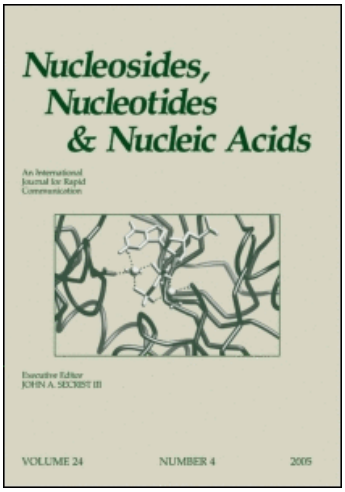

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Drug Binding to Higher Ordered DNA Structures: Ethidium Bromide Complexation with Parallel Quadruple-Stranded DNA

Toshio Shida[a]; Norihito Ikeda[a]; Junichi Sekiguchi[a]
[a] Department of Applied Biology, Faculty of Textile Science and Technology, Shinshu University, Nagano, Japan

To cite this Article Shida, Toshio , Ikeda, Norihito and Sekiguchi, Junichi(1996) 'Drug Binding to Higher Ordered DNA Structures: Ethidium Bromide Complexation with Parallel Quadruple-Stranded DNA', Nucleosides, Nucleotides and Nucleic Acids, 15: 1, 599 — 605
To link to this Article: DOI: 10.1080/07328319608002408
URL: http://dx.doi.org/10.1080/07328319608002408

NUCLEOSIDES & NUCLEOTIDES, 15(1-3), 599-605 (1996)

DRUG BINDING TO HIGHER ORDERED DNA STRUCTURES: ETHIDIUM BROMIDE COMPLEXATION WITH PARALLEL QUADRUPLE-STRANDED DNA#

Toshio Shida,* Norihito Ikeda, and Junichi Sekiguchi
Department of Applied Biology, Faculty of Textile Science and Technology,
Shinshu University, 3-15-1 Tokida, Ueda, Nagano 386, Japan

ABSTRACT: The interaction between ethidium bromide and a parallel G4-DNA, which is a quadruplex composed from four oligonucleotides containing a dG cluster, has been investigated. |d(TTGGGGTT)|4 formed a complex with ethidium bromide, which was assumed to be intercalated between the adjacent guanine tetrads of the quadruplex.

INTRODUCTION

Guanine-rich sequences are found in many locations on chromosomes such as in telomeres, which are the structures at the end of eukaryotic chromosomes. Telomeric deoxyribonucleic acid is composed of a dC-rich strand and a complementary dG-rich strand that is made up of a large number of repetitive dG cluster sequences.[1] The formation of parallel and antiparallel quadruple helices by intra- and intermolecular interactions has been proposed. Parallel quadruplex formation during meiotic prophase under physiological salt conditions has been suggested not only in the telomere regions but also in the dG cluster regions such as the immunoglobulin switch regions.[2] Recently, we have investigated the properties of oligonucleotides containing a dG cluster, $d(T_mG_nT_m)$, which are models of single-stranded parts of telomeric deoxyribonucleic acid and substitutes for poly (dG).[3] Electrophoretic and spectoscopic analyses of the oligomers have indicated that the oligomers can form two alternative structures, single- and quadruple-stranded helices, in solution at room temperature. We have suggested that parallel four-stranded structures of ribopolymers, poly (G), are reproducible within self-associated oligodeoxyribonucleotides containing a dG cluster. Recently, the structure of a parallel-stranded quadruplex formed by the hexanucleotide d(TGGGGT) was determined

#This paper is dedicated to Prof. Yoshihisa Mizuno on the occasion of his 75th birthday.

using X-ray crystallography.[4] The transformation of this quadruple-stranded helix to a single strand was inhibited by the addition of a potassium cation.[5]

In this paper, we report interactions between the parallel quadruplex, [d(TTGGGGTT)]4, and ethidium bromide. Ethidium bromide is known to bind to double helices of DNA and RNA. The interaction is attributable to the insertion of the planar phenanthridium ring of ethidium bromide between adjacent base pairs of the double helix.[6] The intercalation depends on the hydrophobic interactions between the DNA and the dye as well as electrostatic forces exerted between the phosphate residue of DNA and the positively charged nitrogen on the phenanthridinium ring. It is also known to stabilize base pairing of duplexes by intercalation.[7]

MATERIALS AND METHODS

Previously synthesized DNA oligomers[3] were used. Quadruple [d(TTGGGGTT)]4 sample (100 OD/ml, 50 mM NaCl) was prepared by freezing at -20 °C for four days. The oligomer was converted into the quadruplex over 99%. The sample solution was melted at room temperature just before use. The denatured single-stranded sample d(TTGGGGTT) was prepared by heating the quadruplex sample at 95 °C followed by cooling at room temperature.

UV absorption spectra were recorded using a Shimadzu UV-250 spectrophotometer. Circular dichroism (CD) spectra were recorded using a Jasco J-600 spectropolarimeter. For temperature control, a thermo-jacket cell and a circulating bath were used. After sample preparation, UV and CD spectra were immediately measured at room temperature in 0.025 M NaCl - 0.075 M KCl - 0.01 M Na cacodylate (pH 7.0). The molar absorption coefficient, ϵ, and the molar ellipticity, $[\theta]$, are presented in terms of per base residue values. Fluoresence emission spectra were recorded at pH 7.0 with a Shimadzu RF-5000 fluorescence spectrophotometer. The excitation was made at 490 nm. Denaturing (7M urea) electrophoresis was done in 25% polyacrylamide gels (19:1 ratio of acrylamide to bis acrylamide) using 0.4 x TBE buffer (0.36 M Tris-borate, 1 mM EDTA, pH 8.3) at room temperature. DNA bands were visualized by UV shadowing and ethidium bromide staining.

RESULTS AND DISCUSSION

We reported that the transformation of the single-stranded form into the quadruplex form or *vice versa* was undetectable in 0.1 M NaCl at a 4.3 x 10^{-5} M strand concentration at room temperature for a few days. Further, it was shown that the

quadruplex [d(TTGGGGTT)]4, was resistant to denaturation in 7 M urea.[3] The quadruplex was gradually dissociated into the single-strand in the absence of potassium ion.[5] Consequently, the quadruplex dissolved in 0.025 M NaCl - 0.075 M KCl - 0.01 M Na cacodylate (pH 7.0) was used for the spectroscopic analyses. In this condition, the quadruplex was not dissociated into the single-strand at all (data not shown). On the other hand, the single-strand was gradually converted into the quadruplex in 1 M NaCl at room temperature, but the conversion was not observed for at least 7 days in 0.1 M NaCl.

Single-stranded d(TTGGGGTT) and quadruplex [d(TTGGGGTT)]4 were observed as a single band in a denaturing 25% polyacrylamide gel (Fig. 1A, lanes 1 and 2, respectively). This phenomenon indicated that the dissociation of the quadruplex did not occur during the electrophoresis at all. The position of the quadruplex band corresponded to that of a single-stranded 30-40 mer. The band of the quadruplex was stained with ethidium bromide, known as an intercalator, whereas the band of the single-strand was not stained with it at all (Fig. 1A, lanes 4 and 3, respectively). Absorption spectra of ethidium bromide in the visible region were recorded before and after mixing the single-strand and the quadruplex (Fig. 1B). The ethidium bromide solution dramatically exhibited different spectral properties after mixing with the quadruplex. On the other hand, the mixture of ethidium bromide and single-stranded d(TTGGGGTT) did not exhibit these characteristics. Similar spectral shift occurred by the mixing of ethidium bromide with double-stranded DNA and RNA.[8] These results clearly indicated that ethidium bromide bound specifically to parallel quadruplex [d(TTGGGGTT)]4, in contrast to the lack of interaction between ethidium bromide and single-stranded d(TTGGGGTT).

The ethidium bromide-quadruplex complex showed a positive CD band in the 290-350 nm region as shown in Fig. 2. The $[\theta]_{max}$ was at 298 nm. It was suggested that the band was induced by intercalation of the phenanthridium ring of ethidium bromide between the guanine tetrads because ethidium bromide itself has no optical activity. This speculation was supported by the evidence that a similar induced CD spectrum of ethidium bromide by complexation with [d(TGGGGT)]4 was observed (data not shown).

The induced CD band of the ethidium bromide-quadruplex complex was significantly different from the induced CD band by the intercalation of ethidium bromide between standard Watson-Crick base pairs of the duplexes.

The fluorescence spectra of ethidium bromide (1×10^{-5} M) in the presence of DNA (nucleotide concentration: 1×10^{-4} M), which was excited at 490 nm, was measured (data not shown). The emission strength of ethidium bromide interacting with the quadruplex was approximately twice that of ethidium bromide itself at 590 nm. In the case of the short duplex DNA and calf thymus DNA, the strength of the

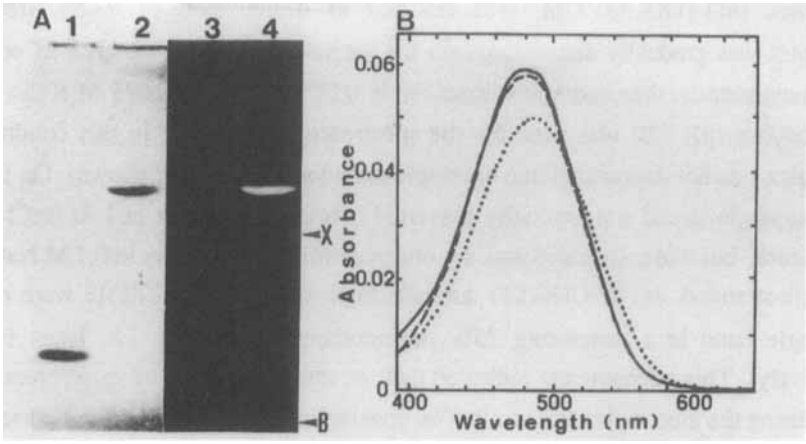

FIG. 1. Demonstration of interaction between [d(TTGGGGTT)]4 and ethidium bromide. A: Denaturing 25% polyacrylamide gel electrophoresis. Lanes 1 and 3, single-stranded d(TTGGGGTT); lanes 2 and 4, quadruplex [d(TTGGGGTT)]4. DNA bands were made visible by UV shadowing (lanes 3 and 4). B: Absorption spectra of ethidium bromide. Oligonucleotide concentration (base-residue concentration), 1 x 10^{-4} M; ethidium bromide concentration, 1x 10^{-5} M. ———, ethidium bromide; – – – – –, mixture of ethidium bromide and single-stranded d(TTGGGGTT); ·····, mixture of ethidium bromide and quadruplex [d(TTGGGGTT)]4.

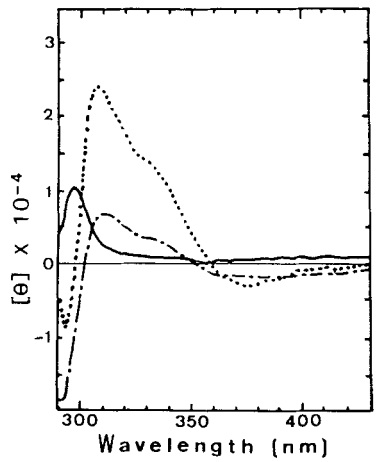

FIG. 2. Induced circular dichroism spectra of ethidium bromide solutions containing DNAs. Difference spectra in the absence and in the presence of ethidium bromide are shown. ———, quadruplex [d(TTGGGGTT)]4; —·—, duplex [d(TTGGGGTT)/d(AACCCCAA)]; ·····, calf thymus DNA.

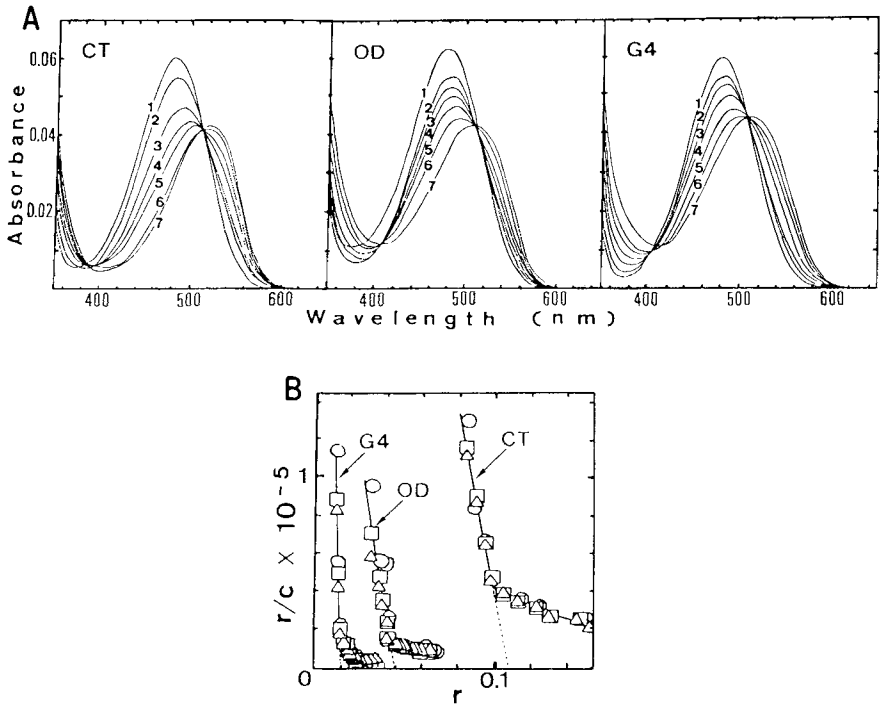

FIG. 3. Binding of ethidium bromide to DNAs. A: Absorption spectra of ethidium bromide (1 x 10^{-5} M) obtained by titration of DNAs. CT (calf thymus DNA): 0 M (1); 1.0 x 10^{-5} M (2); 3.0 x 10^{-5} M (3); 5.0 x 10^{-5} M (4); 7.0 x 10^{-5} M (5); 1.1 x 10^{-4} M (6); 2.5 x 10^{-4} M (7). OD (oligonucleotide duplex, [d(TTGGGGTT)/d(AACCCCAA)]): 0 M (1); 4.0 x 10^{-5} M (2); 6.0 x 10^{-5} (3); 8.0 x 10^{-5} M (4); 1.1 x 10^{-4} M (5); 1.7 x 10^{-4} M (6); 3.4 x 10^{-4} M (7). G4 (quadruplex, [d(TTGGGGTT)]4): 0 M (1); 4.0 x 10^{-5} M (2); 8.0 x 10^{-5} M (3); 2.0 x 10^{-4} M (4); 3.5 x 10^{-4} M (5); 5.0 x 10^{-4} M (6); 8.0 x 10^{-4} M (7). B: Scatchard plots for the interaction between DNA and ethidium bromide. Absorptions were measured at 460 nm (○), 470 nm (△), and 480 nm (□). *c* is the molar concentration of free drug and *r* is the number of drug molecules bound per nucleotide.

ethidium bromide emissions were 4 times and 8 times, respectively, compared with ethidium bromide itself.

Figure 3A shows the effects of increasing the DNAs on the absorption spectra of ethidium bromide in the 350-650 nm region. In each case, the peak progressively shifted towards a limit (spectrum 7) which represented the spectrum of ethidium bromide in fully complexed form. All the spectra passed through an isosbestic point at 510 nm, indicating that they resulted from the contributions of two forms of ethidium bromide, free and bound. Suitable wavelengths for the estimation of the proportions of free and bound

TABLE 1. Binding parameters of ethidium bromide to DNAs [a]

DNA	K [b] (x 10^{-6})	n [c]
Calf thymus DNA	4.74	0.108
d(TTGGGGTT)/d(AACCCCAA) [d]	5.14	0.046
[d(TTGGGGTT)]4 [e]	24.02	0.016

a) In 0.1 M NaCl - 0.01 M Na cacodylate (pH 7.0) at 23 °C. Parameters estimated by the Scatchard plot; ethidium bromide concentration maintained at 1 x 10^{-5} M. b) K: binding constant (M^{-1}). c) n: the number of binding sites per base residue. d) Duplex (OD). e) Quadruplex (G4).

ethidium bromide are 460 nm, 470 nm, and 480 nm. Scatchard plots for different DNA preparations are shown in Fig. 3B. The curvatures of the plots indicate at least two different modes of binding. The association constant, K, of the complex is defined as the following,[9]

$$r/c = (n - r)K$$

where c is the molar concentration of free ethidium bromide, r is the number of ethidium bromide molecules bound per nucleotide and n is the number of binding sites per nucleotide. The values of K and n were obtained in the nearly linear region of each plot at low r values. The binding parameters of ethidium bromide for each DNA are listed in Table I. These parameters are not definite values because the binding parameters are considerably dependent on ionic strength.[10] The association constant for the quadruplex is considerably large, compared with the constants for the duplexes. This difference might be attributable to the hydrophobicities of the guanine tetrads and Watson-Crick base pairs. The number of binding sites per the short duplex and the quadruplex was 0.74 and 0.51, respectively. In the ionic strength condition used in this study, the number of binding sites might be underestimated. The number of binding sites per nucleotides of calf thymus DNA in low salt condition (0.04 M Tris-HCl, pH 7.9)[8] is approximately twice compared with our data. Consequently, the number of binding sites per the quadruplex might be nearly one.

Eventually our data allowed us to reach the following conclusions: (i) ethidium bromide binds to the parallel quadruplex by the intercalation without its dissociation; (ii) ethidium bromide binding to the quadruplex exhibits a lower saturation binding density than ethidium bromide binding to the related short duplex. To clarify the peculiar behavior of the parallel quadruplex and the binding site of the quadruplex, additional study will be needed.

ACKNOWLEDGMENT: This work was partially supported by a Grant-in-Aid from the Nagano Prefecture Techno-Highland Development Organization.

REFERENCES

1. Blackburn, E. H.; Szostak, J. W. *Ann. Rev. Biochem.* **1984**, *53*, 163.
2. Sen, D.; Gilbert, W. *Nature* **1988**, *334*, 364.
3. Shida, T.; Yokoyama, K.; Tamai, S.; Sekiguchi,J. *Chem. Pharm. Bull.* **1991**, *39*, 2207.
4. Laughlan, G.; Murchie, A. I. H.; Norman, D. G.; Moore, M. H.; Moody, P. C. E.; Lilley, D. M. J.; Luisi, B. *Science* **1994**, *265*, 520.
5. Shida, T.; Ikeda, N.; Sekiguchi, J. *Nucleic Acids Sympo. Ser.* **1992**, *27*, 53.
6. Tsai, C. C.; Jain, S. C.; Sobell, H. M. *J. Mol. Biol.* **1977**, *114*, 301.
7. Krugh, T. R.; Reihardt, C. G. *J. Mol. Biol.* **1975**, *97*, 133.
8. Waring, H. J. *J. Mol. Biol.* **1965**, *13*, 269.
9. Scatchard, G. *Ann. N. Y. Acad. Sci.* **1949**, *51*, 660.
10. Douthart, R. J.; Burnett, J. P.; Beasley, F. W.; Frank, B. H. *Biochemistry* **1973**, *12*, 214.