A Comparison of DNA Oligomer Duplexes Containing Formacetal and Phosphodiester Linkers Using Molecular Dynamics and Quantum Mechanics

James M. Veal, Xaolian Gao, and Frank K. Brown*

Contribution from the Structural and Biophysical Group, Glaxo Incorporated, 5 Moore Drive, Research Triangle Park, North Carolina 27709

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Abstract: The replacement of the phosphodiester linker in a DNA duplex by a formacetal linker has been investigated by computational techniques. Conformational energy as a function of torsional angle was initially evaluated for formacetal and dimethyl phosphate anion using the 6-31G* basis set. In these calculations, one COCO or COPO torsional angle was constrained at 60°, and the second was rotated in 30° increments from 0° to 180°. Accurate force field parameters were developed from these potential surfaces. Molecular dynamics simulations, incorporating the force field parameters and NMR-derived restraints, were then conducted for a dodecamer duplex, dCGCGTT$_{OCH_2O}$TTGCGC/dGCGCAAAACGCG, with explicit water and counterions. Several simulations incorporating a range of restraints were conducted for 40–80 ps at 300 K. The results were compared to results from simulations of the unmodified DNA duplex of the same sequence. The molecular dynamics simulations satisfactorily reproduced experimental data obtained from NMR studies, provided a restraint was used for the C5′C4′–C3′–O3′ dihedral angle of the deoxyribose moieties to maintain a B-family sugar conformation. A well-formed helix is observed with normal base stacking and no apparent structural disruptions in the region of the formacetal linker. COCO dihedral values are similar to those seen for COPCO in a B-form duplex. The detrimental effect of the formacetal linker on the T_m of the duplex (–3 °C) is attributed primarily to the rotation of one COCO torsional angle into the –90° to –100° range seen in the simulations and common to B-form nucleic acids. The energy cost of this rotation from an optimum gauche conformation is on the order of 1.0-1.5 kcal/mol higher than a similar rotation for a COPO torsion and is due to a much stronger stereoelectronic effect for COCO. Consequently, this conformational energy difference can explain the observed decrease in T_m caused by incorporation of formacetal linkers.

Introduction

Modification of the sugar–phosphate backbone in nucleic acids is one strategy toward the goal of developing therapeutic agents which possess novel antiviral or antineoplastic activity.[1,2] The objectives in making changes to the nucleic acid backbone are typically to maintain the sequence-specific recognition capabilities inherent in Watson–Crick or Hoogsteen base-pairing schemes while improving duplex or triplex annealing properties, cell permeation through enhanced lipophilicity, and nuclease resistance. These modified nucleic acids may then be able to exert a range of biological effects by complexing with genomic double-stranded DNA via triplex or d-loop formation (gene targeting), or single-stranded mRNA's via duplex formation (antisense). Many structural changes have been reported, ranging from a single methyl, amine, or sulfur substitution of a phosphoryl oxygen in the case of methyl phosphonates, phosphoramidates, and phosphorothioates[3-5] to an entire replacement of the deoxyribose–phosphate backbone as exemplified by peptide–nucleic acids.[6]

Methyl phosphonate and phosphorothioate modifications show improved nuclease resistance,[3,5] relative to unmodified nucleic acids,[7] but confer chirality to the phosphorus. An alternate strategy, for which a number of examples have been reported,[8-15] is to replace the phosphodiester group with a neutral, achiral substitute. We are interested in modified DNA oligomers (ODN's) in which the phosphate linker is replaced by a formacetal linker (Figure 1).[16-19] This modification results in a neutral backbone which may result in improved resistance to nuclease degradation and enhanced cellular uptake characteristics. However, despite the neutral character of the formacetal-modified backbone, ODNs containing formacetal linkers show reduced binding affinity to complementary RNA or DNA oligomers.[17] This reduction in affinity is demonstrated by the temperature at which a native ODN–formacetal-modified ODN duplex is 50% dissociated (T_m). Compared to an unmodified ODN duplex, each incorporation of a formacetal-modified nucleotide results in a 3–4 °C reduction in T_m under conditions of approximately physiological ionic strength.[17]

While T_m results are critical in evaluating the effects of backbone modifications on annealing properties, they provide no insight into the underlying stereoelectronic effects which are responsible for the observed changes in the T_m. Understanding how chemical modifications affect duplex formation is clearly

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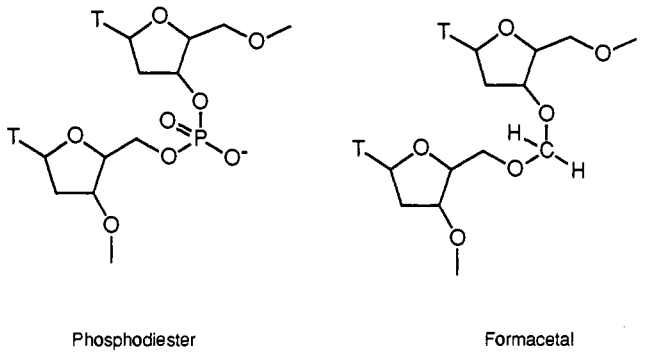
5' - d C G C G T T * T T G C G C - 3'

3' - d G C G C A A A A C G C G - 5'



Phosphodiester Formacetal

Figure 1. Sequence of the DNA dodecamer duplex used in the molecular dynamics simulations and the liners incorporated between bases T6 and T7 (indicated by the asterisk).

useful in evaluation of the relative merit of future backbone changes. To better understand the effect of the formacetal modification, NMR studies were previously conducted on a dodecamer duplex (Figure 1) in which one strand contained a single formacetal linker.[17] The NMR results demonstrate conclusively that the modified duplex retains an overall B-form conformation, including the nucleotides flanking the formacetal, and that the melting is cooperative. This conclusion is based on standard methodologies[20,21] which evaluate the vicinal proton–proton coupling constants and sums of coupling constants determined from phase-sensitive COSY spectra, as well as base to deoxyribose proton distances determined from NOESY spectra.

As a final effort in evaluating the effect of the formacetal modification, we report here the results of computational studies involving the formacetal-modified ODN duplex used for the NMR studies noted above. To evaluate the changes in structure caused by substitution of a formacetal linker for a phosphodiester linker, potential energy surfaces were determined for dimethyl phosphate anion and formacetal (dimethoxymethane) using ab initio quantum mechanics methods (part of the quantum mechanics data for formacetal has been reported[22]). The results of these studies were then used to parametrize appropriate terms in the CHARMm potential energy force field.[23,24] Finally, moleculear dynamics simulations of the dodecamer duplex with solvent, counterion, and a series of different restraints were conducted. The results support the conclusions, on NMR experimental evidence, of an overall B-form geometry and provide rationales for the observed differences in T_m between the unmodified ODN duplex and formacetal-modified ODN duplex.

Methods

Quantum Mechanics. Ab initio calculations were conducted for formacetal and dimethyl phosphate anion using GAUSSIAN90[25] with the 6-31G* basis set.[26,27] One COCO/COPO torsional angle was constrained to 60° for all calculations, and the second COCO/COPO torsional angle was constrained at either 0°, 30°, 60°, 90°, 120°, 150°, or 180° (Figure 2, Table I). The geometry of all other parameters was fully optimized. For dimethyl phosphate anion, full geometry optimization for gauche–gauche, gauche–trans, and trans–trans conformations was performed. The calculations were done on a Cray Y-MP at the North Carolina Supercomputing Center.

Force Field Parameters. Molecular mechanics calculations on formacetal and methyl propyl ether were done using the QUANTA 3.21/CHARMm[23] package on a Silicon Graphics 4D/35 personal iris workstation. Default QUANTA charges were used, and in the case of formacetal, they were compared to those obtained from electrostatic potential fitting using the program CHELP[28] and found to be in reasonable

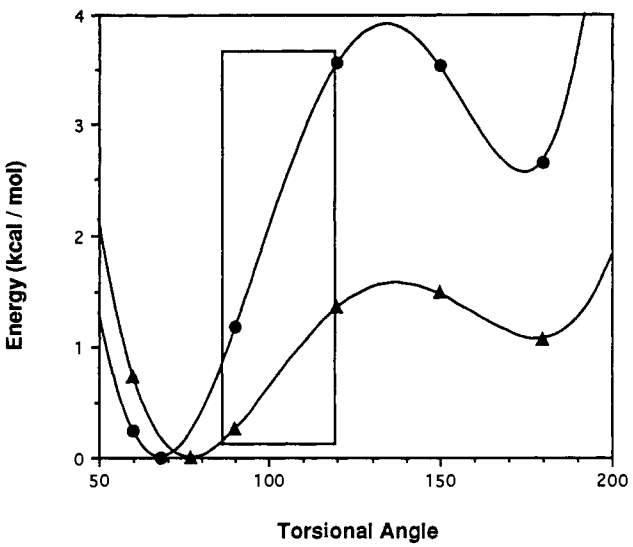


Figure 2. Comparison for formacetal (●) and dimethyl phosphate anion (▲) potential energy surfaces at the 6-31G* basis set level. The graph is for one COCO or COPO torsion constrained to 60° with rotation about the second COCO or COPO torsion at 30° intervals. The rectangle indicates the region typically occupied by one of the COPCO torsional angles in a B-form duplex.

Table I: Comparison of the Formacetal and Dimethyl Phosphate Anion Potential Energy Surfaces at the 6-31G* Basis Set Level[a]

Formacetal			
$COCO_1$	$COCO_2$	energy	rel energy
60	180	−267.950 23	3.03[b]
60	150	−267.948 83	3.90
60	120	−267.948 79	3.93[b]
60	90	−267.952 61	1.54
60	68	−267.954 46	0.37[c]
60	60	−267.954 09	0.60[b]
60	30	−267.944 60	6.56
60	0	−267.936 46	11.67[b]
67	67	−267.954 67	0.00[b,d]
Dimethyl Phosphate Anion			
$COPO_1$	$COPO_2$	energy	rel energy
60	180	−719.518 41	1.57
60	150	−719.517 77	1.94
60	120	−719.517 97	1.81
60	90	−719.519 71	0.73
60	77	−719.520 11	0.47[c]
60	60	−719.518 97	1.19
60	30	−719.510 51	6.49
60	0	−719.503 72	10.75
75	75	−719.520 87	0.00[d]
74	189	−719.519 03	1.15[d]
180	180	−719.516 36	2.83[d]

[a] Energies are in hartrees, and relative energies are in kilocalories per mole. [b] Wiberg and Murcko.[22] [c] Minimum energy conformation with one torsion fixed at 60°. [d] Energies for fully relaxed structures in either gauche–gauche, gauche–trans, or trans–trans conformations.

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Table II. Charges for the Formacetal Linker Incorporated into DNA[a]

atom	charge[b]
T6 C2′	0.093
T6 C4′	0.093
T6 H3′	0.092
T6 C3′	0.053
T6 O3′	-0.360
CTL	0.053
HTL1	0.068
HTL2	0.068
T7 O5′	0.053
T7 C4′	0.093
T7 H5′	0.092
T7 H5″	0.092

[a] CTL, HTL1, and HTL2 refer to the methylene group substituted at the phosphate location. [b] All other charges for atoms of residues T6 and T7 are unchanged from the standard values in the CHARMm residue topology file.

agreement (0.29, -0.37, 0.16 in QUANTA vs 0.25, -0.46, 0.42 determined by CHELP for the terminal CH_3, O, and central CH_2, respectively). All existing CHARMm parameters were used with the exception of those for the COCO, CCCO, and COCC dihedral terms (all dihedral angles were explicitly defined in the residue topology files). For the COCO dihedral term a multiple potential term was used with the first term having a periodicity of 3 and a force constant, $V_3/2 = 0.36$ kcal/mol, and the second term having a periodicity of 2 and a force constant, $V_2/2 = 0.55$ kcal/mol. Multiple potential terms were also used to describe the CCCO ($V_3/2 = 0.15$ kcal/mol, $V_2/2 = 0.40$ kcal/mol) and CCOC ($V_3/2 = 0.36$ kcal/mol, $V_2/2 = 0.40$ kcal/mol) dihedrals. In the above cases, the force constants for the dihedral potential terms having a periodicity of 3 are the standard CHARMm defaults with the 2-fold parameter being added to the force field for fitting of the quantum mechanics results. Fitting was done manually with a focus on the regions most relevant to DNA conformation (see below). For minimizations, a 15-Å nonbonded cutoff and dielectric constant = 1 were used in conjunction with an electrostatic shift function and a van der Waals switch function.[31] The molecules were minimized (Powell conjugate gradient method[29]) to a Δ rms gradient of 0.01 kcal/(mol·Å²).

Molecular Dynamics. Nucleic acid dodecamer duplexes were built using the QUANTA 3.21 modeling software (all dihedral angles were explicitly defined in the residue topology file). The structures were modified to have 5′ OH and 3′ OH groups, and the duplexes had a net negative charge for each phosphate group. The formacetal linker was incorporated via a patch between residues T6 and T7. Charges on the formacetal linker (Table II) were based on QUANTA charges for the formacetal molecule with slight modifications so as to retain charge neutrality. Sodium counterions were placed on the phosphate sp^2 oxygen bisectors at a distance of 4.2 Å from the phosphorus atom. This location positions the sodium at approximately van der Waal's contact distance from each of the sp^2 oxygens. An equilibrated TIP3[30] water box of dimensions 40 Å × 40 Å × 56 Å and containing 2993 water molecules (55.5 M) was constructed. The DNA duplex was centered in this box, and all water molecules with oxygens within 2.8 Å of the duplex or sodium ions were deleted. For the formacetal structure the total number of water molecules in the system was 2606 and the total number of atoms was 8606.

From the solvated structure psf and pdb files were constructed for molecular mechanics and molecular dynamics simulations using the program XPLOR, version 2.11.[31] Calculations were performed on a Cray Y-MP supercomputer. For all calculations an 8-Å nonbonded cutoff and dielectric = 1 were used in conjunction with van der Waals switch and electrostatic shift functions. Periodic boundary conditions were imposed by treating the system as having $P1$ symmetry with lattice dimensions corresponding to the size of the water box. The system was initially minimized (Powell conjugate gradient method[29]) for 1000 cycles with the DNA and counterions fully constrained to remove any bad van der Waals contacts, principally at the lattice interfaces. The system was then fully minimized for another 1000 cycles. Following minimization, the system was heated in 5° intervals to 300 K over the course of 3 ps. An interval step size of 0.001 ps was used, and SHAKE[32] bond constraints were activated for all bonds involving hydrogens.

As noted in the Results and Discussion sections, a range of restraints were applied to different simulations. For hydrogen-bonding restraints, a harmonic potential with a force constant of 2.0 kcal/mol·Å² and an optimum distance of 1.9 Å was used. A square well potential with a force constant of 0.5 kcal/(mol·Å²) and no energy penalty over the range of 3.2–5.2 Å was used for sodium to phosphorus distance restraints. Square well potentials were also used for NOE distance and δ (C5′–C4′–C3′–O3′) dihedral angle restraints. For NOE restraints, a force constant of 2.0 kcal/(mol.Å²) was used for distances greater than ±1 Å from the NOE derived distance. The optimum δ value was assigned at 144° ± 15°, and a penalty force constant of 100 kcal/(mol·rad²) was used for angles outside that range.

Following the heating protocol, the system was equilibrated for 5 ps at 300 K, and simulations were then run for either 40 or 80 ps with coupling to a 300 K heat bath. Interval step sizes of either 0.001 or 0.002 ps were used. Coordinates were written to a trajectory file at 0.2-ps intervals. Following the simulations, the trajectory files were read back into the QUANTA software package for analysis and viewing.

Results

Quantum Mechanics. Ab initio quantum mechanics studies, using the 6-31G* basis set, were undertaken to evaluate the torsional angle potential energy surface for formacetal and dimethyl phosphate anion. A quantum mechanics study on formacetal has been previously published using the 6-31G* basis set,[22] and a study of dimethyl phosphate anion at the STO-3G level has also been published.[33] Our objectives were to focus on the torsional space commonly adopted by the two COPO dihedral angles in oligomer duplexes as determined principally by X-ray crystallographic or NMR spectroscopy studies and to compare formacetal and dimethyl phosphate groups using a common basis set. The two COPO angles are commonly denoted as α (C5′–O5′–P–O3′) and ζ (C3′–O3′–P–O5′) in nucleic acid nomenclature. In B-family oligomer duplex crystal structures, both α and ζ are typically gauche with α near to -60° and ζ ranging between -85° and -125° (BI conformation). On the basis of this knowledge the ab initio studies for formacetal and dimethyl phosphate anion were done holding one COPO or COCO dihedral fixed at 60° and rotating in 30° increments about the other COPO or COCO torsion with fully geometry optimization of all other parameters. Full geometry optimization of dimethyl phosphate anion in the gauche–gauche, gauche–trans, and trans–trans conformations was also done.

The results of the 6-31G* calculations are summarized in Table I and represented in Figure 2. The gauche conformation is lower in energy than the trans conformation for both molecules due to anomeric stabilization: the trans–gauche energy difference is 2.7 kcal/mol for formacetal[22] and 1.1 kcal/mol for dimethyl phosphate anion. Two significant results are apparent in Table I and Figure 2. The barrier to rotation between the gauche and trans states is significantly steeper for formacetal than dimethyl phosphate anion, owing to the overall larger gauche–trans energy difference. Additionally, the localized minimum for the COPO torsion occurs at 77°, whereas for the COCO torsion it is at 68°. Both these results have implications for the formacetal-modified DNA structure given that ζ is normally in the 85° to -120° range (boxed region of Figure 2; note that -85°, -120° is equivalent to +85°, +120°). The energy cost to deviate from an optimal conformation to one reflecting typical α and ζ dihedral values is significantly higher for formacetal (≈1.2–3.6 kcal/mol) than for dimethyl phosphate anion (≈0.2–1.3 kcal/mol).

Molecular Mechanics Parameters. The results of Wiberg and Murcko,[22] in conjunction with those reported here, were used as a guide in constructing potential energy force field parameters

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Table III. A Comparison of the ab Initio Quantum Mechanics and Force Field Potential Energy Surfaces for Formacetal[a]

COCO dihedrals	quantum mechanics[b]	force field[c]
0/0	11.06	10.35
0/60	8.00	7.60
0/120	7.08	5.94
0/180	22.33	22.04
60/60	0.00	0.00
60/120	3.32	3.49
60/180	2.42	2.51
60/−120	3.96	4.73
60/−60	8.27	7.74
120/120	7.01	7.61
120/180	6.25	6.74
180/−120	7.92	8.47
180/180	5.66	5.53

[a] Angles are in degrees, and energies are in kilocalories per mole. [b] From Wiberg and Murcko.[22] [c] Force field values are derived using CHARMm/QUANTA parameters (1–4 interactions not scaled, $\epsilon = 1$) in conjunction with the following dihedral potential terms: XCOX, $V_3/2$ = 0.36 kcal/mol, periodicity = 3, phase = 0°; COCO, $V_2/2$ = 0.55 kcal/mol, periodicity = 2, phase = 0°.

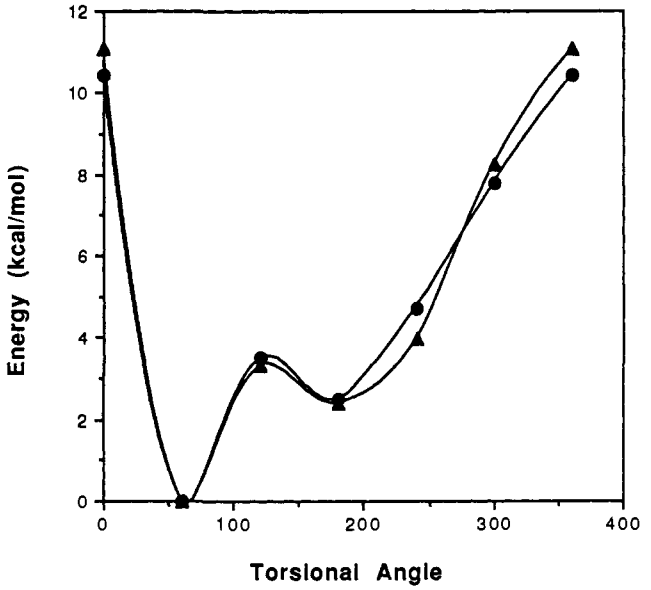

Figure 3. A comparison of the ab initio quantum mechanics (▲) and force field (●) potential energy surfaces for formacetal. The graph is for one COCO torsion constrained to 60° with rotation about the second COCO torsion. See Table II for parameter information.

for formacetal for use in molecular dynamics simulations. These parameters were developed in a straightforward manner, using existing CHARMm parameters for all bond and angle terms and incorporating a multiple dihedral term for the torsional parameter. The comparison between quantum mechanics and molecular mechanics results for formacetal is summarized in Table III and Figure 3. As can be seen in Figure 3, use of two dihedral terms of periodicity 2 and 3 was sufficient to accurately reproduce the potential surface obtained from the ab initio results.

Ab initio studies were reported for methyl propyl ether.[22] Since the component CCCO and COCC torsions occur multiple times in the deoxyribose moiety of nucleic acids, the parameters for these terms were also modified somewhat to reproduce the quantum mechanics results by again incorporating multiple dihedral terms. The results of molecular mechanics calculations on methyl propyl ether are summarized in Table IV.

Molecular Dynamics Simulations. Molecular dynamics simulations of the modified ODN duplex shown in Figure 1 were carried out to further assess the effect of the formacetal modification on duplex structure. A series of simulations were conducted starting with a B-form duplex, as supported by the NMR spectroscopy data. Simulations were conducted employing

Table IV. A Comparison of the ab Initio Quantum Mechanics and Force Field Potential Energy Surfaces for Methyl Propyl Ether[a]

COCC	OCCC	quantum mechanics[b]	force field[c]
180	180	0.36	0.48
77	58	1.28	1.26
80	176	1.74	1.96
179	63	0.00	0.00
60	−60	6.51	6.45
80	−80	4.09	4.72

[a] Angles are in degrees, and energies are in kilocalories per mole. [b] From Wiberg and Murcko.[22] [c] Force field values are derived using the following dihedral potential terms: XCCX, $V_3/2$ = 0.15 kcal/mol, periodicity = 3, phase = 0°; COCC, $V_2/2$ = 0.40 kcal/mol, periodicity = 2, phase = 180°; CCCO, $V_2/2$ = 0.40 kcal/mol, periodicity = 2, phase = 180°. All other terms and conditions are as in Table I.

Table V. Molecular Dynamics Simulations Run on Formacetal-Modified DNA Oligomer Duplex in H_2O with Sodium Counterions[a]

run	length[a]	size[b]	restraints applied
1	40	1	δ angle, all H-bonds, Na^+ to PO_4^-, NOE distances
2	40	2	δ angle, all H-bonds, Na^+ to PO_4^-, NOE distances
3	40	2	all H-bonds
4	40	2	H-bonds for terminal base pairs
5	40	2	δ angle, H-bonds for terminal base pairs
6	40	2	δ angle
7	40	2	none
8	80	2	δ angle for first 40 ps only
9	80	2	δ angle

[a] Lengths of dynamics simulations are in picoseconds. [b] Time step size, in femtoseconds, used for dynamics simulation.

different sets of restraints as summarized in Table V. Restraints on Watson–Crick hydrogen bonding distances, sodium to O1P and O2P distances, NOE-derived distances in the region of the formacetal (bases T6 and T7), and δ (C5′–C4′–C3′–O3′) dihedral angles were incorporated. The objective was to determine which restraints were required to maintain a duplex structure and a structure which was consistent with a B-form conformation over the course of the dynamics simulations. Relatively weak force constants were employed to allow as much conformation flexibility as possible within an overall B-form conformation. Initially, the full set of restraints defined above was employed. Additional simulations were then conducted with decreasing numbers of restraints. The results of the simulations indicate that all restraints with the exception of the δ dihedral angle restraint could be removed and a B-form duplex maintained.

The flexible restraint on the δ angle is designed to maintain a deoxyribose sugar conformation considered part of the B family of sugar conformations, i.e. in the range C1′ exo–C2′ endo–C3′ exo. Removal of this restraint resulted in a consistent transition in the sugar pucker from C2′ endo to C3′ endo for a number of residues (Figure 4) which is inconsistent with the NMR-derived coupling constants.[17] This pseudorotation has been observed previously in molecular modeling calculations using all atom force fields[34] and occurs in our simulations even if the DNA duplex is equilibrated with the δ restraint for 40 ps (dynamics run 8, Table V). Once the transition occurred, it typically remained predominant over the course of the dynamics simulation (Figure 4). Additionally, scaling of 1–4 electrostatic and van der Waals interactions was found to accentuate this effect.

The results for dynamics runs 8 and 9, in which the δ angles were either unrestrained or restrained, are compared with the observed NOE distances in Table VI. The reported proton–proton distances are derived from simple averaging of distances

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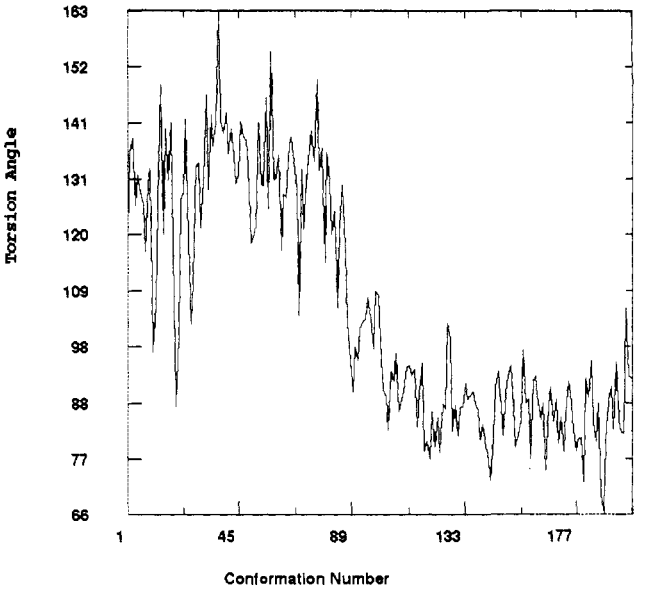

Figure 4. Example of a C2′ endo to C3′ endo transition (base T8) in the absence of a restraint on the C5′-C4′-C3′-O3′ torsional angle. The data points represent 200 structures obtained from 40 to 80 ps at 0.2-ps intervals.

Table VI. Comparison of NMR Derived Distances Versus Those Obtained from Modeling with and without Delta Dihedral (C5′–C4′–C3′–O3′) Restrained[a]

atoms	NMR	restrained[b]	unrestrained[b]
T6 H1′–CTL H1	4.2	4.4	5.5
T6 H1′–CTL H2	4.2	4.2	5.2
T6 H2′–CTL H2	4.1	3.5	3.2
T6 H2″–CTL H2	3.5	2.6	3.4
T6 H2′–CTL H1	null	4.8	4.2
T6 H2″–CTL H1	null	3.7	3.7
T6 H3′–CTL H1	>2.6	3.4	3.5
T6 H3′–CTL H2	2.6	2.4	2.3
T7 H5″–CTL H1	3.2	3.0	2.8
T7 H5′–CTL H2	4.2	3.6	3.5
T7 H5′–CTL H1	3.4	2.3	2.4
T7 H5′–CTL H2	4.2	3.5	3.5
T7 H3′–CTL H2	4.2	4.1	4.2
T6 H1′–T7 H5″	3.5	2.5	3.8
T6 H1′–T7 H5′	4.1	4.1	5.4

[a] Distances are in angstroms. [b] NMR distances are ±1 Å. The unrestrained values are from dynamics run 9, and the unrestrained values are from dynamics run 8.

measured in individual structures observed at 0.2-ps intervals during the 40-ps trajectories. The comparison for the restrained model indicates that distances obtained from the averaged structure are in overall good agreement with the observed NOEs. Specifically, the NMR distances are derived using the isolated spin pair approximation (mixing time of 150 ms) and are not corrected for effects due to spin diffusion, anisotropic tumbling of the DNA molecule, and local internal motion. These effects have all been shown to potentially affect the magnitudes of the NMR-calculated distances,[35-37] and thus the accuracy of these distances is taken at ±1 Å. On the basis of this criteria all of the modeling-derived distances for the restrained simulation (run 9) are within the NMR-defined ranges. The modeling results are thus consistent with the observation that incorporation of the formacetal modification into a duplex structure does not necessitate any significant deviations from an overall B structure (Figure 4).

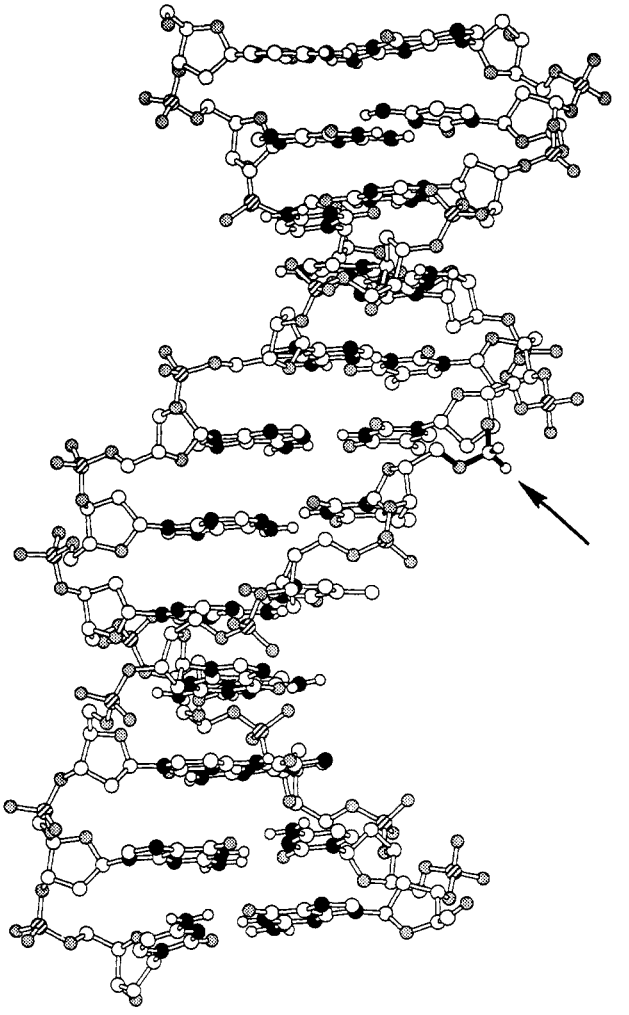

Figure 5. Average structure obtained from molecular dynamics simulation of formacetal-modified DNA duplex (dynamics run 9). The model represents the average of 200 structures obtained from 40 to 80 ps at 0.2-ps intervals. Atom shading: carbon and hydrogen, white; nitrogen, black; oxygen, shaded; phosphorus, diagonal lines. The formacetal linker is indicated by black bonds and an arrow.

For the unrestrained simulation (run 8) in which several sugars show C2′ endo to C3′ endo transitions, three of the average proton–proton distances are greater than 5 Å (T6 H1′–CTL H1, T6 H1′–CTL H2, and T6 H1′–T7 H5′). Protons separated by a distance of more than 5 Å should not show a detectable NOE, and as a result, these distances are in disagreement with the NMR data. The largest deviations (T6 H2″–CTL H2, T7 H5′–CTL H1, and T6 H1′–T7 H5″) for the restrained structure all involve molecular modeling derived distances which are shorter than NMR-derived distances. Thus, for the restrained model, the difference is in the predicted NOE intensity, while for the unrestrained model, the difference is in the presence or absence of the NOE. The negative results, namely, disagreement with NMR coupling constant and NOE data, for dynamics run 8 provide additional support for an overall B-form geometry for the formacetal-modified DNA duplex.

The conformational parameters of the averaged structure from the dynamics simulation run 9 (40–80 ps, δ restrained) were compared to those obtained from a 40-ps simulation of an unmodified ODN duplex of similar sequence (δ restrained). The time-averaged structure of the modified formacetal is shown in Figure 5, and a comparison of the central formacetal-modified T4–A4 tetrad and the central T4–A4 tetrad of the unmodified dimer is shown in Figure 6. The rms deviation between the central tetrads of the two duplexes is 1.33, and 0.37 for the T6–T7 dimers. The small rms deviation indicates that base stacking in the region

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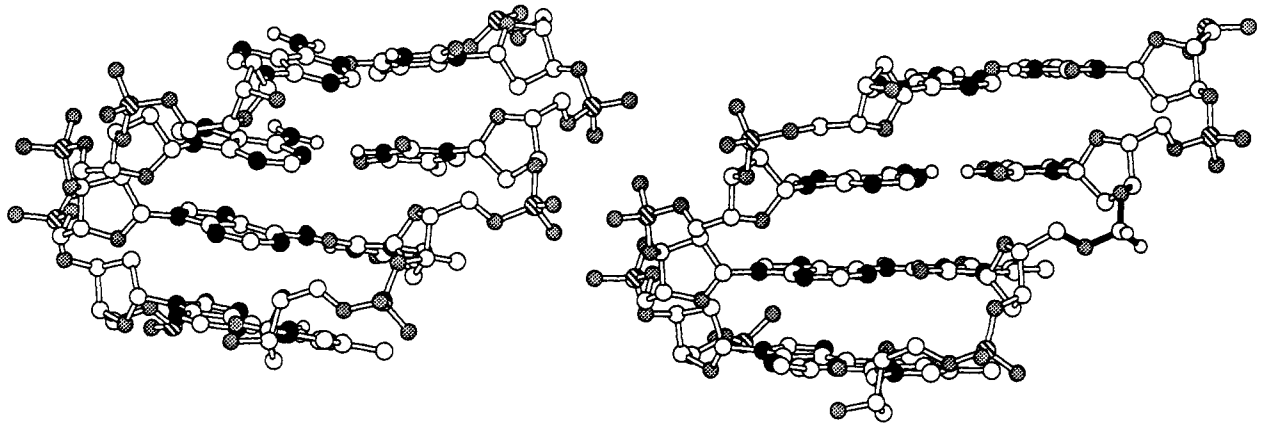

Figure 6. Comparison for the T5–T6–T7–T8 region of the average structures obtained from molecular dynamics simulations of the formacetal-modified DNA duplex (right) and the unmodified DNA duplex (left). The models represent the average of 200 structures obtained from a 40-ps simulation at 0.2-ps intervals. Atom shading is as in Figure 5. The formacetal linker is indicated by black bonds.

Table VII. Conformational Parameterrs of Central T5–T6–T7–T8 Tetrad for Formacetal-Modified and Unmodified Dodecamer Duplexes[a]

base	α	β	γ	δ	ϵ	ζ	χ	P	τ	t	O4′–O4′
					Formacetal-Modified Duplex						
T5	–60	166	59	133	170	–94	–123	140	36	32	8.6
T6	–57	–175	44	135	176	–92[b]	–119	146	36	39	7.8
T7	–63[b]	–174	46	131	172	–103	–114	135	38	33	7.7
T8	–57	177	52	132	173	–109	–123	138	35	37	8.0
					Unmodified Duplex						
T5	–43	139	62	135	175	–88	–120	143	37	40	8.5
T6	–59	172	50	132	171	–95	–114	137	38	37	7.3
T7	–53	171	52	130	170	–105	–119	135	36	39	7.0
T8	–36[c]	179	31[c]	136	177	–102	–116	147	32	34	6.8

[a] α, β, γ, δ, ϵ, ζ, χ are the standard torsional angles describing nucleic acid conformation.[38] P, pseudorotation value; τ, degree of pucker; t, twist angle between base and 5′ neighbor; O4′–O4′, interstrand distance (in Å) for O4′ atoms of base i and opposite strand base $i-3$. [b] α and ζ values corresponding to formacetal modification. [c] Values reflect conformational averaging of an alternate conformation in which α, γ are $+g$, $-g$ rather than the typical $-g$, $+g$.

of the formacetal linker is not disrupted and is similar to that seen for the unmodified ODN. The values of helical parameters for the modified and unmodified duplexes are summarized in Table VII, and these values again reinforce the argument that the substitution of the formacetal linker does not significantly alter the backbone geometry from that of an unmodified duplex. In particular, the values for α and ζ, –63° and –92°, respectively, are in the standard range observed for B-form duplexes. The closest interstrand O4′ atomic distances are also consistent with a minor groove width typical of a B-form nucleic acid.

Discussion

Given that the annealing of two DNA strands requires bringing two polyanionic species into close proximity, the formacetal substitution should be both entropically and enthalpically favored from a simple thermodynamic viewpoint which considers only the substitution of a neutral species for a negatively charged one within a polyanion. There are fewer phosphate–phosphate charge repulsion interactions, which is enthalpically favored, and the entropic cost of a high localized counterion concentration, required to neutralize the phosphate groups, is also reduced. However, the formacetal-modified ODN shows a decreased T_m relative to its unmodified counterpart, indicating that alternative factors are critical in assessing the annealing properties.

The results of the quantum mechanics comparison between formacetal and dimethyl phospohate anion provide a plausible rationale for the source of the detrimental effect on T_m due to the formacetal linker. Rotation of one COCO torsion from its optimum gauche conformation to a conformation characteristic of nucleic acid structures cost significantly more energy than a similar transition for a COPO torsion. This difference, as noted, derives from two factors. First, the degree of anomeric stabilization for the gauche–gauche conformation vs trans–gauche of formacetal is greater than for dimethyl phosphate anion. This larger degree of stabilization in turn results in a steeper potential energy curve for formacetal. Additionally, the value of the dihedral (68°) for the energy minima gauche conformation of one COCO torsion when the other is fixed at 60° is farther removed from the –85° to –125° range seen for nucleic acids than the analogous value for dimethyl phosphate anion (77°). The combined effect of these factors is a torsional energy which is 1–2 kcal/mol higher for a formacetal conformation comparable to that seen typically for COPOC in nucleic acids.

Other properties could also contribute to the reduced T_m. The formacetal linker could form weaker van der Waals interactions than the phosphate linker with either the flanking bases or sugars. The principal difference in the two linkers with respect to van der Waals interactions is the substitution of two hydrogens for two sp^2 oxygens. However, in a B-form duplex, these atoms project into solution and have relatively few van der Waals contacts with the atoms of the flanking bases, which indicates that differences in van der Waals interaction should be limited.

Another source of potential difference between the formacetal linker and the phosphate linker is the degree of solvation. Clearly, the phosphate linker will have a significantly more favorable solvation energy than the formacetal group. With respect to T_m differences, however, the important factor is the relative differences in solvation of the single-stranded state vs the annealed duplex state. This difference can be assessed via free energy perturbation studies, but these studies are problematic due to the difficulty in defining conformation for the single-stranded state.

On the basis of the molecular dynamics simulations in conjunction with the NMR data, the formacetal modification does not significantly distort the duplex structure (Figures 5 and 6). The α and ζ values observed in the modeling studies for the

central T_4 tetrad of the duplex are consistent with the ranges observed for B-form nucleic acids ($\alpha = -45°$ to $-70°$; $\zeta = -85°$ to $-110°$). Given this result, we feel the reduction in duplex T_m due to incorporation of the formacetal group is a result of the energetic penalty for deviation of formacetal from its ideal gauche–gauche conformation. The distortion of ζ to $-90°$ or greater is significantly more unfavorable for the formacetal linker than a normal phosphodiester linker. This more unfavorable distortion must be compensated either by improved base stacking or by some other feature, or it will be manifested in the T_m of the duplex. While it is possible that the formacetal could adopt its optimum conformation which would in turn alter base stacking, the modeling studies up to 80 ps of simulation support essentially normal base stacking with a formacetal conformation comparable with that of the native phosphate backbone. This result is consistent with the NMR data with regard to base stacking which shows that the chemical shifts of T6 and T7 H6 protons differ by less than 0.05 ppm in the formacetal-modified and unmodified ODN duplexes.

Conclusion

Thus, the stronger stereoelectronic effect which alters the torsional energy profile of formacetal, as compared to phosphodiester, is concluded to be primarily responsible for the reduced T_m of the formacetal-modified ODN. Base stacking is not significantly altered by incorporation of the formacetal linker, and the energy cost due to the formacetal linker is thus accounted for locally in a backbone conformation consistent with a normal B-form duplex. While the effect is localized, it, nonetheless, is manifested at macroscopic level in the reduced T_m seen for the formacetal-modified duplex.

Supplementary Material Available: Topology files with full optimized parameters for the quantum mechanics calculations involving formacetal and dimethyl phosphate anion (5 pages). Ordering information is given on any current masthead page.