Solution structure of nucleic acid photoadduct, deoxy-m⁵HO⁶-uridylyl(5-5)(3'-5')deoxyuridine by NMR and restrained molecular dynamics

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ABSTRACT

Sensitized UV-B irradiation (sunlamps) of the dinucleoside monophosphate, d-TpF (F = fluorouracil), produces the usual cyclobutane-type photodimer and an additional defluorinated 5-5 photoadduct, d-T5p5U. In d-T5p5U, the original C5=C6 structure is modified such that the C5 (d-T5p-) is covalently bonded with the C5 (-p5U) (where the fluorine had been) and the C6 (d-T5p-) acquires an OH group. 2D NOE data and the results of J-coupling analysis are used as constraints to refine structures of d-T5p5U in restrained molecular dynamics calculations. The structures obtained show the most probable chiralities of the C5 and C6 atoms of the Thy-portion to be 5R and 6R, respectively. The orientation of the CH₃- and uracil-groups are pseudo-axial and pseudo-equatorial, respectively, with respect to the C5 atom. Glycosidic angles are *high-anti* and *anti* for the d-T5p- and the -p5U residue, respectively. C3'-endo like sugar pucker is predominant in the d-T5p- residue while C2'-endo like pucker is predominant at the -p5U residue.

INTRODUCTION

The effects of ultraviolet light on nucleic acid pyrimidine bases produces a variety of photoproducts (e.g. cyclobutane-type dimers, adducts, and hydrates) depending on experimental conditions (1). If the thymine is replaced with a halogenated-uracil (chloro-, bromo-, or iodo-), other photochemical reactions can occur which may produce additional photoproducts (2, 3). Our previous work (4) has shown that a fluorinated model nucleic acid exhibits enhanced sensitivity to UV-B sensitized irradiation compared to the normal analog. Irradiation using UV-B sunlamps of the dinucleoside monophosphate, d-TpF (F = fluorouracil), in the presence of sensitizer produced the cis-syn cyclobutane-type photodimer and a defluorinated photoadduct [d-T5p5U]. The [photodimer]:[photoadduct] distribution was shown to be pH dependent. The structural characterization of this photoadduct was reported previously (4). In this study the proton coupling constants are reported and interpreted for conformational analysis. In the present study, NOE data, molecular modeling, molecular

mechanics and dynamics studies are collectively used to generate a three-dimensional solution structure of d-T5p5U. These studies will aid in elucidating photochemical mechanism of formation of this unusual photoadduct.

MATERIALS AND METHODS

Materials

d-TpF was chemically synthesized using the solution phosphotriester method described previously (4). A solution containing 10–20% aqueous acetone, 0.1 M Na phosphate (pH 9–10) and 1 mg/ml d-TpF, irradiated with UV-B sunlamps (Westinghouse FS20T12) produced a defluorinated photoadduct, d-T5p5U, as the major photoproduct (4). It was isolated and purified as previously described.

NMR sample preparation

Purified d-T5p5U was repeatedly lyophilized from D₂O, and finally dissolved in 400 μl D₂O(99.9%) containing 0.05 M Na phosphate buffer(pH 7.0).

NMR experiments

A two dimensional NOE spectrum (mixing time of 0.7 sec) was acquired using the TPPI (5) phase sensitive mode on a Bruker AM-400 spectrometer at 298K. Residual water was presaturated only during the relaxation delay with low decoupling power. The carrier frequency was placed at the low-field end of ¹H spectrum to avoid anti-diagonal artifacts (6). The spectral width was 7000 Hz. The t₂ domain contained 2048 data points (32 transients/fid); 512 t₁ values were taken. The data was processed on a Sun-SPARC 4/370 with NMR2 software from New Methods Research, Inc. The data matrix was zero-filled to 2K×2K. Shifted sine bell functions of 90 and 45° were applied to data points along t₂ and t₁ dimension, respectively.

Computational methods

Starting geometries for restrained molecular dynamics were obtained by energy minimization of the initial structure, generated from the CHARMM (7) topology file, by constraining the internal

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coordinates which contain all chemical connectivities and appropriate configurations of model structures.

Model structures were further refined by restrained molecular dynamics calculations using XPLOR (8) with the constraints of NOE distance and NMR-determined torsion angles. Most probable structure was chosen by assessing the refined model structures on the basis of total energy, RMS values of internal coordinates and number of NOE violations.

RESULTS AND DISCUSSION

Assignments of chemical shifts and coupling constants

The ^1H chemical shifts of d-T5p5U were reported previously (3, 9), and the coupling constants are compiled in Table I. The unequivocal assignments of the chemical shifts were accomplished by either 1-D spin-decoupling or 2-D COSY experiments. The accurate determination of the coupling constants (J 's) was performed by using estimated values extracted from resonance multiplicities of expanded spectral plots. These estimated J 's were then highly refined using the iterative spin-simulation program,

PANIC (10). Estimated errors of parameters are included in Table I.

Analysis of the coupling constants

The coupling constants of the sugar protons are interpreted in terms of pseudorotational parameters (Φ, P) and population of N- and S-type conformers. In the thymidine-type portion, C3'-endo like puckering is predominant, while the uridine-type portion shows a preference for a C2'-endo type puckering (Table II). The results of rotamer distribution analysis are summarized in Table III. The exocyclic torsion angle γ ($\text{O5}'\text{-C5}'\text{-C4}'\text{-C3}'$) of the Thy-portion is predominantly g^+ , while a large amount of rotational freedom (between *trans* and *gauche*) of angle γ in the Ura-portion is observed. For the two internucleotide torsion angles β ($\text{P-O5}'\text{-C5}'\text{-C4}'$) and ϵ ($\text{C4}'\text{-C3}'\text{-O3}'\text{-P}$), a predominance of the *trans* conformation is observed.

Analysis of NOE data

Characterization of d-T5p5U and resonance assignment of the ^1H spectrum was done previously in our laboratory (4). The observed NOE's are summarized in Table IV. One of major structural aspects of this system, which is readily deduced from the chemical shift and coupling constant data, is the hybridization of the C5 and C6 atom of the Thy-portion. The original double

Figure 1. Major photoproducts of UV-B sensitized irradiation of d-TpF: (A) cyclobutane type photodimer, d-T[p]F; (B) defluorinated photoadduct, d-T5p5U.

Figure 2. Phase sensitive NOESY spectrum of d-T5p5U at 298 K (mixing time of 0.7 s).

Figure 3. Superposition of STR5 (solid) and STR6 (stippled) of d-T5p5U (RMS fitted with respect to base atoms).

Figure 4. Stereo view of d-T5p5U, STR5 (5R, 6R, g^+).

bond (C5=C6) was modified to a single bond with concomitant bonding of the C5 (d-T5p-) and the C5 (-p5U), and hydroxylation of the C6 (d-T5p-) (4). The NOE cross-peak pattern and intensity distribution can be used to elucidate the chirality at the C5 and C6 atoms. A strong NOE between the two H6 protons (Thy-portion and Ura-portion) and two medium-sized NOE's between the methyl group and each of the two H6 protons are observed. Another important structural aspect of this system is the conformation of the glycosidic bond. The characterization of the sugar-base glycosidic conformational class is straightforward. In the *anti* conformation, a base (H6 or H8) and furanose proton (H2' or H3') are proximal, while the H1' proton is distal. On

the other hand, if the glycosidic angle is *syn*, a base (H6 or H8) and H1' proton are proximal, while the H2' and H3' protons are distal (11). In the Thy-portion, two strong NOEs of H6 with H3' and H2' are observed. In the Ura-portion, only a strong NOE between H6 and H2' is observed. Therefore, for the 5'-side these observations indicate a *high-anti* conformation of glycosidic angle with a large portion of C3'-endo like sugar puckering, and for the 3'-side an *anti* conformation of glycosidic angle with C2'-endo like sugar puckering. It is more difficult to assign a specific set of chiralities for the C5 and C6 atoms among four possible pairs (5R-6R, 5R-6S, 5S-6R, 5S-6S) by inspection, using only molecular modeling and the observed NOE's. Since this dinucleoside monophosphate photoadduct exhibited a correlation time in the fast molecular tumbling limit, only a few cross-peaks could be observed between proton pairs at a distance up to 3.5 Å (12). Hence the number of NOE cross-peaks between proton pairs important in determining the three dimensional structure of this small molecule is limited.

Molecular modelling and structure refinement

To more accurately deduce the structures, eight model structures were generated using CHARMM by considering all chiralities (R-R, R-S, S-R, S-S) of the C5 and C6 atoms of the Thy-portion, and each containing two different torsional forms of γ_2 (*t* and *g*⁺). Pseudorotational analysis showed a predominant C3'-endo like and C2'-endo like puckering of the thymidine-type and uridine-type portion, respectively, so the sugar conformations were fixed. Volume integrated NOE cross peaks, grouped as strong (*d* < 2.5 Å), medium (*d* < 3.0 Å) and weak (*d* < 3.5 Å), were incorporated into the structure calculation as distance constraints. In addition, NMR-determined torsion angles (β_2 , ϵ_1 , γ_1 , τ_0 , τ_2 , γ_2) were also constrained. For NOEs involving the methyl group, an additional 0.5 Å was added to the upper distance limit to account the higher intensity of this resonance (13, 14). The presence of bad contacts in the starting structures were removed by energy minimization with 30-step Powell algorithm before doing restrained molecular dynamics. Restrained molecular dynamics were applied to the eight model structures for 5 ps at 300K using XPLOR. Then an additional restrained energy minimization using the Powell algorithm was done until the energy gradient reached zero.

Table I. ¹H NMR parameters^a of d-T5p5U.

	d-T5p-	-p5U
¹ H chemical shifts (ppm; relative to TSP)		
H1'	6.185	6.424
H2'	2.305	2.490
H2''	2.527	2.236
H3'	4.866	4.678
H4'	3.881	4.059
H5'	3.844	4.105
H5''	3.814	4.030
CH ₃	1.532	—
H6	5.290	7.902
¹ H- ¹ H coupling constants (Hz)		
J _{1'2'}	1.79	10.06
J _{1'2''}	9.50	5.33
J _{2'2''}	-14.44	-13.87
J _{2'3'}	7.54	7.26
J _{2''3'}	10.33	1.87
J _{3'4'}	8.12	3.48
J _{4'5'}	2.50	2.91
J _{4'5''}	2.79	1.73
J _{5'5''}	-12.26	-11.59
¹ H- ³¹ P coupling constants (Hz)		
J _{3',P}	7.93	—
J _{4',P}	—	2.68
J _{5',P}	—	2.66
J _{5'',P}	—	2.07

^aEstimated error, ±0.001 ppm; ±0.02 Hz.

Table II. Furanose distribution of d-T5p5U.

	%C2'-endo ^a	%C3'-endo ^b	Equilibrium ^c <i>f_I</i>	<i>P_I</i>	<i>f_{II}</i>	<i>P_{II}</i>	Φ ^d
T5p-	18	82	0.06	294.4	0.94	24.9	40
-p5U	74	26	0.05	-10.7	0.95	130.5	37

^aEquation used: $f_{C2'-endo} = J_{1'2'}/(J_{1'2'} + J_{3'4'})$ (17); ^bEquation used: $f_{C3'-endo} = J_{3'4'}/(J_{1'2'} + J_{3'4'})$ (17); ^cCalculation are performed by using the program PSEUROT (18). *f_I*, *f_{II}* are mole fraction and *P_I* and *P_{II}* are phase; ^dpuckering amplitude.

Table III. Rotamer distribution of d-T5p5U

	γ(C5'-C4') ^a		β(O5'-C5') ^b				ε(C3'-O3') ^c	
	<i>g</i> ⁺	<i>t</i>	<i>g</i> ⁻	<i>t</i>	<i>g</i> ⁺	<i>g</i> ⁻	<i>g</i>	<i>t</i>
T5p-	1.00	0.00	0.00	—	—	—	0.03	0.84
-p5U	0.53	0.47	0.00	1.00	0.00	0.00	—	—

^aKarplus-type equation used: $J_{HH} = 10.5 \cos^2 \phi - 1.2 \cos \phi$ (19) and rotamer equations from reference 20.

^bEquation used: $J_{HH} = 15.3 \cos^2 \phi - 6.1 \cos \phi + 1.6$ (21) and rotamer equations from reference 20.

^cEquation used: $f_{g-} = (J_{pC2'} - 1.0)/9.2$; $f_t = J_{pC4'}/9.2$ (17).

Table IV. a) The observed NOE and distance constraint as used in energy minimization. b) calculated distances in the refined structures.

				NOE type ^a	Distance	STR5 ^b (5R, 6R, g ⁺)	STR6 ^b (5R, 6R, t)	Ave. ^c
Thy	H6	Thy	CH ₃	m	<3.5 ^d %	3.060	3.047	3.054
Thy	H6	Thy	H1''	a	>3.5 ^e #	3.600	3.541	3.575
Thy	H6	Thy	H2'	s	<2.5	2.050	2.030	2.041
Thy	H6	Thy	H3'	s	<2.5	2.200	2.172	2.188
Thy	H1'	Thy	H2''	m	<3.0	2.730	2.344	2.564
Thy	H2'	Thy	H3'	w	<3.5	2.490	2.453	2.474
Ura	H6	Thy	H6	s	<2.5	1.990	2.021	2.003
Ura	H6	Thy	H2'	w	<3.5	3.530	3.559	3.542
Ura	H6	Thy	CH ₃	m	<3.5 ^d %	3.060	2.614	2.868
Ura	H6	Ura	H1'	a	>3.5 ^e #	3.740	3.711	3.728
Ura	H6	Ura	H2'	s	<2.5	2.110	2.172	2.137
Ura	H1'	Ura	H2''	m	<3.0	2.370	2.380	2.374
Ura	H1'	Ura	H2'	m	<3.0	3.020	3.027	3.023
Ura	H1'	Ura	H4'	w	<3.5	2.990	3.257	3.105
Ura	H2'	Ura	H3'	w	<3.5	2.430	2.446	2.437
Ura	H6	Ura	H5''	a	—	5.246	2.628	4.120
Ura	H5''	Ura	H2'	a	—	3.863	2.371	3.222

^as: strong, m: medium, w: weak.; b) see Table V for calculated structures;^cweighted average of two structures from: d_i(STR 5)×0.57 + d_i(STR 6)×0.43; d) additional 0.5 Å added to the upper distance limit per methyl group (13, 14).**Table V.** Structural assessment based on total energy and RMSD of NOE, bond and angle after restrained molecular dynamics.

Structure	Chirality		γ ₂	RMSD			Total Energy ^c	NOE Violations ^d
	C5	C6		noe ^a	angle ^b	bond ^a		
STR 1	S	R	g ⁺	0.031	3.862	0.011	21.97	3
STR 2	S	R	t	0.039	3.958	0.011	24.55	3
STR 3	S	S	g ⁺	0.039	3.797	0.012	25.47	4
STR 4	S	S	t	0.056	3.988	0.013	31.68	4
STR 5	R	R	g ⁺	0.013	3.859	0.011	17.29	0
STR 6	R	R	t	0.014	3.743	0.011	16.81	2
STR 7	R	S	g ⁺	0.010	3.931	0.011	23.84	2
STR 8	R	S	t	0.013	3.840	0.011	24.04	2

^aÅ; ^bdegree; ^cKcal/mole; ^dnumber of occurrences.

The most probable structures were estimated on the basis of the number of NOE violations, total energy, and the root mean square deviation (RMSD) of NOE constraints, bond angles and bond lengths. The results of restrained molecular dynamics calculation are summarized in Table V. In general, the *trans* conformation of the torsion angle, γ₂, produced common NOE violations among H5'', H6 and H2' of the Ura-portion, irrespective of the configuration on the C5 and C6 atom of the Thy-portion. Except for the structures with 5R-6R chiralities, all other structures showed severe NOE violations (i.e. NOE's inconsistent with the observed NOESY spectrum). In addition, the total calculated energy of the two structures with 5R-6R was lowest (Table V). These results show 5R-6R as the most probable chiralities for the Thy-portion of d-T5p5U.

Analysis of conformation

The results in Table V indicate the structural features of STR 5 are most consistent with the NOE observations and combined with the calculated total energy, represents a refined solution structure. The coordinates of this structure are used to generate a 3-D solution model (Figure 4) and to obtain other structural details. The saturation of the thymine C5-C6 double bond leads to sp³-type hybridization of the corresponding carbons, which

results in a non-planar thymine ring. The configurations at the two thymine carbon (C5, C6) and the NOE obtained distances of the substituents at these sites are major factors determining the pyrimidine-ring conformation. A torsion angle (N1-C6-C5-C4) in the saturated thymine of 21° indicates twisting, while the uracil is effectively planar. Similar twisting of six-membered ring containing sp³ carbon at the C5 and C6 was observed in the refined structure of the 6-4 photoadduct of d-TpT (15). With regard to C5 substituents, the orientation (deduced from Figure 4) of the CH₃-group and the uracil group are pseudo-equatorial and pseudo-axial, respectively. The glycosidic angle of the Thy-portion is in the *high-anti* region where the strong NOE's of H6 with both H2' and H3' are often observed when the sugar has predominant C3'-endo like puckering. In the Ura-portion, the corresponding χ angle has a *anti* conformation and sugar puckering is C2'-endo like. As seen in Table IV, the *trans* conformation of γ₂ is incompatible with the observed NOE pattern, producing two invisible NOE violations, even though rotamer distribution analysis show an equilibrium between *trans* (43%) and *gauche* (g⁺, 57%). However population distributed average interproton distances are quite compatible with observed NOEs, with the exception of the predicted H5''(-p5U)-H2' (-p5U) interaction (Table IV). This result may indicate an

Table VI. Backbone torsion angles of d-T5p5U and other nucleic acids.

	STR 5	STR 6	d-T6p4T ^a	d-T[p]T ^b	B-DNA ^c
γ_1	55	61	69	61	
δ_1	74	83	81	159	
ϵ_1	-132	-138	-125	-153	-169
ζ_2	-70	-72	-70	-138	-108
α_2	-77	146	-76	59	-63
β_2	-179	174	-156	-124	171
γ_2	57	181	25	-171	54
δ_2	142	156	115	81	123
χ_1	-91	-87	-129	61	-117
χ_2	-99	-111	-69	-104	

^aStructure of d-T6p4T from reference 15.^bCrystal structure of cis-syn d-T[p]T from reference 16.^cAveraged values from Dickerson dodecamer (22).

incorrect structure of the *trans* conformer or a lower population of the *trans* conformer (<43%) than calculated from the coupling constants. The refined structures (STR5 and STR6) are compared in Figure 3, and the backbone and glycosidic bond angles are listed in Table VI. These data illustrate the conformational similarity of the two structures. The classical backbone torsion angles of these structures are compared with other photoproducts and normal nucleic acids (Table VI). Comparison of STR5 with the backbone structure of the 6-4 photoadduct, indicates some generally similar conformational features. In both photoadducts, d-T5p5U and d-T6p4T, C3'-endo type and C2'-endo type sugar conformations are observed for 5'-side residue and 3'-side residue, respectively, while in the crystal structure of cis-syn d-T[p]T C2'-endo type and C3'-endo type were observed at 5'-side and 3'-side residue, respectively (16). The two glycosidic angles (5'- and 3'-side) of three photoproducts (d-T5p5U, d-T6p4T, c-s d-T[p]T) show a remarkable difference arising from the different chemical bondings between two bases.

CONCLUSION

The structures of a defluorinated photoadduct of d-TpF, deoxy-m⁵HO⁶-uridylyl(5-5')(5'-3')deoxyuridine (d-T5p5U), were obtained from NMR data and restrained molecular dynamics calculations. In the refined structure, the most probable chirality of the C5 and C6 atoms of the d-T5p-portion is 5R and 6R. The presence of sp³ carbons in the six-membered ring of the d-T5p-portion yields a twisted base. Torsion angle (N1-C6-C5-C4) is 21°. With respect to the sp³ carbon, C5 (d-T5p-), the CH₃-group is pseudo-equatorial, while the uracil-group is pseudo-axial. The *high-anti* conformation of the glycosidic angle and C3'-endo like sugar puckering are observed for the d-T5p-residue, while an *anti* glycosidic angle and C2'-endo like sugar puckering is observed at the -p5U residue.

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REFERENCES

1. Wang, Shih Yi (1976) in *Photochemistry and Photobiology of Nucleic Acids*, Volume I (S.Y. Wang, Ed.), Academic Press, New York, pp. 295-356.
2. Ishihara, H. and Wang, S.Y. (1966) *Biochemistry*, **5**, 2307-2313.
3. Dellweg, H. and Wacker, A. (1964) *Z. Naturforsch.*, **19b**, 305-311.
4. Alderfer, J.L., Soni, S.-D., Arakali, A.V. and Wallace, J.C. (1993) *Photochem. Photobiol.*, in press.
5. Marion, D. and Wüthrich, K. (1983) *Biochem. Biophys. Res. Comm.*, **113**, 967-974.
6. Mehlkopf, A.F., Korbee, D., Tiggelman, T.A. and Freeman, R. (1984) *J. Mag. Resn.*, **58**, 315-328.
7. Brooks, B.R., Bruccoleri, R.E., Olafson, B.D., States, D.J., Swaminathan, S. and Karplus, M. (1983) *J. Comp. Chemistry*, **4**, 187-217.
8. Brünger, A.T. (1988) X-PLOR, Yale University.
9. Soni, S.-D. (1989) *Ph.D. Thesis*, SUNY/Buffalo.
10. PANIC (Parameter Adjustment in NMR by Iterative Caculation) is a minicomputer version of the LAOCOON-type programs, provided in the software packages of NMR spectrometers by Bruker Instruments, Inc.
11. Saenger, W. (1984) *Principle of Nucleic Acid Structure*, pp. 9-28, Springer-Verlag.
12. Koning, T.M.G., Davies, R.J.H. and Kaptein, R. (1990) *Nucleic Acids Res.*, **18**, 277-284.
13. Clore, G.M., Gronenborn, A.M., Nilges, M., Ryan, C.A. (1987) *Biochemistry*, **26**, 8012-8023.
14. Wagner, G., Braun, W., Havel, T.F., Schaumann, T., Go, N., Wüthrich, K. (1987) *J. Mol. Biol.*, **196**, 611-639.
15. Taylor, J.-S., Garrett, D.S. and Wang, M.J. (1988) *Biopolymer*, **27**, 1571-1593.
16. Hruska, F.E., Voituriez, L., Grand, A. and Cadet, J. (1986) *Biopolymer*, **25**, 1399-1417.
17. Alderfer, J.L. and Ts'o, P.O.P. (1977) *Biochemistry*, **16**, 2410-2416.
18. de Leeuw, F.A.A.M. & Altona, C. (1983) *Quant. Chem Prog. Exch. No. 463*, University of Indiana at Bloomington.
19. Altona, C. and Sundaralingam, M. (1973) *J. Am. Chem. Soc.*, **95**, 2333-2344.
20. Davies, D.B. and Danyluk, S.S. (1974) *Biochemistry*, **13**, 4417-4434.
21. Lankhorst, P.P., Haasnoot, C.A.G., Erklens, C. and Altona, C. (1984) *J. Biomol. Struct. Dyn.*, **1**, 1387-1405.
22. Dickerson, R.E. and Drew, H.R. (1981) *J. Mol. Biol.*, **149**, 761-786.