Identification and Characterization of the RNA Chaperone Activity of Hepatitis Delta Antigen Peptides*

Zhi-Shun Huang and Huey-Nan Wu‡

From the Institute of Molecular Biology, Academia Sinica, Taipei 11529, Taiwan, Republic of China

In this study, we identified an activity of the hepatitis delta antigen that both modulates the cis-cleaving activities of hepatitis delta virus (HDV) genomic RNA fragments and facilitates the trans-cleavage reactions between hammerhead ribozymes and the cognate substrates of various lengths in vitro. Hepatitis delta antigen peptides exert their effect by accelerating the unfolding and refolding of RNA molecules and by promoting strand annealing and strand dissociation. In addition, the stimulatory effect of hepatitis delta antigen peptide on hammerhead catalysis is observed whether the peptide is removed or not by phenol/chloroform extraction prior to the initiation of trans-cleavage reaction. Therefore, hepatitis delta antigen peptide acts as an RNA chaperone. The RNA chaperone domain of hepatitis delta antigen overlaps with the coiled-coil domain that is rich in lysine residues. The RNA binding domains of hepatitis delta antigen previously identified are not required for the RNA chaperone activity identified herein. The RNA chaperone activity of hepatitis delta antigen may be important for the regulation of HDV replication in vivo.

Hepatitis delta virus (HDV) is a subviral pathogen that requires hepatitis B virus (HBV) to supply envelope protein for completion of package, secretion, and infection (1–3). The genome of HDV is a single-stranded circular RNA of ~1700 nt and HDV RNA is replicated through a rolling circle mechanism (4). HDV codes one protein of two forms during infection; the small delta antigen (SdAg) contains 195 aa and the large delta antigen (LdAg) has an extra 19 aa at the C terminus (5). Transfection studies with HDV cDNA elucidated that the two protein forms have distinct functions. SdAg initiates genome replication (6) and LdAg promotes package (7). There are two RNA binding domains in each protein form. The first is the arginine-rich sequence near the N terminal, and the second is the arginine-rich motifs (ARMs) located at the middle one-third of the protein (8, 9). The RNA binding activity is important for the function of the two protein forms: the second RNA binding domain of SdAg is required to initiate genome replication (9–12), and the first RNA binding domain of LdAg is responsible for potent inhibition of replication (13). The specific interactions between the hepatitis delta antigen and HDV RNA appear to be involved in the regulation of virus replication although a molecular mechanism has not yet been elucidated.

HDV RNAs of genomic and antigenomic senses cis-cleaved in the absence of protein factors in vitro (14). The ribozyme activity of HDV RNA, which requires a pseudoknot-like structure of the RNA molecule (15, 16) and the catalysis of divalent cations (17), is essential for generating monomeric size RNA molecules during replication (18). Recently, Jeng et al. (19) illustrated that hepatitis delta antigen may enhance, though is not required for, the processing of multiple length HDV RNA in vivo. Conceivably, hepatitis delta antigen per se or together with some other factor(s) acts as an RNA chaperone that modulates the ribozyme activity of HDV RNA.

RNA chaperones are proteins that aid in the process of RNA folding by preventing misfolding or by resolving misfolded species (20). The RNA chaperone activities of several proteins that bind RNA with broad specificity have been explored through their effects on hammerhead ribozyme reactions and group I intron reactions. These proteins, including the nucleocapsid protein (NC) of human immunodeficiency virus (HIV), the C-terminal domain of heterogeneous nuclear ribonucleoprotein A1 (A1 CTD), and Escherichia coli ribosomal proteins, can overcome the general limitations of ribozyme reactions, such as the formation/dissociation of base pairs and the adoption of functional structure, and facilitate ribozyme catalysis (21–24).

Here we analyze the putative RNA chaperone activity of hepatitis delta antigen peptides in vitro. Using the facilitation of trans-cleavage reactions of the previously characterized hammerhead ribozyme HH116 and its 17-nucleotide substrate S (25) as the initial assay, we identify the strand-annealing and strand-dissociation activities of hepatitis delta antigen peptides. We then show that the functional hepatitis delta antigen peptides promote RNA unfolding that stimulates interstranded duplex formation. This activity is able to activate an antisense RNA as well as facilitate trans-acting hammerhead ribozymes to find their targets in cognate substrate RNAs. In addition, hepatitis delta antigen peptides can modulate the cis-cleaving activity of HDV genomic RNA fragments. Hepatitis delta antigen acts as an RNA chaperone and the RNA chaperone domain locates at the N-terminal domain of the protein that contains a high density of basic amino acids. Our findings suggest that the RNA binding domains of hepatitis delta antigen identified previously are, therefore, not required for RNA chaperone activity.

EXPERIMENTAL PROCEDURES

Expression and Purification of Hepatitis Delta Antigen Peptide—The cDNA of the hepatitis delta antigen and its truncated mutants were amplified by polymerase chain reactions with synthetic oligonucleotides as primers. The polymerase chain reaction products were cloned to the Ndel and BamHI sites of vector pET15b (Novagen). The sequence of each recombinant clone was determined by DNA sequencing. The plasmids were transformed into E. coli BL21(DE3) cells for expression purpose (26). The hepatitis delta antigen and its truncated polypeptides were expressed as fusion proteins with a His tag at their N termini. In fusion proteins dAg(1–195), NMDAg(1–143), and NdAg(1–88), the tag

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‡ To whom correspondence should be addressed. Tel.: 886-2-2311-B-001-045-B21.

The abbreviations used are: HDV, hepatitis delta virus; nt, nucleotides; aa, amino acid(s); SdAg, small delta antigen; LdAg, large delta antigen; CTD, C-terminal domain.
had the sequence of Met-Gly-(Ser)2-(His)6-(Ser)2-Gly-Leu-Val-Pro-Arg-
Molecular Dynamics).

Fusion proteins that contain the N-terminal domain of hepatitis delta antigen bound tightly to phosphocellulose resin. These proteins were eluted from phosphocellulose columns by a buffer containing 50
mM HEPES (pH 7.8), 0.2 mM EDTA, 0.9–1.2 mM NaCl, and 20% glycerol. Fusion proteins MdAg and CdAg were purified on nickel columns as described by the manufacturer except that the elution buffer contained 20% glycerol (Novagen). Fractions containing the fusion proteins were frozen in liquid nitrogen and stored at – 70°C. Protein concentration was determined by the Bradford assay. The absorbency at 595 nm from bovine serum albumin was used to establish a standard curve from which the concentration of each purified protein was determined. The yield of purified protein was ~0.2 mg from 50 ml of culture.

Peptide K7 was synthesized by peptide synthesizer using standard solid phase methods. The peptide was purified by high performance liquid chromatography, and the concentration was determined by ninhydrin assay.

Constructs and RNA Synthesis—The 17-nucleotide S (see Fig. 2A) was made by solid-phase chemical synthesis. HH16 was synthesized by T3 RNA polymerase with synthetic DNA as template (27). HDV genomic RNA fragments were run-off transcriptions of polymerase chain reaction-amplified templates. Other RNAs were run-off transcriptions of linearized plasmids. The 5’-end labeled carrier-free S was prepared by incubating the RNA fragment with T4 polynucleotide kinase and excess amounts of [γ-32P]ATP. Other RNAs were internally labeled by incorporating [α-32P]CTP in run-off transcription reactions. The synthetic RNAs were purified on polyacrylamide-7 M urea gels and were ethanol precipitated after being eluted from gels. RNA fragments were resuspended in TE buffer (10 mM Tris-HCl, pH 8, and 0.1 mM EDTA) before use. The concentrations of nonradioactive RNA fragments were determined by assuming a residue extinction coefficient of 260 nm of 6.6
mM

Library of Sa and HH16. S

RESULTS AND DISCUSSION

Hepatitis Delta Antigen Peptides—Hepatitis delta antigen peptides were over-expressed in and were purified from E. coli as fusion proteins (Fig. 1A). dAg contains the full-length small delta antigen, and NMdAg contains the first 143 amino acids of the hepatitis delta antigen. The sequence of aa 1–88 is shown, and the residues of the RNA chaperone domain identified in this study are underlined. B, photograph of the Coomasie Blue-stained 15% SDS-polyacrylamide gel analysis of different peptides. NdAg, NMdAg, and dAg were purified as described (“Experimental Procedures”); MdAg and CdAg of this gel were purified from a phosphocellulose column instead of a nickel column; and 10 μg of protein was used for each lane.

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negligible at 25 and 37 °C, the temperatures at which all of the reactions of this study were carried out.

Effect of Hepatitis Delta Antigen Peptides on the Trans-cleavage Reaction of S and HH16—The reactions of hammerhead ribozyme HH16 and its 17-nucleotide substrate S (Fig. 2A) characterized by Hertel et al. (25) were used as the model system to study the effect of hepatitis delta antigen peptides on RNA-mediated processes.

The release of cleavage products from ribozyme is slower than the chemical step in the trans-cleavage reaction of hammerhead ribozyme HH16 (25). Therefore, under the multiple turnover condition with S in excess of HH16, there was an initial burst of product formation followed by much slower product formation, and one HH16 molecule could barely be used twice in the 4-h incubation period (Fig. 2A). The addition of dAg to the reaction accelerated the dissociation of cleavage products that increased turnover, and there was an enhancement rate of 10-fold when the reaction was carried out in the presence of saturated concentrations of dAg (Fig. 2C). NdAg and NMdAg that contain the N-terminal domain of the hepatitis delta antigen also facilitated the cleavage of S by HH16. In contrast, MdAg and CdAg did not possess this activity (Fig. 2C, and data not shown). Increasing dAg or NdAg concentrations up to 30 ng/μl did not inhibit the trans-cleavage reaction (data not shown). Nevertheless, boiling the functional peptides in the presence of 0.1% SDS caused the elimination of the stimulatory effect of these peptides (Fig. 2C, and data not shown). Therefore, hepatitis delta antigen peptides may promote the single turnover trans-cleavage reactions of S and HH16. In addition, the functional motif appears to reside in the N-terminal half of the hepatitis delta antigen.

Because there is no product dissociation process under single turnover conditions, a pulse-chase experiment was carried out to further address the question of whether the hepatitis delta antigen peptide promotes the annealing of S to HH16. The results shown in Fig. 2D illustrate that dAg may accelerate the association of S and HH16 that overcomes the rate-limiting step of the trans-cleavage reaction of S and HH16. MdAg, the peptide containing the arginine-rich motif of hepatitis delta antigen (9–10) did not possess this property.

In summary, hepatitis delta antigen can overcome the limitations of the reaction of hammerhead ribozyme HH16 by facilitating not only substrate association but also product re-
lease, and these properties were similar to those of the previously identified RNA chaperones (21–24). The functional domain resides in the first 88 amino acids of the hepatitis delta antigen.

**Peptide NdAg Acts as an RNA Chaperone That Facilitates the Trans-cleavage Reactions of Various Substrates and Hammerhead Ribozymes**—The effect of NdAg on stimulating a trans-acting hammerhead ribozyme to find its target was used to evaluate the RNA-unfolding and -refolding activity of this peptide. RNA fragments that had the 17-nucleotide S or the 38-nt HH16 inserted in foreign sequences of different lengths were synthesized, and the trans-cleavage reactions of various combinations of these substrate and ribozyme RNAs were carried out under single turnover conditions for investigating the facilitation effect of NdAg on the reconstitution of hammerhead catalytic domain.

The substrate RNAs 15bS (~60 nt) and PRP19S (~900 nt) contained one copy of S (Fig. 3A). The cleavage of 15bS by HH16 occurred at a rate significantly lower than that of S, whereas PRP19S was hardly cleaved by HH16 (Fig. 3B). Therefore, the accessibility of the 17-nucleotide S decreases dramatically as the substrate RNA elongates. Nevertheless, when the trans-cleavage reaction was carried out in the presence of 2–3 ng/μl NdAg, 15bS as well as PRP19S (0.5 mM of each) could be cleaved to near completion by HH16 (5 mM) at a rate similar to that of S. Higher temperatures were required for the efficient cleavage of PRP19S (37 °C instead of 25 °C) (Fig. 3B, and data not shown). KSS3 (~110 nt) is a substrate RNA containing three tandem repeated S: the first copy has a C to an A substitution at residue 6 of S (C6A), and the other copies are wild type. KSR4 contains four copies of S, wild type. KSR4 contains four copies of S, the first copy has a C to an A substitution at residue 6 of S (C6A), and the other copies are wild type. KSR4 contains four copies of R, B, cleavage of 0.5 mM S (○), 15bS (□), as well as PRP19S (△, ▲) by 5 mM HH16 in the absence of NdAg (the open symbols) or in the presence of 2–3 ng/μl of NdAg (the closed symbols). The reactions of PRP19S were performed at 37 °C, whereas other reactions were performed at 25 °C. The fraction of uncleaved substrate was plotted against reaction time. C, cleavage of 25 mM KSS3 by 25 mM HH16 in the presence of 0.5 ng/μl of NdAg at 25 °C. The cleavage products were resolved by a denaturing polyacrylamide gel and quantitated by PhosphorImager analysis. The cleavage event occurring at each cleavage site of KSS3 was calculated and was plotted against the reaction time. D, cleavage of PRP19S by 5 mM KSR (○) or 5 mM KSR4 (□, ▲) at 37 °C in the absence of NdAg (the open symbols) or in the presence of 6 ng/μl of NdAg (the closed symbols). The cleavage products were resolved from substrate and ribozyme RNAs on a 3.5% polyacrylamide gel containing 7 M urea. 5P and 3P represent the 5’ and 3’ cleavage products, respectively. PRP19S is the precursor RNA and KSR4 is the ribozyme. Ribozyme KSR runs off this gel.

![Graph](image.png)

**FIG. 3. Effect of NdAg on the trans-cleavage reactions of various hammerhead substrate and ribozyme RNAs.** A, schematic diagram of the hammerhead substrate and ribozyme RNAs of this study. The dark boxes represent S, the hatched boxes represent HH16, and the open boxes of each RNA represent the sequences derived from the cloning vectors. KSS3 contains three copies of S, the first copy has a C to an A substitution at residue 6 of S (C6A), and the other copies are wild type. KSR4 contains four copies of R, B, cleavage of 0.5 mM S (○), 15bS (□), as well as PRP19S (△, ▲) by 5 mM HH16 in the absence of NdAg (the open symbols) or in the presence of 2–3 ng/μl of NdAg (the closed symbols). The reactions of PRP19S were performed at 37 °C, whereas other reactions were performed at 25 °C. The fraction of uncleaved substrate was plotted against reaction time. C, cleavage of 25 mM KSS3 by 25 mM HH16 in the presence of 0.5 ng/μl of NdAg at 25 °C. The cleavage products were resolved by a denaturing polyacrylamide gel and quantitated by PhosphorImager analysis. The cleavage event occurring at each cleavage site of KSS3 was calculated and was plotted against the reaction time. D, cleavage of PRP19S by 5 mM KSR (○) or 5 mM KSR4 (□, ▲) at 37 °C in the absence of NdAg (the open symbols) or in the presence of 6 ng/μl of NdAg (the closed symbols). The cleavage products were resolved from substrate and ribozyme RNAs on a 3.5% polyacrylamide gel containing 7 M urea. 5P and 3P represent the 5’ and 3’ cleavage products, respectively. PRP19S is the precursor RNA and KSR4 is the ribozyme. Ribozyme KSR runs off this gel.

**RNA Chaperone Activity of Hepatitis Delta Antigen**
contains a copy of the complementary sequence of S near its 3′ end to be a competitor of HH16. It is a 130-nt RNA that terminates. Nevertheless, the cleavage of S (0.5 nM) by HH16 (5 nM) was not perturbed by the presence of 0.5 to 5 nM of rKSS. PRP19S (0.5 nM) and KSR4 (5 nM) were incubated with 3′-end-labeled groups of the RNA chaperone domain of the hepatitis delta antigen that has been shown to bind HDV RNA specifically (8–10) is required for RNA chaperone activity. The N-terminal arginine-rich sequence nevertheless may increase the nucleic acid binding affinity of hepatitis delta antigen peptides. As a result, NdAg facilitates hammerhead ribozyme catalysis and RNA unfolding and refolding at lower concentrations than those of N5dAg and N7dAg.

The RNA chaperone domain of the hepatitis delta antigen is rich in basic amino acids, especially lysine (Fig. 1A). We next examined if the highly basic peptide KKKKKKK (K7) mimics the effect of hepatitis delta antigen peptides in facilitating the single turnover reaction of S and HH16. The results indicated the action of NdAg was further characterized. rKSS did not have any inhibitory effect if the competitor RNA by itself was pre-mixed with NdAg (condition I) (Fig. 5C) or if NdAg was not mixed with the substrate and competitor RNAs for a certain period prior to the initiation of trans-cleavage (condition II) (Fig. 5B). In contrast, S was cleaved at an elevated rate under both conditions I and II because of the presence of NdAg in the reaction (Fig. 5C). It is likely that because of the longer size and lower concentration, the NdAg unfolded rKSS (130 nt, 0.5 nM) may not be able to compete with HH16 (38 nt, 5 nM) for S binding under condition I. Alternatively, when S is not close by, the NdAg unfolded rKSS may adopt some other structure(s) that prevents the binding of S (condition I). Although rKSS is shorter than most of the other RNAs used in this study, the unfolding of rKSS and the formation of rKSS and S hybrid appear to be relatively slow. As a result, only the stimulatory effect of NdAg on the reconstitution of hammerhead catalytic domain was observed under condition II.

Identification of the RNA Chaperone Domain of Hepatitis Delta Antigen—To further narrow down the functional domain responsible for the catalytic stimulation of the hepatitis delta antigen, truncated versions of NdAg were made, and their effects on hammerhead ribozyme catalysis were examined. Peptides N5dAg and N7dAg are fusion proteins containing aa 14–59 and 24–75, respectively, of the hepatitis delta antigen. These peptides behaved analogously to peptide NdAg at 1) facilitating the cleavage of S (0.5 nm) by HH16 (5 nm), 2) promoting the trans-cleavage reaction of PRP19S (0.5 nm) and KSR4 (5 nm), and 3) stimulating the inhibitory effect of rKSS on the cleavage of S by HH16 (data not shown). The N5dAg and N7dAg concentrations required to achieve the maximum stimulatory effect on each reaction were 5- to 10-fold higher than that of NdAg (data not shown). Therefore, the core of the RNA chaperone domain appears to reside in aa 24–59 of the hepatitis delta antigen, which interacts with non-HDV RNA. Neither the arginine-rich sequence near the N terminus nor the arginine-rich motif of the middle one-third of the hepatitis delta antigen that has been shown to bind HDV RNA specifically (8–10) is required for RNA chaperone activity. The N-terminal arginine-rich sequence nevertheless may increase the nucleic acid binding affinity of hepatitis delta antigen peptides. As a result, NdAg facilitates hammerhead ribozyme catalysis and RNA unfolding and refolding at lower concentrations than those of N5dAg and N7dAg.

The RNA chaperone domain of the hepatitis delta antigen is shorter (data not shown), and the inhibitory effect of rKSS was a function of NdAg concentration rather than a function of time (Fig. 5A). Moreover, with the facilitation of saturated concentrations of NdAg, 0.5 nM rKSS was sufficient to inhibit the cleavage of 0.5 nM S by 5 nM HH16, whereas an HDV cis-cleaving ribozyme did not have any inhibitory effect on the cleavage of S under the same condition (Fig. 5B). The near complete inhibition of S cleavage discloses that most of the S molecules are involved in the formation of S/rKSS hybrid. The substrate/competitor complex appears to be stable and prohibits further interaction of S and HH16. Therefore, NdAg facilitates rKSS becoming an antisense RNA.

molecules are stabilized by the magnesium ion that in turn diminishes the stimulatory effect of NdAg.

We then used the trans-cleavage reaction of PRP19S and KSR4 to study whether NdAg can be removed after it has facilitated the reconstitution of the hammerhead catalytic domain. NdAg (6 ng/μl) was extracted by phenol/chloroform after it has premixed with two RNAs (0.5 nm substrate and 5 nm ribozyme) for 10 min. Trans-cleavage reaction was then initiated by 12 mM MgCl2, and the cleavage of PRP19S was investigated. As shown in Fig. 4B, PRP19S was cleaved rapidly regardless of the removal of NdAg. Therefore, NdAg acts as an RNA chaperone. The phenol/chloroform extraction treatment appears to destabilize the PRP19S/KSR4 complex and decrease the extent of cleavage, and the addition of NdAg to the solution stimulated the cleavage of the remaining PRP19S (Fig. 4B).

Results of these studies regarding the cleavage of various substrates by different hammerhead ribozymes revealed that NdAg may act as an RNA chaperone that rapidly and efficiently facilitates the unfolding and refolding of RNA molecules range from <50 to ~900 nt in length. Consequently, the assembly of the hammerhead catalytic domain gets promoted by NdAg. Ribozyme RNAs may catalyze the cleavage of their cognate substrate RNAs once the trans-cleavage reactions are initiated by the addition of MgCl2.

**Peptide NdAg Activates an Antisense RNA**—rKSS was designed to be a competitor of HH16. It is a 130-nt RNA that contains a copy of the complementary sequence of S near its 3′ terminus. Nevertheless, the cleavage of S (0.5 nm) by HH16 (5 nm) was not perturbed by the presence of 0.5 to 5 nM of rKSS. Moreover, neither the premixing nor the co-denaturation and renaturation of rKSS and S prior to the initiation of trans-cleavage (by the addition of HH16 and MgCl2) affected the cleavage of S (data not shown). Therefore, rKSS appears to have stable intramolecular interaction that buries the complementary sequence of S.

We next investigated if peptide NdAg stimulates the antisense activity of rKSS. When 0.5 nm S was pre-mixed with 5 nm rKSS in the presence of NdAg for 30 min, the cleavage of S by 5 nm HH16 was inhibited. The pre-mixing period could be

**FIG. 4.** The trans-cleavage reaction of PRP19S and KSR4. A, PRP19S (0.5 nm) and KSR4 (5 nm) were incubated with 3′ (○), 12 (●), 24 (□), or 36 mM MgCl2 (▲) at 37 °C for 10 min. Then 6 ng/μl NdAg was added (as indicated by the arrow), and trans-cleavage reaction was carried out for another 20 min. The fraction of uncleaved PRP19S was plotted against the trans-cleavage reaction time. B, PRP19S (0.5 nm) and KSR4 (5 nm) were incubated with 6 ng/μl of NdAg (○) or the reaction buffer (●) was added to the reaction mixture (as indicated by the arrow) for 10 min before the termination of trans-cleavage reaction. For the controls, PRP19S and KSR4 were pre-incubated with 0 ng/μl (●) or 6 ng/μl of NdAg (▲) at 37 °C for at least 20 min before the addition of 12 mM MgCl2. The fraction of uncleaved PRP19S was plotted against the trans-cleavage reaction time.
delta antigen peptides are important for the promotion of hammerhead ribozyme catalysis. Because a peptide containing the RNA chaperone domain of the hepatitis delta antigen is responsible for the coiled-coil multimer formation (28, 29), it is likely that in addition to the high density positively charged groups, the α-helical structure and/or the formation of a peptide multimer are important for RNA chaperone activity. The structural and functional relationship of the RNA chaperone domain of the hepatitis delta antigen requires further elucidation.

**Hepatitis Delta Antigen Affects the Folding of HDV RNA**—Each sense of HDV RNA contains an autolytic domain that may fold into a pseudoknot-like structure and then undergoes site-specific cis-cleavage (15, 16). Previous studies illustrate that presumably because of the highly self-complementary nature of HDV RNA, the sequence surrounding the autolytic domain may cause the formation of alternative structures that interfere or prevent the adoption of the autocatalytic structure. Consequently, the cis-cleaving activities of different HDV RNA subfragments may vary significantly although each of them contains an intact autolytic domain (30). This characteristic of HDV RNA was evident by the HDV genomic RNA fragments synthesized in this study (Fig. 6, A and B): 4–1 (681–775; i.e. nt 681 to nt 775 of HDV genomic RNA), as well as 1–2 (583–800) that underwent cis-cleavage when the reactions were carried out in the presence of 12 mM MgCl₂ at 37 °C. The rate of cleavage of the former was significantly higher than that of the latter; 2–2 (625–800) cis-cleaved slowly and inefficiently under the same condition; and 3–2 (659–800) together with 2–4 (625–860) barely cis-cleaved. However, the cis-cleaving activities of 2–2, 2–4, and 3–2 can be elevated significantly if the RNA molecules have gone through repeated cycles of heat denaturation and renaturation (30) prior to the initiation of cis-cleavage, or if the cis-cleavage reaction is performed in the presence of moderate concentrations of denaturant (at 37 °C) or at higher temperatures (50 °C) (data not shown).

To investigate whether the RNA chaperone activity of hepatitis delta antigen peptides identified through the studies of hammerhead ribozyme catalysis is biologically relevant, we studied the effect of peptide NdAg on the cis-cleaving activities of the HDV genomic RNA fragments described above. As shown in Fig. 6B, the premixing with NdAg prior to the initiation of cis-cleavage altered the ribozyme activity of three fragments: the cis-cleaving activity of 4–1 was abolished; 3–2 became active although it cis-cleaved slowly; and a large portion of the inactive molecules of 2–2 were activated and consequently the extent of cleavage was elevated from <50% to >90%. Preincubation with NdAg did not have detectable effect on 2–4; 2–4 remained inactive and neither the rate nor the extent of cis-cleavage of 1–2 were altered (Fig. 6B). These results disclose that NdAg may modulate the autocatalytic activity of HDV RNA subfragments by stimulating RNA unfolding and refolding.

We next examined the activity of other hepatitis delta antigen peptides on the cis-cleavage of fragment 4–1, which is the smallest RNA fragment containing the autolytic domain of HDV genomic RNA. The results illustrate that similar to that of NdAg, peptides dAg, NMdAg, N5dAg, and N7dAg attenuated the ribozyme activity of 4–1 (data not shown). Peptide CdAg, in contrast, did not have a detectable effect on the cis-cleavage reaction of 4–1 (data not shown). This finding discloses that the...
functional domain resides in aa 24–59 of hepatitis delta antigen. In addition, neither the arginine-rich sequence nor the arginine-rich motif of hepatitis delta antigen that have been shown to bind HDV RNA specifically (8–13) are required for the activity identified herein.

In summary, we have shown that the RNA chaperone domain of the hepatitis delta antigen can modulate the autocatalytic activity of HDV RNA in vitro. Conceivably, the modulation of the ribozyme activity of HDV RNA by hepatitis delta antigen is one of the mechanisms that regulates HDV replication.

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