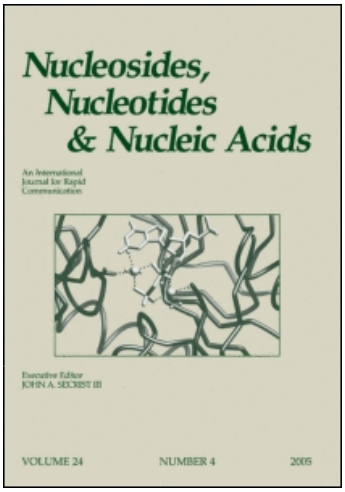

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Determination of The Solution Conformation of A Non-Uniformly Deuterium Labelled (Uppsala 'Nmr-Window') 21Mer Rna Hairpin by Nmr Spectroscopy And Computational Methods.

Anders Sandström[a]; Tatiana Maltseva[a]; Jyoti Chattopadhyaya[a]
[a] Department of Bioorganic Chemistry, Biomedical Centre, Uppsala University, Uppsala, Sweden

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DETERMINATION OF THE SOLUTION CONFORMATION OF A NON-UNIFORMLY DEUTERIUM LABELLED (UPPSALA 'NMR-WINDOW') 21MER RNA HAIRPIN BY NMR SPECTROSCOPY AND COMPUTATIONAL METHODS.

Anders Sandström, Tatiana Maltseva and Jyoti Chattopadhyaya*
Department of Bioorganic Chemistry, Box 581, Biomedical Centre, Uppsala University, S-751 23 Uppsala, Sweden

ABSTRACT: The conformation of a 21mer RNA hairpin, 5'-r(AGCCCGCCUAAUGAG-CGGGCU)-3, was determined by NMR spectroscopy and computer calculations. The 'Uppsala NMR-window' approach was used to overcome the problem of spectral overlap.

The sequence and possibly the folded 3D structure of a 21mer RNA corresponding to residues +114 to +134 within the *trp* leader mRNA transcript in *E. coli* has been proposed to provide the termination and anti-termination signals recognized by RNA polymerase. Due to the biological properties of this molecule an attempt to determine its structure was recently made[1] by 600 MHz NMR spectroscopy, which however gave very little specific information.

To overcome the problem of spectral crowding, the substitution of hydrogen atoms for deuterium has been shown by us to be a useful approach (Uppsala 'NMR-window' concept) for studying the NMR structure of relatively large oligo-DNAs and RNAs [2-8]. There are two variations of the Uppsala 'NMR-window' concept: In 'NMR window I' all sugar protons are exchanged for deuterium (> 97 % ^{2}H incorporation) except in 4'- (85 % ^{2}H) and 1'-positions (20 % ^{2}H), this method is applicable both in the DNA and RNA series. In the 'NMR window II' method the H1' is not exchanged for ^{2}H at all and it is only one of the protons at C2' which is exchanged for ^{2}H (85 % ^{2}H2'/H2" and 15 % H2'/^{2}H2"). The incorporation of ^{2}H at C4' is 65 % in this approach.

In the present work, the 'NMR-window I' concept was used to aid in the determination of the solution conformation of a 21mer RNA hairpin[4], 5'-r(AGCCCGCCUAAUGAGCG-GGCU)-3' (Fig. 1). The stem region of the 21mer was deemed suitable for incorporation of the partially deuterated nucleotides. All nucleotides except the seven residues

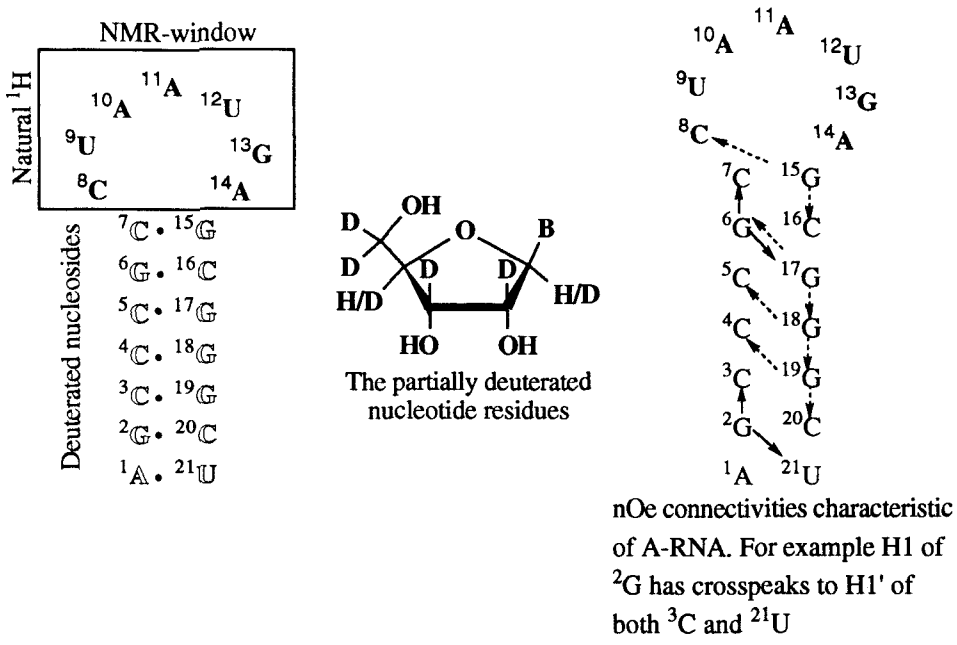

FIG. 1. The 21mer RNA and the partially deuterated building blocks.

(-^{8}CUAAUG^{14}A-) forming the hairpin loop were partially deuterated (Fig. 1). The H1' is not exchanged for ^{2}H in the 'NMR-window I' concept since the H1'-region in NMR spectra are generally not very crowded. The presence of the H1' in the stem residues also made it possible to extract essential structural information from sequential aromatic-anomeric and guanosine imino proton-H1' crosspeaks[9] (Fig. 1), which showed that the stem region (residues 1-7 and 15-21) is indeed in an A-RNA conformation.

Crosspeak volumes for the non-exchangeable protons in the non-deuterated residues were extracted from 500 MHz NOESY spectra with 100, 200 and 300 ms mixing times at 294 K. A total of 143 different crosspeak volumes were extracted and subsequently converted to distances which were used in the structure refinement.

From DQF-COSY spectra of the natural 21mer RNA, the $J_{H1',H2'}$ could be estimated and all residues in the stem region were found to be in the North conformation while the seven loop nucleotides were in a ~50 % North-South equilibrium.

Structure refinement: The first step of the structure refinement procedure was carried out using distance geometry, simulated annealing (SA) and energy minimizations (XPLOR 3.1[10]) with distance constraints (19.9 per nucleotide) derived from nOe volumes from the

three different mixing times using the two-spin approximation. The distance constraints were were divided into four categories depending on their corresponding nOe volumes[11]. In addition to the 143 distance constraints derived directly from the nOe volumes 160 distance and torsional constraints were applied to keep the stem nucleotides in a double stranded A-RNA conformation[12].

Using the 303 constraints (see above) a total of 100 conformers were generated by distance geometry as implemented in XPLOR 3.1[10]. The 100 structures were further refined following the embedding, regularization and initial SA (3 ps at 2000 K followed by cooling to 100 K over 3 ps) by seven cycles of SA.

Each cycle of SA consisted of 6 ps of cooling from 1000 K to 100 K during which the van der Waals energy term was gradually turned on. After the cooling the structures were minimized with all energy terms fully included. The relative weights of the constraint energies were increased from one cycle to the next. The force constant for the planarity constraints was increased from 2 to 20 over the first four cycles, similarly the force constant for the distance constraints was increased from 50 to 400 from cycle 4 to cycle 7.

The MARDIGRAS program[13] was used to calculate theoretical nOe volumes for the 100 conformers and to compare them with the experimental ones. The 100 structures were also evaluated in terms of consistency with the constraints applied and in terms of similarity to each other and to an ideal A-RNA structure (generated by nucgen/AMBER 4.1[14,15]). The two most similar structures showed an rmsd of 1.79 Å when the entire molecules were superimposed while the two most dissimilar structures showed an rmsd of 10.76 Å. Results from MARDIGRAS calculations are shown in Table 1.

Structures that did not display an A-RNA conformation in the stem region were discarded (rmsd of the stem > 2.0 Å to ideal A-RNA). Structures which violated any of the distance constraints by more than 0.4 Å or had more than two constraint violations of more than 0.3 Å were also discarded. By these three selection criteria only 37 conformers remained and they were further refined using the AMBER 4.1[14,15] program package using the distance constraints derived by the RANDMARDI nOe back-calculation program[13].

A total of 3330 MARDIGRAS calculations were performed (37 struct., 3 mixing times, 30 experimental intensity files with randomly added noise) and the distance bounds from these were averaged to yield the final distance constraints. This procedure gave 128 distance constraints for the seven loop nucleotides (18.3 per nucleotide) as well as 11 distance constraints involving residues C7, G15 or C16. These 139 distance constraints were used in the restrained MD[16] and energy minimizations[16] of the structure refinement procedure.

In addition to the distance constraints derived from nOe volumes by the RANDMARDI program, 25 distance and torsional constraints were applied[19]. During all the following

TABLE 1. Comparison of theoretical and experimental nOe volumes using the R-factors calculated by the MARDIGRAS program[13] at different stages of the refinement procedure.

	100 ms				200 ms				300 ms				Energy (kcal.mol^{-1})	
	R	R_2	R^x	$R^x{}_2$	R	R_2	R^x	$R^x{}_2$	R	R_2	R^x	$R^x{}_2$	E_{pot}	E_{constr}
min[a]	0.681	0.775	0.118	0.153	0.651	0.671	0.124	0.159	0.588	0.637	0.115	0.151		
max[a]	0.854	0.954	0.154	0.194	0.867	0.894	0.162	0.212	0.834	0.866	0.152	0.199		
min[b]	0.723	0.790	0.129	0.159	0.670	0.684	0.126	0.162	0.610	0.664	0.116	0.153		
max[b]	0.821	0.954	0.151	0.194	0.843	0.879	0.159	0.211	0.775	0.793	0.145	0.185		
min[c]	0.511	0.649	0.082	0.108	0.431	0.508	0.076	0.104	0.426	0.471	0.085	0.113		
max[c]	0.678	0.817	0.117	0.138	0.589	0.732	0.102	0.131	0.591	0.701	0.110	0.145		
Conf. 1[d]	0.602	0.787	0.101	0.129	0.529	0.703	0.097	0.129	0.488	0.611	0.096	0.124	576.332	148.140
Conf. 2[d]	0.636	0.767	0.110	0.138	0.565	0.651	0.103	0.130	0.531	0.596	0.104	0.131	476.511	109.930
Conf. 3[d]	0.662	0.805	0.113	0.137	0.564	0.688	0.099	0.127	0.549	0.620	0.106	0.133	498.270	108.510
Conf. 4[d]	0.645	0.718	0.116	0.139	0.588	0.628	0.109	0.139	0.552	0.597	0.109	0.137	532.060	157.270
Conf. 5[d]	0.653	0.750	0.121	0.146	0.598	0.669	0.108	0.135	0.542	0.637	0.105	0.131	503.641	116.220
Conf. 6[d]	0.675	0.830	0.112	0.143	0.575	0.688	0.101	0.133	0.518	0.626	0.096	0.128	499.240	117.310
Conf. 7[d]	0.701	0.795	0.127	0.154	0.619	0.688	0.111	0.140	0.564	0.651	0.106	0.132	477.490	119.010

(a) Maximum and minimum R-factors obtained among the 100 structures after XPLOR refinement.
(b) Maximum and minimum R-factors obtained among the 37 selected structures after XPLOR refinement.
(c) Maximum and minimum R-factors obtained among the 37 structures after AMBER refinement.
(d) R-factors and energies for the 7 final structures after AMBER refinement.

TABLE 2. Maximum and minimum rmsd in Å for heavy atoms only between conformers at different stages of refinement

	Entire structure		Res 8-14		Res 2-7 and 15-20	
	min	max	min	max	min	max
XPLOR[a]	1.79409	10.7555	1.14323	6.43056	0.707319	9.14763
XPLOR[b]	1.8002	5.94266	1.52038	5.80847	0.707319	2.60803
AMBER[c]	0.466846	4.16502	0.599842	5.82995	0.115152	0.855278
AMBER[d]	0.74017	4.16907	1.04186	5.21211	0.303352	0.572338

a) Maximum and minimum rmsd (Å) obtained among the 100 structures after XPLOR refinement.
b) Maximum and minimum rmsd (Å) among the 37 selected structures after XPLOR refinement.
c) Maximum and minimum rmsd (Å) among the 37 structures after AMBER refinement
d) Maximum and minimum rmsd (Å) among the 7 final conformers after AMBER refinement.

steps (minimization and MD) except for the very final minimization, only the atoms of residues C7 to G15 were allowed to move. The residues belonging to the stem were completely frozen.

The 37 selected conformers generated by XPLOR (see above) were first belly minimized for 500 steps with the standard force constants[20]. Each of the 37 minimized conformers were then subjected to 7 ps of belly MD at 400 K, 1 ps of cooling to 300 K and 2 ps of belly MD at 300 K. The same constraints and force constants were used as in the initial belly minimization except for the first 2 ps during which the force constants were

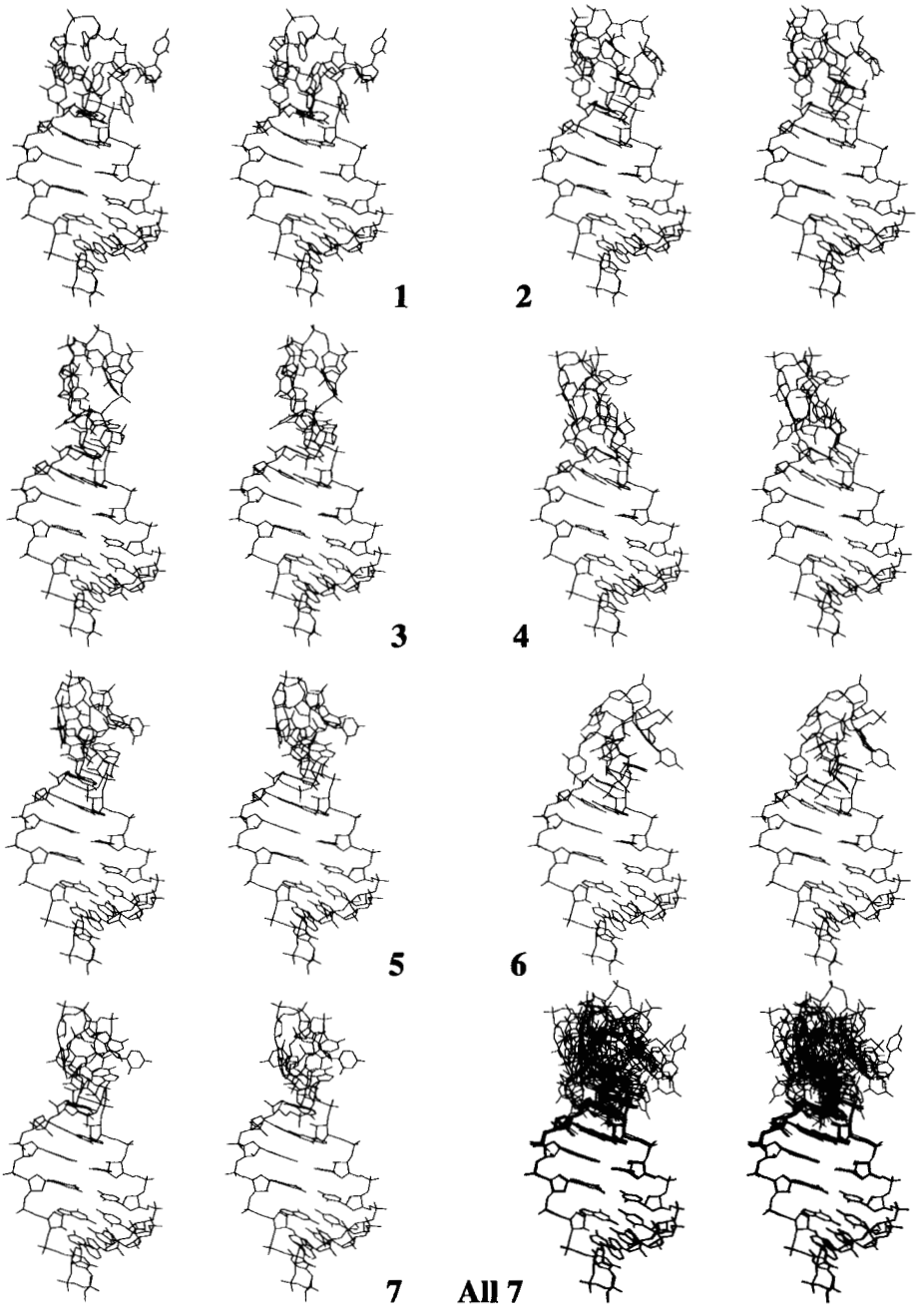

FIG. 2. Stereoviews of the seven final structures and the superimposition of them.

TABLE 3. All backbone torsions of the loop residues of the seven final conformers. See Fig. 2 for stereoviews of the seven conformers.

		C7	C8	U9	A10	A11	U12	G13	A14	G15
α	Conf. 1	-72.9	64.4	-127.6	130.6	-67.0	126.7	-53.8	96.2	89.7
	Conf. 2	-72.5	113.3	-142.6	-150.6	52.1	-101.4	101.1	-56.8	-82.9
	Conf. 3	-70.9	159.4	56.9	162.4	58.1	112.4	-175.1	-61.3	84.9
	Conf. 4	-72.3	-101.4	-82.0	-140.0	58.0	-126.0	147.4	81.2	74.5
	Conf. 5	-71.2	164.1	-142.7	148.8	53.9	-127.7	-81.0	120.34	-143.0
	Conf. 6	-70.6	-94.3	-49.4	-132.8	51.3	-86.0	95.0	101.8	-70.9
	Conf. 7	-71.4	66.3	-71.0	-133.4	61.9	-102.9	-73.4	117.8	83.5
β	Conf. 1	172.5	177.8	-123.5	-104.6	121.3	-112.4	99.1	-177.4	-151.8
	Conf. 2	175.1	-167.4	165.7	-133.1	-97.3	145.6	-143.2	84.5	166.0
	Conf. 3	175.1	-164.0	124.4	150.7	-104.0	172.7	-162.7	82.6	-167.3
	Conf. 4	174.3	-165.7	169.9	172.8	-98.7	175.8	-170.7	-59.5	-111.1
	Conf. 5	175.4	-153.0	-156.0	-121.2	-103.2	165.2	154.5	-79.9	168.7
	Conf. 6	177.4	169.7	107.3	173.5	-96.7	161.2	-137.7	-69.5	173.2
	Conf. 7	175.2	-163.7	-149.9	170.2	-108.8	156.8	140.5	-77.1	-168.9
γ	Conf. 1	56.4	176.2	49.0	79.3	46.2	67.5	61.5	99.6	-173.1
	Conf. 2	52.4	-177.5	60.6	57.6	-124.8	166.2	68.3	-164.7	63.2
	Conf. 3	57.8	173.0	-51.0	57.7	-125.9	73.8	58.1	172.3	170.1
	Conf. 4	57.0	-64.5	17.2	28.9	-121.9	48.4	57.2	-148.5	-174.4
	Conf. 5	57.4	156.1	34.1	62.3	-131.8	84.4	-58.0	-148.4	69.7
	Conf. 6	58.6	30.3	-11.3	10.1	-118.4	155.8	69.8	-132.1	69.4
	Conf. 7	56.8	173.5	37.6	26.9	-136.1	160.4	-49.8	-144.3	-174.9
δ	Conf. 1	70.2	88.7	109.9	155.9	78.8	139.4	87.2	141.9	102.3
	Conf. 2	77.0	73.9	97.2	116.9	76.9	150.4	100.7	83.7	90.4
	Conf. 3	78.1	92.5	120.7	123.9	82.0	137.3	93.4	72.5	102.2
	Conf. 4	81.3	100.6	141.5	126.3	73.3	128.5	75.1	156.8	99.2
	Conf. 5	76.2	87.6	137.6	118.3	70.8	124.1	102.6	63.8	86.4
	Conf. 6	81.9	68.9	119.7	112.1	76.6	149.7	82.9	74.3	87.6
	Conf. 7	65.7	89.9	140.7	111.7	77.1	145.2	103.8	67.0	100.2
ε	Conf. 1	44.6	-161.8	-97.1	-79.7	176.5	54.2	-160.5	35.0	-145.4
	Conf. 2	-170.9	78.3	-170.6	-87.4	169.9	-87.6	-117.0	-163.8	-152.9
	Conf. 3	-177.8	-139.4	54.0	-89.4	34.4	33.8	-110.4	43.5	-147.8
	Conf. 4	25.4	34.1	-57.5	-82.7	166.5	57.7	-120.3	-85.2	-146.0
	Conf. 5	-178.9	170.2	-79.7	-86.3	56.6	-87.7	-165.1	55.1	-150.6
	Conf. 6	176.2	53.7	-74.2	-87.7	-169.1	-86.5	-146.8	-174.7	-155.7
	Conf. 7	59.1	-130.7	-65.5	-86.9	173.9	-117.4	-163.1	36.9	-147.7
ζ	Conf. 1	78.1	-60.8	-116.2	163.4	-153.0	113.1	-57.0	77.2	-66.6
	Conf. 2	-107.1	85.5	-68.6	-86.8	-69.2	154.0	-73.1	-77.6	-65.7
	Conf. 3	-83.4	-118.1	85.3	-91.3	53.4	116.5	-60.2	77.1	-65.0
	Conf. 4	71.9	155.6	82.4	-81.0	-89.1	85.8	-118.3	156.0	-64.7
	Conf. 5	-93.6	36.1	54.4	-88.7	77.9	140.1	-94.0	80.0	-66.9
	Conf. 6	-75.4	177.8	63.0	-86.9	-59.2	144.2	-103.3	-85.0	-68.6
	Conf. 7	81.1	-60.6	82.8	-80.8	-67.7	-177.0	-96.8	74.6	-65.4
χ	Conf. 1	-145.8	-126.9	-82.4	-137.4	177.5	-79.2	-107.0	-147.0	-173.1
	Conf. 2	-145.5	-118.3	-98.8	-175.9	163.0	-149.4	-57.8	-166.3	-165.8
	Conf. 3	-148.7	-133.8	-78.4	-168.0	167.1	-132.6	-137.5	-176.5	-168.1
	Conf. 4	-151.0	-51.6	-133.9	-179.7	157.4	-142.7	-103.3	-145.9	-169.0
	Conf. 5	-147.7	-131.2	-121.4	-176.1	159.3	-137.9	-70.3	-172.7	-168.2
	Conf. 6	-164.9	-93.5	-121.6	169.2	140.4	-148.5	-122.0	-177.2	-165.6
	Conf. 7	-152.1	-127.9	-138.3	177.5	159.2	-142.8	-73.5	-171.3	-171.6

gradually increased from 12.5 % to 100 % of the standard values[20]. Every 250 fs during the final 2 ps of MD snapshots of the molecule were saved and subsequently superimposed and averaged. The averaged structure was then belly minimized for 1000 steps.

After this refinement scheme the 37 conformers were evaluated by comparing theoretical and experimental nOe volumes (Table 1). The similarity between the different conformers was also computed (Table 2). Large conformational differences between conformers were observed. Structures which were best in agreement with the experimental nOe volumes were selected as the 'final' structures. All structures having any of the twelve R-factors (as calculated by MARDIGRAS) worse than 1.5 times the minimum value were discarded. This selection procedure yielded seven conformers which were subjected to 400 steps of minimization. In this minimization all atoms were free to move but several additional constraints were applied; 20 Watson-Crick hydrogen bond distance constraints for all seven basepairs in the stem and 127 torsional constraints (backbone and sugar) for the 14 stem residues. The force constants were rather low[21] to avoid distortions in the structures due to bad balance between the AMBER force field and the applied constraints. The seven 'final' conformers were than evaluated in terms of agreement with experimental data, in terms of energy, and in terms of similarity between each other (Tables 1 and 2). The seven final structures are displayed in Fig. 2 and in Table 3, their backbone torsions and energies are shown.

The agreement, as judged by the different R-factors, between theoretical and calculated nOe volumes for the seven final structures is reasonably good for all seven final structures although they are very dissimilar. The reason for this may be that the loop of 21mer RNA perhaps exist as a dynamic equilibrium of several different conformations, which are rapidly interconverting in the NMR time scale. It is likely that one of the conformers bind to RNA polymerase more tightly than others, followed by a rapid re-equilibration of the pouplation of the conformers. The sugars of the loop residues clearly indicate the existence of multiple conformers since their $J_{H1',H2'}$ are not consistent with either pure North or pure South conformation. The fact that there may be multiple conformers is also consistent with the great variation in conformation of the loop among the seven final structures. No single structure can fulfill the constraints which are derived from a time averaged envelope of conformers present in solution. In order to confirm the great variation in conformation of the loop in the final structures, we have undertaken the synthesis of the 21mer RNA in which the loop (*i.e.* 'NMR-window') residues are additionally ^{13}C labelled. It is likely that the quality of the structures will improve by using time averaged constraints[22-26] and possibly also by computing time average theoretical nOe volumes, which would probably better compare to the experimental volumes. Further work along this line is in progress.

REFERENCES

(1) Ramesh, V. *Nucl. Acids Res.* **1993**, *21*, 5485-5488.
(2) Földesi, A.; Nilsson, F. P. R.; Glemarec, C.; Gioeli, C.; Chattopadhyaya, J. *Tetrahedron* **1992**, *48*, 9033-9072.
(3) Földesi, A.; Nilsson, F. P. R.; Glemarec, C.; Gioeli, C.; Chattopadhyaya, J. *J. Biochem. Biophys. Meth.* **1993**, *26*, 1-26.
(4) Földesi, A.; Yamakage, S.-I.; Nilsson, F. P. R.; Maltseva, T. V.; Chattopadhyaya, J. *Nucl. Acids Res.* **1996**, *24*, 1187-1194.
(5) Glemarec, C.; Kufel, J.; Földesi, A.; Maltseva, T.; Sandström, A.; Kirsebom, L. A.; Chattopadhyaya, J. *Nucl. Acids Res.* **1996**, *24*, 2022-2035.
(6) Yamakage, S.-I.; Maltseva, T. V.; Nilsson, F. P.; Földesi, A.; Chattopadhyaya, J. *Nucl. Acids Res.* **1993**, *21*, 5005-5011.
(7) Agback, P.; Maltseva, T. V.; Yamakage, S.-I.; Nilson, F. P.; Földesi, A.; Chattopadhyaya, J. *Nucl. Acids Res.* **1994**, *22*, 1404-1412.
(8) Földesi, A.; Yamakage, S.-I.; Maltseva, T. V.; Nilsson, F. P.; Agback, P.; Chattopadhyaya, J. *Tetrahedron* **1995**, *51*, 10065-10092.
(9) Heus, H. A.; Pardi, A. *J. Am. Chem. Soc.* **1991**, *113*, 4360-4361.
(10) Brünger, A. T. *XPLOR 3.1*; Yale University: New Haven, CT, 1992.
(11) Distance constraints were divided into the following four regions: (i) 1.8-3.0 Å (strong volumes), (ii) 1.8-4.0 Å (medium), (iii) 1.8-5.0 Å (weak) and (iv) 1.8-7.0 Å (very weak volumes). The volume for the H5-H6 crosspeak of C9 at each mixing time was used as reference volume with a corrsponding distance of 2.46 Å.
(12) Additional distance constraints: 20 hydrogen bond constraints for the seven basepairs in the stem, 12 distance constraints involving the H1-H1' crosspeaks (see Fig. 1) in the stem, six planarity constraints to ensure planarity of the basepairs, and 122 dihedral constraints (α, β, γ, ε, ζ, χ and ν_0-ν_4) to keep the stem nucleotides in an A-RNA conformation.
(13) Borgias, B. A.; Thomas, P. D.; Liu, H.; Kumar, A.; Tonelli, M.; James, T. L. *MARDIGRAS 5.1*; University of California: San Fransisco, CA, 1995.
(14) Cornell, W. D.; Cieplak, P.; Bayly, C. I.; Gould, I. R.; Merz Jr., K. M.; Ferguson, D. M.; Spellmeyer, D. C.; Fox, T.; Caldwell, J. W.; Kollman, P. A. *J. Am. Chem. Soc.* **1995**, *117*, 5179-5197.
(15) Pearlman, D. A.; Case, D. A.; Caldwell, J. C.; Ross, W. S.; Cheatham III, T. E.; Ferguson, D. M.; Seibel, G. L.; Singh, U. C.; Weiner, P. K.; Kollman, P. A. *AMBER 4.1*; University of California: San Fransisco, CA, 1995.
(16) All AMBER calculations were performed in vacuo with a constant dielectric of 4.0 (ε=4.0) and infinite non-bonded cutoffs. Shake[17] was used for bonds involving hydrogen atoms and the time step was 1 fs for all MDs. The Berendsen algorithm[18] was used to control the temperature with τ=0.2 ps.
(17) Ryckaert, J. P. *Molec. Phys.* **1985**, *55*, 549-556.
(18) Berendsen, H. J. C.; Postma, J. P. M.; van Gunsteren, W. F.; DiNola, A.; Haak, J. R. *J. Chem. Phys.* **1984**, *81*, 3684-3690.
(19) Extra five distance constraints: hydrogen bonds in C7-G15 basepair and H1 of G15 to H1' of C8 and to H1' of C16[9], and 20 additional torsional constraints on backbone and sugar torsions of C7 and G15 (α, β, γ, ε, ζ, χ and ν_0-ν_4).
(20) Force constants: 40 kcal.mol^{-1}.Å^{-2} for distances derived from NOESY spectra, 80 kcal.mol^{-1}.Å^{-2} for hydrogen bond distances, 160 kcal.mol^{-1}.Å^{-2} for the two H1-H1' distances, and 400 kcal.mol^{-1}.rad^{-2} for torsions.
(21) Force constants: 5 kcal.mol^{-1}.Å^{-2} for distances derived from NOESY spectra, 10 kcal.mol^{-1}.Å^{-2} for hydrogen bond distances, 2.5 kcal.mol^{-1}.Å^{-2} for the twelve H1-H1' distances, and 50 kcal.mol^{-1}.rad^{-2} for torsions.
(22) Torda, A. E.; Scheek, R. M.; van Gunsteren, W. F. *J. Mol. Biol.* **1990**, *214*, 223-235.
(23) Pearlman, D. A.; Kollman, P. A. *J. Mol. Biol.* **1991**, *220*, 457-479.
(24) Schmitz, U.; Kumar, A.; James, T. L. *J. Am. Chem. Soc.* **1992**, *114*, 10654-10656.
(25) Schmitz, U.; Ulyanov, N. B.; Kumar, A.; James, T. L. *J. Mol. Biol.* **1993**, *234*, 373-389.
(26) González, C.; Stec, W.; Reynolds, M. A.; James, T. L. *Biochemistry* **1995**, *34*, 4969-4982.