can enter the active site. The average dynamics structure of the cyclic CMP complex (Figure 3a), obtained in the simulation that started from the crystal structure (Figure 3b), shows that many of the residues are now hydrogen bonded to water molecules. Lys 41 no longer interacts directly with O2', and the Lys 66-Asp 121 hydrogen bond is no longer present. In the crystal structure, Lys 41 is disordered and the Lys 66-Asp 121 hydrogen bond is also absent. Lys 41 and Lys 7 are linked by a water molecule. In the simulation Lys 7, 41, and 66 are hydrogen bonded to water molecules, most of which are not evident in the crystal due to the high lysine mobility.

As in the crystal structure, His 119 does not interact directly with the phosphate of the substrate. There is one intermediate water in the crystal structure, and there are more than one in the dynamics; one of these waters is likely to be involved in attacking the phosphate. His 119 is no longer bonded to Asp 121 but interacts with water molecules. His 12, which is protonated, now interacts with O2' through a bridging water molecule, instead of making a bifurcated bond to O2' and an equatorial phosphorus oxygen as seen in the crystal structure. The positions of His 119 and His 12 in both the crystal structure and the average dynamics structure are consistent with the conclusions of Eftink and Biltonen²⁶ that the dominant interaction occurs between His 12 and the cyclic phosphate, which does not interact directly with His 119. They found that when His 119 is deprotonated, the affinity for cyclic CMP did not diminish significantly; i.e., the association constant when both His 12 and His 119 are protonated is 2100 M⁻¹ compared to 1700 M⁻¹ with His 12 protonated. Further evidence comes from the increase in the pK_a of His 12 from 5.9 to 9.0 in the RNase A/cyclic CMP complex relative to the free enzyme, whereas there is no change in the pK_a for His 119.

For the product complex with 3'-UMP, the simulation (Figure 4) and the crystal structure have His 12 in the doubly protonated form, hydrogen bonding to O1P rather than O2'. In the crystal

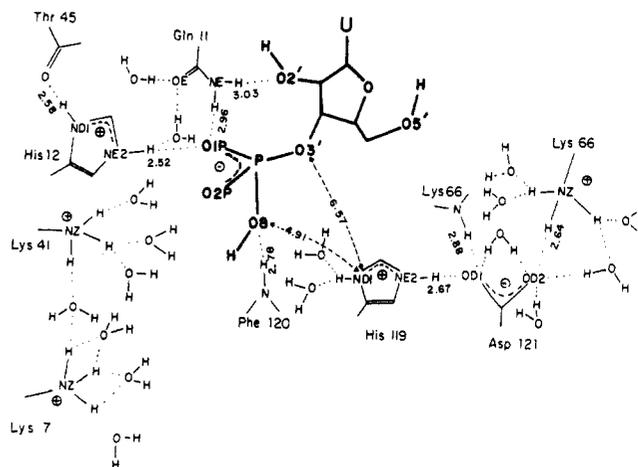


Figure 4. Schematic drawing based on the average structure of RNase A/3'-UMP obtained for 50-ps dynamics (see Figure 1a).

structure of RNase A/2'-CMP,²⁷ His 12 (NE2) also hydrogen bonds to O1P (NE2-O1P distance is 2.41 Å). A number of experimental observations suggest that His 12 is doubly protonated and interacting directly with the phosphate in the enzyme/product complex. The pK_a of His 12 in all mononucleotide phosphates studied² is increased 0.2–2 pK_a units from its value in the free enzyme. Meadows et al.²⁸ found that the C2 proton resonance shift of His 12 seen upon binding 2'-CMP and 3'-CMP is beyond the position of a fully protonated histidine and resembles that seen on formation of an imidazolium-phosphate complex, suggesting direct contact between His 12 and phosphate. Gorenstein et al.²⁹ calculated microscopic pK_a values from the pH dependence of the ³¹P resonance of 2'-CMP, 3'-CMP, and 3'-UMP both when free in solution and when bound to RNase A. The pK_a values of 2'-CMP, 3'-CMP, and 3'-UMP bound to protonated His 12 are perturbed by 1.5–2.0 pH units relative to the free nucleotides. By contrast, the pK_a values of these phosphonucleotides bound to unprotonated His 12 are similar to those of free phosphonucleotides in solution. This implies little interaction between the phosphate and the other protonated groups at the active site, such as His 119 and Lys 41. The latter is supported in the average dynamics structure (Figure 4) where Lys 41 does not interact with

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O2' or the phosphate oxygens and His 119 is bonded to water molecules. In the crystal structure the lysine is highly mobile and His 119 interacts with the hydroxyl oxygen of the phosphate.

Breslow and co-workers^{4,19} proposed a mechanism for hydrolysis of the cyclic phosphate intermediate that involves formation of a phosphorane dianion by His 119 imidazole-assisted attack of a water molecule; the newly formed imidazolium ion of His 119 then protonates the dianion before His 12 donates its proton to catalyze the ring-opening step. The direct interaction between His 119 and a phosphoryl oxygen during hydrolysis appears not to be consistent with the NMR observations nor with the crystal and the average molecular dynamics structures. However, transphosphorylation and hydrolysis need not necessarily follow corresponding pathway. In the enzymatic reaction, the two processes differ in rates by 1 to 4 orders of magnitude (depending on the substrate), and thermodynamically, transphosphorylation is easily reversible while hydrolysis is not. Thus, even though His 119 interacts with the phosphoryl oxygens so as to increase the electrophilicity of the phosphorus during transphosphorylation, this interaction and proton transfer may not be needed during hydrolysis. Further investigations of this question are in progress.

Roles of Other Residues from Crystal Structures and Average Dynamics Structures. In all of the crystal structures as well as in the simulations, the backbone carbonyl oxygen of Thr 45 is hydrogen bonding to ND1 of His 12; this is thought to stabilize the experimentally observed ND1-H tautomeric form of His 12³⁰ and is likely to be important in anchoring His 12 in a fixed position. The decrease in activity of 4-pyridyl-Ala 12 and L-homo-His 12 analogues of RNase S^{31,32} may result from the absence of an interaction that orients the His 12 side chain. This supports a structural role for Thr 45 and ND1 of His 12 in optimizing the orientation of the latter for catalysis.

From chemical evidence Lys 7 appears not to be directly involved in catalysis; e.g., Lys 7 can be substituted by norleucine without loss of enzymatic activity.³³ In all the crystal and average dynamics structures, Lys 7 does not interact with the phosphate or any other part of the substrate. The closest approach of the terminal NZ to any of the phosphate oxygens is greater than 4 Å. NZ of Lys 7 also does not come within hydrogen-bonding distance of any other amino acid residues, except in the native RNase A crystal structure, where it hydrogen bonds to OE1 of Gln 11. Instead, the positively charged amino terminus of Lys 7 is interacting with water molecules. In most cases the same water molecule that hydrogen bonds to Lys 7 also hydrogen bonds to Gln 11 or Lys 41, raising the possibility that it may thus have an indirect effect on catalysis. It may also be involved in "channeling" the negatively charged substrate into the active site.

Asp 121 is conserved in 35 species of mammalian ribonucleases.³⁴ RNase 1-120 exhibits only 0.5% activity,² but its ability to specifically bind nucleotides is not markedly changed. Experiments on semisynthetic RNase 1-118/111-124 with Asp 121 replaced by Asn or Ala also show that the reduced activity results from a diminished catalytic efficiency and not from a decreased affinity for the substrate.³⁵ The greater reduction in activity against cyclic nucleotides compared to RNA may suggest that Asp 121 plays a more important catalytic role in hydrolysis than in transphosphorylation. In all the crystal structures as well as in the X-ray/neutron structure of RNase A/uridine vanadate,¹¹ OD1 of Asp 121 hydrogen bonds to NE2-H of His 119. However, the average dynamics structures show this bond only for the reactant and product analogues but not for the intermediate or transition-state-like structures. Thus, it is not clear if and how

Asp 121 plays a catalytic role in hydrolysis as well as transphosphorylation, and we are currently carrying out further calculations to address this question.³⁶

In all the crystal and average dynamics structures, the backbone N of Lys 66 hydrogen bonds to a carboxyl oxygen of Asp 121. The terminal NH group of Lys 66 hydrogen bonds to the other carboxyl oxygen of Asp 121 in the average dynamics structures; for the other structures, the interaction is less direct (mediated by a water molecule in some cases). It is possible that the proximity of a positively charged terminus of Lys 66 may significantly affect the pK_a of the negatively charged Asp 121 and, thus, play a catalytic role. Umeyama et al.⁷ calculated the potential curves of proton transfer from His 119 to Asp 121 and from water to His 119 using the CNDO/2 method. They found that the transfer from His 119 to Asp 121 decreases the distance between the water molecule and His 119, thus lowering the potential barrier for the proton transfer from water to ND1 of His 119.

Two other residues that interact with the phosphate oxygens are Phe 120 and Gln 11. The peptide N of Phe 120 hydrogen bonds to one of the phosphate oxygens in all structures except in the cyclic CMP complex (Figure 3a) where it hydrogen bonds to O2' via an intervening water molecule. Since it is only the backbone that is involved, it is not surprising that the enzyme retains 13% activity against cyclic nucleotides when Phe 120 is replaced by Leu.³⁷ Gln 11 hydrogen bonds to a phosphoryl oxygen in all the crystal structures. A role of Phe 120 and Gln 11, as in the case of His 12 and His 119, is to increase the electrophilicity of the phosphorus, thus making it more susceptible to nucleophilic attack. This is supported by the calculations of Deakyne and Allen²⁰ who found that the hydrogen bond between the backbone N-H and a phosphoryl oxygen produces a more positive (electrophilic) phosphorus and delocalizes the negative charge on the substrate. In the molecular dynamics simulations the position of Gln 11 is more variable; it is hydrogen bonding to Lys 41 (RNase A/CpA with His 12 neutral), His 12 (RNase A/cyclic CMP), Arg 10 (RNase A/UVC) or O2', and a phosphoryl oxygen (RNase A/3'-UMP). This apparent flexibility raises the possibility that Gln 11 may have varied roles.

4. Concluding Discussion

Active-site simulations of RNase complexed with a substrate, a cyclic phosphate intermediate, and a product have been made to clarify the contribution of various amino acid residues to the enzymatic reaction. Most important is the result that starting with the X-ray structure for deoxy-CpA and a positively charged His 12, a simulation of CpA and neutral His 12 leads to structural changes that position these residues for their role in catalysis. The calculations suggest that a direct interaction between Lys 41 and the substrate's O2' hydroxyl group may be required for His 12 to deprotonate O2' during transphosphorylation. The apparent flexibility of Lys 41 allows it to move away in the hydrolysis step when this interaction is not needed. The molecular dynamics simulations do not support the suggestion^{1,2} that Lys 41 interacts directly with a phosphoryl oxygen of the phosphate group in the transition state. The average dynamics structure of the RNase A/UVC complex shows an indirect interaction between Lys 41 and a phosphoryl oxygen via an intervening water molecule; thus, Lys 41 may help to stabilize the transition state via electric field effects.

His 12 hydrogen bonds to O2' in the first step, as expected because of its role as a base. When it is protonated, it hydrogen bonds to the phosphoryl oxygen O2P. Thus, His 12 may be involved in transition-state stabilization. His 119 hydrogen bonds to the phosphoryl oxygen, O1P, in both CpA simulations. Such an interaction was proposed by Breslow et al. although they required His 119 to protonate the phosphoryl *before* His 12 can deprotonate O2'H in their mechanism. In addition to the His 119-O1P bond, there are hydrogen bonds from the NH backbone

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of Phe 120 (RNase A/CpA simulations) and from NE2 of Gln 11 (RNase/deoxy-CpA crystal structure) as well as from water molecules to the phosphoryl oxygens. These interactions with the phosphoryl oxygens are expected to facilitate the reaction by polarizing the phosphate and making it more susceptible to nucleophilic attack.

Present throughout the reaction are hydrogen bonds from ND1 of His 12 to the backbone O of Thr 45, from Asp 121 to the backbone of Lys 66, from the NH backbone of Phe 120 and/or from NE2 of Gln 11 to the phosphoryl oxygens. In addition, water molecules in the active site provide partial solvation of the lysines, the phosphate, and Asp 121. The calculations indicate that Lys 7 does not hydrogen bond to the substrate or participate directly in the reaction. On the other hand, Lys 66 and Asp 121 may have important roles; this is also suggested by chemical modification studies of these residues.

The simulations reported here represent only one step in an analysis of the reaction dynamics of RNase A. It is hoped that ab initio calculations of the reaction path³⁸ and additional molecular dynamics simulations that are in progress will enable us to address other questions that need to be answered for a full understanding of the detailed dynamics of RNase catalysis.

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Thiol Surface Complexation on Growing CdS Clusters[†]

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Abstract: The growth of small CdS colloidal particles has been initiated by pulse radiolytic release of sulfide from a thiol (3-mercaptopropanediol, RSH) in the presence of Cd²⁺ ions. The kinetics and stoichiometry of the ensuing reactions were followed by conductivity, absorption spectroscopy, and light-scattering techniques. The final CdS product has been identified by electron diffraction. The formation of Cd-thiolate complexes at the surface of the particles is indicated by conductivity and by energy dispersive analysis of X-ray (EDAX) results. The rate of formation of CdS clusters is strongly pH dependent due to the pH effect on the stability of Cd²⁺/HS⁻ complexes. At low pHs (4.0–5.3) the growth mechanism is proposed to be primarily a cluster-molecule process. At this pH range Cd²⁺ ions at the CdS particle surface complex with thiolate ions stronger than in the bulk of the solution. The size control of the particles by thiols is proposed to result from a competition of thiolate ions with HS⁻ ions for cadmium ions at the surface of the growing particles. At neutral and basic pHs growth is primarily a cluster-cluster aggregation process of CdS clusters within the cadmium-thiolate polynuclear complexes. The small particles seem to undergo weak clustering in solution, yet the spectroscopic properties of the small particles is maintained. The utility of the thiol-controlled growth, both in size control and surface modification, is discussed.

Studies of the spectroscopic and photocatalytic properties of semiconductors in colloidal state have gained popularity in recent years, in particular following the observation of the dependence of these properties on size.^{1,2} Consequently, much effort has been expended in the synthesis of these small, quantum size particles. Among the various semiconductor materials, those of the II-VI groups, and especially cadmium chalcogenides, drew much of the attention. Prerequisite for systematic studies of quantum size effects is a general procedure to synthetically control the size of the particles. Particles of CdS were successfully synthesized in a variety of media such as non-aqueous solvents,^{1,3} reversed micelles,⁴ vesicles,⁵ polymer films,⁶ and zeolites.^{7,8} The work of Nosaka et al.,⁹ has demonstrated the remarkable ability of thiols to stabilize small particles. Recently, we synthesized small CdS particles by γ -radiolysis of thiols in the presence of Cd²⁺ ions.¹⁰ It was shown that in addition to serving as a source of sulfide ions, the thiols also play an important role as growth moderators. The latter property was attributed to the ability of thiolate anions (and polynuclear complexes between cadmium ions and thiolate ions) to bind strongly to cadmium ions at the particle surface, thereby effectively inhibiting further growth of small CdS particles. Surface modification of CdSe particles, which also prevents further

agglomeration, has been recently demonstrated by Brus and co-workers in organic solvents using a multistep organometallic synthetic procedure.¹¹

The ability to prepare particles of systematically controlled sizes as a result of a surface complexation reaction process is very appealing. In this paper, we report our results on the formation and properties of colloidal CdS particles from the very early stages of their existence in solution using the pulse radiolysis technique. An advantage of the radiation initiation approach is the ability to study the growth process essentially without any limitations of time resolution. This was demonstrated earlier in a study of silver halides growth.¹² The procedure adopted here is similar

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