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Sequence effects on energetic and structural properties of phosphorothioate DNA: a molecular modelling study

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ABSTRACT

Phosphorothioate (PS) oligonucleotides constitute a new class of potent drugs, resulting from the replacement of one anionic oxygen of the phosphodiester backbone by one sulphur atom. This replacement confers chirality to the phosphorus atom (PS_S or PS_R) and alters the energetic, structural and biological properties of B-DNA. These properties were assessed by molecular mechanics calculations on a set of regular sequences, $d(YR)_8 \cdot d(YR)_8$ and $d(RR)_8 \cdot d(YY)_8$ (R, purine; Y, pyrimidine). Results indicated: (i) destabilisation of both the PS_R and the PS_S oligomers, the loss of total energy being mainly due to a variation in the electrostatic term; (ii) an additional chirality effect, due to van der Waals and backbone angle energies, larger for PS_S oligomers than for PS_R oligomers; (iii) a clear sequence effect on stability, particularly from the base immediately preceding the PS group. Even though the PS group alters the stability of oligomers, it does not significantly modify the conformation. Altogether, our molecular modelling data parallel the available experimental data. Our results reveal that sequence effects on the energetic properties of PS oligomers are local and additive. Therefore, studies of the set of the 10 unique double-stranded modified dinucleotide steps included in regular oligomers could be used to predict the behaviour of any double-stranded PS-DNA.

INTRODUCTION

Replacement of an anionic oxygen by an anionic sulphur in a DNA phosphate group significantly modifies the properties of the DNA (reviewed in 1). Substitution enhances the resistance to degradation by nucleases (1–3) and in many instances favourably alters the biological activity (4,5). Thus, phosphorothioate (PS) oligonucleotides constitute a new class of potent drugs which can be directed against various diseases, including AIDS (6), hepatitis C (7), thrombotic complications (8) and cancer (9,10). The therapeutic approach relies on the inhibition of transcriptional, traductional or enzymatic activities by specific binding to DNA, mRNA or proteins (11).

Even though substitution of an oxygen atom by a sulphur atom is one of the most conservative, it creates a centre of chirality in the DNA phosphate group (PS_R and PS_S) and induces local changes related to a new charge distribution (12). Yet many aspects of the impact of the substitution and chirality on the overall properties of the double helix remain unclear, particularly as most experimental data concern mixtures of diastereoisomers. The available experimental data performed on stereoregular diastereoisomers reveal that no major structural effect can be attributed to the phosphorothioate group. The only known X-ray crystal structure of a self-complementary DNA concerns the hexamer $d(G_{PS}CG_{PS}CG_{PS}C)$, with PS as the *R* conformer (PS_R) (13). Recent solution studies (14–18) and dynamic calculations (19) essentially devolved to PS_S hybrid duplexes. These show little effects of substitution on overall conformations and are only consistent with an increase in flexibility in the sugar backbone, probably in order to accommodate the particular strains specific to hybrid systems.

The situation is more confused in the case of melting temperature data. For circular viral DNA having phosphate groups partially or completely substituted, no change in thermal stability is observed (20,21). In contrast, for polydeoxyribonucleotides of regularly repeating sequences and also DNA oligomers, thermal stability appears generally lower and seems to depend on both the sequence and the PS chirality (14–15,22–25). Unfortunately, the different conditions used in these experiments often preclude detailed comparisons. A clear feature concerns the dependence of melting temperatures on the PS oligomer sequence, reflecting that $A_{PS}T$ and $G_{PS}C$ steps are always less stable than $T_{PS}A$ and $G_{PS}G$ steps respectively.

Here, we used JUMNA for the molecular modelling of a set of stereoregular PS oligonucleotides. The force field Flex associated with JUMNA has recently been parameterised for PS-DNAs (26). Further, in order to take into account solvation effects, all the stable structure energies were recalculated with the finite difference Poisson–Boltzmann program DelPhi. Six repeated regular B-DNA fragments were selected as PO or PS oligomers. These contain all the possible dinucleotide steps, namely $d(YpR)_8 \cdot d(RpY)_8$ and $d(RpR)_8 \cdot d(YpY)_8$, (where R implies purines and Y pyrimidines). They also constitute a set of phosphodiester model oligomers which has been previously studied

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(27). Energetic and structural features were investigated through systematic substitutions of phosphate oxygens by sulphurs, leading either to chiral PS_R or PS_S configurations.

MATERIALS AND METHODS

All calculations were performed with the JUMNA 10 program (28,29). The force field associated with JUMNA, namely Flex (29), was recently parameterised for PS-DNA (26). JUMNA uses a combination of helical and internal variables to treat the flexibility of the nucleic acids. Single bond torsions and valence angles are used to model the flexibility of each nucleotide, while the nucleotides are positioned in space using helical rotations and translations with respect to a reference system. All bond lengths are kept fixed and the junctions between successive 3'-monophosphate nucleotides, as well as closure of the sugar rings, are ensured using quadratic restraints on the O5'–C5' and C4'–O4' distances. This representation leads to the use of roughly 10 times fewer variables than Cartesian coordinate molecular mechanics and thus speeds up and facilitates the conformational search. Solvent effects were modelled by a simple distance-dependent dielectric function of sigmoidal form. The plateau value of the dielectric reached at long distance and the slope of the sigmoidal segment of the dielectric function were respectively set to 78 and 0.16. Counterion damping was dealt with by a reduction in the phosphate charge to –0.5e. This approach seems to be well adapted to model DNA oligomers for which previous work has shown good agreement with experimental data (30–34).

The present study involved a set of six repeated regular oligomers of B conformation (Table 1). These were chosen because they contain all the possible dinucleotide steps which can be built from the standard bases and also because they constitute a set of phosphodiester model oligomers (PO oligomers) already extensively studied (27). Starting from all the stable substates of the PO oligomers, previously obtained by automatic adiabatic mapping on the sugar phase angles, we carried out calculations on their counterpart PS oligomers. Dinucleotide symmetry was applied to these oligomers. This allows reduction of the number of variables and avoidance of end effects.

Table 1. Dinucleotide steps of studied DNA sequences

DNA sequence	Dinucleotide steps
$d(AA)_8 \cdot d(TT)_8$	AA TT*
$d(GA)_8 \cdot d(TC)_8$	GA AG TC* CT*
$d(GG)_8 \cdot d(CC)_8$	GG CC*
$d(AT)_8 \cdot d(AT)_8$	AT TA
$d(GC)_8 \cdot d(GC)_8$	GC CG
$d(AC)_8 \cdot d(GT)_8$	CA AC TG* GT*

It can be noted that although 16 dinucleotide steps can be formed from the four bases, there are only 10 unique double-stranded dinucleotide steps, since the dinucleotides indicated by stars are simply the paired strands of the unstarred dinucleotides on the same line.

These modifications corresponded to substitutions of either all the phosphate groups of the oligomers or the phosphate groups of one given dinucleotide step. The chirality was taken into account and the phosphorothioate groups were either of *R* (sulphur directed toward the major groove of DNA, PS_R) or *S* (sulphur directed toward the minor groove of DNA, PS_S) configuration, although all the studied PS oligomers were stereoregular. For instance, in $d(AC)_8 \cdot d(GT)_8$ the modifications concerned: (i) all the phosphate groups, all-PS $d(AC)_8 \cdot d(GT)_8$; (ii) the phosphate groups either related to the RpY dinucleotide steps ($A_{PS}C \cdot G_{PS}T$) or to the YpR dinucleotide steps ($C_{PS}A \cdot T_{PS}G$); (iii) the phosphate groups of one strand related to the RpY dinucleotide steps ($A_{PS}C \cdot TG$ and $AC \cdot G_{PS}T$) or to the YpR dinucleotide steps ($TG \cdot C_{PS}A$ and $CA \cdot T_{PS}G$); (iv) the phosphate groups related to either the RpY dinucleotide steps on one strand and to the YpR dinucleotide steps on the other strand ($A_{PS}CA \cdot T_{PS}GC$ and $C_{PS}AC \cdot G_{PS}TG$).

Energetic data were discussed in terms of difference of energy between the PS and the PO oligonucleotide divided by the number of modified phosphate groups. Note that Norm. ΔE_{tot} = [(E_{tot} PS oligonucleotide – E_{tot} PO oligonucleotide)/(number of modified phosphate groups)] and it is thus related to the loss of total energy per phosphate group modified. When different substates remained after minimisation, the energies were averaged.

As the main difference between PO and PS oligonucleotides relies on the charge distribution of the internucleotide linkage the electrostatic properties of the optimised structures were studied using the finite difference Poisson–Boltzmann program DelPhi (35,36) and applied as previously described (37). Since finite difference calculations are computationally too expensive to be included in molecular mechanics procedures, the DelPhi calculations were carried out for static conformations optimised with JUMNA. All DelPhi calculations involved five focusing runs. These runs were performed for 20, 40, 60, 80 and 90% of the box filled. The internal dielectric constant was set to 2 and that external was set to 80. Physiological salt conditions were chosen, with an ionic strength of 0.145 and a Stern layer of 2 Å. The probe sphere radius defining the accessible solvent surface was set to 1.4 Å. The charge of each phosphate group was set to –1e. In order to prevent errors that depend on the position and orientation of the system relative to the grid, for each conformation the calculation was repeated six times with the system rotated by different angles with respect to the grid. The results were then averaged.

Drawings were produced with the Insight II program (Biosym/MSI, San Diego, CA).

The accessibility of the atoms was calculated by rolling a probe sphere of 1.4 Å over the van der Waals surface of the atoms as previously described by Lavery *et al*. (38).

RESULTS AND DISCUSSION

Energetic features of phosphorothiate DNA

Replacement at the internucleotide linkage of an anionic oxygen by an anionic sulphur atom produces a loss of total energy. Globally, the loss of total energy per modified phosphate group (Norm. ΔE_{tot}) lies between 0.1 and 2.4 kcal.mol^{-1} and is related to the base sequence and to the chirality of the phosphorothioate group.

The loss of total energy is local and additive. The phenomenon is local as it is essentially concentrated at the step submitted to the modification. It is additive since the more the oligomer is

modified, the more it loses energy. These two features are summarised in Table 2, which illustrates the loss of total energy per modified phosphate group (Norm ΔE_{tot}) for a series of PS oligonucleotides. The loss is additive at 85% for steps $A_{PS}C$, $G_{PS}T$, $C_{PS}A$ and $T_{PS}G$ and can even reach 100% for other steps.

Table 2. Loss of total energy per modified phosphate group (Norm. ΔE_{tot}) for the oligomers derived from the $d(AC)_8 \cdot d(GT)_8$ oligomer

Modified steps	Norm. ΔE_{tot} (kcal.mol^{-1})	
	R configuration	*S* configuration
None	0	0
$A_{PS}C$, $G_{PS}T$, $C_{PS}A$, $T_{PS}G$	0.85	1.7
$A_{PS}C$, $G_{PS}T$	1.0	1.8
$C_{PS}A$, $T_{PS}G$	0.5	1.2
$A_{PS}C$, $T_{PS}G$	0.9	1.55
$C_{PS}A$, $G_{PS}T$	0.7	1.55
$A_{PS}C$	1.1	2.0
$G_{PS}T$	0.8	1.7
$C_{PS}A$	0.5	1.25
$T_{PS}G$	0.4	0.9

As shown in Table 2, the chirality of the phosphorothioate group affects the total energy. For a sulphur atom in the *R* configuration Norm. ΔE_{tot} lies between 0.1 and 1.7 kcal.mol^{-1}, whereas for a sulphur atom in the *S* configuration Norm. ΔE_{tot} is between 0.8 and 2.4 kcal.mol^{-1}.

The analysis of the total energy in terms of different components first shows that the loss of energy is essentially due to the electrostatic term. It was checked that this electrostatic loss is not produced by simple 1–4 interactions with the sulphur atom. Figure 1 represents Norm. ΔE_{tot} as a function of the loss of electrostatic energy per modified phosphate group (Norm. ΔE_{elec}) for both *R* and *S* stereoregular oligomers. The same ratio of electrostatic energy is lost independently of the configuration of the phosphorothioate group (the slopes of the two regression lines are roughly 0.85 and 0.90). Secondly, the same regression lines make explicit the results shown in Table 2 and indicate clearly that the loss of total energy per modified phosphate group is not the same for the *R* and *S* configurations. The difference results from a loss of van der Waals (Lennard–Jones) energy and the adjustment of some backbone angles in *S* oligomers. The impact of chirality on the total energy, although weaker than the global electrostatic effect of the sulphur atom, is significant and makes PS_R oligomers more stable than PS_S oligomers.

Analysis of energetic data reveals a clear sequence effect. Norm. ΔE_{tot} for the 16 *R* and *S* phosphorothioate dinucleotide steps are reported in Figure 2a. For both the *R* and *S* configurations, the total energy loss depends on the nature of the base pyrimidine or purine 5' of the modified phosphate group. Yet, the effect varies as C < T < G < A for the *R* configuration and as Y < G < A for the *S* configuration. The impact of chirality on 3' residues is more selective. In the case of the *R* configuration only, a secondary 3' phosphorothioate sequence effect (C < T < G < A) was

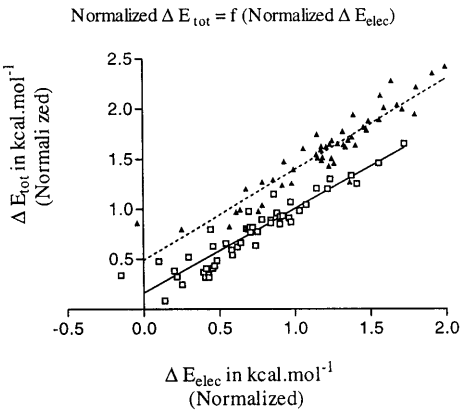

Figure 1. Loss of total energy per modified phosphate group (Norm. ΔE_{tot}) as a function of the loss of electrostatic energy per modified phosphate group (Norm. ΔE_{elec}) for the *R* (square) and *S* (triangle) stereoregular oligomers studied. Linear regression analysis of the energetic data for the *R* oligomers (continuous line: Norm. ΔE_{tot} = 0.85 Norm. ΔE_{elec} + 0.16 with r^2 = 0.90) and *S* oligomers (dashed line: Norm. ΔE_{tot} = 0.90 Norm. ΔE_{elec} + 0.49 with r^2 = 0.88) are also plotted.

detected. All these features are related to the spatial position of the sulphur atom with respect to the base atoms in the DNA grooves. For the *R* configuration, the distances between the sulphur atom and the base atoms either 5' or 3' are approximately equivalent whatever the groove examined and the base position. Thus, in this case the sulphur atom is able to discriminate between the four bases in both the 5' and 3' directions. In contrast, for the *S* configuration the shortest distances occur in the minor groove and concern the 5' position. All the other atoms are found clearly more distant. Thus, the sulphur atom in the *S* configuration is only capable of discriminating between A, G and pyrimidines at the 5' position, in the minor groove.

The Norm. ΔE_{elec} of the *R* and *S* dinucleotide steps are represented in Figure 2b. We observe rather similar sequence effects for the *R* and the *S* configurations, although the *R* conformer does not show discrimination between C and T. This latter point confirms that the Lennard–Jones energy also contributes to loss of the *S* oligomer total energy, probably through an interaction with the methyl group of the thymine.

The data provided by JUMNA showed that the main difference between the PO and PS oligomers rests on loss of electrostatic energy. However, JUMNA calculations do not take into account explicit solvent or counterion interactions. The finite difference Poisson–Boltzmann program DelPhi provides a more realistic approach of electrostatic with solvent/counterion correction and solvation effects. Thus, we decided to study the electrostatic properties of the optimised structures with DelPhi. Data presented in Table 3 show that the Norm. $\Delta E_{elec/DelPhi}$ term does not depend on the chirality of the phosphorothioate group and reveal a 5' phosphorothioate sequence effect. They also confirm the additivity of the phenomenon. Finally, the results obtained from DelPhi parallel those provided by JUMNA.

It seems *a priori* difficult to find any correlation between our energetic data and the thermodynamic stabilities provided by experiments, particularly as the entropic effects are not considered in calculations performed by JUMNA and DelPhi. Typically, DNA duplex stabilities are estimated through melting temperature experiments. Such experiments have been carried out

Table 3. Loss (min–max values) of electrostatic energy per modified phosphate group (Norm. $\Delta E_{elec/DelPhi}$), calculated with the DelPhi program for all the oligomers studied

Dinucleotide step	Norm. $\Delta E_{elec/DelPhi}$[a] (kcal.mol^{-1})	
	R configuration	*S* configuration
$N_{PS}N$	0.2–12.6	0–12.8
$Y_{PS}N$	0.2–7.5	0–7.8
$R_{PS}N$	7.0–12.6	6.5–12.8

with PS oligomers and results have shown that phosphorothioate substitution lowers the T_m of duplexes (14,15,18,22–25). Moreover, despite their limited number, the results reflect a clear sequence effect. The latter is illustrated by the analysis of repeating polymers poly(dA·dT) and poly(dG·dC) carrying selective sequence substitutions by PS_S and PS_R, which reveals altered thermal denaturation midpoints dramatically dependent on the substituted dinucleotide sequence (22). Similar conclusions are drawn from the stereoregular hexamers $d[G_{PS}C(GC)_3G_{PS}C]$ and $d[C_{PS}G(CG)_3C_{PS}G]$ (23). The overall experimental order of stability was $G_{PS}C < C_{PS}G$ and $A_{PS}T < T_{PS}A$, which is similar to that provided by our calculation energies. Moreover, the properties of various duplexes of repeating di-, tri- and tetramers, namely PS_S-DNA·PS_S-DNA, PS_S-DNA·DNA and PS_S-DNA·RNA, were recently studied by circular dichroism by Clark *et al*. (14) and Hashem *et al*. (15). For all these duplexes, either in B-DNA or heteronomous conformations, analysis of melting profiles revealed both a cumulative reduced stability imparted by the phosphorothiate modification and a sequence effect. For example, Hashem *et al*. (15) have observed an order of stabilities in the hybrid structures using two different types of repeating sequences: PS_S-$d(GT)_{12}$·$r(AC)_{12}$ > PS_S-$d(CT)_{12}$·$r(AG)_{12}$ > PS_S-$d(AC)_{12}$·$r(GU)_{12}$ > PS_S-$d(AG)_{12}$·$r(UC)_{12}$. By simple summation of our modified dinucleotide we retrieve the same order with the corresponding DNA–DNA sequences: $PS_S(GT)$·(AC) > $PS_S(CT)$·(AG) > $PS_S(AC)$·(GT) > $PS_S(AG)$·(CT).

A thermodynamic analysis (14) led to the conclusion that the general destabilising effect of the substitution arises from a difference in the entropy upon forming the DNA–DNA duplexes and in the enthalpy upon forming the DNA–RNA duplexes. Although our calculations obviously do not consider the energetic features associated with the complete double-strand/single-strand transitions of DNA, they (i) reveal a general decrease in enthalpy for PS-DNA with respect to PO-DNA; and (ii) underline the presence of a large sequence effect on relative PS oligomer stabilities. The ordering of enthalpies provided by calculations on the various modified sequences rather parallels those derived from experimental T_m values (14,15), suggesting that enthalpy could also participate in the T_m reduction of modified complexes. Our data are, furthermore, able to predict a general effect due to the nature of the 5'-base.

Structural effect of phosphorothioate

From a structural point of view, the phosphorothioate group of PS_S is directed towards the minor groove and forms a crown around the double helix, as shown in the view along the DNA helix axis in Figure 3b. In contrast, the phosphorothioate group of PS is directed towards the major groove and is contained within the DNA helix (Fig. 3c). In this respect it resembles more the PO oligomer (Fig. 3a) than its counterpart PS_S. However, the root mean square deviations (r.m.s.d., PS oligomers compared with the corresponding PO oligomers) display approximately the same values (Table 4) for PS_R and PS_S. r.m.s.d. calculations were performed on the three central base pairs with respect to the symmetry conditions imposed during minimisations. This approach allowed us to take into account

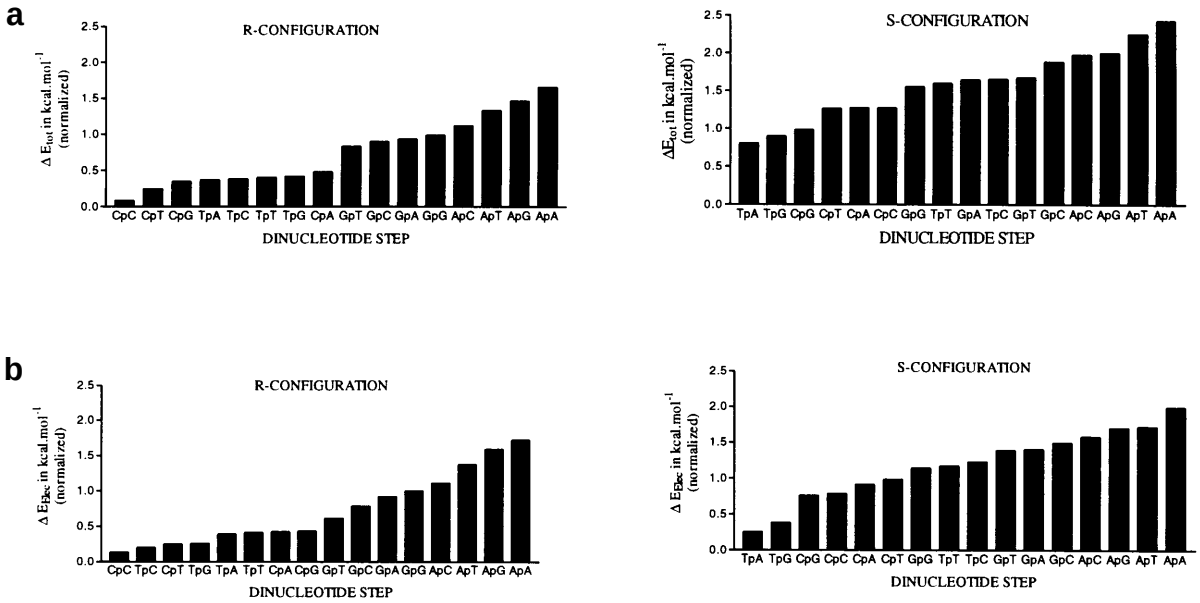

Figure 2. (**a**) Loss of total energy per modified phosphate group (Norm. ΔE_{tot}) for the 16 *R* and *S* dinucleotide steps studied. (**b**) Loss of electostatic energy per modified phosphate group (Norm. ΔE_{elec}) for the 16 *R* and *S* dinucleotide steps studied.

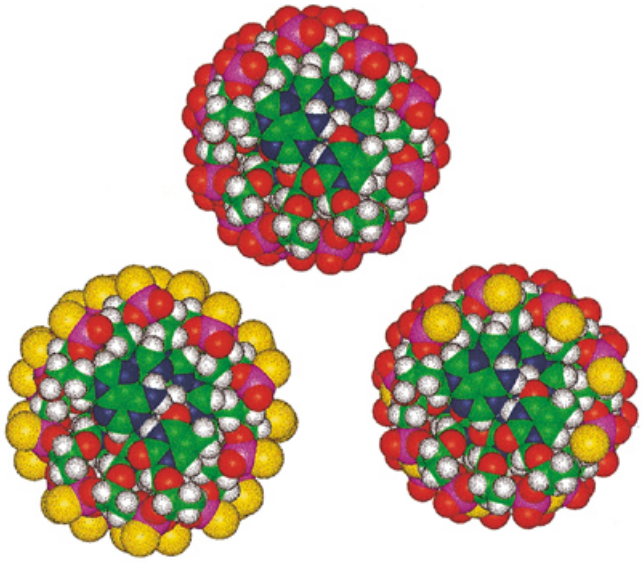

Figure 3. Views along the $d(AA)_8 \cdot d(TT)_8$ helix axis. (**a**) PO oligomer. (**b**) PS_S oligomer. (**c**) PS_R oligomer. Sulphur atoms are in yellow, oxygen atoms in red.

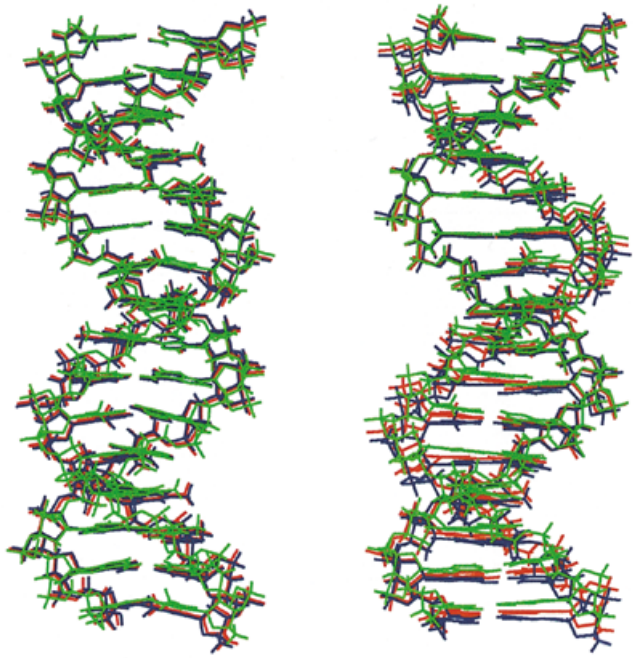

Figure 4. Superposition of unmodified (red), PS_R (green) and PS_R (blue) oligomers for $d(AC)_8 \cdot d(GT)_8$ (left) and $d(AA)_8 \cdot d(TT)_8$ (right).

the effects of the phosphorothioate groups on the neighbouring steps. $r.m.s.d._{max}$ values clearly indicated that the phosphorothioate group does not induce significant structural change. All the helical α parameters and the depth and the width of the grooves of PS oligomers are the same as in PO oligomers. This is illustrated in Figure 4, which shows the superposition of All-PS $d(AA)_8 \cdot d(TT)_8$ and All-PS $d(AC)_8 \cdot d(GT)_8$ with their corresponding PO sequences.

Table 4. Maximal values of r.m.s.d. of the PS oligomers with respect to the PO oligomers

Sequence	$r.m.s.d._{max}$ (Å)	
	R configuration	*S* configuration
ATA·TAT	0.1	0.5
GCG·CGC	0.1	0.1
ACA·TCT	0.5	0.2
GAG·CTC	0.2	0.3
GGG·CCC	0.5	0.2
AAA·TTT	0.1	0.1

r.m.s.d. were calculated for the *R* and *S* configurations on the three central base pairs and for all types of substitutions ($R_{PS}Y$, $Y_{PS}R$, $Y_{PS}Y$ and $R_{PS}R$).

Calculated structures are similar to the unique available crystal structure, i.e. PS-DNA (13). They are also consistent with the spectroscopic results of PS oligomers in solution, which reflect only little modification with respect to PO oligomers (22–24,39).

As the steric accessibility of atoms, related to the overall structure of the oligomers, is likely to be an important factor for biochemical activity, we further calculated the accessible surface for hydrogen bonding and non-specific backbone atoms. As expected, the accessibility of the hydrogen bond donor and acceptor atoms within the grooves varies only slightly in PS oligomers. The van der Waals radius of sulphur atoms is larger compared to oxygen (1.8 versus 1.5 Å), but the sulphur atom is distant enough from the other atoms in the grooves and is unable to disturb their reactivity. In contrast, the non-specific accessible surfaces (i.e. surfaces related to anionic oxygens or sulphurs) vary significantly, as illustrated in Table 5. The ionic sulphur accessible areas are approximately twice as large as the ionic oxygen areas, although the total sphere area of the sulphur is only 1.2 larger compared to the total sphere area of the oxygen. Therefore, in PS oligomers the non-specific surfaces are markedly increased with respect to the specific surfaces.

Table 5. Maximal and minimal values of anionic oxygen and sulphur accessibilities

Atom	Accessibility (Å^2)	
	Minimum	Maximum
O1P	23	32
S1P (R)	45	60
O2P	34	38
S2P (S)	64	69

Accessibilities were calculated for the *R* and *S* configurations and for all types of substitutions ($R_{PS}Y$, $Y_{PS}R$, $Y_{PS}Y$ and $R_{PS}R$).

CONCLUSION

Molecular modelling was used systematically to study the energetic and structural properties of stereoregular PS oligomers that correspond to representative regular B-DNA sequences previously studied as PO oligomers. Calculations performed with both JUMNA and DelPhi revealed that the energetics of PS oligomers differ considerably from the energetics of unmodified PO oligomers. An overall reduced stability is induced by the phosphorothioate alteration, but the *S* conformer systematically leads to a more destabilising effect than the *R* conformer. The energy was analysed in terms of electrostatic, Lennard–Jones, dihedral angle and valence angle components. Results indicated that, whatever the sequence and the conformer considered, the electrostatic component was always responsible for the loss of total energy, although a minor participation of other energetic components was detected in the case of the *S* conformer. Altogether, the destabilising effect was found to be local and additive.

Furthermore, the type of base at the 5' position of the modified PS step appears linked to duplex stability, the loss of energy always being more prominent for a 5' purine base. For PS_R oligomers the 3' purine base also exerts an effect, this being due to the fact that in these oligomers the sulphur atom points towards the major groove and is submitted to the influence of all the base atoms located either in the 5' or 3' position. However, the PS groups do not alter the structural parameters of the double helix whatever the *S* conformer considered.

Globally, experimental data are in accord with our results. Here, we have extended the knowledge on the relative stabilities of PS-DNA and on the related sequence effects, through the systematic study of six stereoregular modified repeating oligomers which contained the 10 unique dinucleotides sequences. Thus, we hope that this theoretical investigation will help to improve the rational design of antisense DNAs and our understanding of the stereo-selective cleavage of PS oligomers (PS_S and PS_R oligomers) by either 3'-exonucleases (1,2,40) or endonucleases (P1 endonuclease for instance) (1,41) or even hammerhead ribozymes (42).

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