Nucleic Acids Research, 1998, Vol. 26, No. 7 **1755–1760**

An RNA aptamer to the xanthine/guanine base with a distinctive mode of purine recognition

Daisuke Kiga, Yasuhiro Futamura, Kensaku Sakamoto and Shigeyuki Yokoyama*

Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo, Hongo, Bunkyo-Ku, Tokyo 113-0033, Japan

Received November 21, 1997; Revised and Accepted February 16, 1998

ABSTRACT

RNAs that bind to xanthine (2,6-dioxypurine) were isolated from a population of 10^{12} random sequences by *in vitro* selection. These xanthine-binding RNAs were found to have a 10 nt consensus sequence at an internal loop in the most probable secondary structure. By trimming one of the xanthine-binding RNAs, a representative xanthine-binding RNA (designated as XBA) of 32 nt residues was prepared. The dissociation constant of this RNA for xanthine was determined to be 3.3 μM by equilibrium filtration experiments. The XBA RNA can bind to guanine as well, whereas it hardly accommodates adenine, cytosine or uracil. The K_d values for various xanthine/guanine analogues were determined, and revealed that the N1H, N7 and O6 moieties of the ligand are involved in the binding with the XBA RNA. The ribonuclease sensitivities of some internal-loop residues changed upon the addition of xanthine, suggesting that the internal loop of the XBA RNA is involved in the ligand binding. Interestingly, the consensus sequence of the xanthine/guanine-binding RNAs is the same as a sequence in one of the internal loops of the hairpin ribozyme, except for a substitution that is neutral with respect to xanthine/guanine binding.

INTRODUCTION

The method of *in vitro* selection (1) or SELEX (2) has been developed to isolate RNA or DNA molecules that bind specifically to a defined target from a large population of random sequences. For example, RNA aptamers to a nucleotide, a nucleoside and a base, such as ATP (3), guanosine (4), flavin mononucleotide (FMN) (5) and theophylline (1,3-dimethyl 2,6-dioxypurine) (6) have already been isolated. In these cases, the isolated RNA aptamers to a ligand have been reported to have a consensus sequence, which is located in an internal loop. By NMR spectroscopy, the solution structures of ligand•aptamer complexes have been determined for the nucleotide/base ligands, ATP (7,8), FMN (9) and theophylline (10), as well as for other types of ligands such as arginine (11), citrulline (11) and tobramycin (12). In each case, the ligand is accommodated in a binding pocket that is formed by the aptamer's consensus sequence through a network of intramolecular hydrogen bonds and/or stacking interactions. Through the structural studies, the RNA aptamers to ATP and theophylline have been shown to recognize base moieties by hydrogen bonding to specific positions of the purine base (7,8,10). These recognized positions are consistent with those suggested initially in the reports of the isolations of these aptamers (3,6). For the aptamers to guanosine, a recognition mode has been suggested (4), but there has been no report on the ligand•aptamer complex structure. Intriguingly, these three aptamers, to ATP, theophylline and guanosine, have their own distinctive modes of purine base recognition.

In order to further explore this ability of RNA to recognize purine bases and/or the purine base moiety of nucleosides, we isolated RNA aptamers to xanthine (2,6-dioxypurine) with high affinity and specificity. We used a xanthine–agarose column, in which the linkage of the xanthine to the agarose is at position 8 of the purine base, and therefore differs from the linkage at position 1 in the theophylline–agarose column previously used for the selection of the above-mentioned RNA aptamer to theophylline (1,3-dimethyl derivative of xanthine). Thus, we isolated RNA aptamers to xanthine with a purine binding mode different from that of the aptamer to theophylline. The isolated RNA aptamer to xanthine bound to guanine with an affinity as high as that for xanthine. The manner of binding to the guanine base is also distinct from that of the aptamers to guanosine.

MATERIALS AND METHODS

DNA manipulation, sequencing and PCR amplification

Standard techniques were used for restriction endonuclease digestion, ligation and gel electrophoresis (13). The nucleotide sequence was determined using a BcaBEST dideoxy sequencing kit (Takara Shuzo, Kyoto, Japan). PCR was performed using AmpliTaq DNA polymerase (Perkin-Elmer) and a DNA Thermal Cycler, PJ2000 (Perkin-Elmer Cetus).

Preparation of RNAs with random sequences

A single-stranded DNA (105 nt), consisting of a random sequence of 60 nt and two flanking primer-binding regions (23 and 22 nt) for PCR and reverse transcription, was chemically synthesized with a Cyclone plus DNA/RNA synthesizer (Millipore). The PCR primers, with the sequences of 5′-AGTAATACGACTCACTA-TAGGTGAGAATTCCGACCAGGATCC-3′, (the T7 promoter sequence is underlined) and 5′-CCGCCCAAGCTTCTACGTC-GAC-3′, were also synthesized. By primer extension analysis, it was found that ~1% of the 105mer DNA sample was of sufficient

*To whom correspondence should be addressed. Tel: +81 3 3812 1805; Fax: +81 3 5689 5609; Email: yokoyama@y-sun.biochem.s.u-tokyo.ac.jp

quality to support the synthesis of the full-length complementary strand. By the use of 5 µg (10^{14} molecules) of this synthetic DNA sample as the template, a double-stranded DNA library, which is considered to contain 10^{12} different sequences, was produced by a 30 ml PCR (15 cycles of amplification). The initial pool of RNAs was prepared from this DNA library by *in vitro* transcription with T7 RNA polymerase, and was dissolved in binding buffer (20 mM Tris–HCl, pH 7.5, 0.3 M NaCl and 5 mM $MgCl_2$).

Selection procedure

The RNAs with random sequences were subjected to affinity chromatography on a xanthine–agarose column (Sigma), where the xanthine is linked to the agarose at its C8 position. The concentration of xanthine in the column was roughly estimated to be 0.1 mM on the basis of the data provided by the supplier. The RNA (10–20 µg in the first round and 5–10 µg in the successive rounds), which had not been renatured, was applied to an agarose precolumn (1 ml) connected to the top of the xanthine–agarose column (0.5–1 ml), which had been equilibrated with >3 ml of the binding buffer. The connected columns were washed with 5 ml of the binding buffer. It was expected that during this procedure, the RNAs that bind to xanthine, but not to agarose, were moved to and retained in the xanthine–agarose column. Finally, the RNAs were affinity-eluted with 3 ml of the binding buffer containing 0.2 mM xanthine. The eluate was concentrated with a Centricon 10 unit (Amicon), and was then precipitated with ethanol. Ten percent of the collected RNA was examined by electrophoresis. The cDNA was synthesized with M-MLV reverse transcriptase (USB) and was amplified by PCR. The RNA pool for the next round was prepared by T7 transcription from the amplified cDNA. The elution profile of the affinity column chromatography was examined by dot-blot hybridization (13). An aliquot of each fraction of the chromatography was spotted onto a Hybond-N nylon membrane (Amersham), and the RNA was detected by annealing to a ^{32}P-labeled PCR primer. The intensity of each spot was measured using a BAS2000 imaging analyzer (Fuji Film Co.).

Ribonuclease mapping experiments

A representative xanthine-binding RNA and its derivative were prepared by T7 transcription from synthetic DNA templates, and were subjected to the following ribonuclease (RNase) mapping and equilibrium filtration assay. The RNA was labeled with 5′-[^{32}P]pCp (111 TBq/mmol, Dupont/NEN research products) at the 3′-end. The labeled RNA, in the binding buffer, was partially digested at room temperature for 5 min with either RNase T_1 or RNase V_1 (Pharmacia Biotech), in either the absence or the presence of 0.2 mM xanthine. Samples were analyzed by electrophoresis on denaturing 20% polyacrylamide gels. The sequence of the RNA was determined as described (14).

Equilibrium filtration

The interactions of xanthine, adenine, cytosine, guanine and uracil with the RNA were analyzed by equilibrium filtration (6), based on the assumption that there is only one binding site per RNA molecule. Each of the tritium-labeled bases (Moravek Biochemical) was added to the RNA sample in the binding buffer (150 µl) at a final concentration of 1 µM. This binding mixture was placed in a Centricon 10 unit, after an incubation at 25°C for 3 min, and was centrifuged at 3000 *g* for 3 min. The filtrate was mixed with the remaining solution again, and the mixture was centrifuged for another 5 min. Aliquots (15 µl) were removed from both the filtrate and the remaining solution, and the radioactivities were measured with a liquid scintillation counter, LSC-700 (Aloka). For various RNA concentrations, the amounts of the RNA-bound ligand were estimated from the differences between the ligand concentrations in the filtrates and those in the remaining solutions. Competitor-dissociation constants (K_dc) of various bases and guanosine against guanine were determined as described (15). Various concentrations of the potential competitors were added to 1 µM of [^{3}H]guanine and 5 µM of RNA in the binding buffer (150 µl). A 1:1 stoichiometry of the competitor to RNA was assumed. A K_d value of 1.3 µM for guanine was used for the calculations.

Hairpin ribozyme

A loop-B fragment, 5′-GGGACCAGAGAAACACACCUUCGG-GUGGUAUAUUACCUGGUAC-3′, derived from a hairpin ribozyme (16), was prepared by T7 transcription from a synthetic DNA template, and was subjected to the affinity chromatography on a column of GMP-agarose, containing 3.7 mM 5′-GMP (Sigma). Elution of RNA from the column was carried out as described above (Selection procedure).

A separated hairpin ribozyme was reconstituted with the loop-A and loop-B domains, and cleavage reactions were performed (17). Various nucleotides and bases were pre-incubated with the 5′-^{32}P-end labeled substrate (1 nM), the non-labeled substrate (15 µM), and the substrate-binding strand (15 µM), where the substrate and substrate-binding strand were annealed to form the loop-A domain. Reactions were then started by the addition of the loop-B domain (10 µM), which was prepared by *in vitro* transcription with T7 RNA polymerase. The pre-incubation and the cleavage reaction were carried out in 50 mM Tris–HCl buffer, pH 7.5 containing 100 mM $MgCl_2$. The reaction products were electrophoresed through denaturing polyacrylamide gels, and were quantitated using a BAS2000 imaging analyzer (Fuji Film Co.).

RESULTS AND DISCUSSION

Isolation of RNA aptamers to xanthine

In vitro selection of xanthine-binding RNAs was started with a pool of RNAs with a random sequence of 60 nt flanked by two primer-binding regions. The complexity of the initial pool was estimated to be 10^{12}. The RNAs in the binding buffer were loaded onto a xanthine–agarose column. The unbound and weakly bound RNAs were washed out of the column. The RNAs that specifically bound to the xanthine–agarose were affinity-eluted with the binding buffer containing xanthine.

In the first three rounds, the amounts of RNA in the affinity-eluted fractions were too small to detect by gel electrophoresis followed by ethidium bromide staining. In the fourth round, however, a significant amount (a few micrograms) of RNA was eluted with the xanthine-containing buffer. Thus, in the fifth round, the eluate was fractionated so the elution profile could be analyzed (Fig. 1). About 40% of the total RNA was eluted with the xanthine-containing buffer.

The isolated RNA aptamers were classified into three groups; one is of the initial length (105 nt), and the other two are of shorter lengths (~85 and 60 nt). This heterogeneity in length may be due to shortening by deletion during reverse transcription, or may

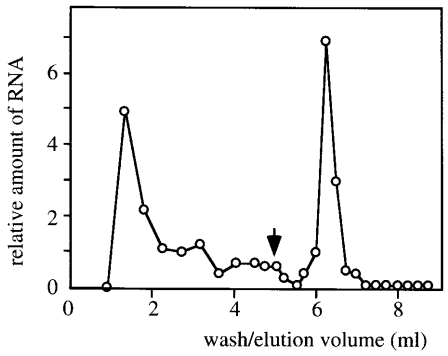

Figure 1. Elution profile of RNA from the xanthine–agarose column for the fifth round of selection. The relative amount of RNA in each fraction was determined by hybridization with a ^{32}P-labeled specific probe. The unbound and weakly bound RNAs were washed out with 5 ml of the binding buffer. Then, the RNAs retained in the xanthine–agarose column were affinity-eluted by the binding buffer containing 0.2 mM xanthine. The arrow indicates the start of the affinity elution.

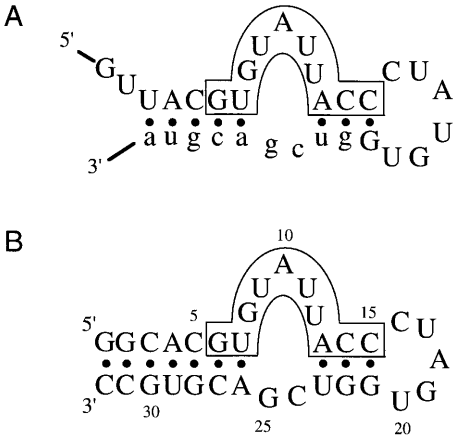

Figure 3. Secondary structures of an aptamer from the shortest class (**A**) and the XBA RNA (**B**). The consensus sequence is enclosed. The nucleotide residues represented by lower case letters are the first 9 nt of the primer binding region at the 3′-end (A).

clone 1	GUUAC	**GUGUAUUACC**	CUAUGUG
clone 2	CUGC	**GUGUAUUACC**	CCCGUG
clone 3	UAU	**GUGUAUUACC**	CAGCGAG
clone 4	UAU	**GUGUAUUACC**	CUAGUG
clone 5	UGC	**GUGAAUUACC**	UGUCUACUC
clone 6	AC	**GUGAAUUAUC**	CGUGAG
clone 7	GC	**GUGUAUUACC**	CUCGUG
clone 8	AC	**GUGUAUUACC**	CUUUGCAG
clone 9	GC	**GUGUAUUACC**	UCAAUG
clone 10	AC	**GUGAAUUACC**	CUUUGUG
clone 11	AC	**GUGAAUUACC**	CAGCGAG

Figure 2. Sequences of the shortest class of aptamers. The sequences of the flanking primer-binding regions are omitted. The consensus sequences of these aptamers are shown in bold letters. The sequence of clone 10 was found twice.

have been caused by hybridization of the PCR primers to the randomized region. For each group, the sequences of 12 clones were determined, and a certain sequence was found more than once. A consensus sequence was found only for the shortest class of aptamers (Fig. 2). In addition, these RNAs are likely to have similar secondary structures, an asymmetric internal loop and two flanking stems (Fig. 3A). In all of the clones, the consensus sequence is located at this internal loop. This secondary structure contains nine residues from the primer binding sequence, and two nucleotides, CG, in the single-stranded region that is opposite from the consensus sequence, are derived from the invariant 9mer sequence.

Ribonuclease mapping of an RNA aptamer to xanthine

We designed the 32mer RNA (designated as XBA) shown in Figure 3B, which has the common secondary structure, including the consensus sequence, of the 60mer aptamers. The guanosine residues at positions 8 and 19 of the XBA RNA were susceptible to RNase T_1, which cleaves the 3′-phosphodiester bonds of guanosine residues in single-stranded regions (Fig. 4A and B). RNase V_1, which cleaves the phosphodiester bonds in double-stranded or stacked regions (18), attacked the 3′-phosphodiester bonds of residues 29 and 30 strongly, and those of residues 6, 11, 24 and 28 more weakly (Fig. 4A and B). These results support the secondary structure of the XBA RNA shown in Figure 3B. The less efficient cleavage at residues 11 and 24 by RNase V_1 may be due to base stacking in the internal-loop region.

In the presence of xanthine, residues 8 and 11 in the internal loop were protected against attacks by RNase T_1 and V_1, respectively, while no significant change in the efficiency of cleavage was found for the other sites (Fig. 4A and B). This decreased sensitivity to RNases may be due to a local conformational change induced by the ligand binding and/or steric inhibition of ribonuclease access by the bound ligand.

Thus, the XBA RNA was shown to have an internal loop carrying the consensus sequence and flanking stems. This type of structural context for a consensus sequence has also been reported for the RNA aptamers to ATP, guanosine, FMN and theophylline (3–6). The importance of the internal-loop region in specific ligand binding also has been demonstrated by chemical modification experiments (3, 4) and NMR studies on the aptamer•ligand complexes (7–10). Taking here the result of the RNase mapping into account, it is concluded that the RNA aptamers isolated in this study recognize the ligands xanthine and guanine, by using the consensus sequence located in the internal loop.

In the solution structures of the ATP•aptamer, FMN•aptamer and theophylline•aptamer complexes (7–10), the internal-loop region of the aptamer folds into a unique conformation that facilitates specific interactions between the ligand and the consensus sequence. Although the ligand-bound conformations of these aptamers are completely different from each other, each aptamer has a purine-rich internal loop; these purine nucleotides form non-canonical base pairs and make stacking interactions with each other, essentially contributing to the unique folded conformations of these aptamers. In contrast, the internal loop of the present xanthine-binding aptamer has a distinct sequence; it predominantly contains U residues. Therefore, specific ligand binding by the aptamer to xanthine probably requires a distinctive folding of the internal-loop.

Dissociation constants of the XBA RNA for various bases

In order to examine the ligand specificity of the XBA RNA, interactions of the RNA with various bases were analyzed. In

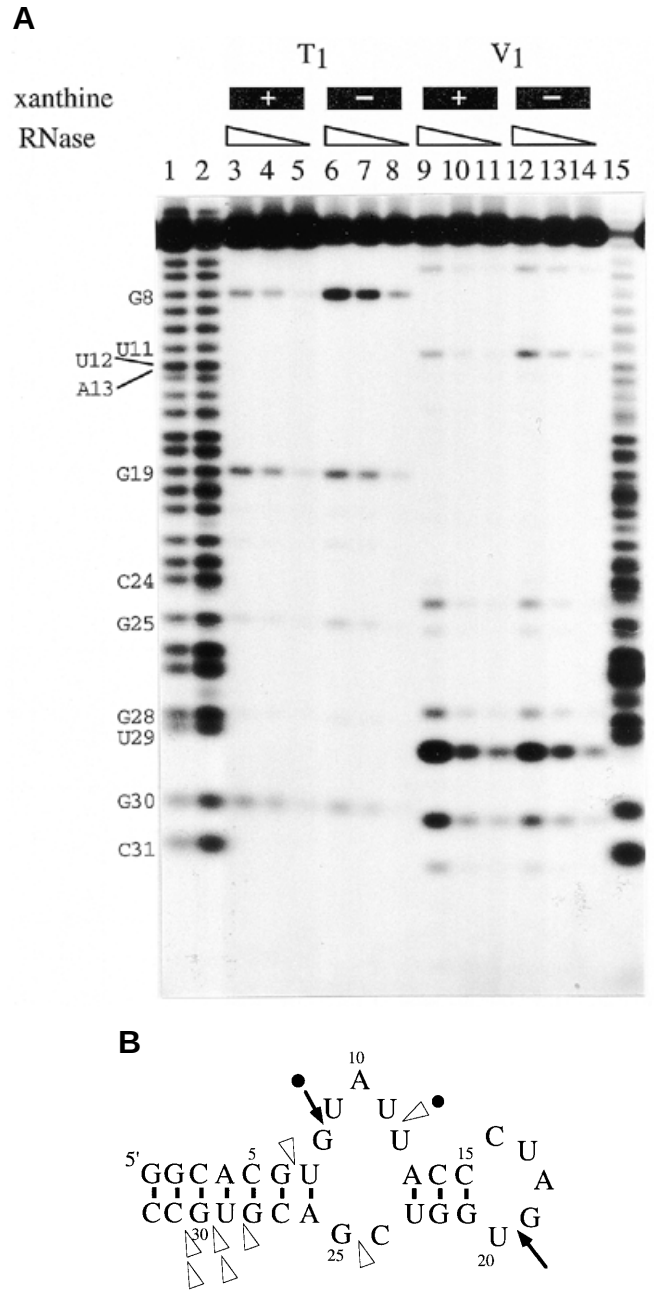

Figure 4. RNase mapping of the XBA RNA. (**A**) Autoradiogram of the 3′-^{32}P-labeled XBA RNA analyzed on a 20% polyacrylamide gel after limited digestion with alkali (lanes 1, 2 and 15), with ribonuclease T_1 (lanes 3–8), and with ribonuclease V_1 (lanes 9–14). RNase digestions were performed in the presence (lanes 3–5 and 9–11) and the absence (lanes 6–8 and 12–14) of xanthine (0.2 mM). Each band was assigned according to the digestion patterns with RNase T_1 and RNase CL3 (data not shown). (**B**) Summary of the cleavage pattern of the XBA RNA. Filled arrows and open arrowheads indicate RNase T_1 cleavage sites and RNase V_1 cleavage sites, respectively. The number of arrowheads represents the efficiency of cleavage. The cleavages with significantly decreased efficiencies in the presence of xanthine are indicated by filled circles.

equilibrium filtration experiments with radioactive ligand (6), the dissociation constant (K_d) of the XBA RNA for xanthine was determined as 3.3 μM. Similarly, the K_d value of XBA for guanine was obtained as 1.3 μM, while those for the other three common bases (adenine, cytosine and uracil) could not be obtained by this method, because of their low affinities for XBA. Therefore, the K_d values for these bases were obtained on the basis of competition with the guanine base for binding to XBA. The competitor-dissociation constants (K_{dc}) thus determined are listed in Figure 5. The XBA RNA has much higher affinities for xanthine and guanine than for the other three common bases (adenine, cytosine and uracil). Since this RNA cannot distinguish between xanthine and guanine, it is evident that position 2 of the purine ring is not involved in ligand binding by XBA. This is confirmed by the observation that hypoxanthine can bind to XBA with an affinity nearly equal to those for xanthine and guanine. On the other hand, as adenine hardly binds to XBA, the 6-carbonyl group is important in the ligand binding by XBA. In order to further investigate the purine recognition mode of XBA, the K_{dc} values were determined for 1-methyl xanthine, 3-methyl xanthine, 7-methyl xanthine and guanosine. It is clear that N1H and N7, but not N3, of the base are important for the ligand binding by XBA. Guanosine has a much lower affinity than xanthine/guanine. Therefore, it is possible that N9 is directly involved in the ligand binding, while it is also possible that the size of the ribose moiety at position 9 causes steric hindrance in the binding pocket.

The RNA aptamer to xanthine/guanine has a distinctive mode of specific purine base recognition

The RNA aptamer to theophylline, the 1,3-dimethyl derivative of xanthine, is another purine base binding RNA (6). Interestingly, the aptamer to theophylline can bind to xanthine with an affinity slightly lower than that of the XBA RNA. However, this aptamer has a symmetric internal loop, while the XBA RNA has an asymmetric internal loop. Furthermore, there is no homology evident between the consensus sequences of these aptamers. Actually, the purine base recognition modes of these two RNA aptamers are different from each other. The aptamer to theophylline recognizes the 2- and 6-carbonyl groups, the 3-methyl group, and N7 (6), while the XBA RNA recognizes N1H, N7, and the 6-carbonyl group. Therefore, the XBA RNA probably cannot bind to theophylline; it has a methyl group at N1. On the other hand, the aptamer to theophylline probably does not bind to guanine, which has an amino group at position 2, while the affinity of the XBA RNA for guanine is nearly equal to that for xanthine.

The purine recognition mode of the XBA RNA is also different from those of the RNA aptamer to guanosine and a variant of this RNA with dual specificity for guanosine/arginine (4). The ligand specificity of the guanosine-binding RNA involves positions 1, 2, 6 and 7 of the guanine base moiety, while the guanosine/arginine-binding RNA exhibits a modified recognition mode for positions 1 and 8. Naturally-occurring group I introns are supposed to recognize the 1-imino and 2-amino groups of the guanosine substrate, while the role of 6-carbonyl group remains unclear (19–22). Among the RNAs that can bind to xanthine, guanine or guanosine, the XBA RNA has a unique feature, in that the binding does not involve position 2 of the purine moiety. Due to this distinctive recognition mode, the present RNA aptamer can bind guanine, xanthine and hypoxanthine equally well, and strictly discriminates against the other three common bases.

ligand	dissociation constant (μM)
xanthine	4.1 ± 0.6 (3.3 ± 0.2)
guanine	1.8 ± 0.4 (1.3 ± 0.1)
hypoxanthine	2.1 ± 0.5
adenine	2900 ± 700
uracil	390 ± 50
cytosine	79 ± 13
1-methyl xanthine	>400
3-methyl xanthine	2.7 ± 0.4
7-methyl xanthine	>400
guanosine	140 ± 10

Figure 5. Dissociation constants (K_d) for various bases. The K_d values for xanthine and guanine that were directly determined using radioactive ligand are also listed in parentheses. The K_dc and K_d values are shown, together with the average errors.

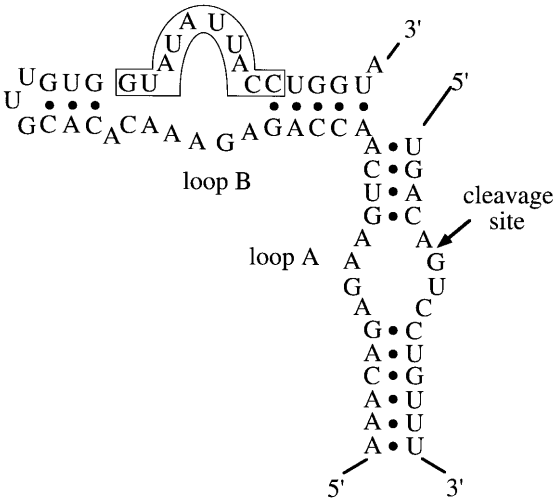

Figure 6. The hairpin ribozyme from tobacco ringspot virus satellite RNA (23). The sequence in loop B that is highly similar to the consensus sequence of the present aptamers to xanthine/guanine is enclosed.

Structural similarity between the RNA aptamer to xanthine/guanine and the hairpin ribozyme

We found that the consensus sequence of the present RNA aptamers to xanthine/guanine is highly similar to a sequence at loop B (23) of the hairpin ribozyme from the tobacco ringspot virus satellite RNA (Fig. 6). This finding prompted us to prepare an XBA variant with the replacement of G8 by A, so it has the same sequence as that of loop B of the hairpin ribozyme. It was found that this XBA variant also binds to guanine, with a dissociation constant of 1.2 μM. Therefore, we now have an RNA aptamer to guanine with significant structural similarity to the hairpin ribozyme.

The hairpin ribozyme undergoes a self-cleavage reaction at a certain guanosine residue in loop A (16), and this G residue, as well as another G in the same region, has been shown to be essential in this cleavage reaction (24–29). Loop B also contains important residues (26–28,30), some of which are included in the sequence common to the aptamer. The loop-A and loop-B domains (17), or domains I and II (31), are required to interact with each other in order to perform the cleavage reaction (17, 31). Recently, a model structure of the hairpin ribozyme was built on the basis of cross-linking data (32). In this model, loops A and B are located close to each other. Therefore, the 10mer sequence in loop B, common to the aptamer, could interact with the base moiety of the G residues in loop A, and contribute to the formation of an interface between these loops.

In order to explore this possibility, we examined the interaction of the loop-B domain with the guanosine nucleotide, and the effects of the guanosine nucleotide on the cleavage reaction of the ribozyme. A 43mer RNA corresponding to the loop-B domain was prepared by run-off transcription, and was subjected to the affinity chromatography on a column of 5′-GMP linked to the agarose. No significant delay in retention from this column was observed for the RNA (data not shown). Since the concentration of GMP in the column matrix was 3.7 mM, the loop-B domain has only low, if any, affinity for GMP, with the K_d being not in the submillimolar range.

Cleavage activity has been reported also for a reconstituted hairpin ribozyme, which comprises the separated loop-A and loop-B domains (17). The intermolecular domain–domain interaction was expected to be more sensitive to base/nucleoside inhibitors than the intramolecular domain–domain interaction in the native form. The guanine and xanthine bases were added to the cleavage reaction up to near-saturation concentrations (10 μM and 150 μM, respectively), but the cleavage reaction of the reconstituted ribozyme was not affected (data not shown). Then, 5′-AMP, 5′-GMP, 5′-CMP and 5′-UMP, which are more soluble than the bases, were added at the final concentration of 20 mM. As expected, GMP was found to inhibit the cleavage reaction significantly (Fig. 7). Interestingly, the other three nucleotides also inhibited the cleavage reaction less efficiently than GMP (Fig. 7). One possibility is that the loop-B domain more specifically recognizes a guanosine residue presented in a specific context of the loop-A domain than the free GMP. On the other hand, we cannot exclude other possiblities, for example, that the

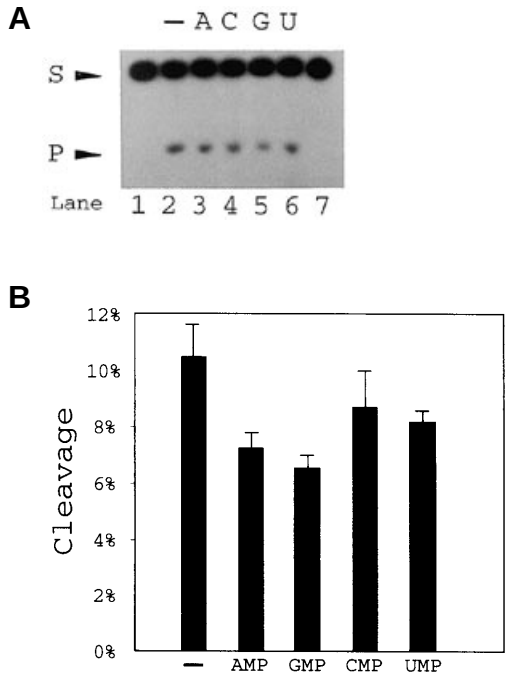

Figure 7. Effects of 5′-mononucleotides on the cleavage reaction of a reconstituted hairpin ribozyme. The reactions were carried out for 30 min at 37°C. (**A**) Autoradiogram of the ^{32}P-labeled substrate, analyzed by the denaturing gel electrophoresis, after incubation with the loop-B domain (lanes 2–6), or without the loop-B domain (lanes 1 and 7). The cleavage reactions were performed in the absence of nucleotide (lane 2), and the presence of AMP (lane 3), CMP (lane 4), GMP (lane 5) and UMP (lane 6). S, substrate; P, 5′ cleavage product. (**B**) Summary of three to four experiments.

interaction between the two domains of the hairpin ribozyme involves A, U and/or C residues of the internal loops, with which the corresponding mononucleotides may compete. Further study on the interdomain interactions in this ribozyme is necessary for testing these possibilities.

ACKNOWLEDGEMENTS

This work was supported in part by a Grant-in-Aid for Scientific Research on Priority Areas (No. 04272103) from the Ministry of Education, Science, and Culture of Japan. D.K. and Y.F. are supported by Research Fellowships of the Japan Society for the Promotion of Science.

REFERENCES

1 Ellington, A. D. and Szostak, J. W. (1990) *Nature*, **346**, 818–822.
2 Tuerk, C. and Gold, L. (1990) *Science*, **249**, 505–510.
3 Sassanfar, M. and Szostak, J. W. (1993) *Nature*, **364**, 550–553.
4 Connell, G. J. and Yarus, M. (1994) *Science*, **264**, 1137–1141.
5 Burgstaller, P. and Famulok, M. (1994) *Angew. Chem. Int. Ed. Engl.*, **33**, 1084–1087.
6 Jenison, R. D., Gill, S. C., Pardi, A. and Polisky, B. (1994) *Science*, **263**, 1425–1429.
7 Jiang, F., Kumar, R. A., Jones, R. A. and Patel, D. J. (1996) *Nature*, **382**, 183–186.
8 Dieckmann, T., Suzuki, E., Nakamura, G. K. and Feigon, J. (1996) *RNA*, **2**, 628–640.
9 Fan, P., Suri, A. K., Fiala, R., Live, D. and Patel, D. J. (1996) *J. Mol. Biol.*, **258**, 480–500.
10 Zimmermann, G. R., Jenison, R. D., Wick, C. L., Simorre, J. -P. and Pardi, A. (1997) *Nature Struct. Biol.*, **4**, 644–649.
11 Yang, Y., Kochoyan, M., Burgstaller, P., Westhof, E. and Famulok, M. (1996) *Science*, **272**, 1343–1347.
12 Jiang, L., Suri, A. K., Fiala, R. and Patel, D. J. (1997) *Chem. Biol.*, **4**, 35–50.
13 Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor University Press, Cold Spring Harbor, NY.
14 Knapp, G. (1989) *Methods Enzymol.*, **180**, 192–212.
15 Martin, R. L., Renosto, F. and Segel, I. H. (1991) *Arch. Biochem. Biophys.*, **284**, 26–29.
16 Buzayan, J. M., Gerlach, W. L. and Bruening, G. (1986) *Nature*, **323**, 349–353.
17 Butcher, S. E., Heckman, J. E. and Burke, J. M. (1995) *J. Biol. Chem.*, **270**, 29648–29651.
18 Lowman, H. B. and Draper, D. E. (1986) *J. Biol. Chem.*, **261**, 5396–5403.
19 Michel, F., Hanna, M., Green, R., Bartel, D. P. and Szostak, J. W. (1989) *Nature*, **342**, 391–395.
20 Michel, F. and Westhof, E. (1990) *J. Mol. Biol.*, **216**, 585–610.
21 Been, M. D. and Perrotta, A. T. (1991) *Science*, **252**, 434–437.
22 Yarus, M. and Majerfeld, I. (1992) *J. Mol. Biol.*, **225**, 945–949.
23 Butcher, S. E. and Burke, J. M. (1994) *J. Mol. Biol.*, **244**, 52–63.
24 Chowrira, B. M., Berzal-Herranz, A. and Burke, J. M. (1991) *Nature*, **354**, 320–322.
25 Berzal-Herranz, A., Joseph, S. and Burke, J. M. (1992) *Genes Dev.*, **6**, 129–134.
26 Berzal-Herranz, A., Joseph, S., Chowrira, B. M., Butcher, S. E. and Burke, J. M. (1993) *EMBO J.*, **12**, 2567–2574.
27 Anderson, P., Monforte, J., Tritz, R., Nesbitt, S., Hearst, J. and Hampel, A. (1994) *Nucleic Acids Res.*, **22**, 1096–1100.
28 Grasby, J. A., Mersmann, K., Singh, M. and Gait, M. J. (1995) *Biochemistry*, **34**, 4068–4076.
29 Shippy, R., Siwkowski, A. and Hampel, A. (1998) *Biochemistry*, **37**, 564–570.
30 Siwkowski, A., Shippy, R. and Hampel, A. (1997) *Biochemistry*, **36**, 3930–3940.
31 Komatsu, Y., Kanzaki, I., Koizumi, M. and Ohtsuka, E. (1995) *J. Mol. Biol.*, **252**, 296–304.
32 Earnshaw, D. J., Masquida, B., Müller, S., Sigurdsson, S. T., Eckstein, F., Westhof, E. and Gait, M. J. (1997) *J. Mol. Biol.*, **274**, 197–212.