Biochimica et Biophysica Acta 1840 (2014) 1163–1170

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagen

Polyaminooligonucleotide: NMR structure of duplex DNA containing a nucleoside with spermine residue, N-[4,9,13-triazatridecan-1-yl]-2′-deoxycytidine

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ARTICLE INFO

Article history:
Received 2 October 2013
Received in revised form 26 November 2013
Accepted 16 December 2013
Available online 20 December 2013

Keywords:
Spermine
Polyamine
Nucleic acid structure
Polyaminooligonucleotide
Nuclear magnetic resonance spectroscopy

ABSTRACT

Background: The nature of the polyamine–DNA interactions at a molecular level is not clearly understood.
Methods: In order to shed light on the binding preferences of polyamine with nucleic acids, the NMR solution structure of the DNA duplex containing covalently bound spermine was determined.
Results: The structure of 4-N-[4,9,13-triazatridecan-1-yl]-2′-deoxycytidine (dCSp) modified duplex was compared to the structure of the reference duplex. Both duplexes are regular right-handed helices with all attributes of the B-DNA form. The spermine chain which is located in a major groove and points toward the 3′ end of the modified strand does not perturb the DNA structure.
Conclusion: In our study the charged polyamine alkyl chain was found to interact with the DNA surface. In the majority of converged structures we identified the presumed hydrogen bonding interactions between O6 and N7 atoms of G4 and the first internal $-NH2^{+}-$ amino group. Additional interaction was found between the second internal $-NH2^{+}-$ amino group and the oxygen atom of the phosphate of C3 residue.
General significance: The knowledge of the location and nature of a structure-specific binding site for spermine in DNA should be valuable in understanding gene expression and in the design of new therapeutic drugs.

1. Introduction

Polyamines are widely distributed in nature and known to be required to support a variety of cellular functions [1]. They are also known to have high binding affinity for negatively charged nucleic acids. Positive charges of the cationic molecules have been shown to improve nuclease resistance, facilitate the formation of secondary and tertiary structures of oligonucleotides and their cellular uptake and therefore represent an interesting class of modifications in nucleic acid technology [2–6]. Since the therapeutic applications of modified oligonucleotides as antisense and antigene agents rely on effective cellular uptake, polyamines and polyamine–oligonucleotide conjugates could be exploited to improve cell permeability [7–16]. Moreover, some properties of the spermine-conjugated oligonucleotides, such as thermodynamic properties and susceptibility of RNA targets for the RNaseH mediated cleavage within complementary heteroduplexes have been also reported [17]. Although the biological function of polyamines and their bioconjugates were the subject of many studies, the exact mechanism of action of polyamines in the cell is still not fully understood. The binding of polyamines to DNA is unquestionable, but there is a discussion concerning the specificity of their interaction and the influence on the structure of DNA [18]. There have been numerous studies attempting to determine these interactions including NMR and Raman spectroscopy, X-ray crystallography and molecular modeling. Polyamines can interact via water molecules, by hydrogen bonding with polar functional groups or with the hydrophobic surfaces of DNA bases by van der Waals forces. Many studies have suggested that polyamines have higher affinity for A- and Z-form [19] than for classical B-DNA. However, the oligodeoxyribonucleotides employed in crystal structure analyses were usually rich in deoxyguanosine and deoxycytidine residues, which could tend to bias crystallization toward either the A or Z conformation. On the other hand, high affinity of polyamines to non-classical forms of DNA may suggest that both sequence and secondary structure of DNA are crucial in polyamine recognition. Till now researchers paid their attention mainly to complexes of polyamines with nucleic acids in order to ascertain the specificity of their interactions [20–28]. However, up till now there were no studies revealing the specificity of interaction between polyaminooligonucleotides and target DNA or RNA. Previous structural studies on the influence of covalently bound polyamine to nucleic acids concerned only simple aminopropyl and aminohexyl modifications [29–32]. The tether cationic chains of these bioconjugates were oriented toward the major groove in the 3′-direction from the modification site. Many factors, such as hydrogen bonding, van der Waals interactions, electrostatic repulsion of phosphate groups and the number of bound water molecules determine the conformation and stability of DNA. Any modification can induce structural changes of

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http://dx.doi.org/10.1016/j.bbagen.2013.12.008

DNA, but the presence of nucleotide residue modified with the cationic chain can affect the structure of nucleic acids primarily by neutralization of phosphate negative charges. The distance between amino groups separated by three and four carbon atoms in spermine is very close to the distance between phosphate anions in DNA backbone, making polyamine–oligonucleotide conjugates perfect compounds for creating zwitterionic structures. Oligonucleotides possessing cationic functional groups have been shown to exhibit promising features facilitating a cellular uptake of DNA in gene therapeutic applications [33]. Therefore we were interested to find out the factors responsible for the interaction between tethered spermine and the DNA molecule. In our opinion, the analysis of such structures could provide important knowledge essential for understanding the physiological role of polyamines and specifying interactions between polyamine and nucleic acid.

The objective of the present work was to explore the interaction between spermine-modified deoxycytidine (4-*N*-[4,9,13-triazatridecan-1-yl]-2′-deoxycytidine, dCSp) and DNA. In order to get insight into the structural changes induced by the presence of dCSp residue we determined the solution structure of two DNA duplexes, 5′-GTCGGCTG-3′/5′-CAGCCGAC-3′ and 5′-GTCGGCTG-3′/5′-CAGdCSpCGAC-3′ (Fig. 1). The presence of the dCSp residue resulted in an increase in the chemical stability of the modified duplex. It is commonly accepted that this stabilization is mainly due to polyelectrolyte effects [34,35,47,50,51]. However, the influence of structural factors could not be excluded.

2. Materials and methods

2.1. Oligonucleotide preparation

In order to obtain an oligodeoxynucleotide with a deoxycytidine conjugate modified at *N*-4 position with polyamine moiety a fully protected phosphoramidite was prepared. The 3′-phosphoramidite of 5′-*O*-dimethoxytrityl-4-*N*-[tris(*N*,*N*′,*N*″-trifluoroacetyl)-4,9,13-triazatridecane-1-yl]-2′-deoxycytidine was synthesized according to the procedure developed by us earlier [36]. The DNA octamers (5′-GTCGGCTG-3′, 5′-CAGCCGAC-3′, 5′-CAGdCSpCGAC-3′) were synthesized using DNA synthesizer Gene Assembler Plus from Pharmacia-LKB (Sweden) or K&A Laborgerate GbR DNA/RNA (Germany), using standard phosphoramidite chemistry. The coupling time used for a modified monomer was the same as for the standard deoxycytidine phosphoramidite. The oligomers were cleaved from CPG-support with 32% ammonium hydroxide solution. The deprotection under standard conditions using concentrated aqueous ammonia at 55 °C overnight allowed to remove all protecting groups including trifluoroacetyls [11,36]. The oligomers were purified by the preparative TLC on Merck 60 F_{254} TLC plates with 1-propanol/aqueous ammonia/water solution (55:35:10, by vol.) as an eluent. The product band (least mobile) was cut out, eluted with water and desalted with a Waters Sep-pak C-18 cartridge. First, the solution containing the oligonucleotide was loaded onto the cartridge and the column was flushed with 10 mM ammonium acetate (10 ml). In the next step the oligonucleotides were eluted by flushing the cartridge with 5 ml of 30% acetonitrile/water solution. The fraction with the product was evaporated to dryness and the purity of oligonucleotides was monitored using HPLC and confirmed by MALDI-TOF spectrometry (Autoflex, Bruker).

2.2. UV Melting curves

UV Melting profiles of the DNA duplexes were obtained in a buffer containing 100 mM sodium chloride, 20 mM sodium cacodylate, 0.5 mM Na_2EDTA, pH 7.0. Duplexes were used in the 10^{-3}–10^{-6} M concentration range. Single strand concentrations were calculated from absorbance above 80 °C with single strand extinction coefficients approximated by the nearest-neighbor model [37,38]. The temperature range, in a heating–cooling cycle, was 0–90 °C with a temperature gradient of 1 °C/min. Thermal-induced transitions of each mixture were monitored at 260 nm with a Beckman DU 650 spectrophotometer with a temperature controller. The thermodynamic parameters were determined from fits of data acc. to a two-state model with the MeltWin 3.5 software [39].

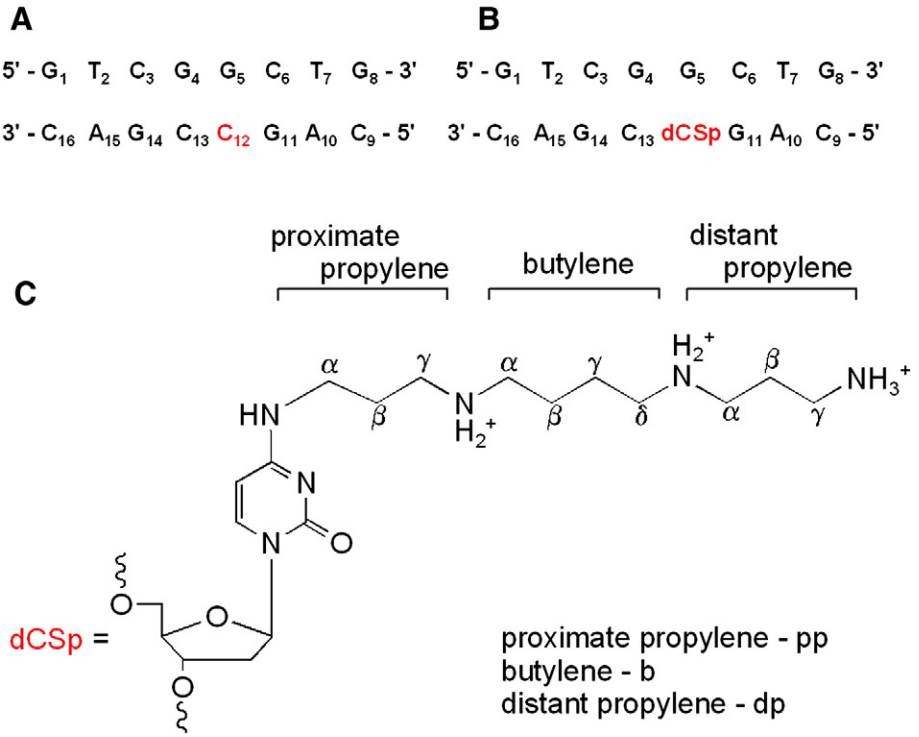

Fig. 1. Sequence of (A) the reference and (B) spermine modified (dCSp = 4-N-[4,9,13-triazatridecan-1-yl]-2′-deoxycytidine) duplexes. (C) Nomenclature of the protons of spermine within dCSp unit used in this paper.

2.3. NMR measurement

Oligodeoxynucleotide 5′-GTCGGCTG-3′ was mixed with 5′-CAGCCG AC-3′ or 5′-CAGdCSpCGAC-3′ in a 1:1 stoichiometric ratio. Fully-complementary oligodeoxynucleotide duplexes were dissolved in a buffer containing 50 mM NaCl, 10 mM NaH_2PO_4 and 0.1 mM Na_2EDTA at pH 7.0 in either 99.996% D_2O or 90%:10% H_2O:D_2O (for the observation of exchangeable protons). The mixture of strands was heated to 80 °C and then allowed to cool slowly to room temperature to achieve a duplex form. NMR spectra were carried out using Bruker Avance 600 MHz spectrometer, processed with TopSpin (Bruker, Inc.) and analyzed with FELIX (Felix NMR, Inc.) software. Exchangeable proton resonances were observed and analyzed using 2D NOESY spectra in 90%:10% H_2O:D_2O obtained at 10 °C with 150 ms mixing time. Data were recorded with 512 real data points in t1 dimension and 2048 real points in t2 dimension with minimum 128 scans per increment. The sweep width was 14,000 Hz in both dimensions. Solvent suppression was accomplished using the 3-9-19 WATERGATE pulse sequence [40]. Two-dimensional NOESY spectra in 99.996% D_2O were acquired with mixing times of 150 and 400 ms. The mixing time of TOCSY experiments (used MLEV-17 mixing sequence for Hartman–Hahn transfer) was 60 ms. For each experiment in D_2O typically 2048 data points in t2 and 512 FIDs in t1 were collected. Spectral widths were 6000 Hz for 2D NOESY and 4800 Hz for DQF-COSY and TOCSY spectra. To facilitate peak assignments (due to slight changes in chemical shift) and acquire additional structural information, 2D NOESY spectra were collected at three different temperatures: 20, 25 and 30 °C. The residual water peak for all homonuclear experiments in 99.996% D_2O was suppressed using a low-power presaturation. Natural abundance ^1H–^{13}C HSQC spectra were acquired within the sweep width of 6000 Hz in ^1H dimension and 25,600 Hz in ^{13}C dimension and GARP sequence for broadband carbon decoupling during acquisition. A total of 512 FIDs with 80 transients each and 2048 complex points were recorded. ^1H–^{31}P COSY experiments were collected with 256 FIDs and about 64 scans with 2048 complex points for each FID. The spectral width was 2400 Hz in the ^{31}P dimension and 1500 Hz in the ^1H dimension.

2.4. Interproton distances and torsion angle restraints

Non-exchangeable interproton distances were derived from the 150 ms 2D NOESY spectra recorded at 25 °C using the isolated spin pair approximation approach. The NOE cross-peaks were standardized to H2′–H2″ cross-peak with H2′–H2″ distance of 1.8 Å. The lower and upper bounds of the restraints were set to be equal to −15% and +30%, respectively, of the calculated NOE distance. For methyl atoms of the thymidine and methylene group of spermine chain pseudoatom corrections of 0.6 Å and 1.0 Å to the NOE intra- and interstrand upper bond were added, respectively. Watson–Crick base pairs were identified on the basis of observation of significantly downfield shifted imino and amino proton resonances and the NOE correlations for G(N1–H)–C(NH_2) or A(H2)–T(N3–H) in 2D NOESY spectra. Hydrogen bond restraints based on the standard geometry of nucleic acids were introduced as a suitable distance restraint to tolerance of ±0.2 Å [41]. These restraints were supplemented by weak planarity restraints of the base pairs. Ribose sugar pucker restraints were determined from J-coupling measurements of E.COSY cross-peaks. Residues with large $^3J_{H1'-H2'}$ couplings around 9 Hz and $^3J_{H1'-H2''}$ around 6 Hz were constrained to the C2′-*endo* conformation of the sugar ring. Thus ranges for all endocycling ν_0–ν_4 torsion angles were set as follows $\nu_0 = -4.2 \pm 15°$, $\nu_1 = 24.9 \pm 15°$, $\nu_2 = -34.9 \pm 15°$, $\nu_3 = 33.2 \pm 15°$, $\nu_4 = -18 \pm 15°$. For residues with couplings indicating intermediate C2′-*endo*/C3′-*endo* sugar puckering or when couplings could not be measured due to overlapping of the peaks sugar conformation was left unconstrained to reflect the possibility of conformational mobility. The glycosidic torsion angles χ which define the orientation of nucleobases with respect to the sugar moiety were restricted to the *anti*-conformation (−119 ± 90°) because of the lack of strong intranucleotide H1′–H6/H8 cross-peaks on 2D NOESY spectra (150 ms). Dihedral angle restraints for the α and ζ backbone angles obtained indirectly from ^{31}P chemical shifts analysis were set to −58 ± 90° (α) and −91 ± 120° (ζ) to exclude *trans* conformation. Information about the ranges of torsion angle (β and γ) restraints was obtained from the observation of long range $^4J_{P-H4'}$ couplings (~4 Hz) in the ^1H–^{31}P COSY spectra. The observation of this long range couplings indicates a planar W-shaped conformation of the atoms P–O5′–C5′–C4′–H4′ and is possible when the β and γ torsions are *trans* (180 ± 90°) and *gauche*$^+$ (41 ± 60°), respectively. A *gauche*$^+$ conformation of the γ is also compatible with weak H4′–H5′/H5″ cross peaks observed in E.COSY spectra whereas *trans* conformation of β was also manifested by weak P–H5′/H5″ cross peaks in ^1H–^{31}P COSY spectra. Furthermore, due to the presence of strong H3′–P cross-peaks indicating J-coupling greater than 5 Hz, dihedral angle ε was loosely constrained to a range of −180 ± 120° to include both *trans* and *gauche*$^-$ conformations. No backbone torsion angle restraints were imposed on the angles α, β, ε and ζ for dCSp residue of the modified duplex.

2.5. Structural calculation and refinement

Structure calculations were performed within the XPLOR-NIH package [42] by simulated annealing protocol in torsion angle space with the use of CHARMM force field. The parameter and topology sets used (dna-rna-allatom.top and dna-rna-allatom.param) were taken from the CNS distribution. For the dCSp residue of modified duplex a distinct topology and parameter files were prepared basing on the model of the dCSp nucleotide created using the Sketcher module of Insight II (Accelrys Inc.). Partial charges on the atoms (introduced to the rMD simulations) were obtained from MOPAC [43] calculation. At the beginning of rMD simulations a template of 100 extended structures with randomized torsion angles was generated and then subjected to the four-step global fold. Through the initial high-temperature stage structures were heated to 20,000 K over 64 ps of torsion angle molecular dynamics (TAMD) [44]. Afterward, the models were cooled during 70 ps of TAMD to 300 K followed by 18 ps dynamics in Cartesian space with temperature gradually scaled from 3000 to 300 K. In the next step 1200 cycles of the restrained Powell minimization were carried out. During the refinement procedure conformational database potentials were turned on for all residues except modified dCSp. In the end, a family of 10 lowest energy structures without violations to the NOE distances (0.2 Å), and dihedral angles (5°) was selected for further analysis. Statistics of structure determination together with a summary of the experimental restraints are placed in the Supporting information (Table S4). The figures representing the NMR solution-state structure were drawn using the program Pymol [45]. The coordinates and NMR restraints have been deposited in the Protein Data Bank as entries PDB ID: *2MCI* and *2MCJ*. Chemical shift assignments for both molecules have been deposited in BioMagResBank under entry codes BMRB ID: *19440* and *19441*.

3. Results

The influence of spermine modification on the thermal stability of DNA duplex was examined. As shown in Table 1, the presence of

Table 1
Thermodynamic data of DNA duplexes (**X** = dCSp).[a]

DNA duplexes	−ΔG°37 [kcal/mol]	T_m [°C]	−ΔG°37 [kcal/mol]	T_m [°C]	ΔΔG°37 [kcal/mol]	ΔT_m [°C]
5′-CAGCCGAC-3′ 3′-GTCGGCTG-5′	9.21 ± 0.10	50.1	9.15 ± 0.08	50.5	0	0
5′-CAG**X**CGAC-3′ 3′-GTCGGCTG-5′	10.0 ± 0.43	54.2	9.65 ± 0.01	54.4	−0.52	+4

[a] Measured in buffer:100 mM NaCl, 20 mM sodium cacodylate, 0.5 mM Na_2EDTA, pH 7.0.

spermine residue does not significantly affect the stability of the duplex and increases thermodynamic stability about 0.52 kcal/mol.

3.1. Assignment of protons in the reference duplex

Assignment of proton resonances in the reference duplex followed well established procedures applied to study regular nucleic acid structures [41]. Nonexchangeable protons were assigned from the analysis of 2D NOESY spectra DNA using intra- and internucleotide H6/H8–H1′ sequential connectivities (Fig. 2). The presence of continuous pathways allowed for an unambiguous assignment of aromatic and anomeric protons and confirmed the formation of the right-handed helical structure. The intensities of H6/H8-H1′ cross-peaks were all weak, indicating anti-conformation of the glycosidic angles. Connectivities for two strands were also traced in the aromatic-to-H3′, aromatic-to-H4′, aromatic-to-H2′/H2″ and aromatic-to-methyl regions. Stereospecific assignment of the H2′/H2″ protons was obtained following the rule that for H1′–H2″ NOE is always stronger than the H1′–H2′. The NOESY spectrum acquired in 90% H_2O/10% D_2O at 10 °C allowed the assignment of the exchangeable protons. Imino protons of T2 and T7 residues were assigned based on the correlation between imino protons of thymines and the H2 protons of A15 and A10, respectively. The terminal imino protons could not be assigned because of the fast exchange of protons with the water. The chemical shifts of imino protons and the corresponding NOE connectivity pattern were in agreement with canonical Watson–Crick base pairing and all NMR data were consistent with the unperturbed B-DNA structure.

3.2. Assignment of protons in the spermine–DNA modified duplex

Protons of dCSp-modified duplex were assigned using the same strategy as for the reference duplex. The NOESY spectra of the modified duplex exhibit the characteristic features of right-handed DNA. All expected H6/H8–H1′ (Fig. 2), H6/H8–H2′/2″ and H6/H8–H6/8 intra- and internucleotide sequential connectivities were observed enabling unequivocal assignment of these resonances.

Assignment of the exchangeable protons was obtained from the NOESY spectra in D_2O/H_2O at 10 °C. The chemical shifts and NOE connectivity patterns of the imino protons of the modified duplex indicated typical Watson–Crick base pairing. The modified deoxycytidine possesses only one hydrogen atom (N4H) which can be involved in hydrogen bond formation with the complementary G5 base. The signal at 8.45 ppm was assigned to N4H of the dCSp residue based on the NOE correlations observed between N4H and imino proton of deoxyguanosine G5 as well as spermine protons. Presence of these correlations (see Fig. S1 in the Supporting information) provided the evidence of the existence of dCSp–G5 pair which forms hydrogen bonds in the Watson–Crick manner.

3.3. Assignment of spermine protons

Additional resonances were observed in the region typical of H2′/H2″ and thymine methyl protons (1–3 ppm) and attributed to the spermine methylene protons [46]. The signals of α and γ methylene protons of propylene chain as well as α and δ methylene protons of the butylene chain were shifted downfield with respect to β or β and γ protons, respectively (Fig. 3).

In the NOESY spectrum cross peaks between the amino-alkyl chain and modified cytosine base as well as the correlations arising from the interactions between polyamine and the neighboring bases were observed. Resonances at 2.67 and 1.99 ppm were assigned to H-1α-pp and H-2α-pp protons, respectively, based on the strong NOE interaction between geminal protons and the presence of NOE between these two protons and H5 of dCSp residue (Figs. 3 and 4). Additionally, the observed NOE to the H6 proton of dCSp in the spectrum recorded with long mixing time (τ_m = 400 ms) resulted from the spin diffusion but was another confirmation of the assignment. Both, H-1α-pp and H-2α-pp resonances revealed additional NOE to H8 of deoxyguanosine G11 suggesting the 3′-arrangement of the spermine chain. Analysis of COSY and TOCSY spectra allowed assigning β-pp and γ-pp protons. Resonances at 1.72 and 1.62 ppm were attributed to H-1β-pp and H-2β-pp protons, respectively, based on different intensities of NOE cross-peaks to H5 of C12 (Fig. 4). Signals at 2.91 and 3.1 ppm were assigned to H-2γ-pp and H-1γ-pp protons, respectively, using the same approach. Assignment of these protons was further confirmed by the observation of NOEs between H-β-pp and H-γ-pp methylene protons with H5 of dCSp residue. Furthermore, NOE contact from H-1γ-pp to the H5 proton of C13 residue was visible and appeared to be crucial in the determination of the direction of the spermine chain (Fig. 4). Chemical shift assignments for spermine protons are listed in Table S3.

Another important, although very weak NOE was observed between H8 of the G4 residue and one of spermine H-α-butylene protons. Assignment of the remaining protons of the spermine chain could not be made specifically due to the lack of corresponding cross-peaks in COSY and TOCSY spectra suggesting small values of their ^3J coupling constants. However, the protons of methylene groups of butylene and distant propylene fragment of the spermine chain have been identified using a combination of TOCSY, COSY, NOESY and ^1H–^{13}C HSQC spectra. Among NOE cross peaks arising from the interactions within the spermine chain those assigned to the proximate propylene fragment exhibited a negative NOE effect (in phase with a diagonal). On the other hand most of the unassigned spermine resonances revealed

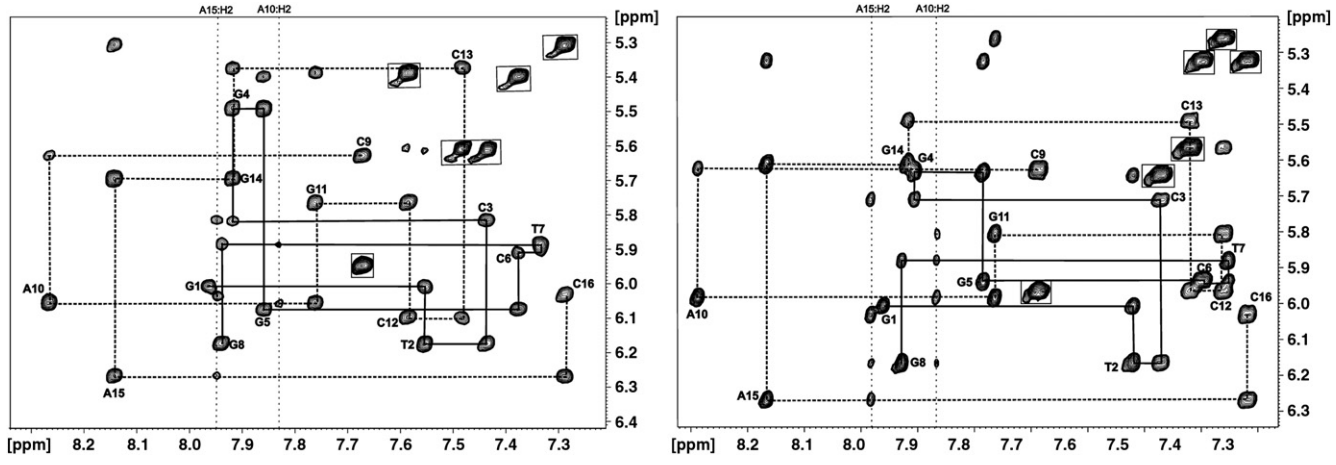

Fig. 2. Base H6/H8-H1′ regions of the 400 ms NOESY spectra. Sequential connectivities are shown for the top (—) and bottom (– – –) strands for the reference (right side) and modified duplex (left side).

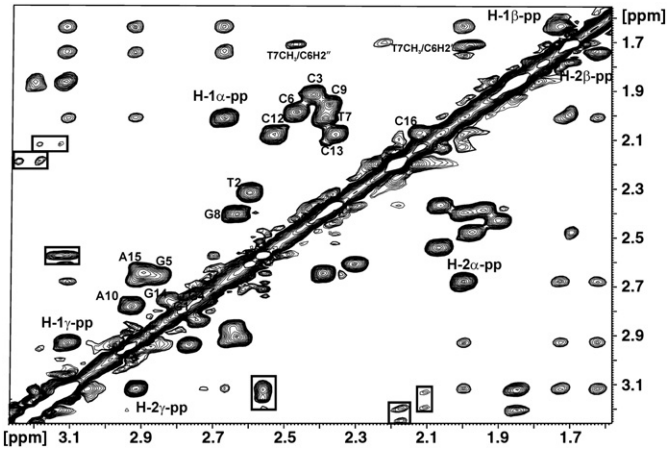

Fig. 3. Region corresponding to H2′–H2″ and spermine interactions. The NOE cross-peak with phase opposite to diagonal are marked with rectangles.

positive NOE (phase opposite to a diagonal) indicating that dynamics of this part of the spermine chain is dominated by fast internal motion of methylene groups. Several NMR studies of spermine–DNA complexes demonstrated that positive intramolecular NOE observed between spermine protons was responsible for the spermine motion that was independent of the rotational tumbling [47] of DNA. Moreover, the negative intramolecular NOEs which were observed when spermine interacts with Z-DNA were interpreted as a slowdown of spermine dynamics [48].

3.4. Chemical shift perturbations

Chemical shift differences between protons of the reference and modified duplex are negligible (<0.10 ppm) for all but H1′, H6 or H8 of the deoxycytidine C12 and residues located in close vicinity of the modification site. The largest absolute values of the chemical shift changes were observed for anomeric protons. In the unmodified strand of the dCSp duplex only C3, G4 and G5 residues exhibited significant chemical shift differences (up to 0.14 ppm) suggesting interaction of spermine rather with residues on the 3′ site of the modification. The nucleotides located more than two base pairs away from the dCSp residue experienced only negligible chemical shift perturbation. In conclusion, the dCSp modification does not cause the substantial perturbation that would extend beyond the neighboring base pairs. Fig. 5 presents the statistical distribution of differences in proton chemical shifts between two duplexes.

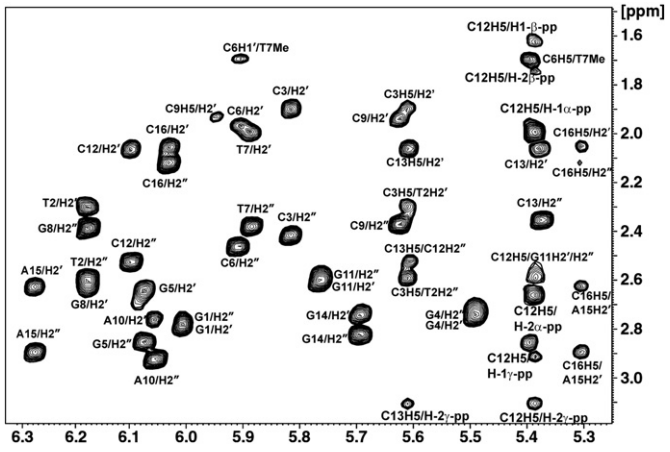

Fig. 4. Region H1′–H2′/H2″ of NOESY spectrum of modified duplex (τ_m = 400 ms).

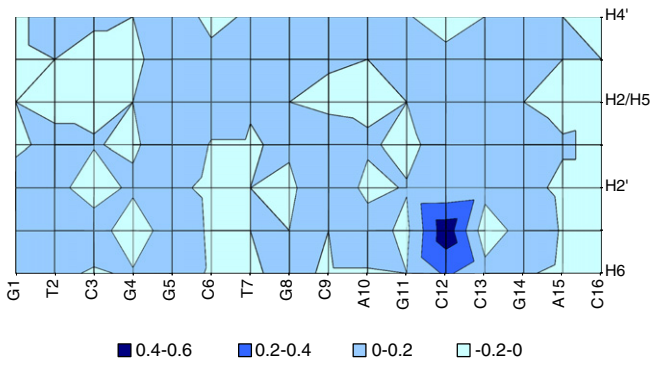

Fig. 5. Statistical distribution of the proton chemical shift changes observed for the dCSp modified duplex.

This statistic illustrated the conclusion that the biggest changes of chemical shifts were limited to dCSp residue and its vicinity. The same observation was further confirmed by the analysis of the chemical shifts of phosphorus atoms (Fig. 6).

The chemical shifts of phosphorus atoms of the G4, G5 and G14 residues undergo the largest changes after introduction of dCSp modification. Upfield ^{31}P chemical shift of 0.33 ppm and downfield chemical shift of 0.30 ppm were observed for the G4 and G5 residues, respectively. Moreover, 0.22 ppm upfield shift for the G14 phosphorus was detected. The observation of the pattern of ^{31}P chemical changes of phosphorus atoms suggested the interaction between spermine amino groups and the phosphodiester backbone of DNA. Based on the comparison of ^{31}P NMR spectra of both duplexes we hypothesized that chemical shift differences of ^{31}P signals could be related to the neutralization of negative charge of phosphate as a result of the orientation of the polyamine chain. The ^{1}H and ^{31}P chemical shifts of reference and modified duplexes are given in the Supporting information (Table S1 and Table S2).

3.5. Structural refinement

The comparison of the chemical shifts between the spermine-modified and unmodified duplexes suggested that introduction of spermine modification does not influence the overall structure. In order to get a more detailed insight into structural perturbation induced

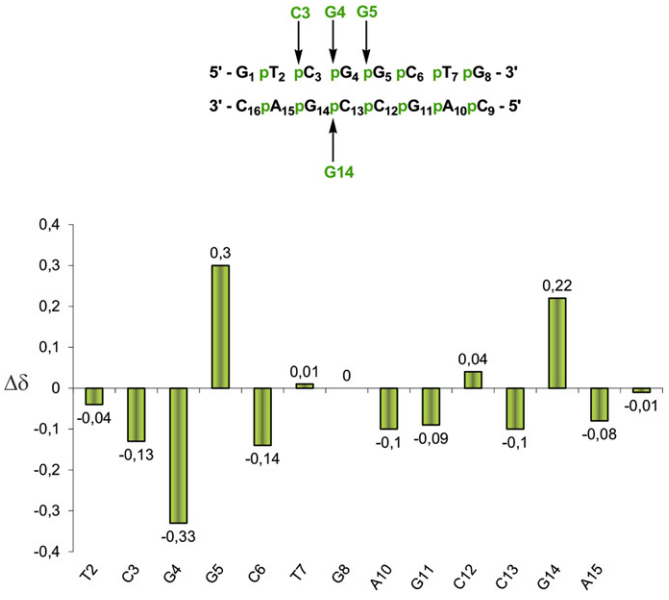

Fig. 6. Chemical shift differences ($\Delta\delta = \delta_{dCSp} - \delta_{ref}$) observed for the phosphorus atoms.

by the presence of spermine, the structure of both duplexes was computed using the restrained molecular dynamics protocol described in the Materials and methods section. Both structures were calculated using the identical protocol [49] and set of parameters with the exception the NOE-based constraints. It was found that the four long range NOEs between H6-dCSp and both H-α-pp, H8-G11 and both H-α-pp, H5-C13 and H-1γ-pp, H8-G4 and H-α-b played an important role in constraining polyamine chain. However, these were not taken as tight constraints. Moreover, to preserve the natural dynamics of polyamine in structural refinement these restraints were omitted which resulted in the clear flexibility of spermine chain. The calculations used the 332 constraints of distance for the reference duplex and 350 for the modified duplex (Table S4). Out of 100 structures 81 for the reference and 69 structures for the modified duplex without violations to the NOE distances (0.2 Å) and dihedral angles (5°) were received. Table S4 placed in the Supporting information summarizes the statistics on the distance and dihedral constraints used in the structure calculations for the reference and modified duplexes.

4. Discussion

The structures of both duplexes are regular right-handed helices with all of the hallmarks of the B-DNA form. This type of the conformation implies the *anti*-orientation of all residues, typical Watson–Crick base pairing and C2′-*endo* sugar conformations. The B-form of both duplexes was supported by the analysis of backbone torsion angles of the family of the 10 lowest energy structures (Fig. S3 demonstrates the superposition of 10 lowest energy structures of both duplexes). Values of all torsion angles are typical for the B-DNA form (see Table S5 and Table S6). Additionally, we characterized both structures by the helical parameters using the CURVES 5.3 software [50]. Selected helical parameters are listed in Table S7 and are in agreement with the canonical B-form. As a consequence of the attachment of spermine residue to deoxycytidine, the polyamine chain is situated in a major groove and points toward 3′ end of the modified strand. In addition, preferential alignement of the spermine chain with the phosphodiester backbone of DNA is illustrated in Fig. 7. The proximate amino-propylene chain is structurally well defined, while the remaining part of the aminoalkyl chain of spermine is poorly converged. This poor convergence might reflect an insufficient number of structural constraints available from NMR data or demonstrate the dynamic nature of the spermine residue. In the NOESY spectrum positive intramolecular NOE connectivities between spermine methylene protons were detected suggesting that part of the spermine alkyl chain had motion which was independent of the dynamics of the duplex. This observation is consistent with the obtained structure of the dCSp–DNA duplex and confirms the relatively high mobility of the spermine chain.

Analysis of the obtained structure reveals that the distances between the first internal $-NH_2^+-$ amino group of the polyamine chain and O6 and N7 atoms of G4 residue are suitable for the formation of the hydrogen bonds. This can be supported by the observation that H-α-butylene protons exhibit negative NOE, which could result from the local hindering of the alkyl chain. The slowdown dynamics of the part of the alkyl chain can be caused by the involvement of the spermine amino group in hydrogen bonds with deoxyguanosine G4 (Fig. 8). We also postulate that this interaction contributes to the increased thermodynamic stability of the modified duplex. Another important observation arising from the analysis of the structure of the dCSp-modified duplex suggests possible interaction between the secondary $-NH_2^+-$ group and the phosphate group of deoxycytidine C3 residue and is corroborated by the observation of the relatively large chemical shift difference of deoxycytidine C3 phosphorus atom between modified and unmodified duplexes.

Incorporation of the cationic moiety into the oligonucleotides provides structural basis for studies concerning the conformational changes of DNA duplexes induced by site-specific modification. This offers a convenient model for understanding of specificity of the interaction between polyamine and nucleic acid, the location of the polyamine and its dynamics. Based on the X-ray [20], NMR [47,51,52] and theoretical studies [18] several spermine–DNA binding models were proposed; however, no definitive evidences were found for the existence of specific binding sites between polyamines and DNA. It was first noted by Tsuboi that the phosphate–phosphate distances in B-DNA match well the separation between the charged amine groups of the biogenic polyamines [53]. In this model the two internal amine groups interact with phosphates on both strands, and the amine group of propyl chains binds to the adjacent phosphate groups. Furthermore, the crystallographic study of complexes of polyamines with A-, Z- and B-forms of DNA showed that spermine ions are placed close to DNA phosphates [54]. On the other hand there are premises, that polyamines have a greater affinity for certain structural types such as Z-DNA [24,51,52], A-DNA [55,56] and some quadruplex structures [46]. This increased affinity probably results from better matching of the distance between the amines in polyamines to the distance between the phosphates than in a typical B-DNA structure. There are several lines of evidence for substantial spermine mobility on all forms of DNA. Unequivocal location of the specific interaction sites was impossible due to considerable spermine dynamics. In our studies immobilization of polyamine chain on the oligonucleotide strand imposes bigger restrictions on its mobility

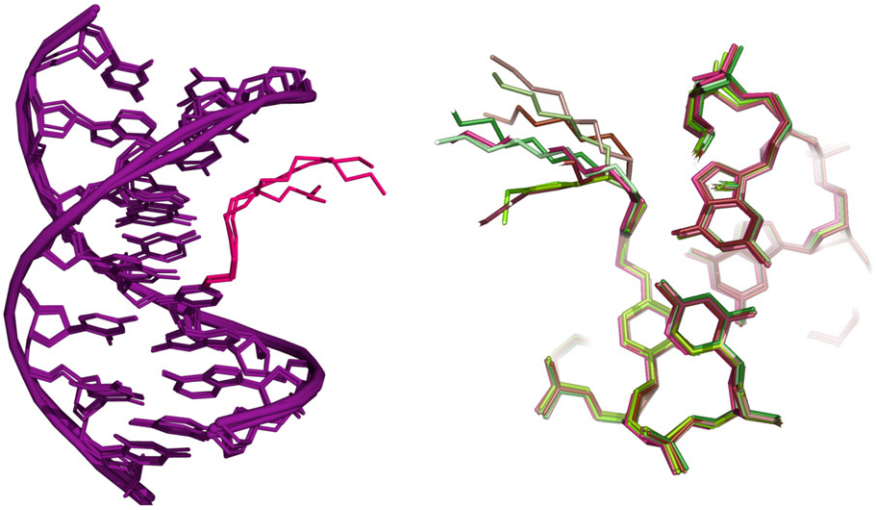

Fig. 7. Spermine chain alignment with the sugar-phosphate backbone. View along the axis of the helix (right side).

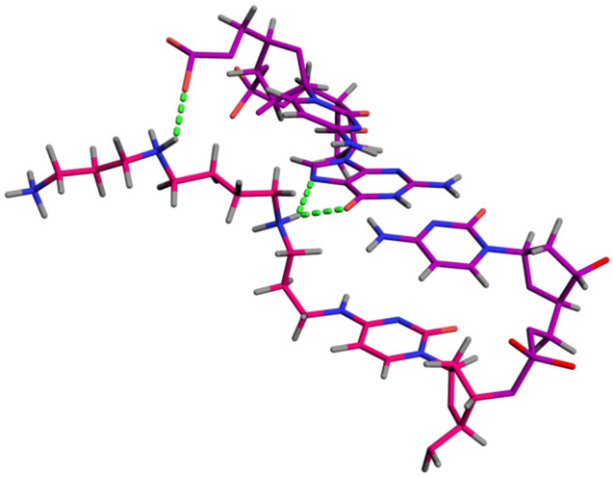

Fig. 8. Interactions between the aminoalkyl chain and the sugar-phosphate backbone of DNA.

than for spermine–DNA complexes. We have shown that the polyamine chain is oriented toward the major groove in the 3′-direction from the modification site. Nevertheless, we determined that the spermine chain exhibits conformational flexibility, and as such, is capable to interact with intrastrand and interstrand residues. These observations are in agreement with the earlier X-ray and NMR studies where incorporation of aminohexyl [32] and aminopropyl [57] chains into the oligonucleotide strands exhibits similar behavior. The dynamic nature of the interaction of spermine with nucleic acids is just as important as its structural recognition properties. Over the years there have been a number of NMR studies of spermine–DNA complexes [47,51,52] which confirmed the spermine high mobility and postulated that spermine chain reorientation is independent of the overall tumbling of the macromolecule. In consequence it was difficult to find spermine–DNA NOE contacts providing the evidence for specific interactions.

In our work, based on the ^{1}H, ^{13}C analysis of NOESY, TOCSY and ^{1}H–^{13}C HSQC spectra, and using literature data we were able to assign all methylene protons belonging to the proximate propylene chain and H-α-butylene protons. The remaining spermine protons were characterized by positive NOE and were not specifically assigned.

Although our data did not give any evidence of tight binding of the spermine chain to DNA, the analysis of chemical shift perturbation of the phosphorus atoms strongly suggests that the spermine chain is loosely aligned with the phosphodiester backbone. Nevertheless, in the majority of converged structures we have identified the putative interaction between the second internal $–NH_2^+–$ amino group and the oxygen atom of the phosphate of C3 residue. This finding was in agreement with the observation obtained from the analysis of NMR spectra, where relatively large perturbation of ^{31}P chemical shifts of phosphorus atoms of C3 residues was observed (Fig. 6) after introduction of spermine modification. This result is consistent with the existing model assuming the electrostatic nature of these interactions. Thus, the positive charges on the spermine chain could attribute to the neutralization of the negative charge on the phosphate backbone and possibly contribute to the stability of Sp-DNA/DNA duplex formation. Besides the electrostatic interactions between spermine and DNA also the possibility of hydrogen bond formation by polyamine should be taken into account when these types of structures are studied.

In the structure of the dCSp modified duplex the first internal amino group $–NH_2^+–$ appears to be close to O6 and N7 electronegative atoms of G4 residue and indicates the possibility of hydrogen bond formation. Similar observation was made based on the analysis of NMR data supported by molecular modeling of DNA dodecamer in which 3-aminopropyl chain was incorporated into an oligodeoxynucleotide [57]. In this study the tethered cation positioned on the floor of the major groove was found proximate to the O6 atom of deoxyguanosine residue. Our results are also in accord with another NMR studies concerning the interaction of spermine and spermidine with tRNA. In this work polyamines were found to bind preferentially to nucleobases through hydrogen bonds generated by the protonated $–NH_2^+–$ group internally present in the polyamine backbone. Moreover, it was noticed that the binding of the primary $–NH_3^+$ groups to tRNA is much weaker than the binding of the secondary $–NH_2^+–$ groups [58,59]. Analysis of our structure indicates that both modes of interaction of polyamines with nucleic acids, to the phosphate groups and to nucleobases, are feasible. Additionally, the obtained structure suggests that interaction of the spermine with DNA results in slowing down the rotational mobility of the amino alkyl chain.

5. Conclusions

Our results indicate that spermine binding does not perturb the DNA structure and seems to have some degree of specificity. Interaction within the major groove appears to be privileged and followed by binding to the phosphates. Additionally, the mobility of the spermine chain is relatively independent of that of the DNA molecule, however, its mobility can be somewhat reduced relatively to the free spermine due to the interactions with DNA bases and phosphate groups.

5.1. Database linking

Structure coordinates have been deposited in the Protein Data Bank as entries PDB ID: 2MCI and 2MCJ. Chemical shift data have been deposited in BioMagResBank (BMRB), entry codes 19440 and 19441.

Acknowledgements

The authors would like to thank Dr. Maria Markiewicz for the syntheses of oligonucleotides. This work was supported by the Polish Ministry of Science and Higher Education and European and Regional Funds under project POIG.02.0100-30-182/09.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbagen.2013.12.008.

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