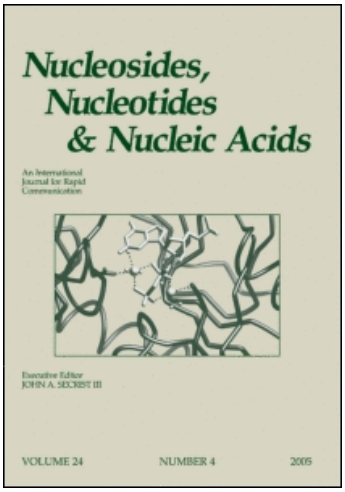

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PHOSPHATE SHIELDING UNDER DIFFERENT CONDITIONS RESULTS IN AN ENHANCED DNA DUPLEX STABILITY

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▫ *Neutralization of charge of the phosphodiester groups in DNA and the significance for transfer of genetic information will be demonstrated. Theoretical models based on proton shielding are elaborated with ab initio level calculations for a Watson-Crick–type dimer. These results are compared with molecular mechanics studies for duplexes of a hexamer with R_P and S_P phosphate-methylated backbones.*

Keywords DNA Backbone, Phosphate Shielding, Ab Initio, Molecular Mechanics

INTRODUCTION

Neutralization of charge of the phosphodiester linkages in DNA will play an important role in the transfer of genetic information. For instance, the intimate ion-pair formation with N-protonated amino acid residues in histone proteins results in highly compacted DNA duplexes. The enhanced stability of DNA in the nucleosome core has been resulted in a variety of conformational structures for duplex DNA.[1]

A unique example of duplex formation based on intimate ion-pair formation with N-protonated amino acid fragments, has been found by us for the self-association in a parallel fashion of natural dT_{10} and dC_{10} in the presence of *N*-protonated octadeca L-lysine.[2] It could be shown that this interaction proceeds with N(3)-H–O(4) and N(4)-H–N(3) hydrogen bridges for T = T and C = C pairing, respectively. Furthermore this duplex formation occurs in a stereospecific recognition of the prochiral phosphate groups. The efficiency of complexation is governed by the location of the peptide in the groove of the duplex.[3] This was demonstrated with octadeca L-ornithine which differs only one CH_2 group in the side chain compared with octadeca L-lysine. In the case of octadeca L-ornithine, no C = C pairing could be established, whereas T = T pairing could be demonstrated. Model studies show that in the case of octadeca L-ornithine complexation must

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occur with one the nonbonded oxygens in the phosphate linkages with the unfavorable O_R prochirality thereby precluding C = C base-pair formation.

Shielding of the phosphates has been studied by Fonseca Guerra et al.[4,5] in their investigation on hydrogen bonding in DNA base pairs at various levels of nonlocal density functional theory. This shielding was based on proton location at the nonbonding phosphate oxygen atoms. Agreement with X-ray crystal measurements was obtained by incorporation environmental elements for the hydrogen bond distances between the nucleic bases. The absence of the repulsive nature through the phosphates in the antiparallel arrangement of natural DNA has been proven by comparison with the Watson-Crick–type dimers separately. For that purpose an overall bond energy was defined as:

$$\Delta E = \Delta E_{\text{prep}} + \Delta E_{\text{int}}$$

in which ΔE_{prep} is the amount of energy required to deform the separate nucleic bases from their equilibrium structure to the geometry for base-pair formation. The interaction energy, ΔE_{int} corresponds to the actual energy change when the prepared fragments are combined as base pair. This energy can be defined as:

$$\Delta E_{\text{int}} = \Delta V_{\text{elstat}} + \Delta E_{\text{Pauli}} + \Delta E_{\text{oi}}$$

in which ΔV_{elstat} corresponds to the classical electrostatic interaction. The Pauli-repulsion ΔE_{Pauli} comprises destabilizing interactions between occupied orbitals and is responsible for steric repulsion. The orbital interaction ΔE_{oi} accounts for charge transfer as among others the HOMO-LUMO interaction. It appeared that the overall bond energy is independent of the backbone structure. For the A = T pairs, ΔE changes from -12.7 to -13.0 kcal/mol, i.e., from perfect proton shielding of the phosphate backbone toward base-pair formation *without* backbone. In the case of the G≡C pairs there was a corresponding change from -25.3 to -26.1 kcal/mol. If two A = T pairs are involved, the value for ΔE is -20.9 kcal/mol, which is less than twice the pairing energy of AT (-25.4 kcal/mol). This difference comes from the strain in the backbone described as ΔE_{prep}. Unfortunately, no calculations could be carried out on the phosphate backbone *without* proton addition because no convergence could be obtained (Baerends, E.J. Personal communication). Interestlingly, our former molecular mechanics studies of parallel and antiparallel methylphosphotriester DNA are in good agreement with the aforementioned results.[6] For the right-handed antiparallel phosphate-methylated $d(G_PC_PG_PC_PG_PC)_2$ we obtained for a one-level G≡C pair -20.20 (unmethylated), -21.54 (counterions), -25.56 (R_P-methylated), and -26.33 kcal/mol (S_P–methylated). For the right-handed parallel phosphate-methylated $d(T_PT_PT_PT_PT_PT)_2$ we calculated for a one-level T = T pair -11.20 (unmethylated), -15.88 (counterions), -18.14 (R_P-methylated), and -17.98 kcal/mol (S_P-methylated). Both examples demonstrate the enhanced stability for increasing shielding of the phosphate linkages. Analysis of ΔE showed that only the methylated systems remain stable at low dielectric constant, whereas the

unmethylated ones become unstable. This result clearly shows the electrostatic contribution with regard to the interstrand P–P interaction. For the slim parallel helix, an effective phosphate shielding is necessary by methylation.

Theoretically and experimentally it has been shown that in the case of T = T pairing there is no preference for R_P and S_P, whereas for C = C pairing only the S_P configuration is active.[3,7,8] On the other hand, the peptide-induced parallel duplexes of oligopyrimidines show that the complexation with the ammonium groups of oligo (L-lysine) and oligo (L-ornithine) is *enforced* by the peptide location in the groove of the duplex.

A biotechnical application of this type of cationic-anionic interactions has been recently found by Wang et al.[9] in their study of molecularly engineered biodegradable polymers used as protecting agents for DNA plasmids. This encapsulation of plasmid DNA, for instance encoding an antigenic sequence, provides a sustained release of DNA and subsequent production of encoded protein antigen for generating prolonged immune responses. Using an additional monomer as *N*-methyldiethanolamine, protonation under the biological conditions takes place which results in a positively charged tertiary amine which binds effectively with the phosphate linkages. The corresponding delay of the DNA release has been resulted in an enhanced immune response in vivo.

From the aforementioned results it is clear that phosphate shielding contributes to the stability of the various duplexes. It is suggested that the enhanced stability is caused by electrostatic interactions, hydrogen bridging, and methyl donation. Summarizing it seems to us that the local environment around phosphodiester groups plays an important role. The presence of organic cations deliver a number of qualifications that are absent in the interaction with inorganic cations. In the latter case the ultimate situation that can be reached is the formation of intimate ion pairs. This involves that the phosphate shielding is far from perfect. For organic cations that are equipped with an amphiphilic character an interaction field is created with a low dielectric constant resulting in an effective shielding of phosphates.[3,9,10] Another aspect is the nearest approach of the interacting charges. A combination of amphiphilicity and nearest approach will be found in methylated ammonium compounds. In histones these cations are present as positively charged ε-methyl ammonium groups of lysines.[11] If the reaction field consists of alkyl ligands as ethyl, propyl, etc., it is to be expected that steric factors result in a decrease of the electrostatic interaction between the ammonium ion and the anionic part of the phosphodiester groups. This is also reflected in the physico-chemical properties of the alkylphosphotriester DNA which is formed after the cationic alkyl transfer to the phosphate linkages. Methylphosphotriester DNA forms a very stable duplex with its natural counterpart, whereas the duplex stability of ethylphosphotriester DNA with natural DNA is strongly reduced in consequence of sterical hindrance by an inward orientation of the ethyl group in the DNA grooves.[12,13] Methyl transfer will be stimulated by phase-transfer processes through change of the medium from polar into nonpolar as is well recognized under biochemical conditions.[3,14,15]

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