Bioorganic & Medicinal Chemistry 22 (2014) 2593–2601

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Interactions of cyclic and non-cyclic naphthalene diimide derivatives with different nucleic acids

Izabella Czerwinska [a,*], Shinobu Sato [a,b], Bernard Juskowiak [c], Shigeori Takenaka [a,b]

[a] Research Center for Bio-microsensing Technology, 1-1 Sensui-cho, Tobata-ku, Kitakyushu-shi, Fukuoka 804-8550, Japan
[b] Department of Applied Chemistry, Kyushu Institute of Technology, 1-1 Sensui-cho, Tobata-ku, Kitakyushu-shi, Fukuoka 804-8550, Japan
[c] Laboratory of Bioanalytical Chemistry, Faculty of Chemistry, A. Mickiewicz University, Umultowska 89b, 61-614 Poznań, Poland

ARTICLE INFO

Article history:
Received 9 February 2014
Revised 5 March 2014
Accepted 21 March 2014
Available online 28 March 2014

Keywords:
Naphthalene diimide derivatives
G-quadruplexes
Telomeric DNA
Double-stranded DNA
Telomerase inhibition

ABSTRACT

Recently, strategy based on stabilization of G-quadruplex telomeric DNA by small organic molecule has been realized by naphthalene diimide derivatives (NDIs). At the same time NDIs bind to DNA duplex as threading intercalators. Here we present cyclic derivative of naphthalene diimide (ligand **1**) as DNA-binding ligand with ability to recognition of different structures of telomeric G-quadruplexes and ability to bis-intercalate to double-stranded helixes. The results have been compared to non-cyclic derivative (ligand **2**) and revealed that preferential binding of ligands to nucleic acids strongly depends on their topology and structural features of ligands.

1. Introduction

Since the structure of double-stranded DNA (dsDNA) has been proposed by Watson and Crick, the scientists have elaborated on DNA model resulting in the identification of three main conformations of DNA double helix (A-form, B-form and Z-form), in which geometry and size of each groove as well as direction of spin vary significantly.[1] At the same time, discovery of the four-stranded DNA structures (alternatively named G-quadruplexes, G4 DNAs, or tetraplexes) have stimulated the great research interest in structural investigations of these interesting DNA assemblies. DNA tetraplexes have been extensively characterized in vitro. In the presence of specific metal ions, different G-quadruplex topologies have been resolved for example a basket-type, a mixed-type or a chair-type structures.[2–8]

Recently, quantitative visualization of G-quadruplex assemblies in human cells have been achieved. G-quadruplex formation by human DNA during cell-cycle have been trapped and visualized due to the complex formation with small organic molecules (ligands).[9] These observations suggest that non-covalent interactions with ligands may play an important role in regulating these life's indispensable molecules. Their stabilization by ligands is believed to serve as an artificial regulation of both transcription and replication-dependent DNA damage, resulting in cell cycle arrest. The involvement of G-quadruplex DNA in site-specific DNA damage, has provided a suggestion that a combination of G-quadruplex ligands either with inhibitors of DNA repair or associated pathways could be a successful strategy that should be taken into account in the future treatment in senescence-mediated, antiproliferative therapy.[10–12] More recently, it was shown that treatment with G4-stibilizing ligands can lead to formation of DNA double strand breaks (DSBs).[13,14] The synthetic lethality in cancer cells was observed by for example exploiting the inherent HR DSB repair deficiency in particular cells suggesting that G4 ligands can be important anticancer agents.

It is also worth mentioning that stabilization of tetraplex structure formed by telomeric DNA may cause inhibition of a telomerase that is active in most cancer cells. It is believed that ligands can stabilize G-quadruplex structure on telomeric DNA (a primer for telomerase), which causes inhibition of this reverse transcriptase. In other words, telomerase cannot effectively elongate DNA leading to telomere shortening and apoptosis of tumor cells.[15–18]

For that reasons small DNA-targeting ligand molecules have been widely investigated and several DNA-binding compounds have been identified as therapeutic agents, especially in the anticancer and anti-pathogenic classes.[19–22] Also a series of naphthalene diimide derivatives (NDIs) has already been investigated as potential anti-cancer agents against pancreatic cancer as well as the selectivity of NDIs towards nucleic acids has been extensively studied.[23–26] It has been proved that the substituents in ligands

* Corresponding author. Tel.: +81 48502509316.
E-mail addresses: izaczerw@amu.edu.pl, izaczerw@yahoo.com (I. Czerwinska).

http://dx.doi.org/10.1016/j.bmc.2014.03.034
0968-0896/

can be an important element of designing telomeric G-quadruplex-directed naphthalene diimide ligands. For NDIs, the nature and size of the side-chain terminal groups, which may provide electrostatic interactions, are important factors in selectivity of binding. Usually ligand bulky groups improve stability of G-quadruplex structure by positioning itself in the G-quadruplex grooves. Since the quadruplex stabilization mainly occurs via end-stacking mode between external G-quartets of G4 DNA and naphthalene diimide large aromatic moiety, the area of G-quartets, loop regions, and groove dimensions are critical elements that influence on the binding selectivity of drug candidates. Therefore, all these parameters and their sensitivity to environmental conditions should be considered during the designing of ligands and their studies.[27–29] Moreover naphthalene diimide derivatives were also investigated as double-stranded DNA binders. Their ability to act as threading intercalating agents were investigated recently by Iverson group.[30,31] The main aim of their studies was to design threading polyintercalators with extended DNA binding sites, more specifically they designed a modular polyintercalation system, in which intercalating naphthalene diimide (NDI) units were connected by flexible linkers that alternated between the minor and major grooves of DNA when bound, which caused very slow dissociation of ligand from DNA helix.

However, to the our best knowledge, no attention has been given to designing tetraplex-targeting NDI molecule with cyclic structure for G-quadruplexes recognition purpose. Here we present cyclic derivative of naphthalene diimide (Fig. 1; ligand **1**) as DNA-binding agent with ability to recognize structures of telomeric G-quadruplexes and ability to bis-intercalate to double-stranded helices represented by oligo- or polynucleotides. Non-cyclic, dimethylamino derivative (Fig. 1; ligand **2**) was investigated as a reference compound. In other words, studies on interactions of these two naphthalene diimide derivatives with specific nucleic acids provided information about the effects of the structural diversity of G-quadruplexes or duplexes on the selectivity of drug binding.

2. Material and methods

2.1. Material

Three DNA oligonucleotides; G-quadruplex: [5′-AGGG(TTA GGG)$_3$-3′] (A-core), and duplex composed of two complementary strands: [(5′-GGGAGGTTTCGC-3′) and (5′-GCGAAACCTCCC-3′)] (dsDNA), were purchased from Genenet Co. (Fukuoka, Japan) and were used without further purification. Calf thymus DNA (ctDNA), poly (dA-dT)$_2$ and poly (dG-dC)$_2$ synthetic polymers were obtained from Sigma Aldrich (St. Louis, MO). Following extinction coefficients were used for quantification of nucleic acid solutions (unit of ε was M^{-1} cm^{-1}): 114,000 for 5′-GGGAGGTTTCGC-3′; 108,600 for 5′-GCGAAACCTCCC-3′; 228,500 for 5′-AGGG(TTAGGG)$_3$-3′; 12,824 for calf thymus DNA, 13,200 for poly (dA-dT)$_2$; and 16,800 for poly (dG-dC)$_2$. Before being used, oligonucleotide solutions were heated to 95 °C and annealed by slowly cooling to the room temperature. Ligands **1** and **2** were synthesized and characterized as previously described.[32,33] The 2.0 M KCl, and 5.0 M NaCl aqueous solutions were obtained from Life Technologies (Carlsbad, CA). 1.0 M Tris–HCl (pH 7.4) buffer was obtained from Sigma–Aldrich (St. Louis, MO). 2-Morpholinoethanesulfonic acid (MES) was obtained from Dojindo (Japan). GoTaq Hot Start polymerase was purchased from Promega (Madison, WI). HeLa cells, and TRAPese kit were obtained from EMD Millipore (Billerica, MA).

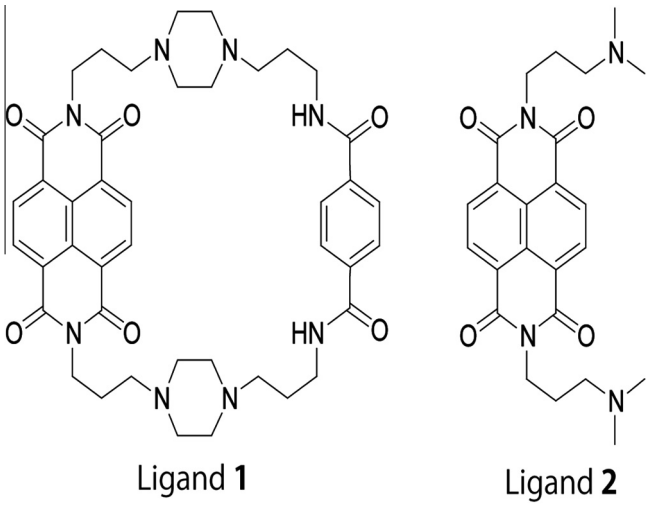

Figure 1. The structures of investigated naphthalene diimide derivatives.

2.2. Methods

2.2.1. CD titration experiments

Circular dichroism (CD) spectra were recorded on a Jasco J820 spectropolarimeter (Jasco Inc., Tokyo, Japan) under the following conditions: response, 2s; sensitivity, 100 mdeg; speed, 20 nm min^{-1}; resolution, 0.1 nm; band width, 2.0 nm; range, 220–500 nm. Each spectrum was obtained by averaging four scans and subtracting buffer baseline scan.

Measurements with oligonucleotides were performed at 25 °C in a 50 mM Tris–HCl buffer (pH 7.4) containing 100 mM NaCl or KCl. Concentration of double-stranded 12-mer oligonucleotide was 2.48 μM/duplex, while concentration of A-core was 1.5 μM/strand. Ligands **1** and **2** were added to DNA solution at increasing concentration from 0.5 to 6.75 μM or at concentration from 0.75 to 11.25 μM.

Measurements with ctDNA and polynucleotides were performed at 25 °C in 10 mM MES buffer (pH 6.25) containing 100 mM NaCl and 1 mM EDTA. Concentration of each DNA was 100 μM/base pair. Concentrations of added ligands **1** or **2** were in the range of 5 to 50 μM.

2.2.2. UV–vis titration experiments

Absorption spectra were measured on a Hitachi U-3310 spectrophotometer with a 0.5 cm path-length quartz cell and were recorded in the 200–600 nm range at 25 °C.

UV–vis absorption titrations were carried out by the stepwise addition of 200 μM A-core or dsDNA solution to a cell containing 5 μM solution of ligands **1** or **2**. All measurements were performed in a 50 mM Tris–HCl buffer (pH 7.4) containing 100 mM NaCl or KCl.

The binding constants of ligands were calculated by evaluation of UV–vis titration using Scatchard analysis or Benesi–Hildebrand method.[34,35]

2.2.3. Thermal melting experiments

Thermal melting experiments were performed with 1.5 μM A-core or with 3.2 μM dsDNA in a 50 mM Tris–HCl buffer (pH 7.4) containing 100 mM NaCl or KCl, using a Jasco J-820 spectropolarimeter in case of A-core DNA and a Hitachi U-3310 spectrophotometer in case of dsDNA, with a 1 cm path-length quartz cell. Measurements were conducted over the temperature range of 25–90 °C with a linear temperature gradient of 0.5 °C/min. Ligand–DNA ratio was set at 2:1.

Absorbance melting method was also used to estimate ΔT_m values for 40 μM poly (dA-dT)$_2$ with increasing concentration of ligand. Measurements were conducted in 1 cm path-length quartz