Theor Chem Acc (1999) 101:269–273
DOI 10.1007/s00214980m153

Theoretical
Chemistry Accounts

Regular article

Determination of a set of parameters for the molecular modelling of phosphorothioate DNA

H.O. Bertrand[1], **A. Pullman**[2], **K. Zakrzewska**[2], **B. Hartmann**[2], **S. Fermandjian**[1]

[1] Département de Biologie Structurale, UMR CNRS 1772, Institut Gustave Roussy, 39 rue Camille Desmoulins, F-94805 Villejuif Cedex, France
[2] Laboratoire de Biochimie Théorique, UPR CNRS 9080, Institut de Biologie Physico-Chimique, 13 rue Pierre et Marie Curie, F-75005 Paris, France

Received: 15 July 1998 / Accepted: 8 September 1998 / Published online: 10 December 1998

Abstract. Phosphorothioate DNAs, have emerged as a new class of potent drugs. They are obtained by the replacement of the anionic oxygens of the phophodiester backbone by sulphur. A set of parameters has been developed for the FLEX force field implemented in JUMNA 10.0 to take into account the influence of sulphur on the structure of the DNA double helix. The consistency of our parameters was tested by modelling a phosphorothioate oligomer namely d(GC)8. d(GC)8. Results, obtained on both R-p_S and S-p_S diastereoisomers, were compared to the phosphodiester counterpart and are in agreement with available experimental data. Thus, our set of parameters seems suitable for further molecular modelling of other phosphorothioate oligomers.

Key words: Phosphorothioate DNA – Force field – Molecular mechanics

1 Introduction

The aim of this work was to develop a set of parameters for the molecular modelling of phosphorothioate oligonucleotides (PS-oligonucleotides). The simple replacement in the phosphate group (PO-oligonucleotides) of an anionic oxygen by an anionic sulphur (Fig. 1) not only enhances the resistance to degradation by nucleases [1] but may also, in many instances, favourably alter the biological activity [2, 3]. PS-oligonucleotides constitute a new class of potent drugs directed against various diseases such as AIDS [4], hepatitis C [5], thrombotic complications [6] and cancer [7, 8] and are currently undergoing clinical trials [2, 3]. The therapeutic approach relies on the inhibition of transcriptional, transductional or enzymatic activities by specific binding to DNA, mRNA or proteins.

From a structural point of view, the substitution, in the phosphate group, of an oxygen atom by a sulphur atom introduces a centre of chirality but also may induce local changes of charge distribution, of steric hindrance and flexibility at the internucleotide linkage (Fig. 1). The impact of the substitution and chirality on the overall properties of the double helix remains unclear. So far, experimental data describing the physicochemical properties of PS-oligonucleotides correlated to their biological activity are scarce and most concern mixtures of diastereoisomers. The main reason is that the synthesis of R- and S- stereoregular diastereoisomers is difficult to carry out. Molecular modelling is a convenient way for studying the fine structure of stereoregular PS-oligonucleotides. Yet, to our knowledge, no parameters related to an anionic sulphur bound to phosphorus are available in the force fields used for the modelling of nucleic acids, namely AMBER 94 [9], FLEX [10] or CHARMM [11].

2 Parameterization

The dimethyl phosphorothioate anion (DMPS) was chosen as a model compound for the parameterization of the phosphorothioate linkage because (1) it simulates the phosphorothioate fragment of DNA and (2) a similar model was used previously for the parameterization of the phosphate group (dimethyl phosphate anion: DMP) (Fig. 2).

The set of parameters was developed for the FLEX force field implemented in the molecular mechanics software JUMNA 10 [10]. JUMNA has been extensively used to model DNA oligomers [inter alia 12–15]. The energy formulation of JUMNA requires the multiplicities and torsion barriers of the backbone dihedral angles ζ and α (Fig. 1), the Lennard-Jones parameters for the anionic sulphur atom and the charge distribution over the phosphorothioate linkage. Geometrical parameters,

Correspondence to: K. Zakrzewska

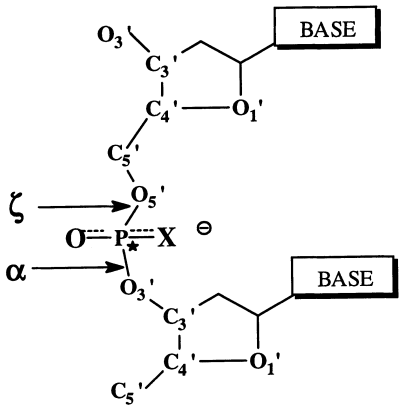

Fig. 1. Atomic representation of a fragment of a polydeoxyribonucleotide chain. X=O implies phosphodiester DNA, X=S implies phosphorothioate DNA

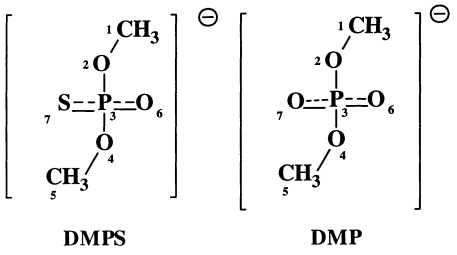

Fig. 2. Model compounds chosen for the parameterization: dimethyl phosphorothioate anion (*DMPS*), dimethyl phosphate anion (*DMP*)

namely the anionic S—P bond length, the anionic O—P bond length and the anionic S—P -anionic O valence angle were set to 1.97 Å, 1.48 Å and 122°, respectively [16].

The multiplicities and torsion barriers of the backbone dihedral angle ζ and α were obtained by simultaneous conformational mapping over the dihedral angles (C_1—O_2—P_3—O_4) and (O_2—P_3—O_4—C_5) of DMPS (Fig. 2). Calculations were carried out with the Discover 3.00 module (Insight II package [17]) using the CFF91 [17] force field which contains parameters for sulphur partially doubly bonded to phosphorus. Results showed that the multiplicity and torsion barriers are the same whether the anion considered is DMPS or DMP. The PS-oligonucleotide values for ζ and α were therefore kept the same as those used for the PO-oligonucleotides (namely 2 for multiplicity and 1 kcal/mol for torsion barrier [10]).

The Lennard-Jones parameters were determined by comparing the oxygen atom classes defined in FLEX [10] (ether —O—, carbonyl =O, anionic —O^-) with the equivalent classes in CFF91 [17] by plotting the Lennard-Jones energy as a function of distance between the pairs of atoms. As the differences between FLEX and CFF91 were systematic, CFF91 Lennard-Jones parameters for anionic sulphur were fitted to be consistent with FLEX. Table 1 contains Lennard-Jones parmeters for sulphur given as equilibrium energies (Eeq) and distances (Req). A selection of Lennard-Jones energies as a function of distance and force field is presented in Fig. 3.

Table 1. Lennard-Jones parameters for sulphur given as equilibrium distances (Req) and energies (Eeq)

Atomic class	Req with anionic sulphur (—S^-) in Å	Eeq with anionic sulphur (—S^-) in kcal/mol
H-aliphatic	2.98	−0.07
H-aromatic	2.71	−0.13
C-tetrahedral	3.63	−0.06
C-trigonal	3.58	−0.08
—NH_2	3.57	−0.07
>NH	3.51	−0.09
—N=	3.48	−0.11
—O—	3.28	−0.10
=O	3.26	−0.14
—O^-	3.26	−0.12
P	3.63	−0.22
—S^-	3.72	−0.09

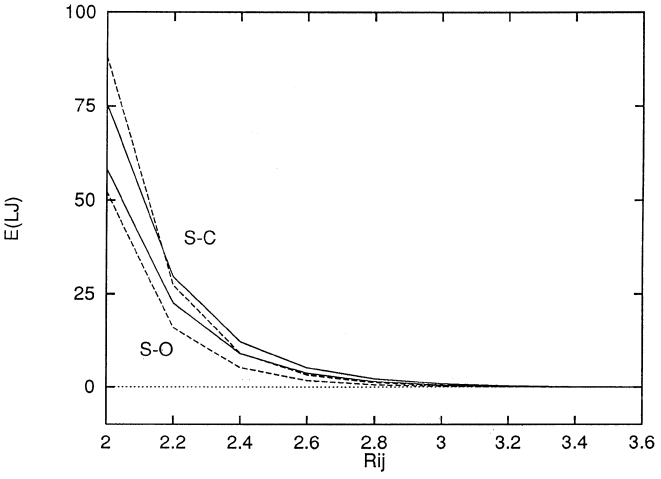

Fig. 3. Lennard-Jones energies, E(LJ) in kcal/mol, as a function of the distances between atoms (Rij, in Å) for sulphur and carbonyl oxygen (S—O), sulphur and *sp*3 carbon (S—C). *Solid lines*: CFF91 force field, *dashed lines*: FLEX force field

The charge distribution of the DMPS was calculated according to a Hückel-Del Re procedure previously parameterized for phosphodiester nucleic acids [18]. This methodology consists of a combination of Hückel [19] and Del Re [20] calculations to treat the π and σ electrons of a molecule, respectively. Thus, the partial charge of an atom i is described as the sum of the σ partial charge and the π partial charge of this atom. The parameters that must therefore be determined are Δ, Γ, for the π calculation and δ, η, γ for the σ calculation. Hückel-Del Re parameters for the anionic sulphur partially doubly bonded to phosphorus were fitted to give atomic monopoles which reproduce the ab initio electrostatic potential over an accessible surface of DMPS (1.6 Å for the probe sphere radius [18]) (Table 2). Electrostatic potential derived charges [18] were chosen instead of Mulliken charges since the latter are unable to reproduce the electrostatic potential on the accessible surface of the molecule. Calculations were performed

Table 2. Huckel-Del Re parameters for sulphur

Hückel-Del Re parameters for sulphur	Conformation 1[a] Basis set 1[c]	Conformation 2[b] Basis set 1	Conformation 1[a] Basis set 2[d]	Conformation 2[b] Basis set 2[d]
Δ	0.85	0.85	0.86	0.88
δ	0.18	0.19	0.21	0.21
Γ	0.37	0.37	0.37	0.37
η	0.32	0.32	0.33	0.33
γ	0.00	0.00	0.00	0.00
Error[e]	0.80	0.67	1.06	1.02

[a] Conformation 1: (C_1—O_2—P_3—O_4) and (O_2—P_3—O_4—C_5) dihedral angles of DMPS were set to 75°
[b] Conformation 2: (C_1—O_2—P_3—O_4) and (O_2—P_3—O_4—C_5) dihedral angles of DMPS were set to −90° and −50°, respectively
[c] Basis set 1: P, S: 10*s*/6*p*/1*d*; C, O: 7*s*/3*p* ; H: 3*s* (ζ_H = 1.2) contracted to minimal
[d] Basis set 2: P: 10*s*/6*p*/1*d*; S: 10*s*/6*p*; C, O: 7*s*/ 3*p*; H: 3*s* (ζ_H = 1.2) contracted to minimal
[e] Error on the potential in %

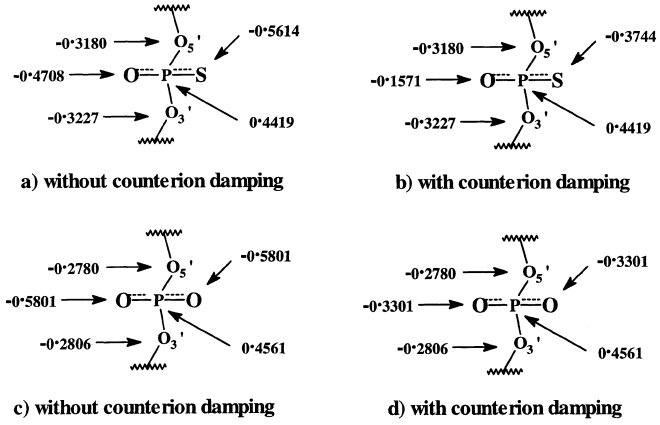

Fig. 4. Charge distribution (in e) for the phosphorothioate and the phosphate groups, with and without counterion damping

with GAUSSIAN 94 [21] using two ab initio basis sets [22] and two DMPS conformations (Table 2). Ab initio basis sets contracted to a minimum were chosen to be consistent with the previous parameterization [18].

The results obtained with the two basis sets are very similar. The results with the first basis set (including *d* orbitals for both P and S) were chosen for future calculations (Table 2). Δ, δ, Γ, η, γ were set to 0.85, 0.19, 0.37, 0.32 and 0.00 respectively. These parameters, obtained from the model, were then used to calculate the charge rearrangement occurring upon the linkage of the phosphorothioate group to the sugar moiety [18]. The charge distribution of the phosphorothioate group is given in Figure 4a. The partial charge of sulphur (−0.5614 e) is more negative than the partial charge of the equivalent oxygen (−0.4708 e). This point seems apparently contradictory if one considers the electronegativity values of oxygen and sulphur 3.5 and 2.5, respectively, in the Pauling scale. However, ^{17}O, ^{18}O and ^{31}P NMR chemical shifts, the vibrational spectra and dissociation constants of phosphoric and thiophosphoric acids provide evidence for a P—S bond order of 1 and a negative charge localized on sulphur for the monoanionic species [23].

Within the FLEX force field, solvent effects are modelled by a simple distance-dependent dielectric function of sigmoidal form and counterion damping is dealt with by a reduction of the total charge of the phosphate group to −0.5 e [18]. Thus, for PO-oligonucleotides, each anionic oxygen is equally dampened and displays a charge of −0.3301 e instead of a charge −0.5801 e (Figs. 4c and 4d). Based on counterion distributions around the molecule, calculated with the finite difference Poisson-Boltzmann program DELPHI [24,25], the partial charges of the anionic oxygen and of the anionic sulphur were differentially dampened and reduced to −0.1571 e and −0.3744 e, respectively (Figs. 4a and 4b). Delphi calculations were performed at physiological salt concentrations (ionic strength of 0.145) with a stern layer of 2 Å. The probe sphere radius defining the accessible solvent surface was set to 1.4 Å.

3 Molecular modelling of phosphorothioate oligomers

The above parameters were implemented in the FLEX force field and applied to the study of a model hexadecamer d(GC)$_8$. d(GC)$_8$. Calculations were performed with JUMNA 10 which uses a combination of helical and internal variables to treat the flexibility of the nucleic acids. This approach leads to 10 times fewer variables than cartesian-coordinate molecular mechanics. In order to locate the stable B- and A-family conformations, the conformational space was explored by scanning the sugar phase angles from −20° to 200°. The oligomer was first studied as a PO-oligonucleotide and then changed into a PS-oligonucleotide. For each modification, the chirality was taken into account and phosphorus atoms were either of R-configuration (sulphur directed toward the major groove of DNA) or of S-configuration (sulphur directed toward the minor groove of DNA). The substitutions were performed at: (1) all the phosphate groups (All-R$_{pS}$ d(GC)$_8$. d(GC)$_8$, All-S$_{pS}$ d(GC)$_8$. d(GC)$_8$); (2) the phosphate groups related to the GpC dinucleotide steps (R-Gp$_s$C; S-Gp$_s$C) and (3) the phosphate groups related to the CpG dinucleotide steps (R-Cp$_s$G; S-Cp$_s$G). Energetic and structural results, for the A- and B-families are given in Tables 3 and 4, respectively.

Analysis of energetic data showed that the more the oligomer is substituted by sulphur, the more it looses

Table 3. Energetic and structural results of the modelling of the B-family phosphorothioate oligomers

Oligomers B-form	$\Delta E^{a}_{tot.}$ in kcal/mol	Normalized[b] $\Delta E_{tot.}$ in kcal/mol	RMSD[c] in Å
PO-d(GC)$_8$. d(GC)$_8$	0.0	0.0	0.0
All-R$_{pS}$ d(GC)$_8$. d(GC)$_8$	20.0	0.7	0.3
All-S$_{pS}$ d(GC)$_8$. d(GC)$_8$	45.6	1.5	0.7
R-Gp$_s$C	14.4	0.9	0.4
S-Gp$_s$C	30.1	1.9	0.3
R-Cp$_s$G	4.9	0.4	0.1
S-Cp$_s$G	13.7	1	0.7

[a] $\Delta E_{tot.}$ = $E_{tot.}$ PS-oligomer − $E_{tot.}$ PO-oligomer [b] Normalized $\Delta E_{tot.}$ = $\Delta E_{tot.}$/(number of modified phosphate groups) [c] Root mean square deviations (RMSDs) were calculated with respect to the PO-oligomer for the overall structure

Table 4. Energetic and structural results of the modelling of the A-family phosphorothioate oligomers

Oligomers A-form	$\Delta E^{a}_{tot.}$ in kcal/mol	Normalized[b] $\Delta E_{tot.}$ in kcal/mol	RMSD[c] in Å
PO-d(GC)$_8$. d(GC)$_8$	0.0	0.0	0.00
All-R$_{pS}$ d(GC)$_8$. d(GC)$_8$	29.7	1.0	0.9
All-S$_{pS}$ d(GC)$_8$. d(GC)$_8$	15.1	0.5	1.3
R-Gp$_s$C	21.4	1.3	0.6
S-Gp$_s$C	14.2	0.9	0.8
R-Cp$_s$G	8.1	0.6	0.3
S-Cp$_s$G	1.5	0.1	0.9

[a] $\Delta E_{tot.}$ = $E_{tot.}$ PS-oligomer − $E_{tot.}$ PO-oligomer [b] Normalized $\Delta E_{tot.}$ = $\Delta E_{tot.}$/(number of modified phosphate groups) [c] RMSDs were calculated with respect to the PO-oligomer for the overall structure

energy, reflecting an additive effect. Moreover, the loss of energy is essentially concentrated at the dinucleotide step where the modification occurs.

Within each family, the replacement of an oxygen atom by a sulphur atom produces a loss of total energy that depends on the nature of dinucleotide step modified (normalized DE_{tot}. Cp_SG < normalized DE_{tot}. Gp_SC). These theoretical results are in line with several experimental data which have shown a clear sequence effect. The influence of the nature of the dinucleotide step modified (i.e. pyrimidine-p$_S$-purine or purine-p$_S$-pyrimidine) was reported earlier on the grounds of melting temperature measurements [26–28]. Experiments which were carried out on d(GC)$_4$. d(GC)$_4$ and d(CG)$_4$. d(CG)$_4$ PS-oligomers have shown less thermal destabilization for the Cp$_s$G dinucleotide step than for the Gp$_s$C step. Similar results were obtained by Eckstein et al. for PS-poly[d(AT)] [27] and PS-poly[d(GC)] [28].

The influence of the phosphorothioate configuration is more complicated. Within the B-structures, the R-conformers are more stable than the S-conformers whereas the reverse order is obtained for the A-structures. This particular result is in line with recent melting temperature measurements on a set of DNA and DNA/RNA duplexes [29]. For PS-DNA or PS-DNA/RNA, with the DNA strand in B- or B-like conformation, the helix stability was found higher for the R-diastereoisomers, whereas for DNA/PS-RNA, with the RNA strand in A-conformation, the helix stability was found higher for S-isomers.

From a structural point of view, our results also suggests that the double helix is altered very little, especially for the B-family since the maximum root mean square deviation between the PO-oligomer and the PS-oligomers studied is 0.7 Å (Tables 3, 4). This result is also in agreement with CD spectra [30] and with the only double strand uncomplexed phosphorothioate DNA structure, resolved so far by crystallography [31].

4 Conclusion

The parameterization we have developed for phosphorothioate DNAs appears to give results in good agreement with known experimental data and should provide a useful basis for studying the conformational properties of these therapeutically promising molecules.

Acknowledgements. H.O. Bertrand wishes to thank the Agence Nationale de Recherches sur le SIDA (ANRS, Paris, France) for their support. The authors also wish to thank R. Savinelli for technical assistance with the CRAY (IDRIS) and C. Etchebest and R. Lavery for helpful discussions.

References

1. Cummins L, Owens S, Risen LM, Lesnik EA, Freier M, McGee D, Guinisso CJ, Cook PD (1995) Nucleic Acids Res 23:2019
2. Matteucci MD, Wagner RW (1996) Nature 384:20
3. Agrawal S (1996) Trends Biotechnol 14:376
4. Tsukahara S, Suzuki J, Hiratou T, Takai K, Kyanagi Y, Yamamoto N, Takakou H (1997) Biochem Biophys Res Commun 233:742
5. Alt M, Renz R, Hofschneider PH, Paumgartner G, Caselmann WH (1995) Hepatology 22:707
6. Stec WJ, Cierniewski CS, Okruszek A, Kobylanska A, Pawlowska Z, Kozolkiewicz M, Pluskota E, Maciaszek A, Rebowska B, Stiasiak M (1997) Antisense Nucleic Acids Drug Dev 7:567
7. Geary RS, Leeds JM, Henry SP, Monteith DK, Levin AA (1997) Anticancer Drug Des 12:383
8. Henry SP, Monteith DK, Levin AA (1997) Anticancer Drug Des 12:395
9. Cornell WD, Cieplak P, Bayly CI, Gould IR, Merz KM, Ferguson DM, Spellmeyer DC, Fox T, Caldwell JW, Kollman PA (1995) J Am Chem Soc 117:5179
10. Lavery R, Zakrzewska K, Sklenar H (1995) Comput Phys Commun 91:135
11. McKerell AD Jr, Wiorkiewicz-Kuczera J, Karplus M (1995) J Am Chem Soc 117:11946
12. Lefebvre A, Mauffret O, Lescot E, Hartmann B, Fermandjian S (1995) Biochemistry 34:12019
13. Lefebvre A, Mauffret O, Lescot E, Hartmann B, Fermandjian S (1996) Biochemistry 35:12560
14. Bertrand HO, Ha-Duong T, Fermandjian S, Hartmann B (1998) Nucleic Acids Res 26:1261
15. Flatters D, Zakrzewska K, Lavery R (1997) J Comput Chem 18:1043
16. Steinke CA, Reeves KK, Powell JW, Lee SA, Chen YZ, Wyrzykiewicz T, Griffey RH, Mohan V (1997) J Biomol Struct Dyn 14:509

17. Biosym/MSI, Insight II, 9682 Scranton Road, San Diego, Calif.
18. Lavery R, Zakrzewska K, Pullman A (1984) J Comput Chem 5:363
19. Hückel EP (1932) Z Phys 76:628
20. Del Re G (1958) J Chem Soc 4:4031
21. Frisch MJ, Frisch A, Foresman JB (1994) Gaussian 94, Gaussian Inc., Pittsburgh Pa., USA
22. Pullman A, Berthod H (1981) Chem Phys Lett 81:195
23. Frey E, Sammons RD (1985) Science 228:541
24. Gilson MK, Sharp KA, Honig B (1987) J Comput Chem 9:327
25. Friedman RA, Honig B (1992) Biopolymers 32:145
26. Cosstick R, Eckstein F (1985) Biochemistry 24:3630
27. Eckstein F, Jovin TM (1983) Biochemistry 22:4546
28. Jovin TM, van de Sande JH, Zarling DA, Arndt-Jovin DJ, Eckstein F, Fuldner HH, Greider C, Greider I, Hamamori E, Kalisch B, McIntosh L, Robert-Nicoud M (1983) Cold Spring Harbor Symp Quant Biol 47:143.
29. Kanehara H, Nishi M, Makino K (1995) Nucleic Acids Symp 34:53
30. Clark CL, Cecil PK, Singh D, Gray DM (1987) Nucleic Acids Res 25:4098
31. Cruse WB, Salisbury SA, Brown T, Cosstick R, Eckstein F, Kennard O (1986) J Mol Biol 192:891