Role of Active Site Residues and Solvation in RNase A

C. BROOKS III,[a] A. BRÜNGER,[a] M. FRANCL,[b]
K. HAYDOCK,[b] L. C. ALLEN,[b] AND M. KARPLUS[a]

[a]*Department of Chemistry*
Harvard University
Cambridge, Massachusetts 02138

[b]*Department of Chemistry*
Princeton University
Princeton, New Jersey 08544

The molecular mechanism of catalysis by RNase A has been investigated using high level *ab initio* molecular orbital calculations in conjunction with molecular dynamics simulations. Despite recent high-resolution X-ray and neutron diffraction studies on RNase,[1,2] the detailed reaction pathway, the roles of active site residues, protonation states, and hydrogen bonding arrangements have remained unclear. His 12 had been thought to be responsible for deprotonating O2′ in order to form the 2′,3′-cyclic phosphate intermediate, but the recent crystal structures show it to be hydrogen bonding to an equatorial phosphate oxygen while Lys 41 H-bonds to 02′. However, since the crystals were formed at low pH, both His 12 and His 119 are protonated. In the active form of the enzyme (at neutral pH) His 12 is expected to be initially unprotonated. We have found that when slightly rotated from its crystallographic position, a deprotonated His 12 can hydrogen bond to O2′. Molecular dynamics simulations of an RNase A/substrate complex with His 12 deprotonated are being undertaken to check this possibility.

Ab initio calculations have been performed to examine further the roles of His 12 and Lys 41 in the deprotonation of O2′. Using imidazole as a model for His, ammonium for Lys, and either water or methanol for the O2′ hydroxyl, we have calculated the optimized geometry, total energy, and proton affinity (*i.e.*, the difference in energy between a deprotonated and protonated molecule). The preferred protonation states can then be ascertained by comparing the proton affinities of individual components that hydrogen bond to each other (TABLE 1) in order to find the most probable position of the shared proton. For example, since the proton affinity of imidazole (*a*) is only 240 kcal/mole whereas that of methanol (*c*) is 409 kcal/mole, if imidazole hydrogen bonds to methanol the shared proton would prefer to stay on imidazole. The use of water (*b*, *d*) rather than methanol as a model for O2′ proved too simplistic. This implies that, without other contributing factors, His 12 could not deprotonate O2′. However, if an ammonium ion representing Lys 41 H-bonds to O2′ (*e*), the proton affinity of O2′ drops considerably. If the geometry of the H_3N–H . . . O2′—CH_3 complex is fully optimized, the proton will transfer from N to O2′ (*f*) and the proton affinity drops to 233 kcal/mole, 7 kcal below that of imidazole. Thus it appears that O2′ can be deprotonated by His 12, but it is simultaneously reprotonated by Lys 41. However, we hypothesize that the O2′ reprotonation by Lys 41 does not occur for two reasons. First, the O2′-P bond will start to form as soon as O2′ starts to be deprotonated by His 12. Second, as soon as His 12 is protonated it will prefer to H-bond to the negatively charged equatorial O6 rather than to O2′, thereby weakening P-O6 and strengthening

TABLE 1. Calculated Proton Affinities of Model Systems[a]

	System	Proton Affinity (kcal/Mole)
(a)	$\text{H–N}\langle\text{imidazole}\rangle\text{N} \xrightarrow{H^+} \text{H–N}\langle\oplus\rangle\text{N–H}$	240
(b)	$^{\ominus}O2'\text{–H} \xrightarrow{H^+} \text{H–O2'–H}$	429
(c)	$^{\ominus}O2'\text{–}CH_3 \xrightarrow{H^+} \text{H–O2'–}CH_3$	409
(d)[b]	$H_3N^{\oplus}\text{–H1}\cdots{}^{\ominus}O2'\text{–H} \xrightarrow{H^+} H_3N^{\oplus}\text{–H1}\cdots\text{H–O2'–H}$	285
(e)[b]	$H_3N\text{–H1}\cdots O2'\text{–}CH_3 \xrightarrow{H^+} H_3N^{\oplus}\text{–H1}\cdots\text{H–O2'–}CH_3$	281
(f)	$H_3N^{\oplus}\cdots\text{H1–}{}^{\ominus}O2'\text{–}CH_3 \xrightarrow{H^+} H_3N^{\oplus}\text{–H1}\cdots\text{H–O2'–}CH_3$	233
(g)	$\text{H–C}(O)_2^{\ominus} \xrightarrow{H^+} \text{H–C}(=O)\text{OH}$	364

[a]All values are based on total energies of fully optimized geometries (unless otherwise indicated) at the 6-31G*/6-32G* level.
[b]The N-H1 bond length was held fixed at 1.034 Å.

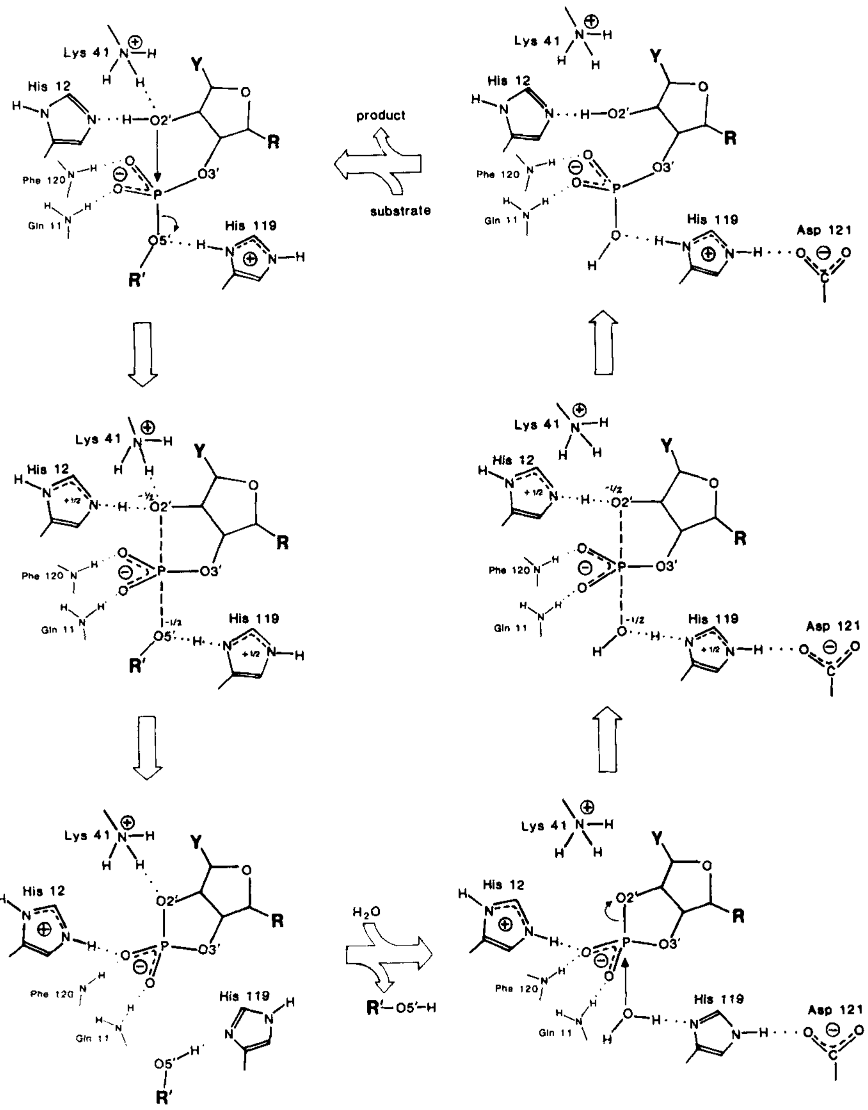

FIGURE 1. The proposed catalytic mechanism of RNase A. In the first step of the reaction, transphosphorylation, O2′ is deprotonated by His 12, with assistance from Lys 41, while O5′ is protonated by His 119. The P-O2′ bond forms as the P-O5′ bond breaks, with a trigonal bipyramid transition state. The protonated His 12 switches over to hydrogen bond to an equatorial oxygen, as is found in the X-ray structures. Hydrolysis of the 2′,3′-cyclic intermediate occurs when the R′-O5′-H leaving group is replaced by a water molecule. The water molecule is deprontonated by His 119, with the assistance of Asp 121. O2′ is reprotonated by His 12, with another trigonal bipyramid transition state leading to the 3′-phosphate product.

P-O2′. We also note that although Lys 41 may enhance the deprotonation of O2 in the transphosphorylation step, it would be expected to inhibit protonation during the hydrolysis step. We have conducted molecular dynamic simulations which suggest that Lys 41 may at times deviate significantly from its observed crystallographic position in which it H-bonds to O2′. This raises the possibility that this H-bond may not exist during hydrolysis.

The formation of the cyclic intermediate also requires the protonation of O5′ by His 119. We have observed that Asp 121 interacts with this histidine, which would assist hydrolysis but hinder transphosphorylation. Initial investigation of the protonation state of this pair of residues has revealed that in the absence of solvation of Asp 121 (as modeled by formate) proton transfer from His to Asp (*g*) would occur. We are in the process of determining the number of additional H-bonds to Asp which are needed to prevent this proton transfer and thus allow His 119 to protonate O5′.

By comparing the geometries and energies of the above interactions and others, we have examined the validity of previously proposed mechanisms and constructed a plausible reaction pathway for RNase A catalysis (as summarized in FIGURE 1).

REFERENCES

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