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Rescue of abasic hammerhead ribozymes by exogenous addition of specific bases

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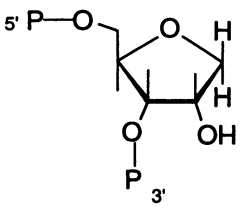
ABSTRACT **We have synthesized 13 hammerhead ribozyme variants, each containing an abasic residue at a specific position of the catalytic core. The activity of each of the variants is significantly reduced. In four cases, however, activity can be rescued by exogenous addition of the missing base. For one variant, the rescue is 300-fold; for another, the rescue is to the wild-type level. This latter abasic variant (G10.1X) has been characterized in detail. Activation is specific for guanine, the base initially removed. In addition, the specificity for guanine versus adenine is substantially altered by replacing C with U in the opposite strand of the ribozyme. These results show that a binding site for a small, noncharged ligand can be created in a preexisting ribozyme structure. This has implications for structure–function analysis of RNA, and leads to speculations about evolution in an "RNA world" and about the potential therapeutic use of ribozymes.**

The interplay between structure and function in RNA has been extensively explored by mutating the sequence of naturally occurring functional RNAs (e.g., refs. 1–3). In several cases, the role of specific nucleotides has been studied in finer detail by using chemical synthesis to incorporate unnatural analogs with specific functional groups removed (for review, see ref. 4). We are exploring a "subtraction mutagenesis" approach in which the entire base of a specific nucleotide is removed rather than altered. This provides a distinct probe of functional RNAs. This type of mutagenesis is made possible by methods that allow inclusion of stable, reduced abasic nucleotides (Eq. **1**) in RNA produced by solid-phase synthesis (5, 6).



[1]

This approach is akin to alanine scanning mutagenesis used in protein studies. Alanine substitutions can perturb the local packing to various extents: the "cavities" that are created may be left unfilled or filled either by solvent or by rearrangements of nearby side chains (7–9). The effects of the analogous subtraction mutagenesis in RNA may reveal how it responds to local changes in packing and flexibility and provide insights into the interrelationship of RNA structure and function.

We have applied this mutagenetic approach to the study of the hammerhead ribozyme (Fig. 1*A*). Its small size renders the hammerhead suitable for chemical synthesis. Furthermore, this ribozyme has been subjected to extensive mutagenetic analysis (for reviews, see refs. 15 and 16), its three-dimensional structure has been solved by x-ray crystallography (17, 18), and a kinetic and thermodynamic framework for its reaction has been established (19). This provides a basis for interpreting the effects of abasic residues and for deriving new functional insights.

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MATERIALS AND METHODS

Materials. Oligonucleotides were prepared by solid phase synthesis. The incorporation of reduced abasic nucleotides and 1-phenyl D-ribose nucleotides in RNA has been described (5, 6, 20). Bases and base analogs used in the rescue experiments were of the best commercially available quality and were not further purified. Thus, rescue at a very low level may in some cases represent an upper limit for the activity of a given base.

Methods. *Single turnover reactions.* The first-order rate constant for the cleavage of the substrate in the ribozyme·substrate complex, k_2, was determined under single-turnover conditions as previously described (19). Briefly, a trace of ^{32}P-5′-end-labeled substrate (≈0.2 nM final concentration) and large excess ribozyme (0.6 μM final) were heated together at 95°C for 2 min in 50 mM Tris·HCl, pH 7.5, and equilibrated at 25°C to allow formation of the ribozyme·substrate complex before initiating reaction with $MgCl_2$ (10 mM final concentration). Control reactions using 2.5 μM ribozyme indicated that the substrate was completely bound in all cases. Nonlinear least squares fitting of the reaction time courses was used to obtain k_2. In agreement with previous results (19), k_2 for the wild-type HH16 was $1.1 \pm 0.2\ min^{-1}$ with an end point of 80–90%. Reactions of variants U4X, U7X, G10.1X, and C11.1X were followed to completion and the data fit well to a single exponential function with end points of ≈90% ($R^2 > 0.99$). For slower variants, the reactions were followed for ≥36 hr and k_2 was obtained from the initial rates, assuming an end point of 90%. The k_2 values varied <25% between independent experiments.

Determination of k_{rescue}. In base-rescue experiments, the observed rate constant for the cleavage of the ribozyme·substrate complex (k_2^{obs}) was determined as described above, except that a variable amount of free base was added. The final concentration of base always greatly exceeded that of the ribozyme·substrate complex. Values of k_2^{obs} obtained at a series of base concentrations were fit by nonlinear least squares to Eq. **2**,

$$k_2^{obs} = \frac{k_2 + k_{rescue} \times [Base]}{1 + [Base]/K_d} \qquad [2]$$

which was derived from the model in Eq. **3** below. K_d is the dissociation constant of the base from the enzyme·substrate·base

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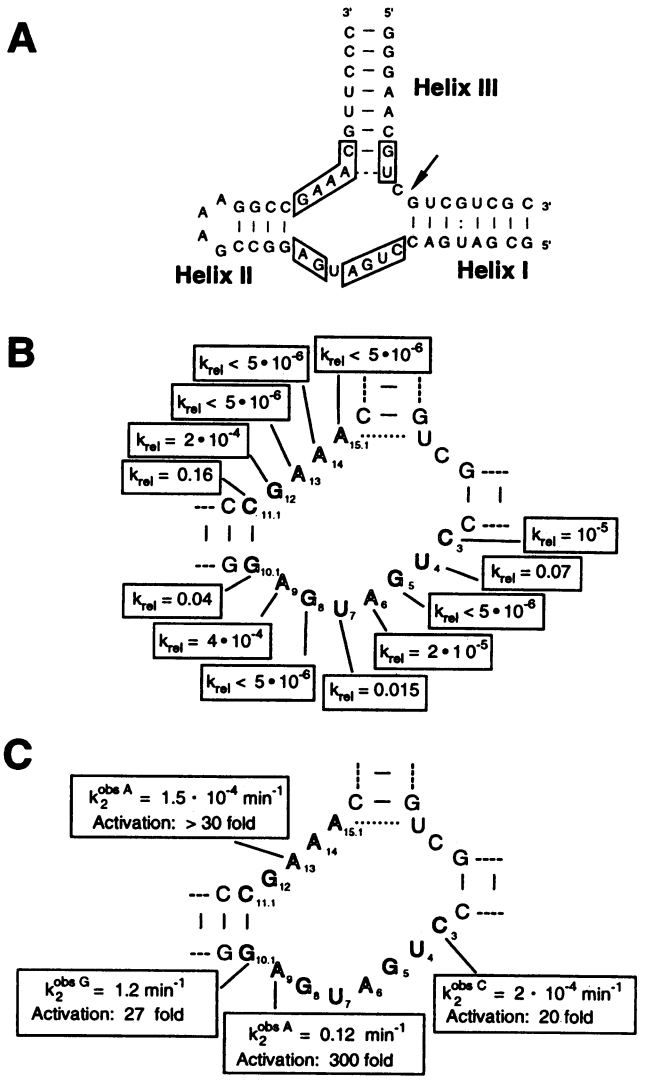


FIG. 1. Effect of abasic residues on the activity of the hammerhead ribozyme and rescue by exogenous bases (*A*) Secondary structure of the hammerhead ribozyme, HH16. The hammerhead consists of three helices and 11 nonhelical residues located in the highly conserved central region (10–12). Conserved residues are boxed (10). The arrow indicates the position of cleavage. (*B*) Reduction of catalysis in abasic HH16 variants. k_{rel} is defined as k_2^{abasic}/k_2^{wt}, where k_2 is the first-order rate constant for the cleavage of the substrate in the ribozyme·substrate complex. Nucleotides are numbered according to the standard hammerhead nomenclature (13). The effects of introducing abasic nucleotides at positions 4 and 7 are larger than reported in earlier studies (5, 6) because under the reaction conditions used in those preliminary studies substrate binding, rather than chemistry, was rate-limiting. Replacing U7 with a tetrahydrofuran residue (a 2′ deoxy abasic) had an effect similar to that observed here (14). (*C*) Upon exogenous addition of the missing base, rescue is observed for the four abasic variants indicated. $k_2^{obs\ base}$ was measured in the presence of 30 μM guanine, 3 mM adenine, 30 mM uracil, or 50 mM cytosine. Under these conditions, the activities of each of the nine other variants and that of wild-type HH16 were unaffected within experimental error (<20%).

ternary complex, k_2 and k_2' are the rate constants for cleavage in the absence and presence of bound base, respectively, and k_{rescue} ($= k_2'/K_d$) is an apparent second-order rate constant for the rescue, incorporating both binding of the base and the subsequent cleavage step.

In most cases plots of k_2^{obs} versus [Base] did not show clear saturation (e.g., see Fig. 3). A decrease in the chemical activity of the base due to aggregation could account for the small deviations from linearity observed at high base concentrations in some cases. Thus, reliable K_d and k_2' values could not be determined.§ Nevertheless, k_{rescue} ($= k_2'/K_d$) is readily determined from fits of the data to Eq. **2**, because it depends only on the linear portion of the dependence. Values of k_{rescue} are therefore used throughout this paper to compare rescue by different bases. Independent determinations of k_{rescue} varied by less than 50%.

RESULTS

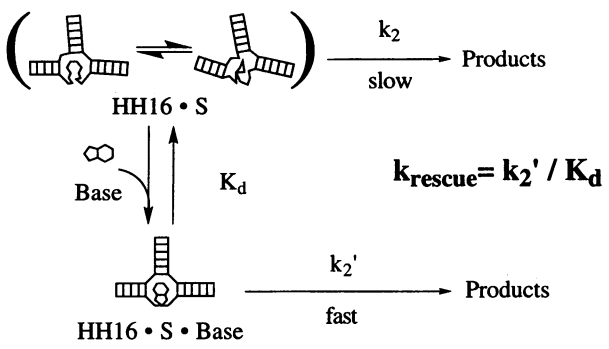
Effect of Abasic Nucleotides on Hammerhead Activity. Thirteen variants of the hammerhead ribozyme HH16 were prepared, each bearing an abasic nucleotide at a specific position of the central core. [Variants are indicated by acronyms analogous to those used for mutant proteins, e.g., A9X is a ribozyme where the A at position 9 has been changed to an abasic (X).] Abasic ribozymes show large decreases in catalysis (Fig. 1*B*), indicating that ablation of the base impairs function in each case. Though some of the bases removed may be directly involved in the catalytic mechanism of the ribozyme, the widespread and rather even distribution of the observed, large effects suggests that most of the effects arise from changes in the conformation of the central core.

Probing the Properties of Abasic Sites Using Free Bases. Deleterious effects from removing side chains in protein cores can occasionally be rescued by small molecules that bind within a cavity created by the mutation (8). The formation of analogous stable "cavities" in RNA might be expected to be less likely, however, due to the absence of a true "hydrophobic core" and to the intrinsic higher flexibility of nucleic acid structures. Nevertheless, even a collapsed cavity could in principle rearrange to accommodate the ablated group.

The ability of bases to rescue activity was tested for the ribozyme by monitoring the reaction of each of the abasic variants in the presence of each of the four bases. Exogenous addition of the missing base had no effect on most of the abasic ribozymes but did increase the activity in four cases (Fig. 1*C*). For variant A9X, catalysis is increased by 300-fold. Even more strikingly, micromolar concentrations of guanine fully rescue the G10.1X variant, increasing the cleavage rate to the wild-type level (Fig. 2). Added bases had no effect on the wild-type ribozyme within experimental error (<20%; data not shown), implying binding of the base to a newly created site. In addition, replacement of the base of G10.1, A9, or C3 with a phenyl group (20) diminished rescue by 5- to 7-fold, presumably by sterically limiting access to the binding site (unpublished results; A13 was not tested). Similarly, nucleosides had much smaller effects than the corresponding bases, as shown by the 200- to 500-fold lower values of k_{rescue} (defined in Eq. **3**) (data not shown). This indicates that the sites created by the abasic introduction discriminate against the larger nucleosides.

A model that accounts for all the results presented above is presented in Eq. **3**. According to the model, removal of a base causes a deformation in the hammerhead core (shown pictorially as the HH16·S structure at the upper right of Eq. **3**) that impairs function. The perturbed structure, however, can sometime rearrange to make the abasic site accessible to the free base (HH16·S structure on the upper left of Eq. **3**). Binding of the base restores the active structure and therefore enhances catalysis ($k_2' > k_2$).

§For bases not showing clear saturation, the highest base concentration used gives a rough lower limit for K_d: 0.03 mM guanine, 3 mM adenine, 50 mM cytosine, 30 mM uracil, 5 mM hypoxanthine, 8 mM 2-aminopurine, 0.25 mM 7-methylguanine, 8 mM 7-deazaguanine, 0.05 mM pterin.



[3]

Specificity of Rescue of the G10.1X Variant. To better understand the molecular basis of the observed rescue, we investigated the specificity of activation of the G10.1X variant in detail. In the wild-type ribozyme, G10.1 forms a Watson–Crick base pair with C11.1 (Fig. 1). Rescue of the G10.1X variant was specific for guanine relative to the other three naturally occurring bases (Fig. 2), consistent with guanine binding in a manner analogous to the ablated base. k_{rescue}, defined in Eq. **3** and in the *Materials and Methods*, is 49,000 $M^{-1}\cdot min^{-1}$ for guanine, whereas k_{rescue} values for adenine, cytosine, or uracil are 190-, 8500-, and ≈50,000-fold lower, respectively.

To further test if the guanine activator binds to the ribozyme via Watson–Crick base pairing, individual hydrogen bonding groups were removed from the base pairing face of guanine (Fig. 3). Removal of one hydrogen bond partner to give hypoxanthine decreases k_{rescue} by 60-fold and removal of two hydrogen bond partners to give 2-aminopurine decreases k_{rescue} by 500-fold, consistent with activation via standard base pair formation.

Changing the Specificity of the Binding Site. If the rescue depends on a Watson–Crick base pair, then the selectivity of the binding site should be modified by changing the base opposite to position 10.1. This was tested by changing C11.1,

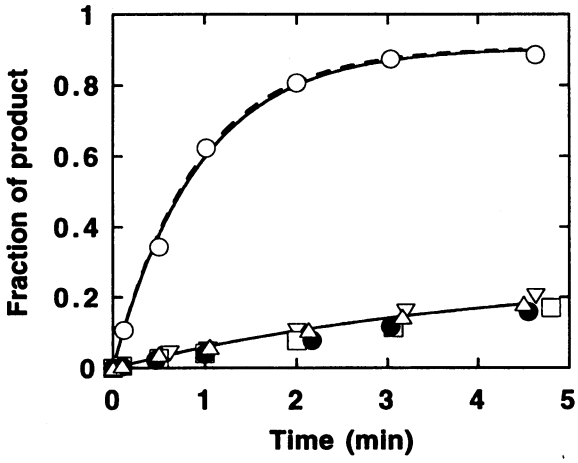


FIG. 2. Specific activation of the abasic variant G10.1X by guanine. Cleavage in the absence of added bases (●) and in the presence of 30 μM guanine (○), 30 μM adenine (▽), 1 mM uracil (□), or 1 mM cytosine (△) was measured under single-turnover conditions. The reaction time course for the wild-type ribozyme is depicted by the dashed line for comparison; it was normalized to the same end point as the abasic variant to facilitate visual comparison (the observed end-points differ by only 10%). The effect of guanine on the G10.1X variant was the same whether the base was added to the ribozyme before folding or at the start of the reaction, indicating that binding of the base and any subsequent structural rearrangements are fast compared with catalysis.

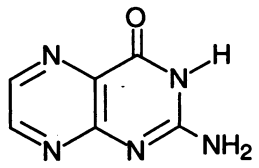
the pairing partner of G10.1, to U. Just as guanine could rescue the activity of G10.1X to the wild-type level, adenine can rescue the activity of the variant G10.1X/C11.1U to the level of its parent ribozyme, G10.1A/C11.1U (data not shown). The role of base pairing was investigated in greater detail by comparing the ability of guanine and 2,6-diaminopurine to rescue the abasic variants with C and U at position 11.1 (Table 1); guanine and 2,6-diaminopurine each form three hydrogen bonds with C and U, respectively. Changing the putative pairing partner changed the specificity for guanine versus 2,6-diaminopurine by >120-fold.

Guanine gave no rescue of the G10.1X/C11.1U variant, but the low solubility of the base prevented determination of whether the specificity in rescue had been reversed upon change of the pairing partner. We therefore investigated rescue by a more soluble guanine analog, hypoxanthine, that makes two hydrogen bonds in a base pair with C (Fig. 3*B*), analogous to the two hydrogen bonds in an A·U pair. As predicted, the G10.1X variant prefers hypoxanthine over adenine, whereas the G10.1X/C11.1U variant prefers adenine over hypoxanthine (Table 2).

The Importance of N7. The x-ray crystallographic structure of the hammerhead ribozyme shows that N7 of G10.1 coordinates a metal ion (17, 18). The 50- and 250-fold decreases in k_{rescue} for 7-methylguanine and 7-deazaguanine, respectively, relative to guanine suggest that an interaction of N7 likewise contributes to the binding of exogenous guanine.

The Importance of Stacking. According to the crystal structure, stacking interactions are present on both faces of the G10.1 base ring (17, 18). Isocytosine bears on its pairing face the same hydrogen bonding groups as guanine, but, being a pyrimidine, stacks less efficiently. The value of k_{rescue} for isocytosine with the G10.1X variant is 5000-fold lower than k_{rescue} for guanine, and 20- and 100-fold lower than k_{rescue} for 7-deazaguanine or 7-methylguanine, respectively, which, like isocytosine, cannot participate in an interaction with N7. This is consistent with a contribution from stacking in the binding of the exogenous base.

Rescue by Pterin. A nonpurine compound that contains the interacting functional groups of guanine and maintains an ability to stack might also be expected to give rescue. Indeed, pterin, which has the functional groups of guanine's base pairing face and a nitrogen positioned analogous to N7 of guanine (Eq. **4**), gave substantial rescue of the G10.1X variant. k_{rescue} for pterin is 2500 $M^{-1}\cdot min^{-1}$, only 20-fold less than that for guanine.



[4]

Saturation of the Binding Site. The above results provide strong evidence for rescue by bases binding to a specific site via standard Watson–Crick base pairing plus additional interactions. According to the model of Eq. **3**, the activating base can saturate its binding site. Nevertheless, the saturation predicted by the model was not observed. The lack of saturation presumably arises because of weak binding and the low solubility of guanine (≈30 μM). Even though adenine is more soluble (≈4 mM), it has one less hydrogen bonding partner than guanine, which is expected to weaken its binding to the G10.1X/C11.1U variant. 2,6-Diaminopurine is both soluble, to ≈10 mM, and can form three hydrogen bonds at its base pairing face. The activation of the G10.1X/C11.1U variant by 2,6-diaminopurine levels off as the concentration of the added

Table 1. The specificity for rescue at site 10.1 depends on the base-pairing partner at position 11.1: Base pairs involving three hydrogen bonds

Residue at position 11.1	k^{G}_{rescue}, $M^{-1}\cdot min^{-1}$	k^{DAP}_{rescue}, $M^{-1}\cdot min^{-1}$	k^{rel}_{rescue} (G/DAP)	Specificity change
C	4.9×10^4	90	540	{540/<4.4}=
U	<415	94	<4.4	>120

The values of k_{rescue} were obtained as described in *Methods and Materials*. The two abasic variants used here showed similar reactivities in the absence of added bases (k_2 values are 0.045 min^{-1} and 0.028 min^{-1} for G10.1X and G10.1X/C11.1U, respectively). However, an HH16 derivative bearing an A·U base pair on top of helix II (variant G10.1A/C11.1U) is intrinsically less efficient in catalysis than the wild type containing a G·C pair at this location (k_2 = 0.11 min^{-1} vs k_2 = 1.1 min^{-1} for the wild type), in qualitative agreement with previous data (2). Thus, the values of k_{rescue} for the G10.1X/C11.1U variant are expected to be 10-fold reduced relative to the G10.1X ribozyme. G, guanine; DAP, 2,6-diaminopurine.

base is increased (Fig. 4). This is consistent with saturation of the binding site created by the introduction of the abasic residue by 2,6-diaminopurine, as predicted (Eq. **3**). The dissociation constant obtained from these data is in the low millimolar range (Fig. 4). Additionally, the apparent affinity of variant G10.1X/C11.1U for 2,6-diaminopurine increases with Mg^{2+} concentration (unpublished results). This suggests that the observed leveling off of rescue at high 2,6-diaminopurine concentrations is due to actual saturation of the binding site rather than to aggregation of the base near its solubility limit. Furthermore, coupling between binding of the base and binding of Mg^{2+} is consistent with coordination of a metal ion to the N7 of the purine in position 10.1, as observed in the crystal structure (17). However, an indirect effect of Mg^{2+} cannot be ruled out.

Rescue of a Ribozyme Variant Not Containing Abasic Residues. Mismatched bases in nucleic acids can sample both intra- and extrahelical positions (e.g., ref. 22), and a "flipped out" base could create a defect in an RNA helix comparable to an abasic site. We tested this by using a hammerhead mutant bearing a U10.1·C11.1 mismatch (variant G10.1U). This mutant is ≈20-fold less active than the wild-type HH16 (data not shown), in reasonable agreement with previous results (2). The G10.1U variant is activated by guanine, with a k_{rescue} only 7-fold less than that for G10.1X (k_{rescue} values are 49,000 and 7000 $M^{-1}\cdot min^{-1}$, respectively, for G10.1X and G10.1U). This indicates that a natural RNA molecule can contain binding sites for bases that are analogous to those in abasic ribozymes.

DISCUSSION

The removal of bases in the context of a structured RNA can provide insights into the interplay between RNA structure, function, and dynamics. Introducing abasics at any of 13 different positions of the hammerhead core has substantial effects on catalysis (Fig. 1*B*), suggesting that the base removal is accompanied by significant changes in the conformation of the central core. This implies that the abasic sites described here should not be thought of as stable, rigid "cavities," but rather as flexible crevices that are apparently collapsed or rearranged most of the time. Nevertheless, some of these crevices can bind the base originally removed, and the rearrangements accompanying binding rescue RNA function.

In one case, the local interactions important for function have been probed individually by measuring activation by a

Table 2. The specificity for rescue at site 10.1 depends on the base-pairing partner at position 11.1: Base pairs involving two hydrogen bonds

Residue at position 11.1	k^{H}_{rescue}, $M^{-1}\cdot min^{-1}$	k^{A}_{rescue}, $M^{-1}\cdot min^{-1}$	k^{rel}_{rescue} (H/A)	Specificity change
C	770	260	2.9	{2.9/0.18}=
U	10.6*	59	0.18	16

The k_{rescue} values are as described in Table 1.

*Hypoxanthine can bind to the G10.1X/C11.1U ribozyme in a wobble pair, forming two hydrogen bonds with U11.1. The fact that adenine is ≈6-fold better than hypoxanthine at activating the G10.1X/C11.1U ribozyme indicates that the Watson–Crick pairing is preferred for rescue at position 10.1. A, adenine; H, hypoxanthine.

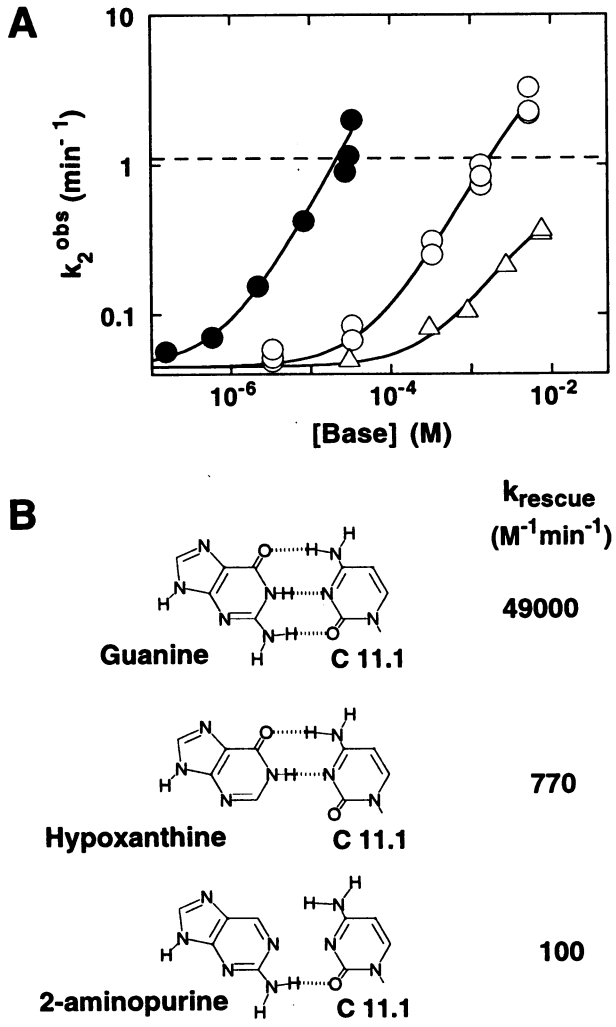


FIG. 3. Specificity for rescue of the G10.1X variant. (*A*) Concentration dependence of base rescue. Single turnover reactions were carried out in the presence of increasing concentrations of guanine (●), hypoxanthine (○), or 2-aminopurine (△). The solid lines represent the least squares fits of the experimental data to Eq. **2**. The wild-type rate is shown as a dashed line for comparison. At hypoxanthine concentrations near its solubility limit (≈5 mM), the rate of the G10.1X variant exceeds that of the wild type by a small, but significant amount (2- to 3-fold). It is possible that removal of the glycosidic bond between the base at position 10.1 and the RNA backbone relieves a conformational constraint that restricts the rearrangement to the catalytically active conformation. Results with the *Tetrahymena* group I ribozyme suggest that the conformational constraint of the ribose ring can also lead to suboptimal alignment (G. J. Narlikar, M. Khosla, N.U., and D.H., unpublished results). (*B*) Interactions for bases binding at position 10.1, assuming a Watson–Crick geometry of the base pair. [2-Aminopurine can potentially form a wobble base pair with C11.1, that would include two hydrogen bonds, as many as in a C·hypoxanthine base pair (21). k_{rescue} for hypoxanthine is ≈8-fold higher than k_{rescue} for 2-aminopurine, suggesting that a Watson–Crick pair is preferred for rescue.]

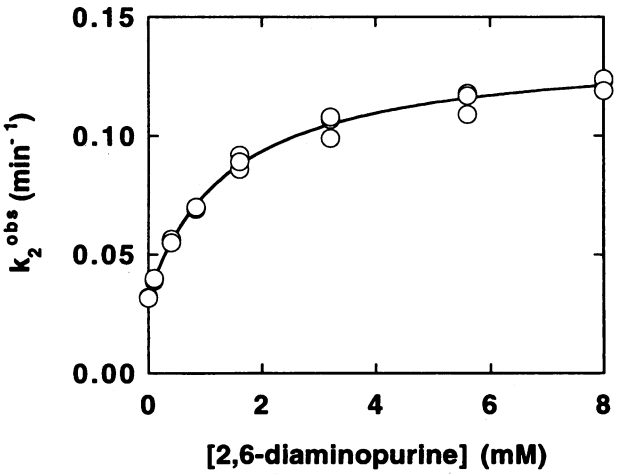


FIG. 4. Binding of 2,6-diaminopurine to the G10.1X/C11.1U variant. Single turnover reactions were carried out in the presence of increasing concentrations of 2,6-diaminopurine. The line represents the best fit of the data to Eq. **2**, giving K_d = 1.5 ± 0.2 mM. A slightly higher K_d of 3 mM is obtained when the small amount of inhibition of G10.1A/C11.1U by high concentrations of 2,6-diaminopurine is accounted for (25% inhibition at 8 mM base).

variety of bases and base derivatives. This technique can be regarded as a noncovalent site-specific mutagenesis and can allow rapid screening of functional interactions in cases where rescue is observed. Analogous rescue experiments may be useful in characterizing functional RNAs and possibly DNAs of unknown structure and in probing the structure–function relationships in RNAs of known structure.

Factors Determining the Ability of Free Bases To Rescue Abasic Ribozyme Variants. Four of the 13 positions examined in this study were rescued. What factors determine whether rescue will be successful? The number of interactions formed with the base appears to be important. Disrupting any of the potential interactions for the base binding at the 10.1 site weakens rescue.

Most of the experiments presented herein have probed groups involved in hydrogen bonding. However, hydrogen bonded pairs between free bases or base derivatives are very weak in water (23). Specific G·C or A·U pairs are not observed at base concentrations as high as 1 M in water (24). In contrast, the low millimolar dissociation constant of 2,6-diaminopurine for the G10.1X/C11.1U abasic hammerhead indicates that the base pair here is substantially stabilized by some other local interactions. In the specific case, one such interaction may be a metal coordination to the purine N7. However, 2,6-diaminopurine can also bind an abasic site facing a U in a simple RNA duplex with affinity of ≈1 mM (unpublished results). This implies the base binding is significantly stabilized by stacking. Indeed, rescue by isocytosine is 20- to 100-fold weaker than rescue by 7-deazaguanine or 7-methylguanine, which presumably form the same hydrogen bonds as isocytosine upon binding, but stack better. In general, rescue by purines may be favored because they stack better than pyrimidines (23).

Another factor affecting rescue may be the rigidity of the site versus its ability to adopt alternative structures upon base removal. The insertion of abasic sites in model DNA duplexes causes local rearrangements, the type and extent of which depends on the context (25). Abasic RNA sites are also likely to be conformationally perturbed (see above), and the extent of the perturbation and the stability of the perturbed structures will dictate the ability to reform a binding cavity for the base.

Finally, rescue is not expected if the glycosidic bond between base and sugar is critical for aligning the RNA for its structure and function.

Comparison with Protein Studies. The rescue of the activity of certain abasic hammerhead ribozymes can be contrasted with previous "rescue" studies in proteins. The deleterious effects of mutating Lys-258 in the active site of aspartate aminotransferase to alanine could be partially rescued by adding exogenous amines, which substituted for lysine as general bases in the catalytic mechanism (26). In the hammerhead system, it appears unlikely that each of the rescued positions is involved in chemical catalysis. Instead, rescue of the hammerhead variants may rather represent the repair of conformational defects introduced by removing the base. This is also in contrast to results with T4 lysozyme, in which mutation of Leu-99 to Ala creates a stable hydrophobic cavity that can bind aromatic ligands (8). The limited packing in RNA and larger number of degrees of freedom in the RNA backbone, compared with the peptide backbone, may make it more difficult for RNA to maintain a rigid cavity.

Speculative Implications for the "RNA World" Hypothesis. *In vitro* selection has been exploited to generate RNA molecules that are capable of specifically binding base derivatives, ribonucleotides, and related biological cofactors from large pools of randomized RNAs (27–30). Such high-affinity motifs are typically present with a frequency of 1 in 10^{10} to 10^{14} random RNA sequences. In contrast, when starting from the preformed hammerhead structure, rescue provides evidence for weak or modest binding at 4 out of 13 abasic sites (Fig. 1*C*). The generalization of these results to other functional RNAs would indicate that low-affinity binding sites for bases and base analogs can be created with surprisingly high frequency in structured RNA.

This suggests a potential pathway for the introduction of metabolic complexity into an RNA world (31): sites of weak or modest affinity may have been created by introduction of defects (e.g., mismatches) into RNAs with preexisting structure, thereby initiating evolution of binding and catalytic sites for substrates and cofactors. Note that pterin, the heterocycle of folic acid, has been shown to bind the abasic ribozyme. Moderate affinity binding sites may have been sufficient for catalysis by primitive ribozymes, as even the highly evolved *Tetrahymena* group I ribozyme has an affinity for its guanosine cofactor of only 0.1 to 1 mM (32).

Speculative Implications for the Therapeutic Use of Ribozymes. Ribozymes are being pursued as therapeutics because of their potential to specifically cleave deleterious RNAs (33). The present results showing that binding of small molecules to ribozymes can be coupled to large changes in catalytic activity suggest a possible means of controlling these potential drugs allosterically. Allosteric ribozymes may be obtained, either by design or by *in vitro* selection, that are specifically activated by small molecules or "codrugs." After introduction of the ribozyme via gene therapy or conventional delivery approaches, the activity of such ribozymes could be controlled temporally—by administration of the codrug, or spatially—by using a codrug specifically absorbed by a certain tissue. The bases that serve as activators in the present study are small and rather hydrophobic, generally desirable properties for drugs. Indeed, 6-thioguanine, which effectively rescues the G10.1X variant (unpublished results), is currently used in leukemia therapy (34).

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