Crystal Structure of an RNA Purine-Rich Tetraplex Containing Adenine Tetrads: Implications for Specific Binding in RNA Tetraplexes

Baocheng Pan,1 Yong Xiong,2 Ke Shi,1 Junpeng Deng,3 and Muttaiya Sundaralingam1,∗
1Departments of Chemistry and Biochemistry
The Ohio State University
200 Johnston Lab
176 West 19th Avenue
Columbus, Ohio 43210
2Department of Molecular Biophysics and Biochemistry
Yale University
New Haven, Connecticut 06511
3Department of Biochemistry and Biological Structure
University of Washington
Seattle, Washington 98195

Summary
Purine-rich regions in DNA and RNA may contain both guanines and adenosines, which have various biological functions. Here we report the crystal structure of an RNA purine-rich fragment containing both guanine and adenine at 1.4 Å resolution. Adenines form an adenine tetrad in the N6-H-N7 conformation. Adenines are known to form A tetrads in the guanine tetraplex and do not change the global conformation but introduce irregularity in both the hydrogen bonding interaction pattern in the groove and the metal ion binding pattern in the central cavity of the tetraplex. The irregularity in groove binding may be critical for specific binding in tetraplexes. The formation of G-U octads provides a mechanism for interaction in the groove. Ba2+ ions prefer to bind guanine tetrads, and adenine tetrads can only be bound by Na+ ions, illustrating the binding selectivity of metal ions for the tetraplex.

Introduction

Guanine-rich regions exist in telomeric DNA (Blackburn, 1991; Wright et al., 1997) and RNA (Sundquist and Hopkins, 1993; Christiansen et al., 1994). Recent research has shown the importance of these regions in RNA. For instance, they are involved in the mRNa turnover process (Bashkirov et al., 1997) and packaging of HIV RNA (Sundquist and Hopkins, 1993) and may play a regulatory role in cellular metabolism (Shafer and Smirnov, 2001). The mechanism of downstream frameshifting in translational recording of mRNA arises from the guanine tetraplex structure (Horsburgh et al., 1996). The G-rich fragments form tetraplex structures in both DNA and RNA (Patel et al., 1999a). More and more evidence shows that the purine-rich regions containing adenosines play an important role in cellular processes. For example, a protein binding site in the 3′-untranslated region of chicken elastin mRNA, UGGGGGGAGGGAGGGAGGGA, may play a potential role in the regulation of elastin mRNA stability (Hew et al., 2000). The fragile X mental retardation protein binds specifically to a purine-rich fragment containing both guanine and adenine in its mRNA (Schaeffer et al., 2001). Mutations in HIV type 1 RNA showed that GA-rich sequences are required for efficient RNA packaging and dimerization, but G-rich segments are not important in these processes (Russell et al., 2003). The Shine-Dalgarno (SD) sequence also contains GGAGG, which plays an important role in the initiation of protein biosynthesis (Bonham-Smith and Bourque, 1989). Human telomeres containing repeats of d(TTAGGG) may function in regulated recombination and genomic evolution (Sun et al., 2001).

Adenines and guanines display different behavior in forming structures of purine-rich sequences. Solution studies indicate that adenines can form various conformations, while guanines usually adopt the guanine tetrad. For instance, adenines are involved in TTA loops in d[AGGG(TTAGGG)3] (Wang and Patel, 1993) and adopt two different conformations of adenine tetrad (A tetrad) in d(AGGGT) and d(TAGGGT) (Patel et al., 1999b). Furthermore, they participate in the formation of an A-G-G-G pentad in d(GAGGTG) (Matsugami et al., 2001). Similar conformation of the A-G-G-G-G-A hexad has also been observed in an RNA oligomer, r(GGAGGUUUGGAGG) (Liu et al., 2002). Re- cently, crystal structures of d(TAGGGTTAGGGT) and d([AGGG(TTAGGG)]) show that adenines do not form A tetrads but are involved in TTA loops and TATA tetrads (Parkinson et al., 2002). All these results indicate that adenines are quite flexible in conformation in purine-rich sequences. Even though there are many crystallographic studies investigating the characteristics of guanine tetrads (Kang et al., 1992; Laughlan et al., 1994; Phillips et al., 1997; Deng et al., 2001; Parkinson et al., 2002; Haider et al., 2002), we still do not have any crystals containing adenine tetrads in either DNA or RNA. Since they have a different hydrogen bonding pattern from guanines, adenosines form one cyclic hydrogen bond in the A tetrad. Because the A tetrad has more than one conformation with one cyclic hydrogen bond, it is essential to determine the conformation of the A tetrad and its relationship with the interactions with medium. This structural information will be helpful in uncovering the biological functions of adenosines in purine-rich regions. Here we report a crystal structure containing A tetrads in the RNA tetraplex structure. The N6-H…N7 conformation is preferred in A tetrad formation. The substitution of an A tetrad in a G tetraplex does not change the global conformation of G tetras but introduces irregularity in the groove binding interaction pattern and metal ion binding pattern in the central cavity of the tetraplex.

Results and Discussion

Overall Structure of Tetraplex
The hexamer (6dU)r(GAGGG) crystallizes in the tetragonal space group P42, with four independent strands...
Figure 1. Crystal Structure of (BrdU)r(GAGGU)

(A) Schematic diagram and numbering scheme of the tetraplex showing the interaction with metal ions. The numbers indicate the distance between two atoms, in angstroms.

(B) Stereo view of the crystal packing of the four independent tetraplexes interacting with Ba\(^{2+}\) ions (red) and Na\(^{+}\) ions (blue).

in the asymmetric unit (where BrdU is 5-bromodeoxyribo-uridine) (Figure 1A). Each independent strand and its three symmetry-related strands form a parallel tetraplex, which packs along the c axis in the 3' to 3' and 5' to 5' pattern and generates pseudocontinuous columns of tetraplexes (Figure 1B). The groove width in each tetraplex varies between 3.5 Å and 5.2 Å. The five bases in r(GAGGU) form base tetrads, while the 5'-termini BrdUs adopt two different conformations, forming a U tetrad inside the tetraplex (in strands 2 and 4) and swinging out of the tetraplex to engage in G-U octad formation (in strands 1 and 3). The different conformations of the terminal uridines do not affect the conformation of the central purine tetraplex r(GAGG). The average twist and rise for r(GAGG) are 28.5° and 3.7 Å, corresponding to 12.6 tetrads per turn and a pitch of 46.8 Å. The RNA purine tetraplex is underwound and extended compared with the canonical RNA duplexes with a twist angle of 33° and rise of 2.9 Å. All of the residues are in the anti glycosyl conformation and all the purines adopt the C3'-endo sugar pucker, while the terminal uridines adopt three different sugar puckers: C2'-endo (U1, U12, U13, and U18), C3'-endo (U7 and U19), and C1'-exo (U6).

Adenine Tetrads

Adenines in the four tetraplexes form four A tetrads with similar conformation (Figure 2A). There is only one cyclic hydrogen bond, N6-H...N7 (2.9 Å). A Na\(^{+}\) ion is located above the 5' side of the A tetrad plane, but there is no metal ion on the 3' side. The average distance between N6(A) and the Na\(^{+}\) ion is 3.3 Å, which is longer than the average value of 3.0 Å for O6(G) and the Na\(^{+}\) ion in the present structure. The weaker interaction between N6(A) and the Na\(^{+}\) ion is due to the NH\(_2\) of adenine, implying different behavior between adenine and guanine in interaction with metal ions in the cavity of tetraplex. A water bridge connecting O2', N3, and the adjacent O2P is conserved in all the four A tetrads. All four adenines adopt the trans/trans conformation for α/γ, which is different from the gauche /gauche' conformation for guanines in G tetrads. The A tetrad only has small overlap in stacking with its 5' side G tetrad at the twist angle of 36° (Figure 2B) but stacks well on its 3' side G tetrad at the twist angle of 27° (Figure 2C).

Superposition of the three G tetrads in the present structure, (BrdU)r(GAGGU), with the corresponding G tetrads in r(UGGGGU) (Deng et al., 2001) (rmsd of 0.26 Å) shows the different orientation of the A tetrad from that of the G3 tetrad in r(UGGGGU) (Figure 2D). Comparison indicates that adenine rotates toward the groove to participate in the N6-H...N7 interaction and to expose the N3 atom to the groove. The A tetrad can adopt either the cyclic N6-H...N1 or N6-H...N7 hydrogen bonding scheme (Patel et al., 1999b). The G tetrad in Figure 2D happens to be in the same orientation as the A tetrad in the N6-H...N1 scheme. Thus, the present structure illustrates that the A tetrad in the N6-H...N7 scheme exposes the N3 atom into the groove more than that in the N6-H...N1 scheme. In other words, the A tetrad in N6-H...N7 conformation is favorable for interaction with
The A Tetrad and G-U Octad in RNA Tetraplex

Figure 2. Conformation and Stacking of the A Tetrad
(A) |2Fo – Fc| electron density map of the A tetrad in the N6-H…N7 hydrogen bonding scheme with a Na+ ion (blue) in the center and three water molecules (green) connecting O2', N3, and O2P, contoured at 1 α.
(B) The G2 tetrad (cyan) stacks with the A3 tetrad (red).
(C) The A3 tetrad (red) stacks with the G4 tetrad (yellow).
(D) The different orientations of the A tetrad (red) in (5'dU)r(GAGGU) and G tetrad (cyan) in r(UGGGGU).

ligands in the groove and, thus, is more stable than the N6-H…N1 scheme. Theoretical calculation shows that the shortest distance between the hydrogen atoms of amino groups is 2.9 Å for the N6-H…N7 scheme and 2.3 Å for the N6-H…N1 scheme (Deng et al., 2001). Such steric consideration favors the N6-H…N7 scheme because hydrogen atoms in amino groups are less repulsive when the A tetrad is in the N6-H…N7 conformation.

G Tetrad and Parallel G-U Octad
Guanines adopt the G tetrad conformation with two cyclic hydrogen bonds, N1-H…O6 (2.9 Å) and N2-H…N7 (2.9 Å) (Figure 3A). G2 and G14 tetrads further interact with U1 and U13, respectively, to form the G-U octad with two hydrogen bonds, N2(G)-H…O2(U) (3.0 Å) and O2'(G)-H…O4(U) (2.7 Å) (Figure 3B). A water molecule above the G-U octad plane connects N3(G) and N3(U). Because uridines associated with the base octads come from parallel strands relative to the G tetrads, we call the base octads “parallel G-U octads.”

The G-U octads observed here are different from those in r(UGGGGU), in which uridines come from the antiparallel strands relative to the G tetrad (Deng et al., 2001), which we call the “antiparallel G-U octad.” Comparison of these two different G-U octads shows that uridines usually use their Watson-Crick sides for interactions in the minor groove of guanines. It is clear from the structures that N2(G), phosphate groups, and 2'-hydroxyl groups are very important in the groove binding interaction. The different orientations and hydrogen bonding schemes for the parallel and antiparallel G-U octads reflect the strong interaction potentials between G tetrads and uridines.

U Tetrads
Two different conformations of the U tetrad have been observed in the present structure. The two 5’-terminal uridines, U7 and U19, form a novel U tetrad conformation that does not have any base-base hydrogen bonds (distance between O4 and N3 is 3.7 Å) (Figure 3C). The base stacking does not contribute greatly to stabilization of
the U tetrad because there is only a small overlap with the two adjacent G tetrad. The conformation is mainly stabilized by the interaction with metal ions on both sides of the tetrad plane. The O4(U) atom interacts with the Na\(^+\) ion with a bond length of 3.2 Å on the 5′ side of the tetrad plane and with the Ba\(^{2+}\) ion with a bond length of 2.7 Å on the 3′ side (Figure 3E). A water bridge connecting O2 and O5′ further stabilize this conformation. This conformation is different from that of the U tetrads observed in the NMR and crystal structure of r(UGGGGU), in which a base-base hydrogen bond is observed (Cheong and Moore, 1992; Deng et al., 2001). For the 3′ side U tetrads, a base-base hydrogen bond, N3-H...O4 (2.74 Å), is observed (Figure 3D). Also, a water bridge connects O2, O2, and the anionic oxygen atom of the phosphate group of neighboring uridines. However, U tetrads interact with metal ions only on their 5′ side (Figure 3F). A Ba\(^{2+}\) ion interacts with the U6 tetrad, while a Na\(^+\) ion interacts with the U12 tetrad and U18 tetrad, respectively (Figure 1A).

The inward base buckles have been observed for the U tetrads. All these buckles are related to the interaction with metal ions. The 3′-terminal U tetrads have larger buckles (average of 28°) than the 5′-terminal U tetrads (average of 15°) because the 3′-terminal U tetrads interact with metal ions only on their 5′ side (Figure 3F), but the 5′-terminal U tetrads interact with metal ions on both sides (Figure 3E). The unbalanced interaction with metal ions on two sides of U tetrads leads to larger buckles in the 3′-terminal U tetrads. The 5′-terminal U tetrads have buckles because the Ba\(^{2+}\) ion has stronger interaction with U tetrads than does the Na\(^+\) ion. The U6 tetrad has the largest buckle (35°), since it only interacts with the Ba\(^{2+}\) ion on the 5′ side, while U12 and U18 have similar buckles (24° and 25°) because of their similar interaction with the Na\(^+\) ion.

### Metal Ion Binding

There are eight Ba\(^{2+}\) ions and eight Na\(^+\) ions in the present structure. A Ba\(^{2+}\) ion, hydrated with six water molecules, sits outside of the tetraplex and interacts with U18 at O2′ (2.9 Å) and O3′ (2.8 Å) (Figure 1B). All the other Na\(^+\) and Ba\(^{2+}\) ions locate in the central axis of the tetraplex (Figure 1). The average distances between O6(G) and Ba\(^{2+}\) ions, O6(G) and Na\(^+\) ions, and N6(A) and Na\(^+\) ions are 2.8 Å, 3.0 Å, and 3.3 Å, respectively. It should be mentioned that, even though the amino group of adenine itself is somewhat positively charged, cations above or below the A tetrad plane can still interact with the amino group through the lone pairs of electron in N6. However, the present structure indicates that the amino group can only have interaction with one cation on the 5′ side of the A tetrad. Indeed, A tetrads are quite different from G tetrads in interacting with metal ions, and more studies are needed to investigate the binding behavior of metal ions to A tetrads.

Metal ions are critical for the formation of tetraplexes. Guanine tetraplexes selectively chelate metal ions with suitable ionic radii (Guschlbauer et al., 1990). Previous solution studies showed that tetraplexes cannot form without proper monovalent cations (Williamson et al., 1989) and that tetraplexes may adopt different conformations when they interact with Na\(^+\) and K\(^+\) ions (Balagurunmoorthy and Brahmacari, 1994; Ketani et al., 1998; Bouaziz et al., 1998). Crystal structures showed that Na\(^+\) and K\(^+\) ions locate between every G tetrad plane along the central axis of DNA tetraplexes (Laughlan et al., 1994; Phillips et al., 1997; Parkinson et al., 2002; Haider et al., 2002). However, divalent cation Sr\(^{2+}\) ions locate between every other G tetrad plane in an RNA tetraplex (Deng et al., 2001), implying different characteristics of monovalent and divalent cations.

In the present structure, monovalent and divalent cations co-exist in the axis of the purine tetraplex, which provides us an opportunity to compare the binding preference of monovalent and divalent cations. Metal ions in the present structure locate between every tetrad plane, except at the 3′ side of the A tetrads (Figure 1). G tetrads can interact with both Ba\(^{2+}\) and Na\(^+\) ions, and tandem G tetrads always interact with a Ba\(^{2+}\) ion. By comparison, the A tetrad only interacts with the Na\(^+\) ion. This result indicates that Ba\(^{2+}\) ions prefer to bind tandem G tetrads, and A tetrads can only be bound by Na\(^+\) ions. A close inspection of the metal ion binding reveals that electrostatic interaction plays an important role in the alignment of metal ions in the core of tetraplexes. Divalent cations have stronger electrostatic attraction with carbonyl groups of guanine than monovalent cations, and, thus, better stabilize the formation of G tetrads. However, the electrostatic repulsion between divalent cations is bigger than that between monovalent cations. Further separation to every other tetrad plane, instead of every tetrad plane, for divalent cations reduces the repulsion but at the same time decreases the number of attractions between cations and base tetrad (e.g., 24 attractions are reduced to 16 for a tetraplex of four consecutive G tetrads). Crystal structures of r(UGGGGU) show that the repulsion between divalent cations is so strong that they have to separate farther away to reduce repulsion at the cost of decreasing the number of attractions (Deng et al., 2001). In the present structure divalent cations usually have monovalent cations as their neighbors, except in the 3′-terminus of tetraplex 1, which has the longest distance in all the metal ion separation (Figure 1). This result indicates that the repul-
sion between divalent and monovalent cations is still not strong enough to separate them to every other tetrad plane. In other words, repulsion between two divalent cations compels the divalent cations to separate to every other tetrad plane, but divalent and monovalent cations can still remain in every tetrad plane, in spite of their repulsion. The final alignment of metal ions at the core of tetraplexes is the balance of the attraction between metal ions and base tetrad s and the repulsion between metal ions along the central axis.

The central cavity of a G tetraplex possesses the regular geometry and regular binding pattern of eight O6 groups of two adjacent G tetrad s. Accordingly, only one kind of metal ion interacts with a G tetraplex, as shown in the binding of Na⁺ or K⁺ ions to the DNA G tetraplex (Laughlan et al., 1994; Phillips et al., 1997; Parkinson et al., 2002; Haider et al., 2002) and Sr²⁺ ions to the RNA G tetraplex (Deng et al., 2001). When an A tetrad is introduced into the G tetraplex, the regular chelating pattern is disrupted at the A tetrad, where the chelating site is formed by four O6 groups (G tetrad) and four N6 groups (A tetrad). The N6 group is not as favorable as the O6 group for interaction with metal ions, especially for multivalent cations. Also, the N6 group is bulkier than the O6 group, and, thus, the A tetrad is expected to be more suitable than the G tetrad for interaction with metal ions of small ionic radii, like the Li⁺ ion. These differences result in the selectivity for metal ions to bind tetraplexes containing both guanines and adenines.

Hydration
A total of 113 water molecules are clearly located in the electron density maps, with an average B factor of 22.0 Å². The grooves of the four tetraplexes are highly hydrated. The four tetraplexes have similar hydration, except in the 5'-end of the tetraplex, in which the hydration sites are occupied by uridine bases in tetraplex 1 and 3 (Figures 3G and 3H). Two common hydration patterns are observed in the present structure: (1) water bridges linking O2P and 2'-hydroxyl groups across the strands and (2) water bridges linking O2P in the neighboring phosphate groups in the same strands (Figures 3G and 3H).

Because the 2'-hydroxyl groups are exposed to the groove and available for interaction with water molecules, the hydration patterns observed here are quite different from those in DNA tetraplexes. The cross-strand water bridges in DNA tetraplexes connect anionic oxygen atoms of phosphate groups with O4' atoms in d(TGGGGT) (Phillips et al., 1997) and mainly with N2 atoms of guanines in d(GGGGTTTTGGGG) (Haider et al., 2002). Even though the average intrastand separation between adjacent phosphate groups in the present structure is similar to that in d(TGGGGT) (Phillips et al., 1997) (6.6 Å compared with 6.2 Å in the present structure), water molecules in DNA tetraplexes cluster around individual phosphate groups, and only a few water bridges connect the adjacent phosphate groups (Phillips et al., 1997; Haider et al., 2002). In the present structure water bridges link all the adjacent phosphate groups in the same strand. A careful examination indicates that 2'-hydroxyl groups mainly make up the difference. The distances between O2P atoms and 2'-hydroxyl groups in the adjacent strands range from 4 Å to 6 Å in the central region of the tetraplex and from 7 Å to 8 Å near the ends of the tetraplexes. These distances and the positions of exposed 2'-hydroxyl groups are suitable for forming water bridges between phosphate groups and 2'-hydroxyl groups. These water bridges in turn stabilize the water bridges between the neighboring phosphate groups in the same strand. In fact, some of the water molecules participate in both bridges. Bridges of one and two water molecules are observed in the central region, while bridges of two and three water molecules exist near the ends of the tetraplexes.

Comparison with r(UGGGGU)
Both NMR and crystal structures showed that the hexamer r(UGGGGU) adopts the G tetraplex conformation (Cheong and Moore, 1992; Deng et al., 2001). Comparison with the crystal structure r(UGGGGU) (Deng et al., 2001) shows that the 3'-terminal uridines in r(UGGGGU) adopt the similar buckled U tetrad conformation but that its 5'-terminal uridines have different conformations, swinging out of the tetraplex to engage in antiparallel G-U octads. Superposition of three corresponding G tetrads in r(UGGGGU) and ("dU)r[GAGGU], gave an average rmsd of 0.26 Å, indicating that replacement of guanine by adenine does not change the global conformation of the G tetraplex.

Biological Implications
Tetraplexes provide four grooves for interaction with proteins, nucleic acids, and ligands. Hydration patterns of the A tetrad (Figure 2A) and G tetrad (Figure 3A) suggest that the interaction sites in the grooves of RNA tetraplexes are N3 (acceptor) for adenine and N2 (donor) and N3 (acceptor) for guanine. The formation of G-U octads (Figure 3B) supports the suggestion for the G tetrad. A parallel tetraplex containing G tetrads has the same hydrogen bonding pattern of N2 (donor)-N3 (acceptor) along the groove and displays the same recognizable pattern for hydrogen bonding interaction. The present structure shows that the replacement of an adenine in this G tetraplex can provide a different hydrogen bonding pattern at the site of the A tetrad by changing N2 (donor)-N3 (acceptor) to N3 (acceptor). Such change in the interaction pattern provides specific binding sites, which is characteristic of A tetrads. This structural information may be very helpful for designing new drugs that target specific sites of RNA tetraplexes.

G tetrads can interact with uridines to form antiparallel G-U octads (Deng et al., 2001) and parallel G-U octads, as observed in the present study. In these two conformations, the G tetrad keeps the same conformation and hydrogen bonding scheme. This result indicates that the G tetrad with two cyclic hydrogen bonds is very stable, and we may expect that it will not change its conformation during the interaction with proteins and nucleic acids.

Purine-rich regions in RNA are involved in various biological processes, and adenines play crucial roles in some instances (Schaeffer et al., 2001; Russell et al., 2003). Determination of the structural behavior of ade-
Table 1. Data and Statistics of (\textsuperscript{\textit{Br}}dU)r(GAGGU)

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MAD Phasing Statistics at 2.0 Å Resolution

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Refinement Statistics

| Resolution range used (Å) | 30.0–1.4 |
| Number of reflections used \(\text{[}\ell\text{]}\) | 22,556 |
| \(R_{\text{work}}/R_{\text{free}}(\%)\) | 16.6/18.4 |
| Final model | Nucleic acid atoms | 496 |
| Na\textsuperscript{1} | 8 |
| Ba\textsuperscript{2+} | 8 |
| Water molecules | 113 |
| Rmsd bonds (Å) | 0.015 |
| Rmsd angles (°) | 2.0 |

\*Friedel pairs unmerged.

...and the effect of adenines embedded in consecutive guanines is essential to unravel the biological properties of these purine-rich regions. The present structure shows that an adenine embedded in consecutive guanines forms an A tetrad in the N6-H...N7 conformation, which exposes the N3 in the groove for interaction. Substitution of an A tetrad in the G tetraplex does not change the global conformation but introduces irregularity in both patterns of hydrogen bonding interaction in the groove and ion binding in the core of the tetraplex. The irregularity in hydrogen bonding pattern in the grooves seems to be crucial for specific recognition and interaction in some cellular processes. For instance, A tetrads may play some specific roles in the binding of the fragile X mental retardation protein to the purine-rich fragment in its mRNA (Schaefker et al., 2001). All these results may imply that G tetrads mainly provide the structural framework and that A tetrads provide specific binding sites in some biological process.

**Experimental Procedures**

**Synthesis and Crystallization**

The DNA/RNA chimera (\textsuperscript{\textit{Br}}dU)r(GAGGU) was synthesized by the phosphoramidite method with Applied Biosystem DNA synthesizer 391 and incubated in the 3:1 (v/v) solution mixture of triethylamine Tris(hydrofluoride) and N,N-dimethylformamide at 55° for 3 hr to deprotect the 2'-hydroxyl groups. The sample was purified by ion exchange chromatography. Crystallization was carried out by the hanging drop vapor diffusion method at room temperature (293 K). Primary trials of crystallization were made with Nucleic Acids MiniScreen (Hampton Research) to screen different metal ions. The result showed that Ba\textsuperscript{2+} and Na\textsuperscript{+} ions have the best effect in crystallization. The best crystals were grown under the condition of 1 mM (single-strand) RNA, 20 mM sodium cacodylate buffer (pH 6.0), 90 mM NaCl, 10 mM BaCl\textsubscript{2}, 6 mM spermine tetrachloride, and 2% (v/v) methyl-2,4-pentanediol (MPD) against 15% MPD in the reservoir. Crystals of dimensions 0.2 mm × 0.2 mm × 0.3 mm were obtained in about 2 weeks.

**Date Collection and Structure Determination**

Three data sets for one crystal were collected at three different wavelengths near the bromine absorption edge at beamline 14BM-D of the Advanced Photon Sources (APS) in the Argonne National Lab. The data were processed with DENZO and SCALEPACK (Otwinowski and Minor, 1997) (Table 1). The structure was determined with experimental phases obtained from the MAD phasing method with the bromine in the 5-bromouracil nucleotide as the anomalous scatterer. The electron density maps clearly showed the structure of the tetraplex with the SOLVE and RESOLVE software package (Terwilliger, 2001). The CHAIN program was employed for model building and fitting (Sack and Quioto, 1992). The data set collected at the bromine remote site \(l = 0.800 \text{ Å}\) was used in the refinement. The torsion angle simulated annealing with slow cooling in the program package CNS (Brunger et al., 1998) was used to reduce the model bias in manual building and fitting. Iterative positional and B factor refinements were performed in the resolution range of 30.0–1.4 Å. The base of U24 is disordered and cannot be located in electron density maps. The metal ions and water molecules were added to the structure according to the electron density map of \(|2F_o - F_c|\) at 1 σ and \(|F_o - F_c|\) at 3 σ contour. The electron density map for Ba\textsuperscript{2+} is much bigger than that for Na\textsuperscript{+}, and it is not difficult to identify...
the Ba$^+$ from the Na$^+$ ion in the map. The final $R_{	ext{work}}/R_{	ext{free}}$ was 16.6%/18.4%. The refinement statistics are summarized in Table 1.

Acknowledgments

We greatly thank Dr. R.V. Hosur for his helpful discussion on the A tetrad conformations in NMR studies and Drs. Florante A. Quiocho and Alex Nickitzendo for providing the new version of the CHAIN program. We acknowledge the support of this work by the National Institutes of Health grant GM-17378 and the Board of Regents of Ohio for an Ohio Eminent Scholar Chair and Endowment to M.S. Use of the Advanced Photon Sources was supported by the U.S. Department of Energy, Basic Energy Sciences, Office of Science, under contract number W-31-109-Eng-38.

Received: January 14, 2003
Revised: March 18, 2003
Accepted: April 9, 2003
Published: July 1, 2003

References


Accession Numbers

The atomic coordinates and the structure factors have been deposited in the Nucleic Acid Database with accession code UR0023 and Protein Data Bank ID 1J6S (Berman et al., 1992).