Impact of Pyrophosphate and *O*-Ethyl-Substituted Pyrophosphate Groups on DNA Structure

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Received: September 12, 2006; In Final Form: October 27, 2006

Design of novel DNA probes to inhibit specific repair pathways is important for basic science applications and for use as therapeutic agents. As shown previously, single pyrophosphate (PP) and *O*-ethyl-substituted pyrophosphate (SPP) modifications can inhibit the DNA glycosylase activities on damaged DNA. To understand the structural basis of this inhibition, the influence of the PP and SPP internucleotide groups on the helical parameters and geometry of a double-stranded DNA was studied by using molecular modeling tools including molecular dynamics and quantum mechanical−molecular mechanical (QM/MM) approaches. Native and locally modified PP- and SPP-containing DNA duplexes of dodecanucleotide d(C1G2C3G4A5A6T7T8C9G10C11G12) were simulated in aqueous solution. The energies and forces were computed by using the PBE0/6-31+G** approach in the QM part and the AMBER force-field parameters in the MM part. Analysis of the local base-pair helical parameters, internucleotide distances, and overall global structure at the located stationary points revealed a close similarity of the initial and modified duplexes, with only torsion angles of the main chain being altered in the vicinity of introduced chemical modification. Results show that the PP and SPP groups are built into a helix structure without elongation of the internucleotide distance due to flipping-out of phosphate group from the sugar−phosphate backbone. The mechanism of such embedding has only a minor impact on the base pairs stacking and Watson−Crick interactions. Biochemical studies revealed that the PP and SPP groups immediately 5′, but not 3′, to the 8-oxoguanosine (8oxodG) inhibit translesion synthesis by a DNA polymerase in vitro. These results suggest that subtle perturbations of the DNA backbone conformation influence processing of base lesions.

1. Introduction

Synthetic fragments of nucleic acids (NA) and their analogues containing modified units are widely employed in solving various problems of molecular biology, biotechnology, and medicine. Synthetic analogues of nucleic acids bearing reactive groups are of special importance. These compounds can form covalent bonds with the reactive groups of biopolymers, and they are promising tools for topography studies of protein active centers, their functional features, and molecular mechanisms of NA−protein recognition.

Several types of nucleic acids analogues containing pyrophosphate (PP) and reactive *O*-alkyl-substituted pyrophosphate (SPP) internucleotide groups have been developed and studied experimentally.[1,2] Because of their ability to form covalent bonds with nucleophilic amino acid residues of NA-recognizing proteins, the SPP-containing compounds are successfully used for studies of nucleic acids metabolism as well as for affinity modification and probing binding sites of enzymes, transcription factors, and other regulatory proteins.[2−7] DNA duplexes containing PP and SPP internucleotide groups are considered as promising reagents for in vivo applications including the design of antiviral and antitumor drugs due to inhibition of gene expressions in viral or oncogenic cells.[8,9]

Despite wide applications, little is known about structures of DNA duplexes modified by pyrophosphate groups. Low-resolution methods based on the temperature melting technique and circular dichroism spectroscopy have shown very high similarity to their parent all-phosphate oligomers.[10] The thermodynamic and spectroscopic studies provide no information about the structural details of the modified DNA. However, knowledge on structures of the PP and SPP analogues of NA is required for correct and unambiguous interpretation of experimental data.

In this work, we apply modern molecular modeling tools including quantum mechanical−molecular mechanical (QM/MM) approaches[11−19] to provide a quantitatively accurate atomic-level description of the PP and SPP group influence on the helical parameters and geometry of double-stranded DNA. An important issue of the present work is that the modified DNA structures were studied in solution under physiologically relevant conditions. Successful applications of previous QM/MM-based modeling of proton transfer in model DNA base-pair,[20] gas-phase DNA oligonucleotide structures,[21] and mixed ab initio Car−Parrinello/classical molecular dynamics studies of anticancer drug−DNA complexes[22−24] provide credit to the

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10.1021/jp065947n CCC: $37.00
Published on Web 12/22/2006

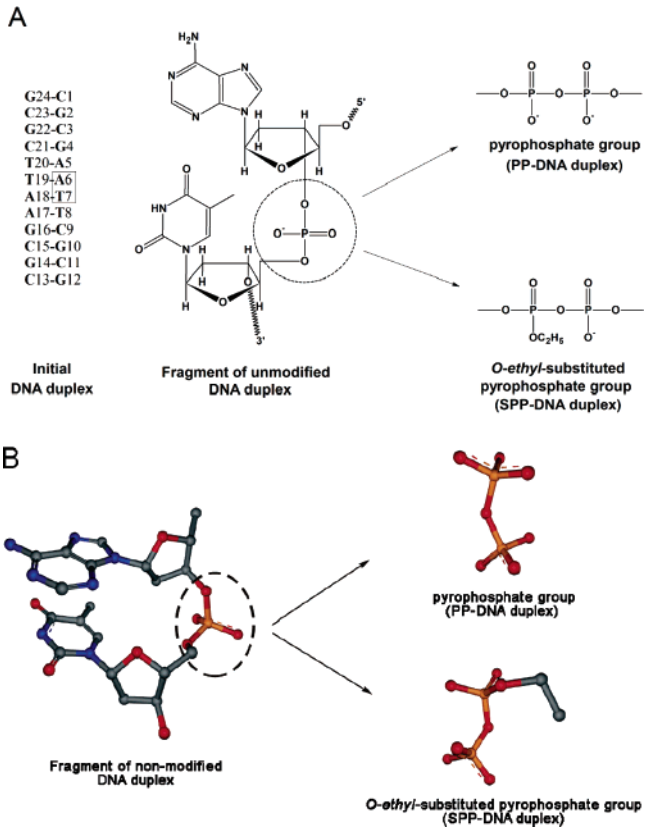

Figure 1. Structure of initial DNA duplex and modified fragments of DNA duplexes studied in this work: ChemDraw scheme (A) and ball-and-stick model (B).

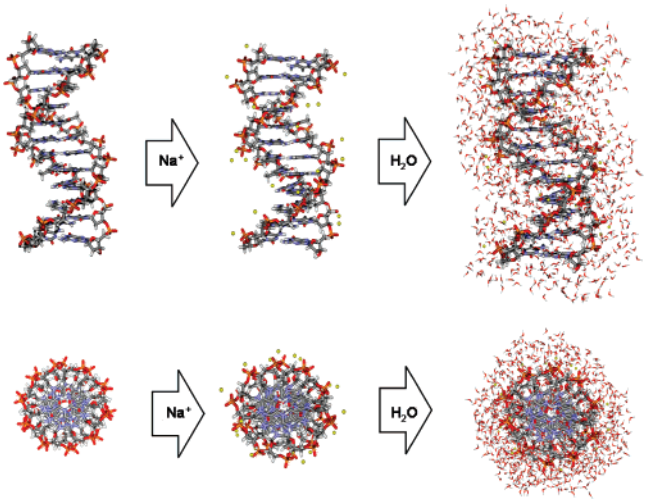

Figure 2. Preparation of the model systems.

present theoretical estimates. Other theoretical approaches included the empirical valence bond and hybrid QM/MM free energy simulations of catalytic action of T7 DNA polymerase[25,26] and 8-oxoguanine-DNA glycosylase.[27] Target-related applications of first principles quantum chemical methods in drug design are described in the most recent review.[28]

To measure the biological impact of the modification, a synthetic oligonucleotide containing a single PP group was used as a template for primer extension reactions catalyzed by the Klenow fragment of *Escherichia coli* DNA polymerase I. The role of the DNA backbone conformation in recognition of base damage is discussed.

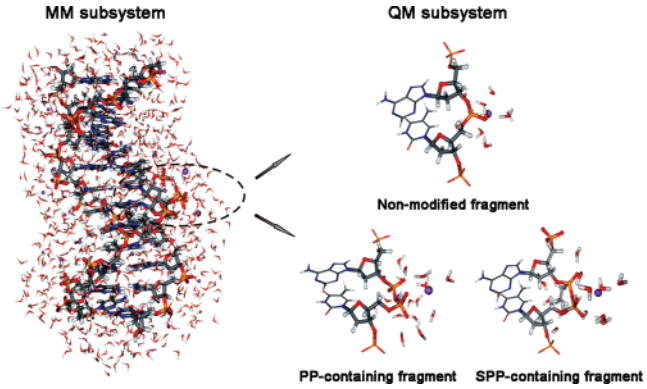

Figure 3. Partitioning of the solvated DNA duplexes into the QM (ball-and-sticks and bold sticks for water molecules included in QM part) and MM (thin sticks) subsystems. A dashed line circles the QM subsystem.

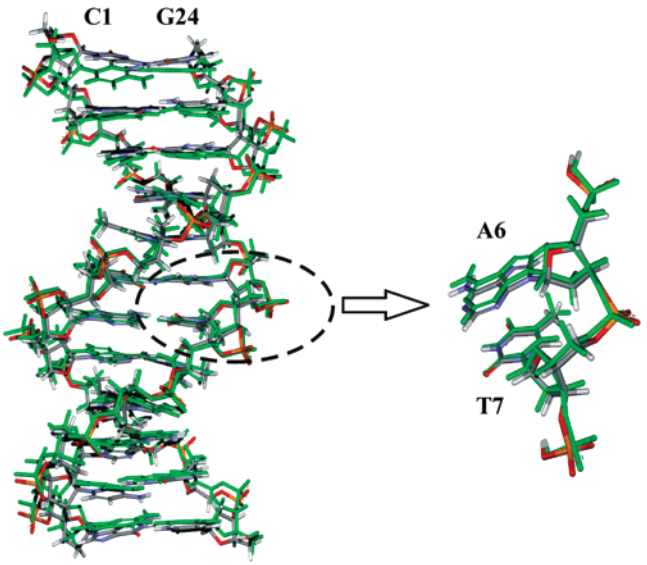

Figure 4. Superposition of the simulated d(CGCGAATTCGCG)$_2$ structure (green) with the original NMR structure of nonmodified DNA duplex (colored by elements), PDB code 1NAJ. Water molecules are omitted for clarity.

2. Methods

2.1. Computational Details. Calculations were based on the NMR structure of the DNA duplex d(CGCGAATTCGCG)$_2$ reported by Wu et al.[29] This structure was further modified to include PP or SPP groups. Starting coordinates of the modified DNA structures were generated manually by considering different orientations of the newly inserted functional groups. To each complex, a total of 22 (23 for the PP duplex) neutralizing sodium ions were added at the bifurcation positions of the O−P−O angle at a distance of 4.0 Å from the phosphorus atom. The DNA duplexes with counterions were then uniformly solvated by water molecules by using the computer program VMD.[30]

The DNA duplex environment was thermally equilibrated at 298 K during 12 ps with a time step of 1 fs and gradually relaxed to 0 K during 4 ps by using the molecular dynamics (MD) technique with the standard AMBER99[31,32] force-field parameters. The molecular modeling package TINKER (J. W. Ponder, http://dasher.wustl.edu/tinker) was utilized in these MD simulations.

Equilibrium geometry configurations of the entire molecular complexes were further optimized by using the mechanical embedding quantum mechanical−molecular mechanical (QM/

TABLE 1: Local Helical Parameters of the Nonmodified, PP-, and SPP-Containing DNA Optimized Structures[a]

parameter	same strand P−P bond distances, Å				rise *h*, Å				twist *t*, (deg)			
base pair	NMR	nonmod.	PP-DNA	SPP-DNA	NMR	nonmod.	PP-DNA	SPP-DNA	NMR	nonmod.	PP-DNA	SPP-DNA
1 C/G					3.3	3.0	2.9	3.0	33.2	35.4	36.5	39.1
2 G/C	6.9	7.0	7.2	7.0	3.2	3.3	3.3	3.4	38.2	34.9	32.0	29.9
3 C/G	6.8	6.4	6.5	6.5	3.3	3.1	3.1	3.2	33.9	36.0	37.6	38.5
4 G/C	6.9	7.2	7.0	7.0	3.1	3.1	3.1	3.2	36.9	42.9	40.3	41.5
5 A/A	7.0	7.2	7.2	7.2	3.1	3.2	3.3	3.2	37.8	37.0	35.2	33.5
6 A/T	**6.9**	**6.8**	**6.3**	**6.1**	**3.0**	**3.1**	**3.2**	**3.2**	**34.8**	**32.2**	**32.8**	**33.2**
7 T/T	6.7	6.5	**7.0**	**7.8**	3.1	3.0	3.0	3.0	37.8	34.8	37.3	40.5
8 T/C	6.7	7.0	7.0	6.8	3.2	3.0	3.2	3.1	37.0	41.5	42.9	41.0
9 C/G	6.8	6.8	6.5	6.6	3.4	3.1	2.8	2.7	34.1	30.7	30.0	31.3
10 G/C	6.8	7.0	7.0	7.0	3.2	3.4	3.3	3.4	38.1	42.4	41.6	39.8
11 C/G	6.6	6.7	6.6	6.8	3.3	2.8	3.1	3.2	33.0	35.1	35.1	38.7

[a] The results are compared to the experimental NMR structure.

TABLE 2: Backbone and Glycosyl Torsion Angles, Amplitudes (t_m), Pseudorotation Phase Angles (*P*), and Puckering of the Sugar Ring in the Central Part of the Investigated DNA Structures

	torsion angles (deg)								
nucleotide	α* (P1−$O_{5''}$)	α (P−$O_{5'}$)	β ($O_{5'}$−$C_{5'}$)	γ ($C_{5'}$−$C_{4'}$)	δ ($C_{4'}$−$C_{3'}$)	ϵ ($C_{3'}$−$O_{3'}$)	ζ ($O_{3'}$−P)	ζ* ($O_{5''}$−P2)	χ ($C_{1'}$−N)
nonmodified DNA duplex (NMR)									
A (6)		−71.3	178.3	57.8	125.6	−178.7	−92.1		−111.4
T (7)		−60.5	172.6	52.5	109.5	−178.0	−87.4		−121.8
nonmodified DNA duplex (QM/MM)									
A (6)		−74.4	−172.7	60.0	123.5	169.8	−88.5		−118.2
T (7)		−62.0	−179.5	53.1	97.6	172.0	−78.0		−130.7
DNA duplex, containing PP group between A(6) and A(7) (QM/MM)									
A (6)		−47.8	154.5	47.2	108.8	−133.2	−152.4		−129.3
T (7)	−65.0	−63.0	148.9	−28.0	80.2	163.4	−78.6	112.1	−130.0
DNA duplex, containing SPP group between A(6) and A(7) (QM/MM)									
A (6)		−51.3	155.4	46.7	110.2	−109.4	−166.6		−131.8
T (7)	−64.2	−72.6	171.1	−14.3	91.2	167.1	−111.2	−72.6	−133.9

nucleotide	ν_0 ($O_{4'}$−$C_{1'}$)	ν1 ($C_{1'}$−$C_{2'}$)	ν2 ($C_{2'}$−$C_{3'}$)	ν3 ($C_{3'}$−$C_{4'}$)	ν4 ($C_{4'}$−$O_{4'}$)	amplitude, t_m	phase, *P*	sugar puckering
nonmodified DNA duplex (NMR)								
A (6)	−27.4	30.2	−22.9	8.1	11.7	30.3	139.0	$C_{1'}$-*exo*
T (7)	−35.4	27.1	−10.4	−9.3	26.9	33.7	108.0	$C_{1'}$-*exo*
nonmodified DNA duplex (QM/MM)								
A (6)	−33.1	32.7	−20.5	2.0	19.4	34.0	127.2	$C_{1'}$-*exo*
T (7)	−35.6	22.4	−2.5	−18.4	34.1	36.0	93.9	$O_{4'}$-*endo*
DNA duplex, containing PP group between A(6) and A(7) (QM/MM)								
A (6)	−42.6	30.8	−9.1	−15.2	35.9	41.5	102.6	$O_{4'}$-*endo*
T (7)	−40.3	17.4	10.0	−34.9	47.1	46.5	77.5	$O_{4'}$-*endo*
DNA duplex, containing SPP group between A(6) and A(7) (QM/MM)								
A (6)	−45.1	33.8	−11.6	−14.1	36.8	43.7	105.4	$O_{4'}$-*endo*
T (7)	−46.5	28.6	−1.7	−25.8	45.1	47.4	92.1	$O_{4'}$-*endo*

MM) technique[15] as implemented by J. Kress and A. A. Granovsky within the PC GAMESS computational package (http://lcc.chem.msu.ru/gran/gamess/index.html).[33] The conventional link hydrogen atom approach was applied to interface the QM and MM regions. The energies and forces in the QM subsystem were computed with the hybrid PBE0 functional and 6-31+G** basis set. The remaining part was modeled by using the classical molecular mechanical approximation (AMBER99[31,32]). Efficient geometry optimizations were performed by applying novel implementations of large-scale minimization algorithm incorporated to PC GAMESS. The truncated Newton SYMMLQ algorithm was used to locate the stationary point on the combined multidimensional potential energy surface. A complete set of geometrical parameters for all investigated structures was calculated using the 3DNA program (V1.5, Nov 2002).[34] Bending of the helical axis was calculated using the CURVES program.[35]

2.2. Primer Extension Assay. To generate a double-stranded matrix for DNA synthesis, a 29-mer oligonucleotide template containing G, Gpp, oxoG, oxoGpp, and ppoxoG at position 14 was annealed to a 5′-[^{32}P]-labeled primer (PR-10) at a 1:1 molar ratio as previously described.[36] The standard reaction mixture (20 μL) contained 10 nM 5′-[^{32}P]-primer/template, 10 mM Tris-HCl, pH 7.5, 1 mM dithiothreitiol, 5 mM $MgCl_2$, 100 μM each of dNTP, and 1 unit of Klenow fragment. Reactions were performed at 37 °C for 5 min, and products were analyzed as described.[37] Gels were exposed to a Fuji FLA-3000 Phosphor Screen and analyzed using Image Gauge V3.12 software.

TABLE 3: Global Helical Parameters of the Nonmodified, PP-, and SPP-Containing DNA Optimized Structures[a]

	NMR	QM/MM modeling		
helical parameters	nonmodified	DNA duplex	PP-DNA duplex	SPP-DNA duplex
H, Å	33.8	32.3	32.2	31.6
h, Å	3.4	3.2	3.3	3.2
n, b.p.	10.0	10.0	9.9	9.8
t, deg	36.0	36.2	36.5	36.5
bending, deg	11.9	16.8	15.0	19.2

[a] The results are compared to the experimental NMR structure.

3. Results and Discussion

3.1. Molecular Modeling. Much work on the details underlying DNA structure and its variability derives from experimental and modeling studies of the DNA duplex d(C1G2C3G4A5A6T7T8C9G10C11G12)$_2$. Atomic resolution structure for this dodecamer was first determined by Dickerson et al.[38] using single-crystal X-ray diffraction and then later, at a higher resolution, by Williams and co-workers.[39−41] The same palindromic DNA was also studied extensively by molecular dynamics simulations.[42,43] It was also the subject of many NMR studies.[44,45] In our work, this B-form double helical structure of the DNA duplex was chosen for the investigation of the influence of PP and SPP internucleotide groups on geometrical parameters and conformation of DNA double helix.

Initial coordinates of the nonmodified DNA double helix were taken from experimental structural data obtained by high-resolution NMR spectroscopy in aqueous dilute liquid crystalline phase (Protein Data Bank code 1NAJ).[29] Modified PP or SPP groups were incorporated between the A6 and T7 residues (Figure 1). Sufficient number of counterions was added to DNA duplex to provide electroneutrality of the system. The DNA with counterions was then uniformly solvated by water molecules (Figure 2). The overall size of the system is equal to ∼3500 atoms (Figure 2).

The QM part included a fragment of sugar−phosphate backbone, containing modified or native phosphate group, counterion of this group, and water molecules from the first solvation shell (Figure 3). The rest was modeled within the classical molecular mechanical approximation.

Figure 4 illustrates comparison of the simulated d(CGCGAATTCGCG)$_2$ structure and the initial NMR structure of this dodecamer (1NAJ[29]). Values of the optimized local and global helical DNA structure parameters together with experimental NMR data[29] are summarized in Tables 1−3. Such comparison shows an encouraging similarity between theoretical results and experimental observations. All torsion angles associated with the central base pairs fall within the range of ±20° relative to those found in the NMR structure (1NAJ). As expected, the largest discrepancies of parameters are located in the terminal nucleotides (Figure 4). As seen from Table 1, changes of the characteristic distances between phosphorus atoms of the same strand and heterocyclic bases of neighboring nucleotides do not exceed 0.4 Å. The backbone and glycosyl torsion angles, as well as the pseudorotation phase angles and amplitudes calculated for both NMR and QM/MM structures, are consistent with the B-DNA conformation (Table 2). The global QM/MM structure seems to be more compressed as compared to the NMR structure (PDB code 1NAJ). However, an accurate global DNA structure determination by even new experimental NMR techniques, such as residual dipolar coupling and chemical shift anisotropy, still represents a challenge and critically depends on the choice of mean-force interaction potentials used in the

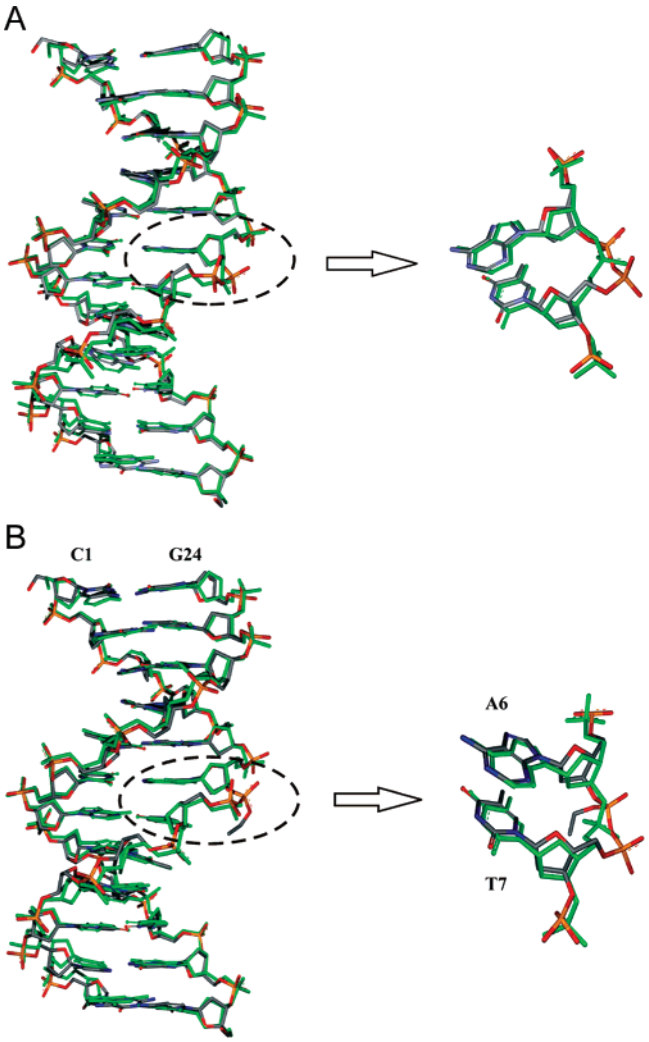

Figure 5. Superposition of the nonmodified DNA duplex (green) with the PP-containing DNA duplex (colored by elements) (A) and the SPP-containing DNA duplex (colored by elements) (B). Water molecules are omitted for clarity.

NMR refinement procedure.[46] Therefore, the small deviations between the experimental and theoretical results may be expected not only because of the accuracy limit of simulation methods used, but also as a result of some uncertainty in the determination of the structural parameters by NMR technique. Nonetheless, the structure as a whole obtained in molecular modeling represents quite well the experimental one, indicating the reliability of the simulation tools used in further studies of the modified DNA duplexes.

Structural effects of PP and SPP groups were examined by comparison of the helical parameters of both modified and nonmodified DNA duplexes. Figure 5 shows superposition of the duplex, containing either pyrophosphate or substituted pyrophosphate group, and regular duplex. The helix parameters of modified DNA duplexes are listed in Tables 1−3. Data obtained indicate that introduction of pyrophosphate or substituted pyrophosphate group does not affect the overall oligomer structure and global properties, including DNA bending, which is critical for packaging, recognition, and regulation of DNA transcription.[47,48] Furthermore, such local parameters as base rise (Table 1) and distance between $C_{1'}$ atoms of neighboring nucleotides flanking the modified group are much the same as those of the nonmodified DNA duplex. Therefore, stacking interaction and hydrogen bonding between these base pairs

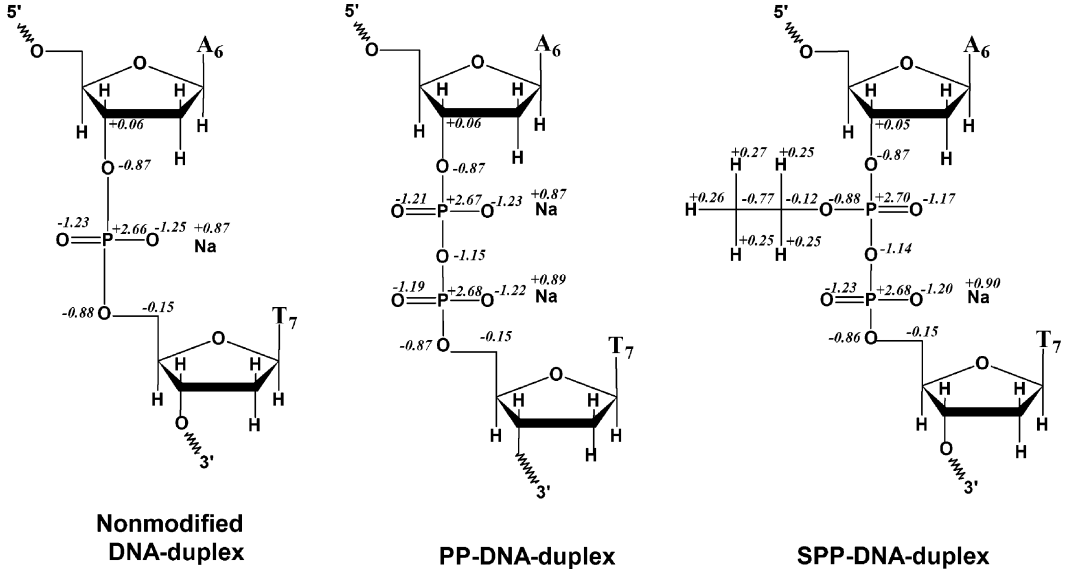

Figure 6. Charge distributions for the nonmodified, pyrophosphate, and *O*-ethyl-substituted pyrophosphate internucleotide groups.

remain invariable. According to simulation results, the pyrophosphate and *O*-ethyl-substituted pyrophosphate internucleotide groups build into DNA structure without elongation of internucleotide distance. This is achieved by means of flipping-out of (P_2) phosphate linked to $C_{5'}$ atom of deoxyribose of pyrophosphate group due to rotation around $C_{4'}-C_{5'}$, $C_{5'}-C_{5'}$ and $C_{3'}-O_{3'}$, $O_{3'}-P_1$ bonds, as reflected in the change of the torsion angles γ and β of 7-nucleotide and ϵ and ζ torsion angles of A6-nucleotide, respectively (Figure 5, Table 2). Phosphorus atoms of PP and SPP groups, which are connected to $C_{3'}$- and $C_{5'}$-atoms of deoxyribose (P_1 and P_2, respectively), are located from the phosphorus atom of the nonmodified phosphate at 1.0 Å and 2.4 Å for the PP group and 0.9 Å and 2.3 Å for the SPP group, respectively. As can be seen from Table 2, backbone, glycosyl torsion angles, and endocyclic torsion angles of furanose calculated from the lowest energy structure are consistent with B-DNA conformation. The presence of the PP or SPP group did not affect the sugar conformation significantly as indicated by the dihedral torsion angle δ, which mainly affects the sugar pucker state (Table 2).

Based on equilibrium geometry configurations of DNA duplex, containing PP and SPP groups, the electron density analysis and estimation of atomic charges were performed by using natural bond orbital (NBO) analysis.[49] Charge distributions of the nonmodified phosphate, pyrophosphate, and *O*-ethyl-substituted pyrophosphate groups are illustrated in Figure 6. We notice that introduction of ethyl substitute does not affect the charge distribution of the pyrophosphate moiety.

3.2. Effect of a Single Modified Internucleotide Group on Bypass of 8oxodG Residue by a DNA Polymerase. We have shown previously that DNA duplexes containing PP or SPP internucleotide groups 3′ next to a damaged base are noncleavable substrate analogues for the base excision repair enzymes, such as human 8-oxoguanine-DNA glycosylase, *E. coli* formamidopyrimidine-DNA glycosylase, and *E. coli* uracil-DNA glycosylase.[7,50] The simulation studies described in this work suggest that the type of conformation changes induced by PP or SPP groups in the DNA duplex that can lead to inhibition of the various DNA glycosylases are mainly torsion angles of the modified nucleotide. To examine whether these conformational changes also influence DNA synthesis, the effect of PP DNA modification on a DNA polymerase using primer extension assays was studied.

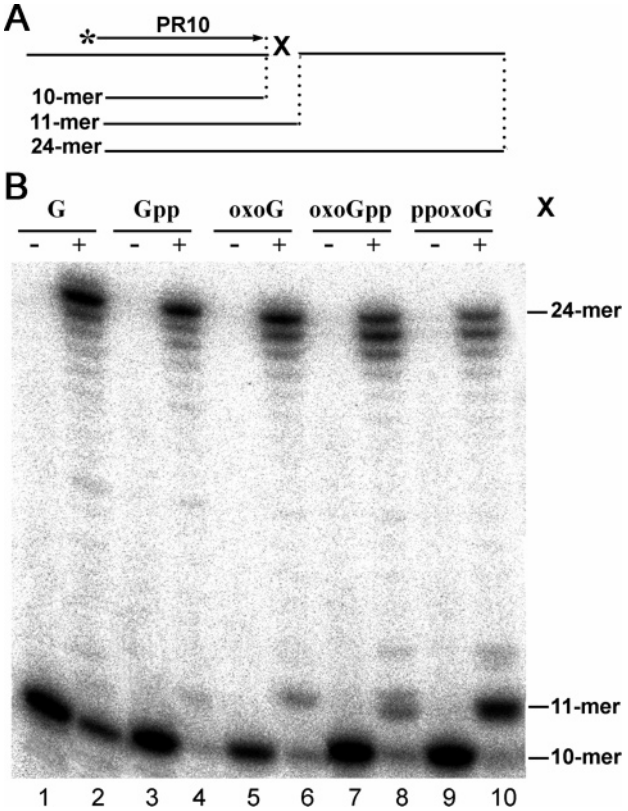

Figure 7. Effect of PP group on DNA synthesis in primer extension assay. (A) Schematic representation of the DNA template containing G, Gpp, oxoG, oxoGpp, or ppoxoG and possible elongation products: 24-mer, full-size product; 11-mer, oxoG termination product; 10-mer, 5′-[^{32}P]-labeled primer. (B) 10 nM 5′-[^{32}P]-labeled G (lanes 1, 2), Gpp (lanes 3, 4), oxoG (lanes 5, 6), oxoGpp (lanes 7, 8), and ppoxoG (lanes 9, 10) primer/template were incubated with (even lanes) or without (odd lanes) 1 unit of Klenow fragment, and the primer extension reaction was performed for 5 min at 37 °C.

DNA template containing either G or oxoG base was used to study the effect of PP and SPP groups on Klenow fragment DNA polymerase I activity (Figure 7A). As shown in Figure 7B, when present in DNA, PP and SPP can be bypassed by DNA polymerase. Elongation of the 5′-[^{32}P]-labeled primer annealed to nonmodified G-template by Klenow fragment

generated full-sized 24-mer product (lane 2), and introduction of the PP group had no effect on polymerization (lane 4). Under the reaction conditions used, the DNA polymerase bypasses efficiently the oxoG template but was slightly blocked in the subsequent extension step after the lesion generating 11-mer fragment (lane 6). Interestingly, the presence of the PP group downstream from 8oxodG creates only a weak termination site for the DNA polymerase. In contrast, the PP group upstream from 8oxodG strongly blocks DNA synthesis 1 base after the lesion (lane 10). These results suggest that interaction of the phosphate group 5′ next to a damaged base with the polymerase active site is critical for translesion synthesis.

4. Conclusion

Molecular simulations described above show that structures of DNA duplexes containing PP and SPP internucleotide groups are extremely close to those of the nonmodified DNA. Atomic level description of structural deviation induced by sugar−phosphate modification is of great interest for more precise determination of single atoms interaction in DNA−protein contacts within the active site and binding regions of an enzyme. The electron density analysis and estimation of charge distribution within the modified DNA moiety permit envisage of the molecular targets for the attack by protein's nucleophile groups. Theoretical studies of both the equilibrium geometry configurations and the transition states[51] may provide direct support to experimental findings of the chemical reactivity of phosphate moiety of DNA.[1−9,52]

Structural analysis of DNA−protein interactions is greatly facilitated by constructing modeled substrate analogues.[53,54] Here, we demonstrated that subtle conformational changes in DNA backbone are critical in the DNA damage recognition process by DNA glycosylases and DNA polymerases. In the present study, in silico modeling combined with biochemical studies identify new determinants of substrate specificity in damaged DNA and in the enzyme.

Abbreviations. NA, nucleic acids; PP group, pyrophosphate group; SPP group, *O*-ethyl-substituted pyrophosphate group; QM/MM, quantum mechanics/molecular mechanics; NBO analysis, natural bond orbital analysis; 8oxodG, 7,8-dihydro-8-oxo-2′-deoxyguanosine; 8oxoG, 7,8-dihydro-8-oxoguanine.

Acknowledgment. The substantial contributions of J. Kress and A. Granovsky to the QM/MM extended PC GAMESS version are greatly acknowledged. This work was supported by the INTAS Young Scientist Fellowship No. 04-83-2888 to M.V.R., by the Russian Federal Science and Innovation Agency State contract 02.442.11.7435 to A.V.B., and by the European Community Grant RISC-RAD FI6R-CT-2003-508842, ARC and EDF (to M.K.S.).

Supporting Information Available: Cartesian coordinates of all optimized structures. This material is available free of charge via the Internet at http://pubs.acs.org.

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