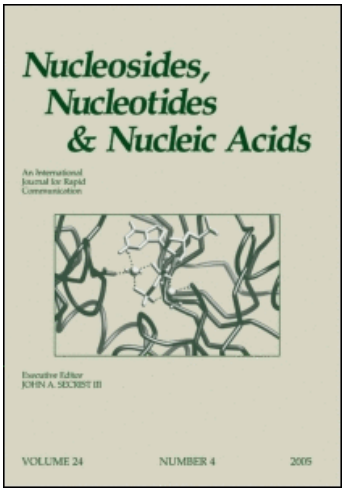

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Effect of Putrescine and PEG on a Structural Transition of DNA G-Quadruplex

Daisuke Miyoshi[a]; Akihiro Nakao[a]; Naoki Sugimoto[ab]
[a] Department of Chemistry, Faculty of Science and Engineering, Higashinada-ku, Kobe, Japan [b] High Technology Research Center, Konan University, Higashinada-ku, Kobe, Japan

Online publication date: 09 August 2003

To cite this Article Miyoshi, Daisuke , Nakao, Akihiro and Sugimoto, Naoki(2003) 'Effect of Putrescine and PEG on a Structural Transition of DNA G-Quadruplex', Nucleosides, Nucleotides and Nucleic Acids, 22: 5, 1591 — 1594
To link to this Article: DOI: 10.1081/NCN-120023041
URL: http://dx.doi.org/10.1081/NCN-120023041

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 22, Nos. 5–8, pp. 1591–1594, 2003

Effect of Putrescine and PEG on a Structural Transition of DNA G-Quadruplex

Daisuke Miyoshi,[1] Akihiro Nakao,[1] and Naoki Sugimoto[1,2,*]

[1]Department of Chemistry, Faculty of Science and Engineering, and
[2]High Technology Research Center, Konan University,
Higashinada-ku, Kobe, Japan

INTRODUCTION

We investigated quantitatively a structure, stability, and function of duplex, triplex, quadruplex, and non-Watson-Crick base pairs of DNA, RNA, and PNA.[1–4] However, it is still difficult that biomacromolecules such as a protein and nucleic acid are designed to form a native structure and function in a living cell containing high concentrations of solutes, because in a cell, the solutes occupy $30 \sim 40\%$ of the cellular volume.[5,6] Here, we demonstrate quantitatively an effect of molecular crowding on structures and stabilities of G-quadruplex, which is one of the important highly-ordered structures of DNA in vivo.

MATERIALS AND METHODS

DNA oligonucleotide used here, $d(G_4T_4G_4)$, was synthesized and purified as described previously.[7,8]

*Correspondence: Naoki Sugimoto, Department of Chemistry, Faculty of Science and Engineering, 8-9-1 Okamoto, Higashinada-ku, Kobe 658–8501, Japan; Fax: +81-78-435-2539; E-mail: sugimoto@konan-u.ac.jp.

DOI: 10.1081/NCN-120023041
1525-7770 (Print); 1532-2335 (Online)

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CD spectra of an antiparallel G-quadruplex structure had positive and negative peaks near 295 nm and 265 nm, respectively, while a parallel G-quadruplex structure had positive and negative peaks near 260 nm and 240 nm, respectively.[7] In this study, a structural type of G-quadruplex was determined with this information.

All simulations of d($G_4T_4G_4$) structure were performed with a QUANTA 97.1/CHARMm 23.2 (Molecular Simulation Inc.) as described previously.[1] The initial antiparallel G-quadruplex structure of d($G_4T_4G_4$) was obtained from the NMR data. Energies of the structures of putrescine, PEG (poly(ethylene glycol) Mw: 300), and d($G_4T_4G_4$) were individually minimized. The polycation and PEG were added manually to the groove of the energy-minimized G-quadruplex structure. The energy of complexes was further minimized as described previously.[1]

RESULTS AND DISCUSSION

It was reported that guanine-rich oligonulcotide used here, d($G_4T_4G_4$), forms the antiparallel G-quadruplex in the presence of Na^+.[9] Fig. 1 shows the CD intensity of 50 μM d($G_4T_4G_4$) at 260 nm with various concentrations of PEG or putrescine in a buffer containing 100 mM NaCl and 50 mM MES (pH 6.1). The CD intensity changes indicate that molecular crowding with PEG induced a structural transition from antiparallel to parallel G-quadruplex, although that with putrescine did not alter the antiprallel G-quadruplex. Binding constants of putrescine for d($G_4T_4G_4$) in the absence and presence of Na^+ are calculated to be 277 and 2.5 M^{-1} at 5°C, respectively. This shows that putrescine coordinates d($G_4T_4G_4$) with electrostatic interactions. On the other hand, the midpoint of the structural transition induced by molecular crowding with PEG in the presence of 100 mM NaCl is about 1.3 M, and therefore the observed binding constant of PEG for d($G_4T_4G_4$) is estimated to be 0.77 M^{-1} at 5°C. This indicates that the interaction between PEG

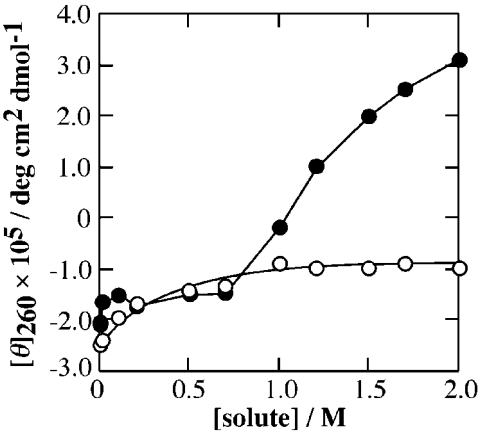

Figure 1. CD intensity of 50 μM d(G4T4G4) at 260 nm and 5°C for various concentrations of putrescine (open circle) and PEG (closed circle) in a buffer containing 100 mM NaCl and 50 mM MES (pH 6.1).

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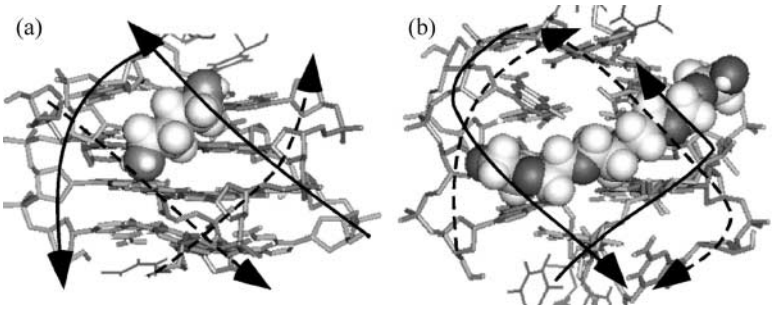

Figure 2. Calculated structures of (a) d(G4T4G4)-putrescine and (b) d(G4T4G4)-PEG complexes. The strands were traced by lines and dotted lines for clarity. Arrows indicate the strand directions. Putrescine and PEG were drawn with VDW model.

and $d(G_4T_4G_4)$ is thermodynamically unfavorable, and therefore, structural transition from antiparallel to parallel G-quadruplex was induced by the excluded volume.

The calculated putrescine-$d(G_4T_4G_4)$ structure suggests that putrescine can coordinate to the wide groove of the antiparallel G-quadruplex and does not disrupt the G-quadruplex structure (Fig. 2a). This tight putrescine coordination for the antiparallel G-quadruplex is consistent with the results of thermodynamic analysis. The electrostatic interactions inhibit the structural transition of $d(G_4T_4G_4)$. In contrast, a calculated PEG-$d(G_4T_4G_4)$ structure shows that the antiparallel G-quadruplex structure is disrupted by PEG (Fig. 2b), indicating that PEG does not interact chemically with $d(G_4T_4G_4)$. This is also consistent with the thermodynamic analysis of the PEG-$d(G_4T_4G_4)$ interaction. The thermodynamic analysis and molecular simulations showed that both volume excluded by solutes and chemical interaction between DNA and the solutes affect the structure of G-quadruplex.

ACKNOWLEDGMENT

This work was supported in part by Grants-in-Aid from the Ministry of Education, Science, Sports and Culture, Japan to N.S.

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