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Does a Single Metal Ion Bridge the A-9 and Scissile Phosphate Groups in the Catalytically Active Hammerhead Ribozyme Structure?

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We have constructed a model structure that we believe represents the strongest possible physically and chemically reasonable representation of a hypothesized catalytically active hammerhead ribozyme structure in which a single divalent metal ion bridges the A9 and scissile phosphate groups. It has been proposed that such a structure arises from a conformational change in which the so-called ground-state structure (as observed by X-ray crystallography) rearranges in such a way that the *pro*-R oxygen atoms of both the A9 and scissile phosphate groups are directly coordinated by a single divalent metal ion in the transition-state of the hammerhead ribozyme cleavage reaction. We show that even the small subset of possible model structures that are consistent with these requirements, and that are stereochemically and sterically reasonable, are contradicted by experimental evidence. We also demonstrate that even a minimal subset of assumptions, i.e. that stems I and II are helical and that the two phosphate groups are coordinated by a divalent metal ion in the standard octahedral geometry, are sufficient to lead to this contradiction. We therefore conclude that such a mechanism of hammerhead ribozyme catalysis is untenable, at least in its present formulation.

Keywords: hammerhead ribozyme; hammerhead RNA; RNA catalysis; catalytic RNA; ribozyme mechanism

Introduction

Recently, it has been proposed that the hammerhead ribozyme (Prody *et al*., 1986; Uhlenbeck, 1987; Hasellof & Gerlach, 1988; reviewed by Wedekind & McKay, 1998) rearranges from the structure observed by X-ray diffraction (Figure 1; Pley *et al*., 1994; Scott *et al*., 1995) to a significantly different structure that is catalytically active (Peracchi *et al*., 1997, 1998; Wang *et al*., 1999). In this proposed structure, two phosphate groups that are approximately 20 Å apart in the crystal structure join together to form a single metal binding pocket. One of these phosphate groups is that of A9 and is observed to bind a divalent metal ion in the crystal structure (Pley *et al*., 1994; Scott *et al*., 1996) through direct coordination with the non-bridging *pro*-R oxygen atom (Figure 2). The other, the scissile phosphate group, is also said to coordinate the same divalent metal ion directly through its *pro*-R oxygen atom. The strongest evidence for this mode of metal binding was obtained from simultaneous phosphorothioate substitutions at each of the phosphate *pro*-R oxygen atoms (Wang *et al*., 1999). The deleterious effect of these substitutions upon the Mg^{2+}-catalyzed hammerhead ribozyme cleavage reaction are ameliorated by including a thiophilic metal ion, such as Cd^{2+}, in the reaction mixture.

Each of the two individual phosphorothioate substitutions can be ''rescued'' such that the cleavage rate of the modified ribozyme exceeds that of the wild-type ribozyme in reaction mixtures containing both Mg^{2+} and Cd^{2+}. When both the A9 and scissile phosphate groups are simultaneously replaced with the phosphorothioate molecules, the doubly modified RNA can again be rescued with reaction mixtures containing both Mg^{2+} and Cd^{2+}, albeit to 1% of the wild-type activity (Wang *et al*., 1999). This observation has been offered as strong evidence that these two phosphate molecules form a single metal-ion binding site that assembles upon a proposed transition of the hammerhead ribozyme to a catalytically competent structure from

Abbreviations used: NOE, nuclear Overhauser enhancement.

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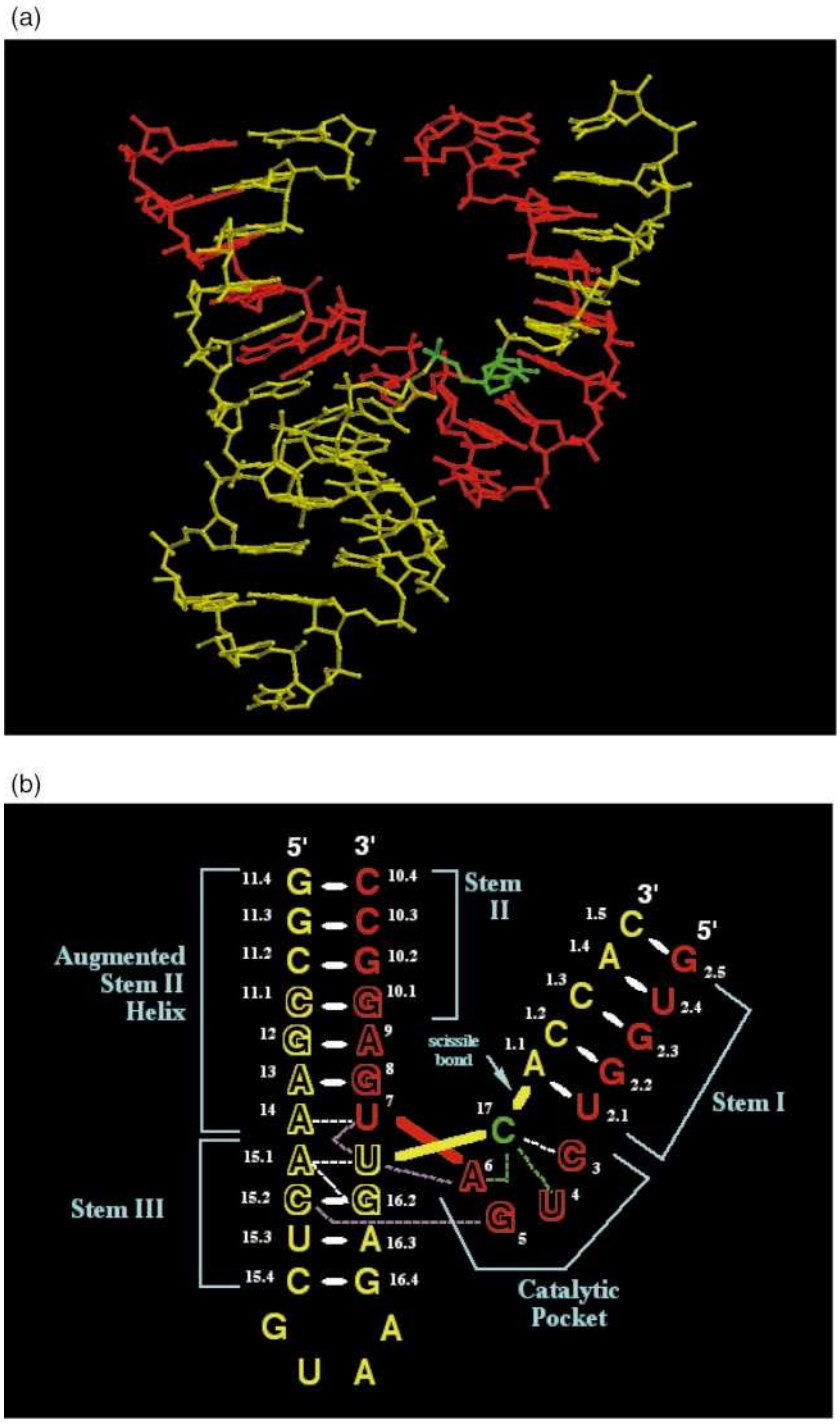

Figure 1. (a) The three-dimensional structure of the hammerhead ribozyme as determined by X-ray crystallography. (b) Schematic diagram of this structure designed to complement (a). The enzyme strand is shown in red, the substrate strand in yellow, and the cleavage-site base in green. Base-pairing is indicated by white lines (with broken lines indicating non-canonical single H-bond contacts). The numbering scheme used throughout the text is shown here.

that observed in the crystal (the so-called ground-state). These experiments also lead to the suggestion that the relevant metal ion is bound by the A9 phosphate group and N7 of the adjacent nucleotide, G10.1, in the ground state (as is observed in the crystal structure) and that the scissile phosphate group also becomes coordinated to this metal ion when the conformation changes to that of the catalytically active structure, leading to the formation of a transition-state in which the N7 of G10.1 and both phosphates, *via* their *pro*-R oxygen atoms, are directly coordinated to the divalent metal ion (Wang *et al*., 1999). (An example of such a structure is shown in Figure 3). In other words, the stem II helix and those nucleotides augmenting it (domain II) bind the metal ion and remain unchanged. In the transition to the active structure, the stem I helix, the cleavage-site residue (C17) and the conserved bases that surround it (the uridine turn or domain I) must therefore change conformation (from the ground-state crystal structure) relative to the unchanged part of the molecule in such a way as to enable binding of the scissile phosphate group to the same metal ion. The scis-

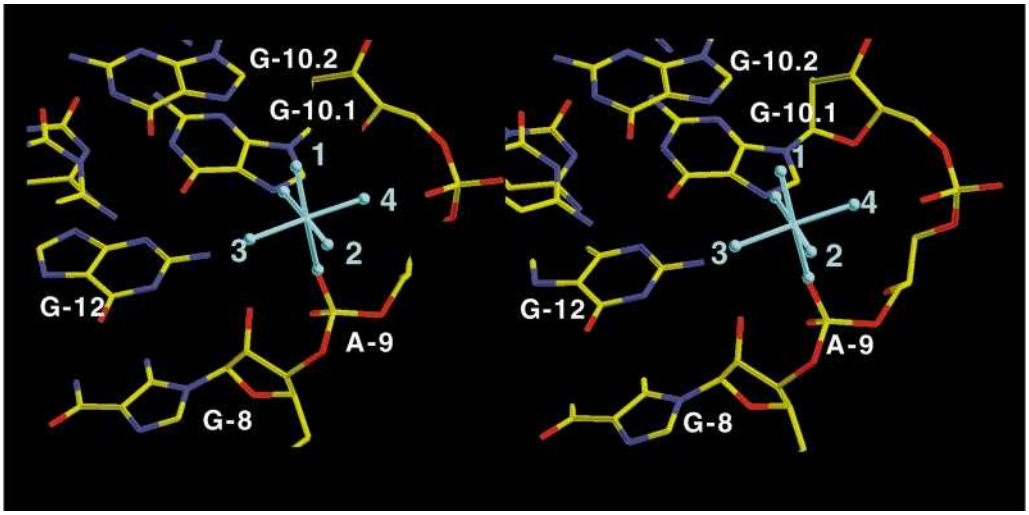

Figure 2. Stereo view of the A9 phosphate metal binding site as it appears in the crystal structure of the hammerhead ribozyme in the presence of manganese.

sile phosphate group, according to this scheme, must travel approximately 20 Å from the position that it occupies in the crystal structure.

To better understand the structural basis of the proposed conformational change to a catalytically active structure (Wang *et al.*, 1999), we have attempted to construct a family of model structures that are simultaneously compatible with the octahedral coordination geometry of the bridging divalent cation, the hammerhead ribozyme stem II and domain II structure determined by X-ray crystallography, the known requirement for an in-line attack mechanism, and the stereochemical constraints that are inherent to the RNA molecule. We found that the set of possible structures simultaneously satisfying all these criteria (i.e. only those stipulated by Wang *et al.*, 1999) is quite restricted.

Results and Discussion

Constructing physically plausible model structures consistent with the proposal

If one first considers the crystallographically observed ground-state interaction between the hammerhead RNA and the divalent metal ion, two inner-sphere contacts are made between the RNA and the metal ion (Pley *et al.*, 1994). One is with the N7 of G10.1, and the other is with the *pro*-R oxygen atom of the A9 phosphate, 90° from it, as shown in Figure 2. Each of these bonds is about 2 Å in length, as expected for octahedrally coordinated oxygen and nitrogen ligands. These interactions unambiguously fix the position and orientation of the octahedral metal ion complex uniquely in space (Fraüsto da Silva & Williams,

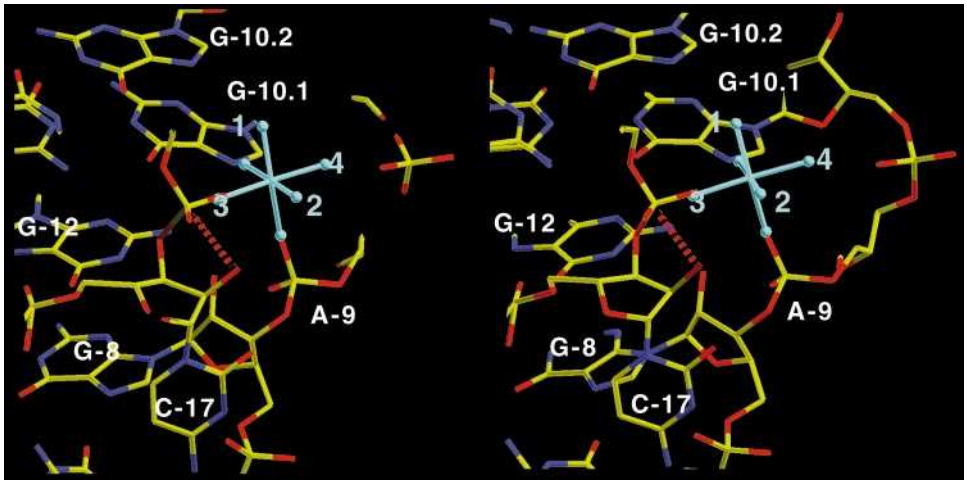

Figure 3. Stereo view of the A9 phosphate metal binding site as it appears in our model of the hammerhead ribozyme when the same metal also coordinates the scissile phosphate group adjacent to C17 at ligation site 3 in the octahedral complex. Models in which the scissile phosphate group is coordinated at either position 1, 2 or 4 invariably lead to stereochemical clashes and unphysical bond distances.

1993). Four discrete potential RNA coordination sites therefore remain for the scissile phosphate group to bind to; these are presumably occupied by water molecules in the ground-state structure. These potential coordination sites are labeled 1 to 4 in Figures 2 and 3. We will therefore consider four classes of structures corresponding to coordination of the scissile phosphate *pro*-R oxygen atom at each of these four potential coordination sites in turn. We find that three of the four possible coordination sites can be eliminated immediately on stereochemical grounds, enabling us to concentrate our examination upon the one remaining coordination site.

We constructed representatives of each of the four classes of models by manually docking the stem I helix and its attached C17 (arranged in such a way that the attacking 2′-oxygen atom is in-line with the scissile phosphorus atom and the 5′-oxygen atom leaving group of residue 1.1) to each of the four potential coordination sites of the metal ion depicted in Figure 2, in turn. Each of these models was constructed in such a way as to: (a) optimize the geometry of the scissile phosphate *pro*-R oxygen-metal ion coordination; (b) minimize the distance between the 5′-phosphate group of C17 and the 3′-oxygen atom of U16.1, to which it must be covalently bound; and (c) minimize the steric clashes (or van der Waals repulsive forces) within the resulting RNA model. These models were initially constructed manually, and then were refined using a rigid-body simulated annealing molecular dynamics minimization protocol as a robust method for finding minimized structures.

Based upon these exercises, we immediately eliminated all models in which the scissile phosphate group was bound at coordination site 4, as this site is made sterically inaccessible by the location of the phosphate group of G10.1, approximately 3.2 Å from the potential position 4 oxygen coordination site. (It is therefore likely that the metal ion makes a through-water contact to the G10.1 phosphate group at this position). Although sites 1 and 2 are sterically accessible, all models that coordinate at positions 1 or 2 can also be eliminated purely on the basis that the required distance between C17 and the covalently attached U16.1 would be far too great to be spanned by the phosphate backbone, even if it were completely distended (coordination site 4 models suffer from this defect as well). The only set of model structures that cannot immediately be eliminated on the basis of the distance between C17 and U16.1 (i.e. a constraint inherent to the stereochemistry of any RNA molecule) are those structures in which the scissile phosphate group coordinates at position 3. If the scissile phosphate group is coordinated at position 3, a subset of all such structures can, in addition, form a stereochemically plausible link between C17 and U16.1 and can also avoid steric clashes if U16.1 is allowed to unpair from A15.1 and the phosphate backbone of U16.1 is allowed to become almost completely distended. Since A15.1 and U16.1 form a base-pair of only one hydrogen bond in the crystal structure, and since the phosphodiester backbone between 16.1 and 17 is already rather distended in the crystal structure, such an arrangement is not entirely implausible. These conclusions were tested and double-checked using a series of simulated annealing molecular dynamics rigid-body energy-minimizations designed to maximize the radius of convergence of the calculation by defining each helix as a rigid body (see below).

Optimizing the most plausible model

The subset of model structures that simultaneously satisfy the requirements imposed by the geometry of coordination of the scissile phosphate ligand to the complex metal ion, the phosphate backbone connectivity between C17 and U16.1, and that avoid steric clashes between the stem I helix and the remainder of the RNA molecule is even more limited. By combining these requirements with the requirement that the stem I helix also smoothly join residues 3 through to 6 (domain I), we obtained an energy-minimized model structure that we believe constitutes the strongest detailed formulation of the hypothesized catalytically active structure within the requirements imposed by Wang *et al*. (1999). By eliminating all of the model structures that violate stereochemistry, that have steric clashes or that do not conform to the requirements imposed by coordination to the divalent metal ion that bridges the A9 and scissile phosphate groups, we arrived at a unique model structure (or one family of very closely related model structures). The most plausible model structure was obtained by first fixing the stem II and stem III helices, as well as domain II and the bound metal ion in space, and then by allowing the stem I helix (defined as a rigid body, apart from residue 1.1) and the remaining residues to adopt a minimum-energy conformation following rigid-body simulated annealing molecular dynamics and conventional Powell minimization of the model in XPLOR 3.8. (This procedure was also used to confirm our finding, based upon manual model building and inspection, that models in which the scissile phosphate group was coordinated at any of the other possible positions, were stereochemically not allowed.) Apart from maintaining reasonable stereochemistry, no constraints were placed upon the connecting residues (3 through to 6 and 16.1). Also, apart from maintaining a geometry compatible with the known in-line attach mechanism, and the coordination of the *pro*-R oxygen atom of the scissile phosphate group , no constraints were imposed upon residues C17 and A1.1. Following this first round of structural refinement, against a potential that included terms governing ideal bond lengths, angles, torsion angles, base planarity and perhaps most importantly a van der Waals repulsion term, a final energy minimization was performed following release from pos-

itional constraints, except as imposed by the metal ion coordination. The resulting model is illustrated in Figure 4.

To confirm the uniqueness of this solution, we systematically altered the starting model for the rigid-body molecular dynamics refinement by rotating the stem I helix in 90° increments, using the metal-bound scissile phosphate oxygen atom as a fixed point, to sample each of the five other directions. Without fail, both the rigid-body molecular dynamics minimization protocol and even conventional least-squares rigid-body minimization quickly returned to the original model structure (with essentially negligible differences). It is most likely then that the distance constraints imposed by the phosphate backbone connectivity to the stem III helix and those imposed by the bridging divalent metal ion, in conjunction with the requirement for eliminating spatial overlap, uniquely define a model structure that is physically realizable.

The main features that arise as a consequence of the three sets of criteria described above are that maintenance of the phosphodiester backbone connectivity between U16.1 and C17, when combined with the requirements imposed by the geometry of the metal ion complex to the two phosphate groups, imposes the requirement that the stem I helix is approximately parallel with the stem II helix. It essentially becomes nested in the minor groove of the stem II helix, but is at the same time staggered with respect to the stem II helix by a distance corresponding to about four *A*-form helical base-pairs. As a result, the stem I helix is positioned significantly higher than the stem II helix (as shown in Figure 4) in order to accommodate placement of the scissile phosphate group at the level of the metal ion bound to the A9 phosphate group. Any significant deviation from this arrangement results in steric clashes, violation of the U16.1 to C17 distance constraint, or other significant distortions of the stereochemistry of the RNA. We therefore believe that this structure (or the family of very closely related structures) constitutes the only physically reasonable model structure that is entirely consistent with the hypothesis that both the A9 and scissile phosphate groups are bound by a single metal ion and with the physical requirements for maintaining a stereochemically reasonable RNA molecule. If other such models exist,

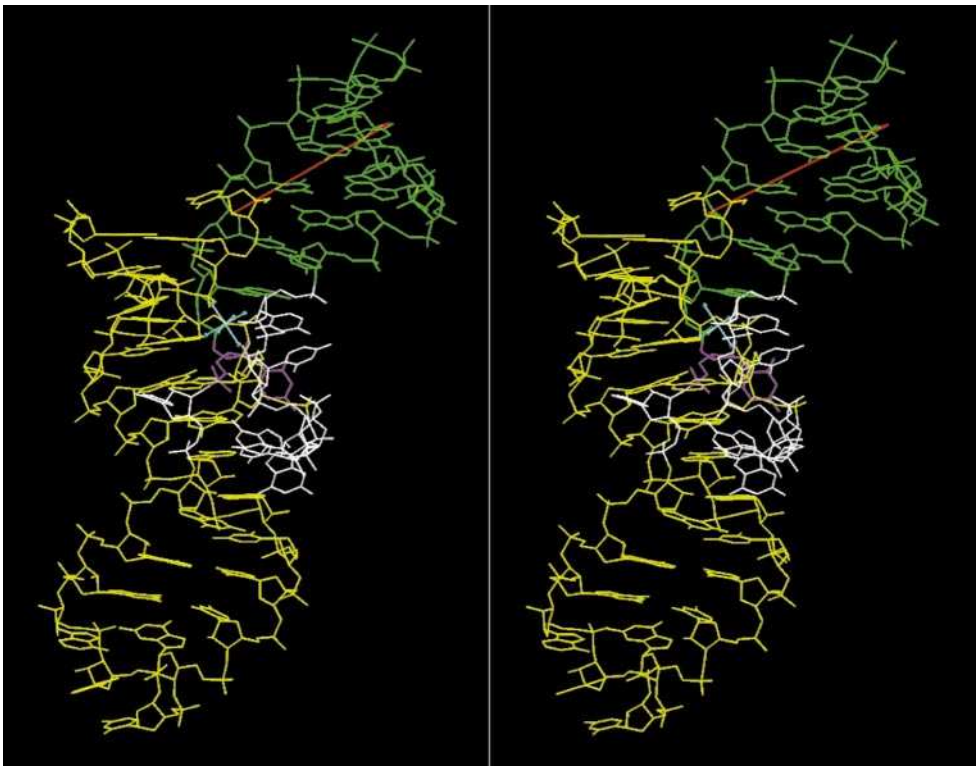

Figure 4. Stereo view of the most plausible model structure consistent with the requirements of the double phosphorothioate experiments and their interpretation. The parts of the RNA shown in yellow (stem II, domain II and stem III, apart from U16.1) and green (stem I) were treated as separate rigid bodies allowed to achieve their most favorable orientations subject to the constraints imposed by the divalent metal ion coordination geometry (blue) and the requirement for maintaining the connectivity of the phosphate backbone. The residues allowed to vary in position to achieve this connectivity are shown in white, and the cleavage site base is shown in pink. The distance required to be spanned by a chemical crosslink less than or equal to 16 Å in the catalytically active molecule is in red. The actual distance in the model cannot be forced to be less than 20 Å without unwinding the helices. Moreover, the stem I helix would be forced to unwind completely in order to prevent a steric clash with the crosslinking moieties.

they do so at the expense of maintaining the structural integrity of the helices and the divalent metal-ion site. These entities are again believed to remain intact, according to the formulation under consideration. Although residue 16.1 and residues 3 through to 6 adopt specific conformations in this energy-minimized model, we have made no attempt to ascribe any significance to these conformations other than the fact that they maintain reasonable phosphate backbone connectivity, and we have made no attempt to model their conformations in detail. As a consequence, our examination of the properties of this model does not include an analysis of the detailed conformation of these residues, but instead focuses upon aspects independent of these regions.

Does the most plausible model structure conflict with experimental observations?

In addition to being internally consistent, the best model structure must also stand up to experimental tests if it is to be considered to be a true representation of the hammerhead ribozyme. A number of previous experiments performed upon the hammerhead ribozyme are in fact appropriate to consider in this context. Perhaps the most definitive of these is one in which the hammerhead ribozyme can be reversibly crosslinked using a chemically engineered disulfide linkage between non-essential residues in the stem I and stem II helices (Sigurdsson *et al*., 1995). When two 2′-NH_3-modified nucleotides are incorporated into the hammerhead ribozyme, these allow linkage of the 2′-N atoms to moieties (3-aminobenzyl mercaptan derivatives) that in turn can form disulfide crosslinks between the 2′-NH_3-modified nucleotides in the RNA molecule. In the original experiment, this technique was used to test between two model hammerhead structures (one based on FRET measurements (Tuschl *et al*., 1994), and the other based on the crystal structure) in a decisive manner. Two hammerhead ribozymes were synthesized for this experiment. One permitted residue 2.1 in the stem I helix to be crosslinked to residue 11.2 in stem II. The 2′-OH groups of these nucleotides are about 13 Å apart in the FRET model, but are 33 Å apart in the crystal structure. The other permitted residue 2.6 of the stem I helix to be crosslinked to L2.4, a nucleotide occupying a tetraloop terminal position analogous to residue 11.5 in stem II. These are 11 Å apart in the crystal structure, but are 32 Å apart in the FRET model. Both ribozymes were active within a factor of 2 of wild-type activity when the disulfide bond was reduced. Upon oxidation, the hammerhead ribozyme crosslinked in a manner consistent with the crystal structure maintained at approximately wild-type activity, whereas the activity of the ribozyme crosslinked in a manner consistent with the FRET structure decreased 300-fold. As a further control, a ribozyme with a crosslink between residues 2.1 and 10.4 in stem II showed a similar decrease in activity, despite again having near wild-type activity when the disulfide bond is reduced. These experiments revealed unambiguously that the distance between the 2′-OH group of residue 2.6 and that of residue 11.5 (or its equivalent) is less than or at most equal to the fully extended length of the crosslink, 16 Å, and that the space between these residues must be unoccupied for the crosslink to form. This covalent distance constraint is compatible with the 11 Å distance observed in the crystal structure and incompatible with the FRET-based model structure.

The disulfide crosslinking experiment does not prove that the crystal structure of the hammerhead ribozyme is the catalytically active structure. It merely proves that in the catalytically active structure, there exists a rigid distance constraint consistent with that observed in the crystal structure. If the crystal structure rearranges to form another structure that is catalytically active, this structure too must have the distance between the 2′-OH group of residue 2.6 and that of residue 11.5 (or its equivalent) less than or at most equal to 16 Å. We therefore measured this distance in the model of the most plausible active structure that we constructed based upon the Herschlag experiments and analyses. In this model, the distance between the 2′-oxygen atom of residue 2.6 and that of 11.5 (assuming stems I and II continue as standard *A*-form RNA helices) is 21 Å. Moreover, a line passing through these two 2′-oxygen atoms passes through the stem I helical axis diagonally, meaning that even if the covalent crosslink were stretched to 21 Å, it would also be required to pass through the RNA helix, an obvious impossibility. The only way in which the model structure can be forced to accommodate the crosslink without steric clashes and without stretching beyond 16 Å is for the stem I helix to unwind fully. It is of course rather unlikely that the stem I helix will fully unwind in such a way as to resemble an antiparallel ladder, especially as a prerequisite to forming a catalytically active structure. Therefore we conclude that all of the remaining physically plausible model structures that are consistent with Wang *et al*. (1999) can be eliminated based upon the disulfide crosslink-imposed distance constraint.

We again tested the validity of these conclusions, based upon manual molecular modeling, using the rigid-body molecular dynamics minimization protocol. This was carried out as before, but the crosslink was also included in the potential energy function in the form of an NOE constraint that required the distances between the relevant 2′-oxygen atoms to be less than or equal to 16 Å. A square well potential was used to model this constraint. If the crosslink distance constraint was weighted heavily, bond distances and angles in the molecule were significantly distorted, and the end result, upon lifting the rigid-body criteria for final Powell minimization, was that the stem I helix would have to unwind completely in order to simultaneously satisfy all of the imposed criteria. If the

crosslink distance constraint was weighted more appropriately, a spatial frustration occurred during rigid-body molecular dynamics, and no reasonable minimized structure could be obtained.

What is the minimal set of assumptions that leads to a contradiction of the hypothesis?

So far we have only demonstrated that the previous chemical crosslinking data contradict the assumptions made by Wang *et al*. (1999), i.e. that the A9 phosphate group and N7 of G10.1 coordinate the metal ion that binds to the scissile phosphate group, that the augmented stem II helix (stem II and domain II) remain in the conformation observed in the crystal structure, and that stem I must change in conformation relative to stem II in order to allow the metal ion to coordinate the scissile phosphate group. If one of these assumptions used in the construction of our models is false, perhaps the hypothesis that the A9 and scissile phosphate group are bridged by a single coordinating metal ion may nevertheless be true. For example, if a conformational change takes place within domain II, perhaps this will allow formation of the hypothesized transition-state structure. For that reason, we sought to determine the minimum set of assumptions that would lead to a contradiction between the model and the crosslinking data. We found that if one disregards the hammerhead ribozyme structure except for the stem I helix, the stem II helix, and the two phosphate groups bridged by a common octahedrally coordinating metal ion, and if one assumes that these helices remain as rigid *A*-form entities (with the exception of the two phosphate groups in question and their adjacent ribose molecules), these conditions alone are incompatible with the distance-constraint imposed by the crosslink. Even under this minimal set of constraints, convergence to an energetically reasonable structure is impossible unless one or both helices are allowed to fray significantly, or to unwind. In other words, we found that the helical structures of stem I and stem II and the known coordination geometry of magnesium are all that need to be assumed to arrive at a contradiction with the crosslinking data. Any model structure compatible with the crosslinking data must involve significant disruption of one of these helices, either through fraying of more than one base-pair or through an energetically unfavorable unwinding or denaturation of the *A*-form helix.

Other experimental evidence that contradicts the hypothesis

Although the disulfide crosslinking experiments appear to be quite sound and have been corroborated elsewhere (O. C. Uhlenbeck, personal communication), it is fair to ask if the most plausible model would be acceptable if the disulfide crosslinking experiments were somehow in error.

A separate set of experiments that place distance and orientational constraints upon the stem I helix relative to stem II have recently been reported (Stage-Zimmermann & Uhlenbeck, 1998). Circular substrate molecules having five, two or zero nucleotides bridging a seven base-pair stem I helix to a seven base-pair stem II helix in a hammerhead ribozyme construct were all catalytically active. The most active of these was the circular substrate with no bridging nucleotides, then the molecule with two bridging nucleotides, and finally the molecule with five bridging nucleotides. The circular substrate with five bridging nucleotides was still reasonably catalytically active, but about fivefold slower than the circular substrate construct with no bridging nucleotides. Based upon our best-refined crystal structure of the hammerhead ribozyme (Scott *et al*., 1996), in which stems I and II are extended to seven base-pairs using least-squares superimposed seven base-pair *A*-form RNA helices, we find that the distance between the 5′ and 3′ termini of the substrate corresponds to approximately one nucleotide. Stage-Zimmermann & Uhlenbeck (1998) report this distance to be larger, based upon a different model that appears to have helices distorted from typical *A*-form geometry (cf. their Figure 1(b)). These results appear to be at least consistent with the crystal structure, and again place a distance and orientation constraint upon any conformational change that is likely to represent the active structure. Although the longest linker can, in theory, accommodate this distance in the model structure we constructed if the substrate wraps back around one of the helices or unwinds, it is clear from the experimental data (Stage-Zimmermann & Uhlenbeck, 1998) that the optimum distance corresponds to less than that of two nucleotides (about 12 Å), a distance clearly incompatible with the model structure.

Other experiments that appear to be consistent with the disulfide crosslinking experiments include transient electric birefringence results (Amiri & Hagarman, 1996), gel mobility assays and FRET experiments (Bassi *et al*., 1996, 1997, 1999). If we add to that the observation that the hammerhead ribozyme can exhibit catalytic turnover in the crystal (Scott *et al*., 1996; Murray *et al*., 1998a), and, as in the case of the circular hammerhead substrate, the cleavage reaction equilibrium is shifted to favor further product formation over re-ligation (J.B.M. & W.G.S., unpublished results), the case for ruling out model structures in which the scissile phosphate group is coordinated by the bridging metal ion bound to the A9 phosphate group is further substantiated.

Concluding remarks

Based upon the experimental observations, interpretations and assumptions made by Herschlag and colleagues (Wang *et al*., 1999), combined with the stereochemical, steric and metal ion coordination geometry requirements, we have con-

structed what we assert to be the most physically plausible model structure of the catalytically active hammerhead ribozyme, and therefore a detailed structural formulation of the hypothesis that the A9 and scissile phosphate groups are coordinated by a single metal ion in the active structure in the strongest, most robust and valid manner possible, consistent with the physical and chemical requirements imposed by the RNA molecule. This model structure therefore represents a concrete, testable hypothesis of the claim that a single metal ion binds both the A9 and scissile phosphate groups in the active hammerhead ribozyme. We have found that the currently existing disulfide crosslinking data, as well as several other sets of experimental results, conflict even with the most plausible structures that are based upon the observations and proposals in the Herschlag analysis. We therefore suggest that a hammerhead ribozyme catalytic mechanism in which the A9 and scissile phosphate groups are bridged by a single divalent metal ion that coordinates both phosphate groups is structurally unsound. Seen in this light, several experimental observations now have fairly straightforward interpretations. For example, we have observed previously that divalent metal ions are not strictly required for hammerhead ribozyme catalysis (Murray *et al*., 1998b). This lack of a divalent metal ion requirement would be hard to reconcile with a requirement for a metal ion that bridges the A9 and scissile phosphate groups for catalysis. The extremely limited rescue (to 1% of wild-type activity) of the hammerhead ribozyme containing phosphorothioate molecules at both the A9 and scissile phosphate groups (Wang *et al*., 1999) can be easily explained if the metal is in fact stabilizing a catalytically inactive conformation (with the residual activity accounted for in terms of acknowledged sample impurity). In addition, the lack of a thio-effect in hammerhead ribozymes in which the scissile phosphate group has both the *pro*-R and the *pro*-S oxygen atoms simultaneously replaced with sulfur atoms (O.C. Uhlenbeck, personal communication), become understandable if there is no requirement for a divalent metal ion to bind to the scissile phosphate group. Finally, the acceleration in the rate of cleavage and enhancement of the extent of cleavage of a hammerhead ribozyme sequence upon crystallization (Murray *et al*., 1998a) becomes more understandable if a large-scale conformational rearrangement that would require disruption of the crystal lattice is not required for catalysis. As we have recently obtained the structure of the hammerhead ribozyme cleavage reaction product within the confines of the crystal lattice, we now have direct evidence that lattice disruption is not required for hammerhead ribozyme cleavage (Murray *et al*., 2000).

Materials and Methods

Our best-refined unmodified (i.e. catalytically active) hammerhead ribozyme crystal structure with Mn^{2+} bound (Scott *et al*., 1996) was used as a starting point for all molecular modeling. All models were initially constructed manually using the graphics display program O (Jones & Kjeldgaard, 1997), and then were refined in X-PLOR 3.8 (Brünger, 1993). Both conventional and simulated annealing rigid-body dynamics were performed using the canonical helices (stems I, II and III), or portions thereof as described in the text, as rigid bodies for refinement. Rigid-body molecular dynamics refinement of the models was performed using temperature coupling to a bath held at 298 K for 40,000 time-steps of 0.005 ps duration each. Although stems I and II were treated as rigid bodies, the ribose and phosphate atoms of both the scissile phosphate group and the A9 phosphate group were allowed to move freely within the limitations imposed by direct metal ion coordination. In practice, the metal ion coordination geometry was maintained by fixing the initial positions of atoms O1P and P of each of the two phosphate groups and the N7 of G10.1 with regard to the metal ion, thus maintaining a 2 Å distance and octahedral geometry for these ligands. By so doing the stem I and stem II helices could freely rotate about the single phosphorus atom of each that was fixed in space. When employed in the refinements, the crosslinking distance constraint was modeled as a square-well potential with an energy penalty of zero when the crosslinking distance was between 0 and 16.0 Å, and of 100 to 10,000 kcal/mol if the distance exceeded 16 Å. The potential energy function used (Parkinson *et al*., 1996) contained terms for standard bond lengths, angles, torsion angles, base planarity, van der Waals repulsive forces, and (when relevant) the square-well distance constraint. An electrostatic repulsion term was not included due to the difficulty of accurately modeling the counterion environment of the RNA in solution.

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ERRATUM

Does a Single Metal Ion Bridge the A-9 and Scissile Phosphate Groups in the Catalytically Active Hammerhead Ribozyme Structure?

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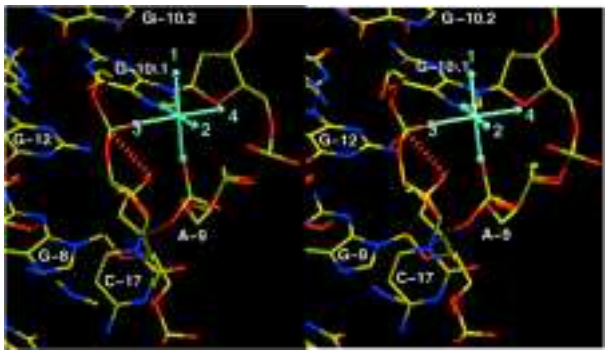
Figure 3 of the above paper shows the divalent metal ion coordinated to the pro-S nonbridging phosphate oxygens of A-9 and C-17 in the proposed hammerhead ribozyme conformational change that we modeled. The metal ion should in fact be bound by the pro-R oxygens of these phosphates, as is now shown in the revised Figure 3 provided. This revision causes only very minor changes in model (as may be seen by comparing the two figures) and does not affect the refinements and optimizations (which we repeated with the corrected starting model) or the analysis and conclusions presented in our original paper. We regret any inconvenience that this error may have caused and thank D. Herschlag for alerting us to it. Coordinates for the corrected model have been deposited as PDB 1FG1.



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