π -stacking effects on the EPR parameters of a prototypical DNA spin label

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The character and value of spin labels for probing environments like double-stranded DNA depends on the degree of change of the spin Hamiltonian parameters of the spin label induced by the environment. Herein we provide a systematic theoretical investigation of this issue, based on a density functional theory method applied to a spin labeled DNA model system, focusing on the dependence of the EPR properties of the spin label on the π stacking and hydrogen bonding that occur upon incorporating the spin label into selected base pair inside DNA. It is found that the EPR spin Hamiltonian parameters of the spin label is only negligibly affected by its incorporation into DNA, when compared to the its free form. This result gives theoretical ground for the common empirical assumption regarding the behaviour of spin Hamiltonian parameters made in EPR based measurements of distance between spin labels incorporated into DNA.

1 Introduction

Conformational changes of DNA, manifested as double-strand breaks, mispairings, or modifications in the nucleosides, can be related to diseases, ageing or cell death^{1–4}. One way to detect these conformational changes is by employing electron paramagnetic resonance (EPR) techniques which can provide structural information^{5–8}. Among these techniques, the pulsed electron-electron double resonance, PELDOR, also known as double electron-electron resonance DEER, is most suitable for investigations of biomolecules as it does not require the availability of crystalline samples⁵. However, a direct investigation of unmodified DNA or other biomolecules using this method is rarely possible, since these molecules are diamagnetic and thus are EPR silent⁹. One way to circumvent this obstacle is to employ the so-called site-directed spin labeling (SDSL) method^{10–12} in which stable radicals are incorporated, typically of nitroxides family, into the DNA by spin labeling specific nucleoside targets. The SDSL technique is not solely used for labeling nucleic acids, but also for a wide range of macromolecules, e.g. proteins¹³ and amyloid fibrils¹⁴ to mention a couple of many examples. However, upon incorporating such nitroxide spin labels into the targeted site several mechanisms that may render EPR silence can occur, thus hampering the EPR measurements. Out of these mechanisms, the one-electron reduction of the nitroxide radical to the corresponding EPR silent hydroxylamine^{15–17} and the two-electron

cellular bioreduction¹⁸ are most frequently encountered in *in vivo* as well as in *in vitro* environments. These chemical processes have been extensively studied and several ways have been proposed to extend the *in vivo* lifetime of nitroxide spin labels.^{19–22}

Differently from the chemical processes responsible for the reduction of nitroxide spin labels to EPR silent diamagnetic molecules, the behaviour of magnetic properties of spin labels in DNA environment has been scarcely studied. Furthermore, among a vast number of theoretical studies^{23–32} devoted to environmental effects on EPR spin Hamiltonian parameters of nitroxides, only a few have ventured beyond the investigation of simple aprotic and protic solvent environments. Recently, we carried out such studies of magnetic properties of nitroxide spin labels bound to proteins³³ and encapsulated in “guest-host” complexes^{31,32}. From these studies emerged the fact that the EPR spin Hamiltonian parameters of the spin labels undergo various modifications upon changes in their local environment. Thus, we found that the empirical assumption made in a typical analysis of EPR measurements may not always hold for those systems.

In this work we investigate the behaviour of a nitroxide spin label in a DNA environment, focusing on the impact of incorporation of the spin label into DNA on its EPR spin Hamiltonian parameters, i.e. electronic g-tensor and nitrogen hyperfine coupling (hfcc) constant. To narrow the scope of our investigation, we focus on an unexplored part of environmental effects on nitroxide spin labels, namely the influence of π stacking on the EPR parameters. Overall, the impact of π stacking on EPR parameters has so far only scarcely investigated and only a few studies have been carried on the benzo-

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Fig. 1 Model of the spin labeled DNA consisting from two (G-C) base pairs and one spin labeled base pair (G-C) used in this work.

Fig. 2 Geometrical and electronic structure of spin label C. On the left side two distinct parts of spin label are depicted i.e. nitroxide part, featuring $R_2NO\cdot$ moiety, and phenoxazine derived group with large π electrons system. On the right side, graphical representations of n and π molecular orbitals, which play key role in defining EPR parameters of spin label C, are given.

quinone anion and similar radicals in the context of theoretical studies of photosystem I.^{34,35} Thus, this work not only aims to address the question about the role played by π stacking in defining the behaviour of EPR parameters of nitroxide spin labels in a DNA environment, but it also provides further insight into the overall importance of this interaction in the description of electronic structure and properties of radicals in complex environments.

2 Computational Details

We investigate the π stacking effects on EPR parameters of nitroxide spin labels in a DNA environment by building a prototypical DNA model consisting from three guanine-cytosine (G-C) base pairs, in which cytosine in the central pair is substituted by a nitroxide spin label C (see Fig. 1). The proposed model is thus minimalistic in its design, but is still capable to capture the major part of the π stacking interaction

between DNA and a nitroxide spin label C (closest G-C pairs around the G-C pair are accounted for), while still being sufficiently small for computation of EPR parameters at the full DFT level. In this model we selected the spin label C, which has been investigated experimentally^{36–38}, to be a representative DNA spin label for the following reasons: (a) it is a cytosine analogue which is capable of making a base pair with guanine³⁶; (b) it was shown that the effect of the spin label C on the DNA stability and conformation is negligible³⁷; (c) the extended π system of this spin label is well suited for providing coupling between a π orbital located on the nitroxide spin label RNO $\cdot$ moiety and the π orbital system of the G-C base pairs surrounding G-C pair (see Fig. 1). The initial structure of the spin labeled DNA model was built using typical geometrical parameters of B-type DNA (base pair rotation angle 35.9 degrees), and by substituting cytosine in the central base pair with the spin label C, the geometry of which has been optimized at the B3LYP-D3/6-31G* level.^{39–42} In order to account for local relaxation of the geometrical structure of the spin labeled DNA, the initial geometrical structure of our model system has been relaxed using a partial geometry optimization procedure in which atoms which would connect the nucleotides to the sugars in the real DNA have been frozen. Similarly to the free spin label C, the partial geometry optimization of the model system has also been carried out at the B3LYP-D3/6-31G* level, where dispersion interactions are accounted for using Grimme's dispersion correction^{43,44} as indicated by the addition of "D3" to the B3LYP abbreviation of the exchange-correlation functional. The obtained geometry of our spin labeled DNA model has been used as input for the subsequent calculations of the electronic g-tensor and nitrogen hfcc of spin label C in the DNA environment.

The EPR parameters - electronic g-tensor and nitrogen hfcc - are evaluated using a computational procedure which has been extensively used by us in investigations of EPR parameters of nitroxides.^{45,46} The electronic g-tensor calculations were thus carried out with spin-restricted density functional response theory with the following particularities: (a) the two-electron spin-orbit operator has been approximated using the mean field approach⁴⁷; (b) the two-electron gauge correction to the g-tensor shift has been neglected; (c) the electronic charge centroid method has been employed to reduce the gauge invariance error. The nitrogen hfcc calculations were carried out using our restricted-unrestricted density functional response theory method⁴⁶, which is free from spin contamination and allows strict separation between the direct spin density and spin polarisation contributions to the hyperfine coupling constants. All these calculations were performed using the B3LYP exchange–correlation functional, where for the electronic g-tensor calculations we employed the Huz-II basis set⁴⁸ and, for the nitrogen hfcc calculations, the N07D basis set^{49–51}, respectively. The selection of these smaller basis

sets (conventionally in studies of nitroxides we use Huz-III and Huz-IIIsu3 basis sets^{48,52}) is motivated by the favourable computational cost/accuracy ratio compared to larger basis sets suitable for EPR spin Hamiltonian parameters calculations. This computational scheme has been applied to determine electronic g-tensor and nitrogen hfcc of free and DNA incorporated spin label ζ , as these data are needed to determine the total shift of the electronic g-tensor and nitrogen hfcc of spin label ζ in the DNA environment. Apart from these calculations, we also carried out a series of EPR parameters calculations for various fragments of our spin labeled B-DNA model system in order to decompose the total DNA induced shift of the EPR parameters into geometry relaxation, hydrogen bonding and π stacking parts. More specifically, the electronic g-tensor and nitrogen hfcc calculations were performed on the following fragments extracted from our spin labeled DNA model system (see Fig. 1): a) single spin label ζ ; b) single base pair guanine-spin label (G- ζ). All the above described calculations of EPR spin Hamiltonian parameters have been performed using the DALTON program⁵³, while the geometry optimizations were carried out using the GAMESS-US program.⁵⁴

3 Results and Discussion

The rigid nitroxide spin label ζ consists of two distinct parts - the nitroxide moiety $R_2NO\cdot$ and the phenoxazine derived group, fused together (see Fig. 2). It represents quite a large group of molecules used for DNA labelling, which achieve nucleoside specific targeting of DNA by replacing a specific nucleoside in a base pair. Due to this DNA targeting mechanism, the structure of such spin labels are quite different from conventional spin labels used for proteins labelling, which typically consists of a linker and a small nitroxide. These structural features of spin label ζ lead to a specific arrangement of molecular orbitals which is not encountered in conventional nitroxide spin labels. More specifically, the π orbital (see Fig. 2) located on the nitroxide moiety $R_2NO\cdot$, which contains the single unpaired electron, shares the nodal plane with the large π electron system on the phenoxazine derived group. Due to the rigidity of the spin label itself the interaction and overlap between this π electron system is maximised. Therefore, the influence of the DNA environment on the phenoxazine derived group, which functions as the substitute for cytosine, will be directly sensed by the π orbital located on the nitroxide moiety due the interaction with the π electrons of the phenoxazine derived group, and this is the main mechanism of direct influence by the DNA environment on the magnetic properties of the spin label ζ . Thus, the direct influence of the DNA environment on the EPR spin Hamiltonian parameters should overall be relatively small, as according to the described DNA and spin label interaction mechanism, where only the π orbital

on the nitroxide moiety (see Fig. 2) is directly affected by the presence of DNA in the spin labeled B-DNA complex. Furthermore, due to the indirect nature of the interaction between the spin label ζ and the surrounding base pairs, the two additional mechanisms which could lead to environmental shifts of EPR parameters according to Stone theory^{55,56}, namely a change in size of the spin-orbit coupling matrix element between n and π orbitals on the nitroxide moiety $R_2NO\cdot$ and the redistribution of spin density in the π orbitals, are expected to be ineffective. Therefore, the issue of a direct influence of the DNA environment on the EPR parameters reduces to a question on how much the various interactions between the spin label and the DNA base pairs can change the structure and energetics of the π orbital on the nitroxide which contains the single unpaired electron. To answer this question we carried out a series of calculations of the electronic g-tensors and nitrogen hyperfine coupling constants using the above described spin labeled DNA model and estimated the total environmental shift of these spin Hamiltonian parameters by comparing the results obtained for the free spin label ζ and for the spin label ζ incorporated into DNA. To gain more insight into the shift mechanism we also decomposed the total environmental shift into three parts:

$$\Delta P = \Delta P^{geom} + \Delta P^{hyd} + \Delta P^{\pi}, \quad (1)$$

where ΔP is the total environment shift of property P due to the DNA environment, ΔP^{geom} is the contribution from the geometry relaxation of the spin label due to its incorporation into DNA, ΔP^{hyd} is the hydrogen bonding contribution to the environmental shift due to the hydrogen bonding of the spin label with guanine in the base pair, and ΔP^{π} is the part of the environmental shift due to the π stacking between the base pair containing the spin label with the closest surrounding base pairs.

After settling the dominant mechanics for the environmental shift of the spin label EPR parameters caused by the DNA and giving the proposed decomposition scheme of these shifts into individual contributions, it is relevant to analyze the actual behaviour of these parameters in the DNA environment, see 1 collecting the results for the free and the DNA model with incorporated spin label ζ . The environmental shift of the isotropic g-tensor shift, Δg_{iso} , is given as -102 ppm, thus only around 3% of the total Δg_{iso} value. This result is in good agreement with our prediction that the DNA environment has limited impact on the EPR parameters as based on an analysis of the interaction between the base pairs in DNA and the nitroxide moiety of the spin label ζ . According to 1, the π stacking interaction between the spin labeled base pair (G- ζ) and the two surrounding (G-C) base pairs in the model system is responsible for half of the environmental shift of Δg_{iso} , and constitutes thus one dominant interaction for the environmental shift. The remaining half of the environmental shift

Table 1 Electronic g-tensor of free and DNA incorporated spin label $\dot{\text{C}}$ computed using spin-restricted density functional response theory.^a

Δg_{ii}^b , ppm	Free	B-DNA			
	$\dot{\text{C}}$	$\dot{\text{C}}$	G- $\dot{\text{C}}$	(C-G)(G- $\dot{\text{C}}$)(G-C)	(C-G)(G- $\dot{\text{C}}^p$)(G-C)
Δg_{xx}	3368	3313	3290	3209	3342
Δg_{yy}	6967	6982	6941	6975	6883
Δg_{zz}	-338	-369	-389	-493	-383
Δg_{iso}^c	3332	3308	3281	3230	3281
Type ^d	total	geom.	hyd.	π	planarity ^e
$\Delta \Delta g_{iso}^{type}$, ppm	-102	-24	-27	-51	51

^a All calculations carried out using B3LYP exchange–correlation functional and Huz-II basis set.

^b Electronic g-tensor shift component i.e. $\Delta g_{ii} = g_{ii} - g_e$, where g_{ii} is the electronic g-tensor value along principal axis and g_e is the free electron g-factor.

^c Isotropic electronic g-tensor $\Delta g_{iso} = 1/3(\Delta g_{xx} + \Delta g_{yy} + \Delta g_{zz})$.

^d Decomposition of Δg_{iso} environmental shift due to DNA environment into three contributions (geometry relaxation, hydrogen bonding and π stacking) according to Eq. 1, and ^e the contribution arising from the planarity of the spin-label $\dot{\text{C}}$ when compared to the tilted one, as found in the optimized model structure.

origins in contributions of almost equal size from spin label geometry relaxation upon its incorporation into the DNA and from the internal hydrogen bonding between guanine and spin label $\dot{\text{C}}$ in the modified base pair (G- $\dot{\text{C}}$). Before concluding the g-tensor analysis, we should address the issue of structural conformation of the spin label adopted in the B-DNA model structure. Recently, Edwards et al.⁵⁷ have reported the crystal structure of the rigid spin label $\dot{\text{C}}$ within a small molecule crystal lattice as well as incorporated in a double stranded A-form DNA. It is found that within a small crystal lattice the spin label exhibits a $\sim 20^\circ$ bend at the oxazine linkage, while a planar conformation of the spin label is found within the A-DNA system. Moreover, it is stated that the planar and bent forms are close in energy, and a small degree of flexibility is expected around the oxazine linkage. From our theoretical investigation, it appears that the spin label within the B-DNA model system displays a $\sim 9^\circ$ bend at the oxazine linkage, due to the pronounced π -stacking interaction occurring between the spin label and the upper cytosine unit (See. Fig. 1). To address the planarity influence on the EPR parameters we performed a different calculation in which the bent spin label is replaced with a planar one. These results are shown in the last column in 1, evidencing a negligible effect of the planar structure on the g-tensor, when compared to the bent form (that corresponds to the optimized geometry of the B-DNA model). Taking this into account, we conclude that the direct influence of the DNA environment on the electronic g-tensor, due to the spin label interaction with base pairs in DNA, is rather limited as the DNA can affect the nitroxide moiety $\text{R}_2\text{NO}^\cdot$ only indirectly via interaction with the π electron system of the phenoxazine derived group in the spin label $\dot{\text{C}}$.

After discussing the behaviour of the electronic g-tensor of

spin label $\dot{\text{C}}$ in the DNA environment, we turn to the second important parameter which characterises the spin label, namely the nitrogen hyperfine coupling tensor in the nitroxide moiety $\text{R}_2\text{NO}^\cdot$, see principal values of this tensor and its isotropic value for the free and the DNA model with incorporated spin label $\dot{\text{C}}$ in 2. As one can see from direct comparison of our results for the two systems, the overall influence of the DNA environment on A_{iso} is negligible and can thus safely be neglected in the analysis of EPR measurements. Thus, the spin density distribution as well as structure of orbitals on the nitroxide moiety are not affected by the incorporation of the spin label into the DNA. We point out that this result is in line with the proposed mechanism of interaction between the nitroxide moiety of the spin label with the base pairs, and, taken together with our findings for the electronic g-tensor, it strongly suggest that this mechanism is the dominant interaction between spin label and B-DNA.

It is also relevant to compare the influence of the DNA environment on the EPR spin Hamiltonian parameters with respect to two other environments studied by us previously, see 3 collecting the environmental shifts of Δg_{iso} and nitrogen A_{iso} in DNA, in a “guest-host” complex and in water environment^{26,27,31,32}. From the presented data it is clear that the water protic solvent induces the largest environmental shift for both the electronic g-tensor and the nitrogen hyperfine coupling constants, while comparing the remaining two environments the electronic g-tensor and the nitrogen isotropic hfcc are apparently more affected by incorporation into DNA than by encapsulation into the hydrophobic “host” cavity upon formation of the studied “quest-host” complex. Similarly to the case of the “guest-host” complex which we extensively studied in our previous work, the significant additional environ-

Table 2 Nitrogen hyperfine coupling constants of free and DNA incorporated spin label $\dot{\text{C}}$ computed using restricted-unrestricted density functional theory method.^{a,b}

A_{ii}^c, G	Free	B-DNA			
	$\dot{\text{C}}$	$\dot{\text{C}}$	G- $\dot{\text{C}}$	(C-G)(G- $\dot{\text{C}}$)(G-C)	(C-G)(G- $\dot{\text{C}}^p$)(G-C)
A_{xx}	2.58	2.58	2.56	2.61	2.53
A_{yy}	2.76	2.76	2.74	2.78	2.71
A_{zz}	28.41	28.36	28.26	28.48	28.46
A_{iso}^d	11.25	11.24	11.19	11.29	11.23
Type ^e	total	geom.	hyd.	π	planarity ^e
$\Delta A_{iso}^{type}, \text{G}$	0.04	-0.01	-0.05	0.10	-0.06

^a Nitrogen atom is located in R_2NO moiety of spin label $\dot{\text{C}}$.

^b All calculations carried out using B3LYP exchange–correlation functional and N07D basis set.

^c Hyperfine coupling constant component A_{ii} along principal axis, which consists from Fermi contact and spin-dipolar contributions. ^d Isotropic hyperfine coupling constant, $A_{iso} = 1/3(A_{xx} + A_{yy} + A_{zz})$, which corresponds only to Fermi contact contribution due to traceless nature of spin dipolar contribution.

^e Decomposition of A_{iso} environmental shift due to DNA environment into three contributions (geometry relaxation, hydrogen bonding and π stacking) according to Eq. 1, and ^e the contribution arising from the planarity of the spin-label $\dot{\text{C}}$ when compared to the tilted one, as found in the optimized model structure.

mental shift of the EPR parameters of the spin label in DNA is caused by altered patterns of the hydrogen bonding and dynamics around the nitroxide moiety $\text{R}_2\text{NO}\cdot$ of the spin label due to the presence of DNA rather than by the direct influence of the DNA itself.

Table 3 Environmental shifts of EPR spin Hamiltonian parameters of prototypical nitroxide spin labels in various environments.

Environment	B-DNA ^a	GH complex ^b	Water ^c
$\Delta\Delta g_{iso}, \text{ppm}$	-102	-64	-637
$\Delta A_{iso}, \text{G}$	0.04	-0.45	2.36

^a Environmental shifts corresponds only to direct DNA influence on EPR spin Hamiltonian parameters upon spin label incorporation into B-DNA. $\Delta\Delta g_{iso}$ and ΔA_{iso} values computed in this work.

^b Environmental shift corresponds to encapsulation shift of TEMPO like nitroxide into Cucurbit[8]uril into aqueous environment^{31,32}. $\Delta\Delta g_{iso}$ and ΔA_{iso} values have been taken from our previous work.

^c Environmental shift corresponds to solvation in water. $\Delta\Delta g_{iso}$ and ΔA_{iso} values have been taken from our previous works^{26,27}.

4 Conclusion

In order to validate crucial assumptions made in EPR spin label techniques, in this work we investigated the EPR spin Hamiltonian parameters of a spin labeled DNA-like system,

thereby complementing earlier studies of water and “guest-host” complex environments for the same parameters. We focused on the dependence of the shift of the electronic g-tensor and the nitrogen hyperfine coupling constant on the stacking and hydrogen bonding interactions that occur between a spin label and the adjacent nucleobases, which manifests upon incorporating the spin label into DNA. We found that, although the electronic structure of the spin label in DNA changes when compared to the one of the free spin label, the significant orbitals which define the overall size of the electronic g-tensor shift as well as the nitrogen hfcc change only slightly, and these changes have little effect on the nitroxide part of the spin label. Consequently, both spectroscopic parameters are only weakly influenced upon incorporating the spin label into DNA. An important result that emerges from these findings is related to the distance measurement experiments in DNA which involve nitroxide spin labels. Indeed, the empirical assumption that the performance of the EPR probe in such an environment is not affected is theoretically sustained in the present study. Although our earlier, similar, theoretical studies have indicated that the EPR spin Hamiltonian parameters of the spin labels indeed can undergo various modifications upon changes in their local environment in the cases of proteins and encapsulation complexes, thus that the empirical assumption made in typical analysis of EPR measurements may not always hold in those cases, the present study gives credibility to this important experimental assumption in the case of DNA.

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