at 120 °C for 17 h. On cooling, the adduct precipitated and was removed by filtration (22 mg, 17%, or 27% based on recovered **11**): white solid, mp > 250 °C, consisting of a 94:6 mixture of isomers (^{1}H NMR analysis); IR (CCl_4, cm^{-1}) 1710; ^{1}H NMR (300 MHz, $CDCl_3$) (major isomer) δ 7.46-7.06 (m, 10 H), 5.54 (s, 2 H), 5.50 (d, J = 6.6 Hz, 2 H), 3.26 (dt, J = 3.0, 6.6 Hz, 2 H), 3.05 (br s, 2 H), 2.99 (dd, J = 3.1, 8.0 Hz, 2 H), 2.90 (dd, J = 2.8, 8.0 Hz, 2 H), 2.78 (s, 4 H), 2.72-2.67 (m, 4 H), 2.15-2.03 (m, 4 H); ^{13}C NMR (63 MHz, $CDCl_3$, ppm) 178.2, 177.7, 150.9, 139.6, 132.0, 129.2, 128.6, 128.2, 126.5, 119.0, 44.8, 44.2, 41.4, 39.6, 36.3, 30.0, 24.4; MS m/z (M^+) calcd 606.2519, obsd 606.2514.

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Supplementary Material Available: Figure of the second molecule of **12** and crystallographic experimental procedures and tables of X-ray crystal data, bond lengths and angles, final fractional coordinates, thermal parameters, and least-squares planes for **11** and **12**, as well as final computed coordinates for **11′** and **11″** (16 pages). Ordering information is given on any current masthead page.

Thiolation of Uridine Carbon-2 Restricts the Motional Dynamics of the Transfer RNA Wobble Position Nucleoside

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Abstract: Thiolation of transfer RNA wobble position uridines produces a preferred conformation of the nucleoside in solution at ambient temperature that is of biological significance to codon recognition [Sierzputowska-Gracz, H.; Sochacka, E.; Malkiewicz, A.; Kuo, K.; Gehrke, C.; Agris, P. F. *J. Am. Chem. Soc.* **1987**, *109*, 7171-7177]. We investigated and compared, by proton nuclear magnetic resonance (NMR) spectroscopy, the thermodynamic stability of the conformations of 2-thiouridine and five biologically important 5-position derivatives and the six analogous uridines. Under physiological conditions, there were 4.8 times larger values of enthalpy and an average change of 1 kcal/mol, ΔG, for the C(2′) to C(3′) endo transitions of the 2-thiouridines, found to favor the C(3′) endo conformation, than for the respective non-thiolated uridines, found preferentially in the C(2′) endo conformation. The effect of an adjacent nucleoside on the structures and dynamics of 2-thiouridine and uridine was studied by analyzing the dinucleoside s^{2}UpU. Within the dinucleoside the individual nucleosides neither differed in structure nor dynamics from their respective mononucleosides. Therefore, the 2-position thiolation, and not the 5-position modification, produced a significantly more stable, motionally more restricted, C(3′) endo, gauche plus, anti conformer. This thermodynamically preferred structure may be best suited for anticodon base stacking and loop and stem stability. The result in tRNA is a modified-wobble selection of adenine as the only suitable third base of the codon.

Uridines (U) in the first or wobble position of transfer RNA anticodons are many times found to be naturally thiolated at carbon-2 and otherwise modified at carbon-5.[1] Thus, codon recognition by the tRNAs wobble position modified uridine may be dependent on the nucleoside's conformation as conferred by its 2- and/or 5-position modifications. We found that at ambient temperature thiolation of uridine produced a C(3′) endo, gauche plus, anti structure irrespective of the 5-position modification and postulated that this conformation is biologically significant.[2] Mutations producing a dramatic decrease in uridine thiolation and the loss of 2-thio-5-[(methylcarboxy)methyl]uridine had been found to inactivate the UGA and UAA suppressor serine tRNAs of *Schizosaccharomyces pombe* in vivo.[3,4] However, the biologically important conformation of modified uridine in tRNA may be that which is most thermodynamically stable. Therefore, we compared the enthalpy and energy requirements (ΔG) for ribose C(2′) endo to C(3′) endo transitions of 2-thiouridine (s^{2}U) and five differently modified 5-position s^{2}Us to that of the analogous U's (Figure 1). In addition, we analyzed the conformation and thermodynamic stability of s^{2}U and U in the dinucleoside s^{2}UpU (Figure 1). Here we report that the thio group imparted to the nucleoside, alone or in the dinucleoside, a thermodynamically stable C(3′) endo, gauche plus, anti conformation.

Experimental Section

Synthesis of Nucleosides and Dinucleoside. All nucleosides were chemically synthesized with the exception of uridine (U) which was purchased from Sigma Chemical (St. Louis). The chemical syntheses and purification of the modified nucleosides (compounds II-XII, Figure 1) were reported previously: II, 2-thiouridine, s^{2}U; III, 5-[(methylamino)methyl]uridine, mnm^{5}U; IV, 2-thio-5-[(methylamino)methyl]-uridine, s^{2}mnm^{5}U; V, 5-methoxyuridine, mo^{5}U; VI, 2-thio-5-methoxyuridine, s^{2}mo^{5}U; VII, uridine-5-oxyacetic acid methyl ester, mcmo^{5}U;

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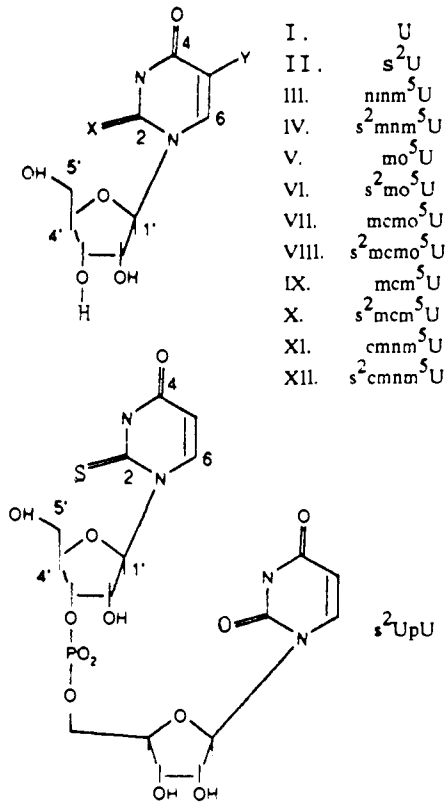

		X	Y
I.	U	O	H
II.	s^2U	S	H
III.	nmm^5U	O	CH_2NHCH_3
IV.	s^2mnm^5U	S	CH_2NHCH_3
V.	mo^5U	O	OCH_3
VI.	s^2mo^5U	S	OCH_3
VII.	$mcmo^5U$	O	OCH_2COOCH_3
VIII.	s^2mcmo^5U	S	OCH_2COOCH_3
IX.	mcm^5U	O	CH_2COOCH_3
X.	s^2mcm^5U	S	CH_2COOCH_3
XI.	$cmnm^5U$	O	CH_2NHCH_2COOH
XII.	s^2cmnm^5U	S	CH_2NHCH_2COOH

Figure 1. Molecular structures of the six uridines, their 2-thio analogues, and the dinucleoside s^2UpU. The figure shows the numbering of atoms in the pyrimidine and ribose rings. Uridine (U), 2-thiouridine (s^2U, s=S), and five 5-position derivatives of each were investigated as well as the dinucleoside s^2UpU.

VIII, 2-thiouridine-5-oxyacetic acid methyl ester, s^2mcmo^5U; IX, 5-[(methylcarboxy)methyl]uridine, mcm^5U; X, 2-thio-5-[(methylcarboxy)methyl]uridine, s^2mcm^5U; XI, 5-{[(carboxymethyl)amino]methyl}uridine, $cmnm^5U$; and XII, 2-thio-5-{[(carboxymethyl)amino]methyl}uridine, s^2cmnm^5U.[2] The dinucleoside s^2UpU was synthesized via the phosphotriester method[5] and purified by HPLC, as were the mononucleosides.[2]

NMR Spectroscopy. Solutions of the mononucleosides and the dinucleoside were prepared in phosphate buffered saline in D_2O, PBS (10 mM $MgCl_2$, 15 mM KH_2PO_4, 10 mM Na_2HPO_4, pH 7.2, 100 mM NaCl). Sample viscosity and the temperatures at which spectra were collected did not vary significantly among the 12 mononucleosides and the dinucleoside. NMR spectra were obtained with temperature regulation of ±0.5 °C and with the use of two different instruments: a Nicolet 300 MHz (^{1}H) Fourier transform spectrometer with a Nicolet 1280 computer and a GE Omega 500 MHz spectrometer. Both spectrometers were equipped with Oxford Instrument magnets and single frequency 5 mm ^{1}H and ^{13}C probes. In addition, the 500 MHz instrument was equipped with a 5-mm broadband probe here used for ^{31}P NMR.

One-dimensional proton data were typically collected with 8K data points and enough scans to obtain a good signal-to-noise ratio. One-dimensional carbon spectra were recorded at 125 MHz with a spectral width of 25 000 Hz, 8K data points, and broadband proton decoupling with WALTZ modulation applied for the duration of the experiment. Homonuclear two-dimensional experiments such as 2D phase-sensitive COSY and NOESY were collected with presaturation of the residual HDO peak and with hypercomplex phase cycling. The data were acquired with 31–128 scans, a 90° pulse of 7 μs, in 512 blocks and 1024 data points per block. The spectral window was 5000 Hz. The data were typically processed with the GE Omega software, using a phase shifted sine bell in the evolution dimension and exponential apodization in the acquisition dimension. The data were zerofilled twice to give a final data set of 1K × 1K. Total correlation spectroscopy was also used to aid in signal assignments. The HOHAHA (homonuclear Hartman–Hahn) experiment yielded all the correlations for a given spin system, enabling one to trace connections around the ribose ring, for example. Two-dimensional HOHAHA spectral data were collected with hypercomplex phase cycling, solvent presaturation, and MLEV 17 synchronous decoupler modulation. The data were acquired with a 22 μs 90° pulse on the decoupler channel, which was used for spectral excitation. The spectral window was 4866 Hz. Acquisitions numbering 32 were collected in 512 or 1024 blocks over 1024 data points. The data were processed with a phase shifted sine bell function in both dimensions and zerofilled to give a final data set of 1K × 1K. For ^{31}P decoupling of the dinucleoside the proton spectra were collected at 500 MHz with a spectral width of 5000 Hz over 8K or 16K data points using a dedicated reverse polarization transfer probe. The phosphorus frequency was determined by collecting a spectrum at 202 MHz with detection through the decoupling coil. For the ^{31}P decoupled spectrum, broadband phosphorus decoupling with WALTZ 16 modulation was applied for the duration of the experiment. Two-dimensional reverse-detected heteronuclear shift correlation (HMQC) spectra with hypercomplex phase cycling were collected using the reverse polarization transfer probe. The data were optimized for a one-bond scalar coupling constant of 160 Hz. The experiment was carried out with a proton observation pulse of 10 μs; carbon modulation is carried out with a 90° pulse of 35 μs. The spectral window in the proton dimension (f2) was 3300 Hz; in the carbon dimension (f1) 21 000 Hz. Blocks, 512, of 64 acquisitions were collected over 1024 data points. The data were processed with a phase shifted sine bell function in both dimensions and zerofilled to a 1K × 1K square matrix. Two-dimensional reverse detected multiple bond heteronuclear shift correlation (HMBC) was carried out in the magnitude calculation mode. The dedicated reverse polarization transfer probe was used. The 90° pulse on proton observation and carbon decoupling channels were 10 and 35 μs, respectively. The experiment was set for a one-bond scalar coupling interaction of 160 Hz and a multiple-bond interaction of 7 Hz. No ^{13}C decoupling was applied during acquisition. The spectral window in the proton dimension was the same as in the HMQC experiment; in the carbon dimension a larger window, 25 000 Hz, was used. The data were processed with a phase shifted sine bell function in both dimensions and zerofilled to a square matrix.

For the mononucleosides and the dinucleosides the ribose protons chemical shifts and spin coupling constants were determined within 0.4 Hz by spectral simulation, and the puckering equilibrium of the ribose ring was analyzed as previously.[2] The free energy (ΔG), enthalpy (ΔH); and entropy (ΔS) differences between the two forms were obtained from the temperature dependence of the equilibrium constants (K_{eq} = C2′-endo/C3′-endo).[6]

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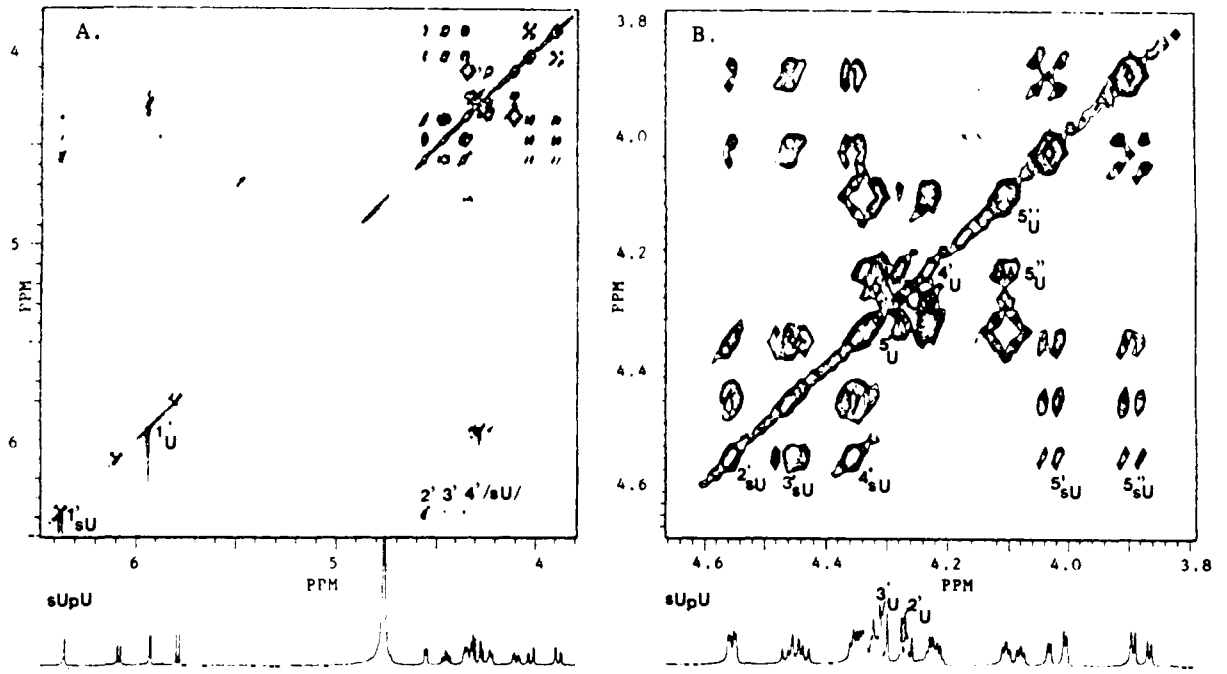

Figure 2. The 500 Hz homonuclear Hartman–Hahn (HOHAHA) spectrum of the dinucleoside s^2UpU. The region of the spectrum containing the ribose proton signals are shown. (A) Chemical shifts between 3.8 and 6.5 ppm contain all of the ribose resonances. The spin system connectivity between the ribose H1′ of s^2U and the 2′, 3′, and 4′ protons of the same residue is shown. (B) The region of the spectrum bound by 3.8–5.0 ppm is enlarged. The spin system connectivities of the s^2U ribose 2′, 3′, 4′, 5′; and 5″ protons, and that of the U ribose 4′, 5′, and 5″ protons, are indicated. The corresponding high resolution one-dimensional spectra are shown at the bottom of each figure.

Results

Temperature Dependence of the Conformation of Modified Uridines and Their 2-Thio Analogues. The thermodynamic stability of wobble position uridines in tRNA may be effected by thiolation at C(2) or the modification of C(5).[2,7] Enthalpy (ΔH) and entropy (ΔS) differences and the energy barrier, ΔG, between the C(2′) endo and C(3′) endo forms of each of six uridines and their 2-thio analogues (Figure 1, compounds I–XII) were obtained from the temperature dependence of the equilibrium constants for the ribose ring puckering.[6] The temperature dependence of the vicinal coupling constants, $J_{1'2'}$ and $J_{3'4'}$, was determined for the 12 mononucleosides by measuring the coupling constants at 22°, 37°, 57°, 67°, and 82°. The fractional populations of the ^{2}′E and ^{3}′E forms were then calculated for each temperature.[6] Enthalpy differences and ΔG of the ^{2}′E to ^{3}′E transitions for all of the 2-thiouridines were larger than that of the uridines (Table I). The ΔG for the conformational transition of each 2-thiouridine was about 1 kcal/mol larger than that of the corresponding uridine. The 5-position modification had insignificant influence on ΔG and ΔH. The relatively larger ΔG for the s^2Us is evidence that the C3′-endo conformer is significantly more stable than the C2′-endo form of the 2-thiolated nucleoside. The smaller ΔG of the sugar pucker transition for the uridines is indicative of their preference for the C2′-endo form.

Conformation and Dynamics of s^2UpU. The conformation and dynamics of the constituent nucleosides of the dinucleoside s^2UpU were determined to ascertain if the immediate environment may have an effect on structure. Signal assignments were accomplished with the aid of HOHAHA, COSY, and NOESY experiments and with two-dimensional reverse-detected heteronuclear shift correlation spectra with hypercomplex phase cycling (HMQC). Figure 2 demonstrates the application of the HOHAHA experiment in differentiating and assigning the 1′, 2′, 3′, 4′, 5′, and 5″ proton resonances of the two ribose residues (sU and U) of the dinucleoside. The crowded 3.8–4.6-ppm region is shown in detail to demonstrate the separation of spin systems. Proton and carbon signal assignments and spin coupling constants (J_{HH}) are shown in Table II. The proton chemical shifts associated with the ribose 3′ and 5′ carbons in the phosphodiester bond have significant downfield shifts compared with the analogous protons from the mononucleosides (Table II, section A). This result, plus the comparison of ^{31}P coupled and decoupled ^{1}H spectra aided greatly in making unambiguous assignments.

Spin coupling constants for the dinucleoside (Table II, section C) were determined with the aid of spectral simulation, as were those of the mononucleosides. Differences in $J_{(HH)}$ couplings between the dinucleoside and the mononucleosides occurred for both s^2U and U but were more pronounced for U. Coupling

Table I. Differences in Uridine Free Energy (ΔG), Enthalpy (ΔH), and Entropy (ΔS) for the Ribose C3′ to C2′ Transitions[a]

nucleoside	ΔG^b	ΔH^c	ΔS^d
U[d]	0.1	0.8	274
s^2U	1.0	4.7	214
mnm^{5}U	0.1	0.6	140
s^2mnm^{5}U	1.1	5.0	238
mo^{5}U	0.2	1.0	71
s^2mo^{5}U	0.9	4.0	131
mcmo^{5}U	0.3	1.4	235
s^2mcmo^{5}U	0.9	3.6	86
mcm^{5}U	0.2	0.9	65
s^2mcm^{5}U	0.9	4.1	292
cmnm^{5}U	0.2	1.2	452
s^2cmnmp^{5}U	0.9	3.9	3

[a] Solutions of mononucleosides were prepared as previously described.[2] NMR spectra were obtained with a Nicolet 300 MHz (^{1}H) Fourier transform spectrometer with an Oxford Instruments wide bore magnet. Proton NMR spectra of all of the nucleosides were recorded at the frequency of 300 MHz with a 5-mm probe and with variable temperature. The temperature was controlled within 1 deg. For the ribose protons, chemical shifts and spin coupling constants were determined within 0.3 Hz by spectral simulation. The puckering equilibrium of the ribose ring was analyzed as previously.[2] The free energy, enthalpy, and entropy differences between the two forms were obtained from the temperature dependence of the equilibrium constants (C2′-endo/C3′-endo) with an accuracy corresponding to that of the coupling constants, 0.4 Hz. [b] ΔG = kcal/mol. [c] ΔH = kcal/mol. [d] ΔS = j/deg·mol.

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Table II. Proton and Carbon Chemical Shifts, Spin Coupling Constants, and Ribose Conformation for s^2UpU, s^2U, and U

	S^2U–p–U		s^2U	U
	A. Proton Chemical Shifts (ppm)			
H5	6.10	5.79	6.01	5.90
H6	8.21	7.94	7.98	7.90
H1′	6.31	5.93	6.47	5.85
H2′	4.56	4.27	4.25	4.32
H3′	4.45	4.32	4.04	4.21
H4′	4.36	4.23	4.04	4.11
H5′	3.89	4.10	3.72	3.76
H5″	4.03	4.34	3.87	3.90
	B. Carbon Chemical Shifts (ppm)			
C2	175.9	151.5	176.8	152.3
C4	162.3	165.5	163.9	167.3
C5	106.1	101.8	107.3	103.4
C6	141.4	141.0	142.5	143.0
C1′	93.9	89.0	94.2	90.6
C2′	73.2	74.0	75.4	74.8
C3′	71.1	68.5	69.3	70.6
C4′	82.8	82.0	84.6	85.5
C5′	50.9	63.6	60.7	62.0
	C. Coupling Constants (H_z), Glycosidic Bond Conformation, and Sugar Pucker			
J1′2′	1.6	3.5	2.5	4.6
J2′3′	4.9	2.4	4.0	5.2
J3′4′	6.6	3.8	6.0	5.2
J3′P	6.2			
J4′5′	2.3	2.4	1.6	2.8
J4′5″	3.2	2.7	3.0	4.3
J5′5″	13.4	11.9	13.5	12.5
J5′P		2.4		
J5″P		2.4		
χ[a]	anti	anti	anti	anti
%S/%N[b]	0.24	0.92	0.41	0.88

[a] χ = conformation around the glycosidic bond given by the dihedral angle χ = C(6)–N(1)–C(1′)–O(1′). [b] %S/%N is derived from the K_{eq} of the fractional populations ratio C(2′) endo/C(3′) endo obtained from $J_{1'2'}/J_{3'4'}$.[2,8]

constants for uridine were lower in the dinucleoside in comparison to the corresponding coupling constants of the mononucleoside, whereas the opposite was true for s^2U. The sugar puckers and glycosidic bond angles of s^2U and U within the dinucleoside were calculated to be similar to that of the mononucleosides. However, s^2U in the dinucleoside preferred the C(3′) endo conformation (%S/%N = 0.24) to a greater degree than the mononucleoside (%S/%N = 0.41). Uridine in the dinucleoside preferred the C(2′) endo conformation (%S/%N = 0.97) to a greater degree than the mononucleoside (%S/%N = 0.88). Glycosidic bond angles for the dinucleoside and the corresponding mononucleosides were all anti. The temperature dependence of the dinucleoside ribose conformations was determined, as was that of the mononucleosides, from the temperature dependence of the K_{eq} of C(2′) endo/C(3′) endo. K_{eq} values at five temperatures for each of the ribose residues are shown in Table III. The ΔG, ΔH, and ΔS for the C(2′) to C(3′) endo transition were then calculated for each ribose and are shown in Table III. There was 1 kcal/mol ΔG difference between the s^2U and U transitions in the dinucleoside as was found for the mononucleosides (Table I).

Discussion and Conclusion

To understand the influence of thiolation on the conformational dynamics of tRNA wobble position uridines and their codon recognition, we investigated the motional dynamics of 2-thiouridine and five biologically important derivatives and the six analogous uridines. Coupling constants ($J_{1'2'}/J_{3'4'}$) were used to arrive at the K_{eq} for the C(2′) to C(3′) endo transition at five different temperatures from which ΔG, ΔH, and ΔS were determined for each of the 12 nucleosides. Conformational analysis of the sugar ring in nucleosides using a generalized Karplus equation allows for an accurate description of sugar pucker from coupling constants

Table III. Differences in Uridine Free Energy (ΔG), Enthalpy (ΔH), and Entropy ($T\Delta S$) for the Ribose C2′ to C3′ Transitions of S^2UpU[a]

A. Temperature Dependence of K_{eq}

	temperature (°C)				
	22	28	37	52	67
S^2U	0.24	0.27	0.36	0.36	0.37
U	0.92	0.91	0.82	0.83	0.83

B. Thermodynamic Parameters

	ΔG[b]	ΔH[c]	$T\Delta S$[d]
S^2U	1.1	4.9	357
U	0.1	0.2	86

[a] NMR spectra of a solution of the dinucleoside were taken at five different temperatures. The puckering equilibrium (K_{eq} = C(2′) endo/C(3′) endo) of the ribose ring was analyzed at these five temperatures. [b] Free energy (ΔG) in kcal/mol. [c] Enthalpy (ΔH) in kcal/mol. [d] Entropy ($T\Delta S$) in j/deg·mol were obtained from the temperature dependence of the K_{eq}.

even when substituents of varying electronegativity are being compared.[12] Under physiological conditions, there were 1 kcal/mol larger ΔG values and 4.8 times larger values of ΔH for the ^{2}′E to ^{3}′E transitions of the 2-thiouridines, found to take the C(3′) endo conformation, than for the respective non-thiolated uridines, found preferentially in the C(2′) endo conformation. The 5-position substituents may be important to restricting the conformations of thiolated and non-thiolated uridines.[7] Therefore, we investigated the motional dynamics of the nucleosides through a study of the 5′-position substituents. Internal motions of the C–H internuclear vectors of the 5-position modifications were characterized by order parameters (S^2) and effective correlation times (τ_e) in the range of that of small molecules, and thus indicate little or no involvement in restricting conformation of the nucleosides. An investigation of the dinucleosides s^2UpU showed that an adjacent nucleoside and the adjoining phosphodiester bond had little effect on the conformations and dynamics of the ribose moieties which were found to be similar to that of the corresponding individual nucleosides s^2U and U. Therefore, the 2-position thiolation of the wobbles base of tRNA, not the 5-position modification or the 5′-phosphate of the nucleotide,[7] produced a significantly more stable, motionally more restricted, C(3′) endo, gauche plus, anti conformer.

Chemical modification of 2-thiouridine in tRNA with cyanogen bromide and other reagents reduces the ability of the tRNA to be aminoacylated.[13,14] The presence of 2-thiouridine in the wobble position of bacterial, yeast, and animal tRNAs has been shown, in vitro and in vivo, to influence codon recognition in favor of codons ending in adenosine.[3,4,12,15–20] Significant differences are

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found in codon recognition patterns for the two types of wobble position uridines, U vs s^2U. For instance, in triplet-dependent binding to the ribosome, $tRNA^{Glu}$ with wobble position s^2mnm^5U recognized predominantly the codon GAA, whereas thio deficient $tRNA^{Glu}$ recognized GAG and GAU as well as GAA.[15] Those tRNA anticodons with non-thiolated (but 5-position modified) uridines at the wobble position exhibit recognition of codons ending in G as well as A (Yokoyama, S., personal communication). Thus, we conclude that for those tRNAs containing wobble position s^2U (vs U or 5-position modified U), the relatively more restricted dynamics and conformation of s^2U are responsible for the tRNAs preferential recognition of codons ending in adenosine.

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A Solid-Support Methodology for the Construction of Geometrical Objects from DNA

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Abstract: A solid-support procedure has been developed for the synthesis of geometrical stick figures from branched DNA. The method permits control over the synthesis of individual edges of an object. Control derives from the restriction endonuclease digestion of hairpin loops forming each side of the new edge. Restriction sites are destroyed when the edge forms. Each cycle of the procedure creates an object that is covalently closed and topologically bonded to itself. This features permits destruction of incompletely ligated edges by exonuclease digestion, thereby purifying the growing object while it is still on the support. The use of the solid support permits convenient removal of reagents and catalysts from the growing product. The solid support also isolates individual objects from each other, thereby eliminating a class of potential side products. The strategy permits the separate execution of steps involving additions and cyclizations, which are optimized under different conditions. A technique is presented to rescue those hairpin loops that fail to be digested by restriction endonucleases. The synthesis of a quadrilateral from three-arm junctions utilizing this protocol is reported. In principle, the methodology can be used to combine more complex components, in order to fabricate polyhedra and two-dimensional or three-dimensional arrays.

Introduction

There is considerable interest in the development of macromolecular chemical systems with well-defined structural properties for use as molecular scaffolding that orients and juxtaposes other molecules. The motivations for pursuing these constructions include the formation of "macromolecular zeolite" lattices to enable diffraction analysis of complex guest molecules that do not readily crystallize,[1-3] the caging of active biological macromolecules to form new multifunctional enzymes,[4] the development of drug delivery systems for therapeutic macromolecules,[5] the achievement of mechanical control on the nanometer scale,[6] and the assembly of molecular electronic components.[7,8] Branched nucleic acid molecules offer one of the most direct routes to these ends. DNA is particularly well-suited for use as a scaffolding medium, since it is a thick (2-nm diameter), stiff[9] molecule over the range of a few nanometers, a molecule whose structure is unlikely to be perturbed markedly by tethering to it smaller noninteractive molecules.

In living cells, DNA is found almost exclusively in the form of a linear duplex molecule; the molecule may be supercoiled,[10] stressed,[11] or even knotted,[12] but its helical axis is unbranched. Nevertheless, DNA branched junction structures may be observed transiently as intermediates in the process of recombination.[13] Naturally-occurring branched structures are inherently unstable because of their homologous (2-fold) sequence symmetry.[14,15] Since 1983, it has been possible to model these molecules in stable oligonucleotide systems[16] containing minimal sequence symmetry.[1,2,17] In principle, the attachment of a series of cohesive ("sticky") ends to a DNA branched junction converts it to a valence cluster whose ends are specifically available for binding only complementary cohesive ends.[1-3]

For several years, we have conducted experiments involving the ligation of branched DNA molecules to form larger structures. Stick figures are produced in which the vertices are the branch points of junctions and the edge are double helical DNA. We have oligomerized three-arm[18] and four-arm[19] branched junctions into macrocycles, and we have synthesized a specific quadrilateral from four separate junctions.[4] Recently, we have constructed a

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