Investigation of B-Z Transitions with DNA Oligonucleotides Containing 8-Methylguanine

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Abstract

Among various Z-form DNA inducers, such as transition metal complexes, polyamines and high ionic concentrations, 8-methylguanine have received attention as efficient chemical modifications. Although it is clear that m8–modified guanine base markedly stabilizes the Z conformation of short oligonucleotides under physiological salt conditions, how sequence composition affects the preference of Z-DNA is still not well established. In this study, various oligomers of $d(CG)_n$ or $d(GC)_n$ containing either 8-methylguanine in a different position were synthesized and their capacity of stabilizing Z-DNA were evaluated by CD spectra and then compared with each other. It is was found out that the Z-DNA stabilizing effect depend on the order of arrangement of m^8G and m^8rG in DNA strands and the center position is the most effective to stabilize the Z-DNA and promote the B to Z transition.

Introduction

It is well known that DNA possesses a considerable conformational flexibility.[1] Its polymorphic nature and the local conformational heterogeneity suggest that DNA should be regarded as a dynamic molecule, instead of a static molecule. The discovery of the molecular structure of DNA, right-handed double helix, provided us critical information how DNA maintains its secondary structure and participates various biological event as a genetic material. Since then, besides the predominant right-handed B-form DNA, a variety of secondary structures such as A-form, Z-form, and quadruplexes were found in specific conditions. Particularly, left-handed Z-form DNA has gained remarkable attention due to its opposite chirality. Since its discovery in 1979, many experiments were carried out in order to unveil the biological functions of the exceptional left-handed form.[1,2] It has been shown that Z-DNA has a high correlation with transcription, while over the few years, Rich and co-workers have found several proteins with a Z-DNA binding domain, including ADAR1, E3L, and PKZ.[3,4] Although the improvement of NMR studies and X-Ray crystallization has made a great contribution to relevant studies, there is still a bottleneck due to the stability of Z-DNA in a physiological condition. In fact, Z-DNA is more like a transient structure, which could be triggered by a biological event. In vivo, the conformation is mainly stabilized by negative supercoiling, which can be generated by translocation of RNA polymerase during a transcription process. Due to the narrower minor grooves and shorten distance among phosphate groups, steric hindrance and repulsion were elevated in Z-DNA. Therefore, extrinsic conditions such as high salt concentrations were necessary to overcome these limits, especially in short oligonucleotide model systems. In this context, various approaches such as chemical modifications, incorporation of metal complexes, small molecules have been investigated to stabilize the Z-form DNA under physiological salt conditions.[5] In the previous study, we have reported that the introduction of a methyl group at the guanine C8 position produces a stable m8–modified guanine base, 8-methyl-2'-deoxyguanosine (m^8G) and 8-methyl-guanosine (m^8rG) and markedly stabilizes the Z conformation of short oligonucleotides of a variety of sequences under physiological salt conditions.[6,7] In the light of these findings we have extended our investigation on the B-Z transition of m^8G-

and m^8rG-containing DNA oligonucleotides. In this study, we focused the relevance between the positions of the incorporated methylated guanine in a DNA strand and their corresponding stabilizing effects for Z-form DNA. A variety of CG and GC oligomers [$d(CG)_3$, $d(GC)_3$, $d(CG)_4$ and $d(GC)_4$] containing m^8G and m^8rG in a different position were synthesized and evaluated their ability to stabilize DNA in the Z-form. Herein, we report a systematic trend how sequence composition of the Z-stabilizer (m^8G or m^8rG) affects the inducing B-Z transition.

Results and Discussion

Circular dichroism spectroscopy is a common method in determining DNA secondary structure. B-Z transition of the DNA can be observed in a straightforward matter, as the B-form and Z-form possess opposite chirality. The B-form DNA contains a positive cotton effect at about 260–280 nm and a negative effect around 245 nm, while the Z-form DNA has a significant negative band around 290 nm and a positive band around 260 nm. The relative ratio of B and Z form DNA was determined by the change in the ellipticity at 295 nm, as there is negligible amount of single stranded DNA in 5 °C. A variety of m^8G- and m^8rG-containing DNA oligonucleotides were synthesized for this study. The CD spectra of m^8G- and m^8rG-containing hexamers under various NaCl concentrations are shown in Figure 1 and their midpoint NaCl concentrations for B-Z transition are summarized in Table 1. As shown ODN2–7 in Table 1, every hexamer containing m^8G and m^8rG has a lower midpoint NaCl concentration compared to the unmodified DNA oligomers, $d(CGCGCG)_2$. It is apparent that the incorporation of m^8G and m^8rG into DNA sequences significantly stabilizes the Z form DNA as we have previously reported.[6-7] With respect to the superior stabilization effect by m^8rG compared with m^8G, we have suggested that the introduction of a hydrophilic group to the solvent-exposed site stabilizes the Z form.[7] With regard to the order of arrangement of m^8G and m^8rG, we observed that the center position of m^8G and m^8rG in the DNA strand gave the lowest midpoint NaCl concentration and was the most effective to promote the B to Z transition and stabilize the Z-DNA.[8,9] In addition, it was found if m8–modified guanine base is placed further from the center of the oligomer, the stabilizing effect decreases consecutively. Notably, the results of CG hexamers and GC hexamers were dramatically different, as GC hexamers possess a much higher midpoint than CG

hexamers. For example, the midpoint NaCl concentration of oligomer $d(GCGCGC)_2$ is undeterminable (Table 2, entry 1 and Figure 2h). It didn't undergo B-Z transition even when NaCl concentration in the buffer was added until saturated, and showed its positive cotton effect around 295 nm-it still maintained B-DNA form. With regard to the significant difference in B to Z transition between $d(CG)_n$ and $d(GC)_n$, $(GC)_3$ is more stable as B-DNA under physiological salt condition due to the three GpC steps. The base stacking energy of GpC is the most stable step in B-DNA due to its extensive stacking interactions.[10,11] In contrast, the CpG has relatively higher stacking energies compared with GpC, so it is probable that the $d(GC)_3$ prefers three GpC steps to two CpG steps as B-DNA. Even though $d(GC)_3$ is not intrinsically favorable to be Z-form, m8–modified guanine base has powerful influence on the B to Z transition (Table 2, entries 3 and 6). In addition, the DNA octamers, $d(CG)_4$ and $d(GC)_4$, containing m^8G and m^8rG in a different position were synthesized and investigated. As shown in Table 3 and Figure 3, the consistent results with hexamer series were observed.

From the following results, we can certify that sequence composition largely alters the effect of the Z-form stabilizer. For the different behavior of the present oligodeoxynucleotide series, we have compared the solvation free energy with relevance to the conformational stability of Z-DNAs with different order of m^8G.[12,13] Solvation force is the important factor to affect the conformation of biomolecules. The three-dimensional reference interaction site model (3D-RISM) theory was utilized to investigate the value of the solvation free energy.[13-17] The energy minimized models for $d(CG)_3$ Z-DNA structure are shown in Figure 4 and the calculated solvation free energies of Z-DNAs are summarized in Table 4. The solvent analysis data indicates that $d(CGCm^8GCG)_2$ gives the lowest value of the solvation free energy. Furthermore, it was found if m8–modified guanine base is placed further from the center of the oligomer, the solvent free energy increases in the order of arrangement of m^8G. These results suggest that the center position of m^8G in the DNA strand might contribute to the stability of Z-DNA conformation by making favorable hydration structure.

Conclusion

In conclusion, we have synthesized DNA oligomers of $d(CG)_n$ or $d(GC)_n$ containing either 8-methylguanine in a different position and investigated their capacity of

stabilizing Z-DNA. It was found that the Z-DNA stabilizing effect depend on the order of arrangement of m^8G and m^8rG in DNA strands and the center position is the most effective to stabilize the Z-DNA and promote the B to Z transition. In addition, if m8–modified guanine base is placed further from the center of the oligomer the stabilizing effect of m8–modified guanine bases decreases consecutively. This study reaffirms the fact that the m^8rG is one of the exceptional Z-DNA stabilizers until now. We expect that the present results will provide the helpful information for the application of various Z-DNA-specific reactions. Further studies are under way to elucidate the correlation between the chemical modifications of bases and B to Z transition.

Materials and Methods

Oligonucleotide synthesis.

Various oligonucleotides containing 8-methylguanine were designed, and then synthesized on controlled pore glass supports by phosphoramidite method through an ABI DNA synthesizer (Applied Biosystems 3400, Foster City, CA). The phosphoramidite monomers were purchased from Proligo and Chemgenes. After the automated synthesis process, oligonucleotides were detached from the support by NH_3/CH_3NH_2 wash for 30 minutes. The deprotection was done by heating the wash at 95 °C for 30 minutes. Oligomers that contain m^8rG require an additional process: removal of the TBDMS-protecting group. It was done with 0.2 ml of 1 M TBAF in THF solution at room temperature, overnight. The reaction was then quenched by addition of 2 mL of 1 M TEAA solution and was desalted and purified on an OPC cartridge (Applied Biosystems).[18]

Then, oligonucleotides were purified by high-performance liquid chromatography, freeze-dry collected, then identified by electrospray ionization mass spectrometry (Perkin Elmer SCIEX API 165 mass spectrometer, negative mode). Through a Nanodrop device ND 1000 (Nano-drop Technologies, Wilmington, DE), the concentration and purity of the oligomer were determined.

CD spectra

The oligomers were first dissolved into buffers (0.10 mM base concentration in 5 mM sodium cacodylate buffer, pH 7.0, at various NaCl concentrations). Then, an annealing process was performed by heating 1 minute-long at 95 °C, cooling down to room temperature, then placed on ice ready for use. The CD spectra was then were recorded from 220 nm to 320 nm on a JASCO J-805 spectropolarimeter by using a 1 cm path-length cell under 4 °C.

Molecular Modeling Studies

Molecular modeling was carried out using the MOE (Molecular Operating Environment) software package. The coordinate of Z-DNA was taken form the Protein Data Bank, 1TNE, and then hydrogen atoms were added at proper positions. Based on this PDB data, 4 types of $d(CG)_3$ duplexes containing m^8G in a different position were constructed and minimized. The energy minimizations were performed with amber force field parameters, a distance-dependent dielectric constant of $\varepsilon = 4r$ (where, r is the distance between two atoms) and convergence criteria having an RMS gradient of less than 0.001 kcal mol^{-1} Å. The solvation free energy was estimated using MOE solvent analysis method based on the 3D-RISM (the three-dimensional reference interaction site model) theory. For energy minimization and solvent analysis water molecules were added to produce distance of 10 Å from the solute to droplet sphere boundaries and sodium counter ions were added to neutralize the system.

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Table 1. Midpoint NaCl Concentrations for B-Z Transitions in Modified $d(CGCGCG)_2$ Derivatives

entry	Oligonucleotides	NaCl (mM)
1	$d(CGCGCG)_2$	2600[a]
2	$d(Cm^8GCGCG)_2$	115
3	$d(CGCm^8GCG)_2$	30[a]
4	$d(CGCGCm^8G)_2$	227
5	$d(Cm^8rGCGCG)_2$	19
6	$d(CGCm^8rGCG)_2$	0[a]
7	$d(CGCGCm^8rG)_2$	28

[a] References 6 and 7. Midpoint NaCl concentration was determined from CD measurements at 10 °C at various NaCl concentrations.

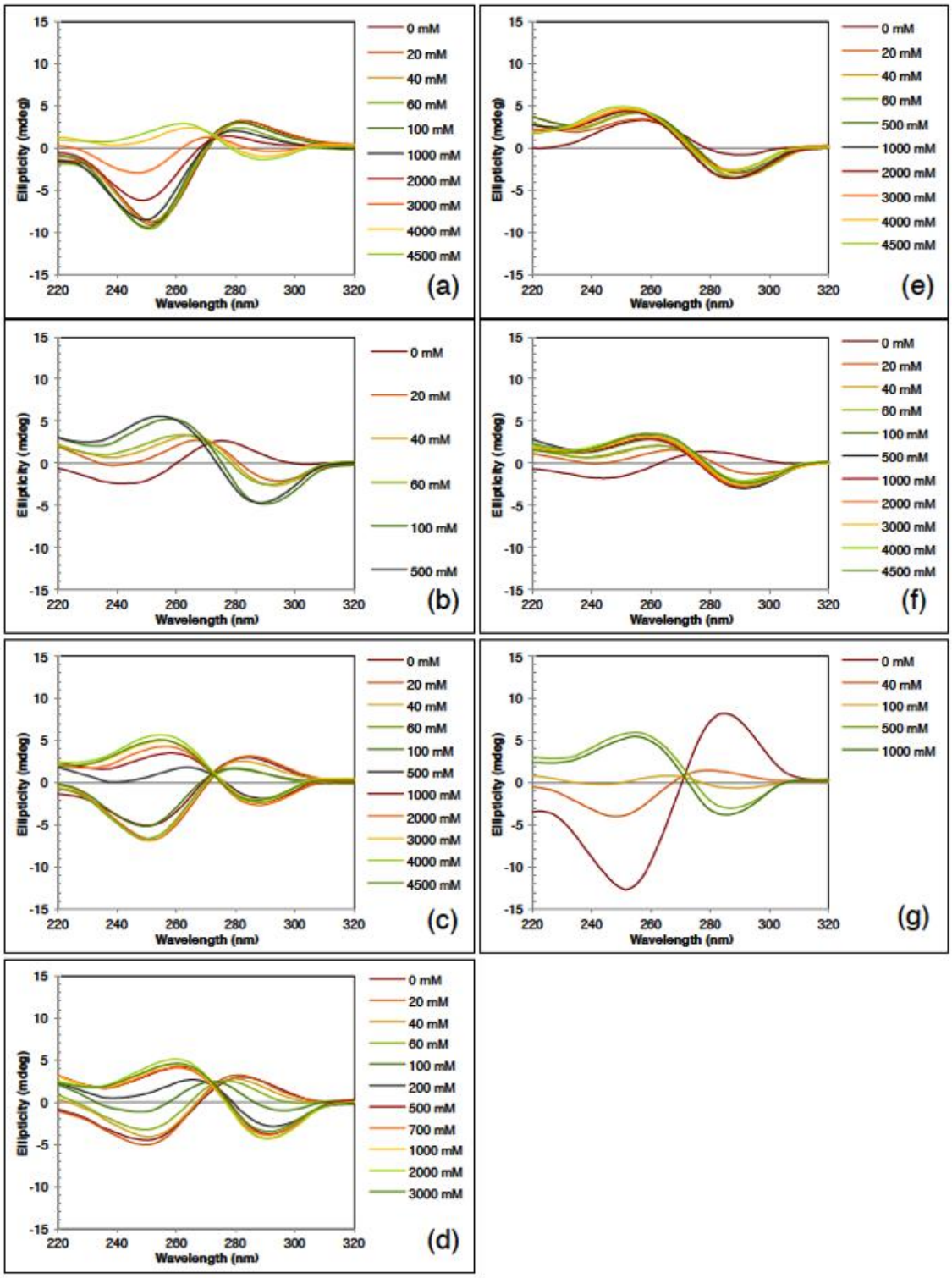

Figure 1. CD spectra of modified $d(CG)_3$ strands. (**A**) $d(CGCGCG)_2$; (**B**) $d(CGCm^8GCG)_2$; (**C**) $d(Cm^8GCGCG)_2$; (**D**) $d(CGCGCm^8G)_2$; (**E**) $d(CGCm^8rGCG)_2$; (**F**) $d(Cm^8rGCGCG)_2$; (**G**) $d(CGCGCm^8rG)_2$; Midpoint NaCl concentration was determined from the 290 nm of CD spectra at 4 °C, under various NaCl

concentrations.

Table 2. Midpoint NaCl Concentrations for B-Z Transitions in Modified $d(GCGCGC)_2$ Derivatives

entry	Oligonucleotides	NaCl (mM)
1	$d(GCGCGC)_2$	undeterminable
2	$d(m^8GCGCGC)_2$	2500
3	$d(GCm^8GCGC)_2$	96
4	$d(GCGCm^8GC)_2$	1370
5	$d(m^8rGCGCGC)_2$	1320
6	$d(GCm^8rGCGC)_2$	59
7	$d(GCGCm^8rGC)_2$	1000

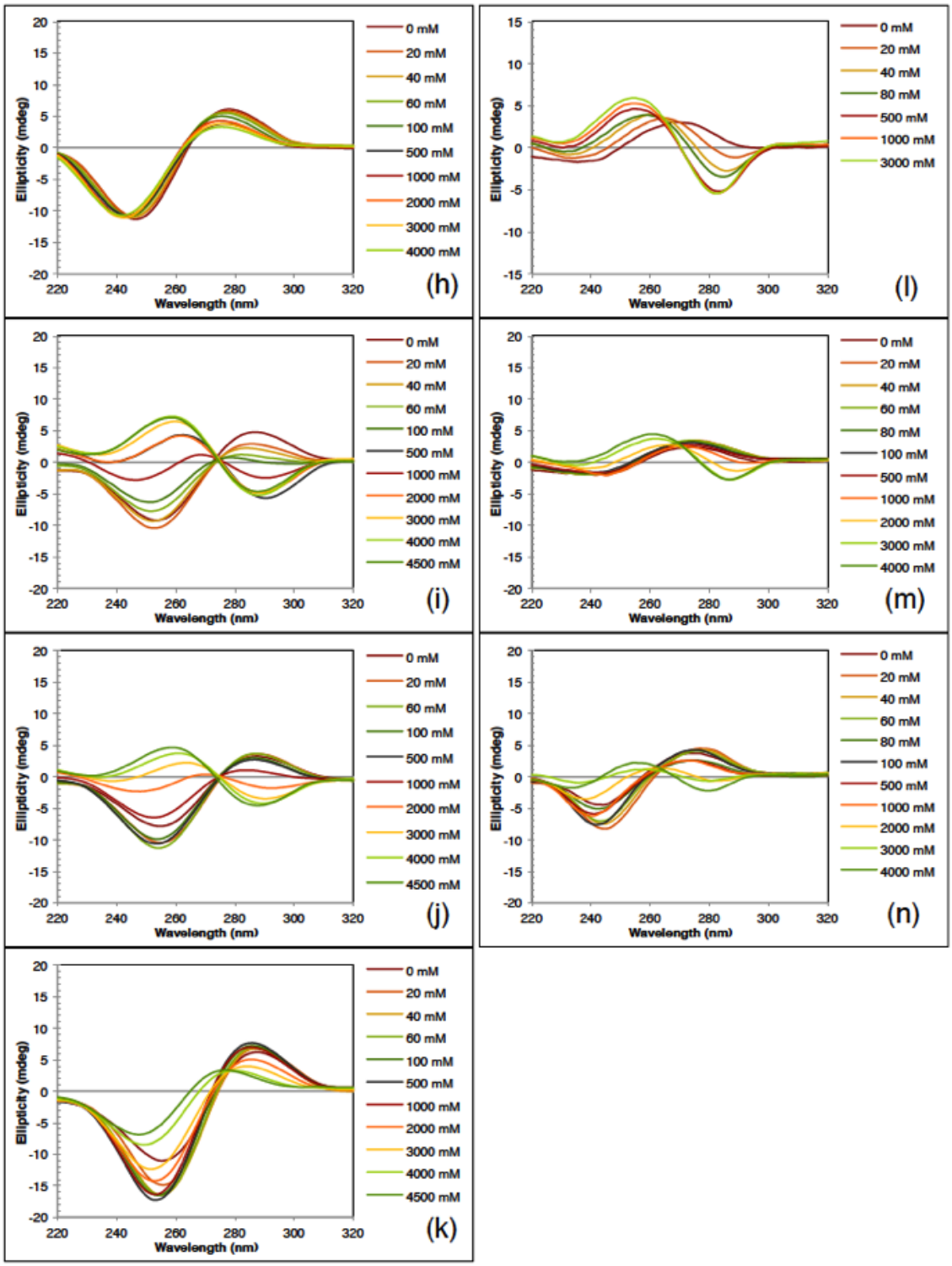

Figure 2. CD spectra of modified $d(GC)_3$ strands. (**H**) $d(GCGCGC)_2$; (**I**) $d(GCm^8GCGC)_2$; (**J**) $d(GCGCm^8GC)_2$; (**K**) $d(m^8GCGCGC)_2$; (**L**) $d(GCm^8rGCGC)_2$; (**M**) $d(GCGCm^8rGC)_2$; (**N**) $d(m^8rGCGCGC)_2$; Midpoint NaCl concentration was

determined from the 290 nm of CD spectra at 4 °C, under various NaCl concentrations.

Table 3. Midpoint NaCl Concentrations for B-Z Transitions in Modified $d(CGCGCGCG)_2$ and $d(GCGCGCGC)_2$ Derivatives

entry	Oligonucleotides	NaCl (mM)
1	$d(CGCGCGCG)_2$	1690
2	$d(CGCm^8GCGCG)_2$	34
3	$d(CGCGCm^8GCG)_2$	519
4	$d(GCGCGCGC)_2$	undeterminable
5	$d(GCm^8GCGCGC)_2$	1040
6	$d(GCGCm^8GCGC)_2$	1380

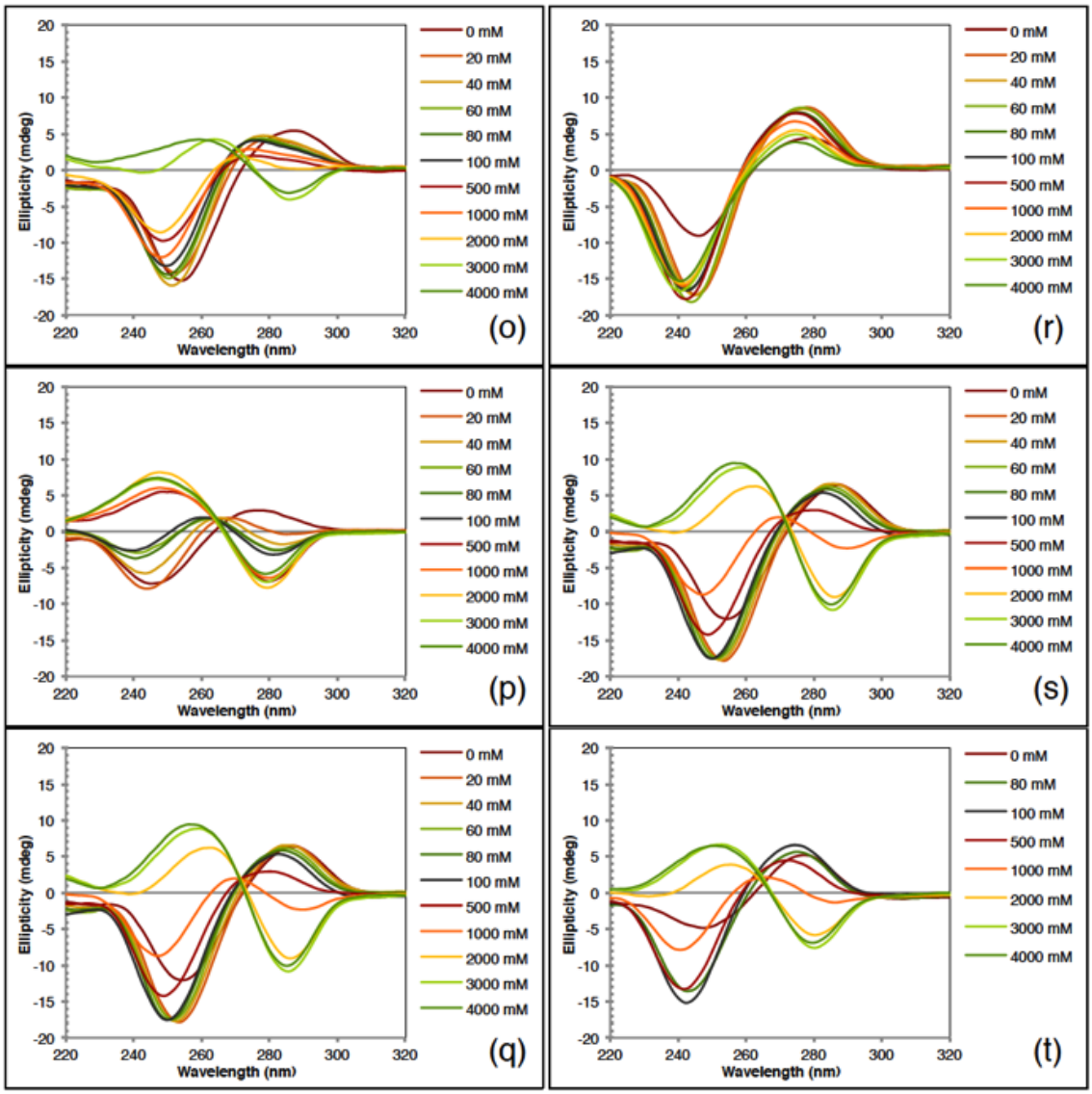

Figure 3. CD spectra of modified $d(CG)_4$ And $d(GC)_4$ strands. (**A**) d(CGCGCGCG)2; (**O**) $d(CGCm^8GCGCG)_2$; (**P**) $d(CGCGCm^8GCG)_2$; (**Q**) $d(GCGCGCGC)_2$; (**R**) $d(GCm^8rGCGCGC)_2$; (**S**) $d(GCGCm^8rGCGC)_2$.

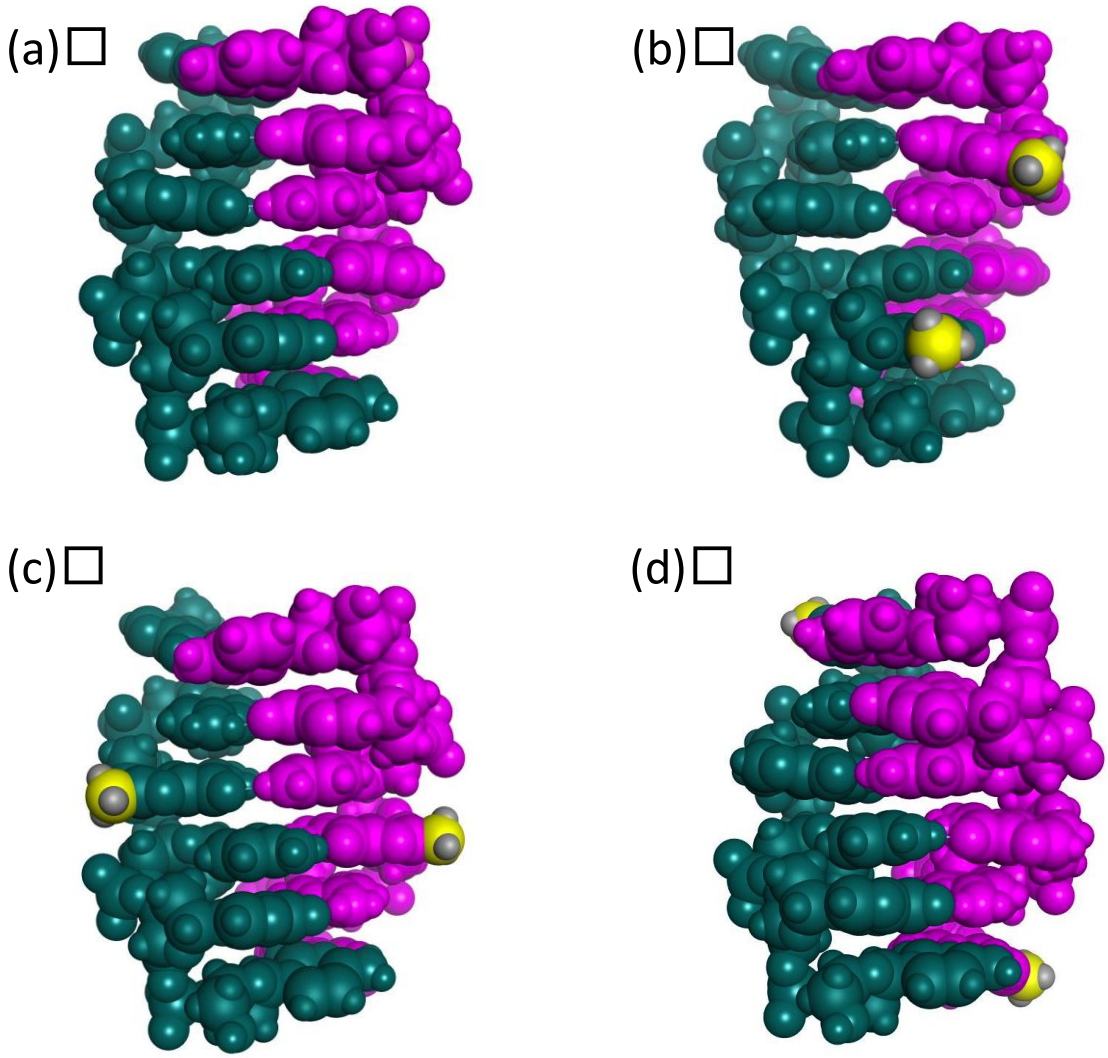

Figure 4. The optimized structures of modified $d(CG)_3$ strands. (a) $d(CGCGCG)_2$; (b) $d(CGCm^8GCG)_2$; (c) $d(Cm^8GCGCG)_2$; (d) $d(CGCGCm^8G)_2$.

Table 4. Midpoint NaCl Concentrations for B-Z Transitions in Modified $d(CGCGCGCG)_2$ and $d(GCGCGCGC)_2$ Derivatives

entry	Oligonucleotides	solvation free energy[a]
1	$d(CGCGCG)_2$	–9390
2	$d(Cm^8GCGCG)_2$	–9489
3	$d(CGCm^8GCG)_2$	–9539
4	$d(CGCGCm^8G)_2$	–9414

[a] Energy unit is kcal/mol. The solvent analysis was performed using the MOE (Molecular Operating Environment) under 500 mM NaCl concentration.