Structure Analysis of an RNA Aptamer Binding to a Stem-loop RNA

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RNA structure can be analyzed by biophysical or biochemical methods. X-ray crystallography or NMR (nuclear magnetic resonance) involved in biophysical method has not been popular so far because it is not easy to make RNA crystal for X-ray crystallography and the small size of RNA only is available for the structural analysis with NMR.[1] So biochemical methods using structure specific enzymes and chemicals have been used widely.[2-5] Enzymes and chemical which have been used for probing RNA structure in solution, are double-strand-specific RNase V1, single-strand-specific nuclease S1, RNase T1 which has a specificity for a guanine in single strand region, and kethoxal (3-ethoxy-1,1-dihydroxy-2-butanone), which modify the N1 and N2 of guanine in the single strand. Hydroxyl radical (·OH) has also been used for the structural analysis of RNA. Exposed nucleotides are damaged by hydroxyl radical while nucleotides involved in tertiary contacts are protected from damage, making it a favorable approach for establishing exterior/interior relations for RNA.[6-12] Radicals are generated from Fe(II)-EDTA with hydrogen peroxide (H_2O_2). Ascorbate (or DTT) is added to reduce Fe(III) to Fe(II). Hydrogen abstraction from the ribose 4' carbon leads to strand scission. Enzymes whose size is above 100 Å, may have an accessibility problem to target RNA because of their big size. To overcome this problem, sometimes radical or chemicals whose size is below 10 Å, can be used.

RNA aptamers which bind to a stem-loop RNA, which plays an important role in the synthesis of gag-pol fusion protein in HIV-1 were selected from a random-sequence RNA library.[13] In this work, the structure of the RNA aptamer 13-1-3, one of the selected RNA aptamers was found by the MFOLD program accessed on the internet (www.bioinfo.rpi.edu/applications/mfold/old/rna) and also supported by RNA structural probes such as RNase T1, RNase V1 and nuclease S1.

The structure of RNA aptamer 13-1-3 was probed in binding buffer with RNase T1, RNase V1, and nuclease S1 (Fig. 1). G13, G14, G15, and G28 were cleaved by RNase

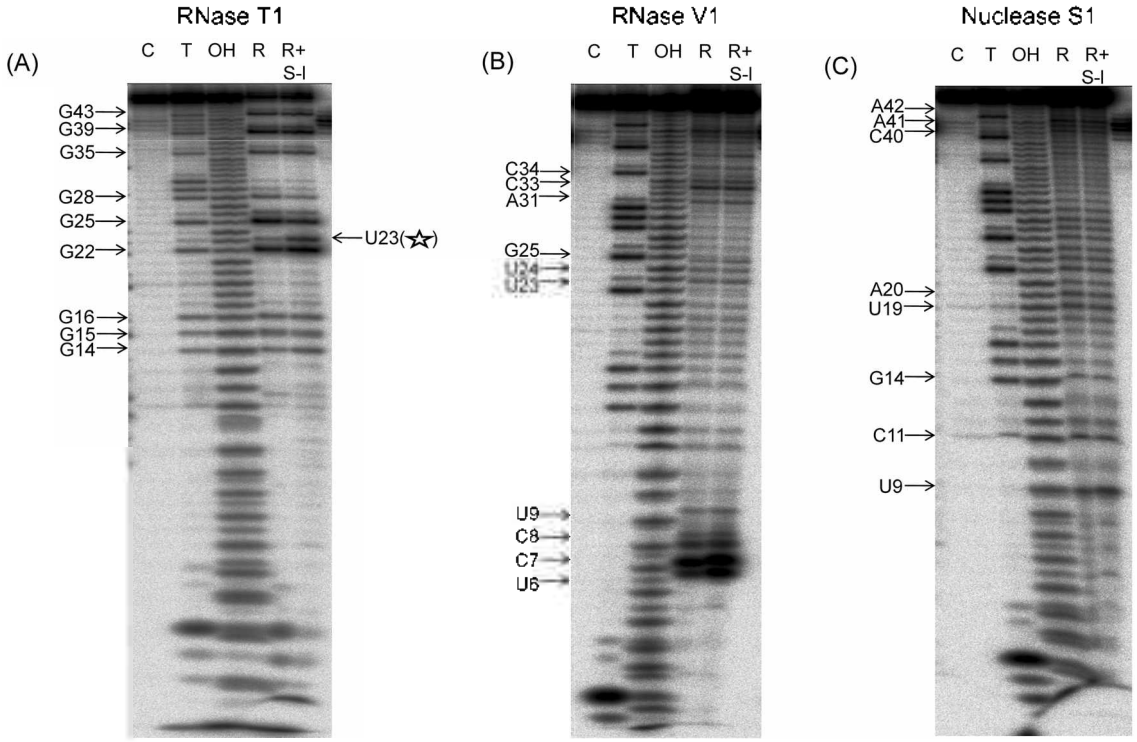

Figure 1. Enzymatic probing of RNA aptamer 13-1-3 labeled at the 5'-end. The RNA was partially digested with RNase T1 (A), RNase V1 (B) and nuclease S1 (C). The cleaved nucleotides are indicated by arrows. Residue U23 protected from RNase T1 digestion by stem-loop RNA was marked with a star. Lane C, control; lane T, the denatured RNA treated with RNase T1; lane OH, partial alkaline ladder; lanes R and R+S−1, enzymatic cleavages in the absence of and the presence of stem-loop RNA, respectively.

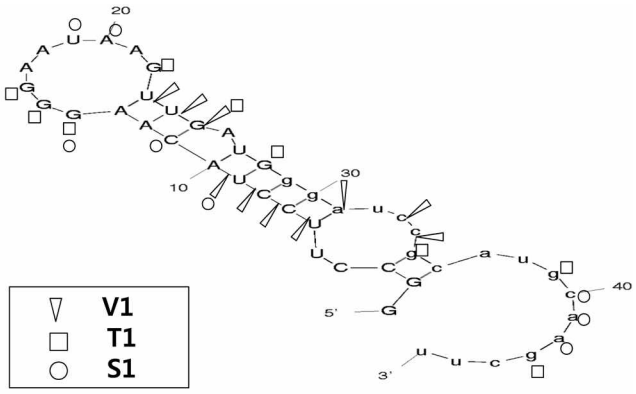

Figure 2. Schematic representation of possible secondary structure of RNA aptamer 13-1-3. Triangles indicate the sites cleaved by RNase V1, squares indicate the sites cleaved by RNase T1 and circles indicate the sites cleaved by nuclease S1.

T1, especially strong cleavages at G20, G25, G35, G39 and G43, so these guanines are thought to be in single-strand region of the secondary structure model of RNA aptamer 13-1-3. Residue U23 marked with a star, was protected from RNase T1 digestion by the stem-loop RNA, a ligand used for selection of this RNA aptamer 13-1-3. Footprinting studies are in progress to evaluate which sequence of RNA aptamer 13-1-3 is important for the interaction between the RNA aptamer and the stem-loop RNA ligand, and will be discussed later.

Regions U23UG25 and A31 were cleaved by RNase V1, especially strong cleavages at region U6CCU9 and so these regions are thought to be located in double strand. But the region C33C34 which was thought to be in single strand, was susceptible to RNase V1, suggesting that this region was stacked from intramolecular interaction and became accessible to RNase V1 in solution.

Residues U9, C11, G14, U19A20 and C40AA42 were cleaved by nuclease S1, so these regions are thought to be in single-strand region of the secondary structure model of RNA aptamer 13-1-3. The residue C11 was susceptible to nuclease S1 and residue G25 was attacked by both RNase T1 and RNase V1, and so C11:G25 base pair is thought to be unstable because of the adjacent presence of a bulge A26.

In conclusion, the structure of RNA aptamer 13-1-3 was analyzed in solution with probes such as RNase T1, RNase V1, and nuclease S1 and its possible secondary structure was represented (Fig. 2). The result suggested that 1) this molecule has three single strand regions and two double strand regions, a long double strand region of the two has a bulge A26, and 2) C11:G25 base pair is unstable because of the adjacent presence of a bulge A26.

Experimental Section

Preparation of RNA. RNA aptamer 13-1-3 was synthesized by run-off *in vitro* transcription with T7 RNA polymerase from the DNA template to which the T7 promoter was annealed and purified by gel elution of the crush and soak method.[14] The resulting RNA was treated with CIP (calf intestinal alkaline phosphatase) to remove 5' end phosphate and then labeled at the 5' end using [γ-^{32}P]ATP and T4 polynucleotide kinase.

Enzymatic Cleavage Reaction. 5'-terminal radiolabeled RNA aptamer 13-1-3 was heated in binding buffer (30 mM Tris-acetate, pH 7.5, 60 mM magnesium acetate, 120 mM potassium acetate, and 120 mM ammonium acetate) at 70 °C for 5 min and allowed to cool to RT (~21 °C). Then 0.1-1 unit of nuclease S1 (Boehringer Manheim GmbH, W.-Germany) or 0.001-0.01 unit of RNase V1 (Pierce Molecular Biology, Perbio) or 0.1-1 unit of RNase T1 (Industrial Research Limited) was added to the above mixture and then the reaction mixture was incubated for 20 min at RT. The reaction volume included an additional 1 mM $ZnCl_2$ for nuclease S1 cleavage. The cleavage products were recovered by ethanol precipitation and separated on a 15% polyacylamide gel in 90 mM Tris-borate (pH 8.3) and 2.5 mM EDTA containing 7 M urea.

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