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Cyclic ferrocenylnaphthalene diimide derivative as a new class of G-quadruplex DNA binding ligand

Md. Monirul Islam [a], Shinobu Sato [a,b], Shingo Shinozaki [a], Shigeori Takenaka [a,b,*]

[a] Department of Applied Chemistry, Kyushu Institute of Technology, Kitakyushu 804-8550, Japan
[b] Center for Bio-microsensing Technology, Kyushu Institute of Technology, Kitakyushu 804-8550, Japan

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ABSTRACT

To identify an effective ligand that binds to a G-quadruplex structure but not a double-stranded DNA (dsDNA), a set of biophysical and biochemical experiments were carried out using newly synthesized cyclic ferrocenylnaphthalene diimide (cFNDI, **1**) or the non-cyclic derivative (**2**) with various structures of G-quadruplex DNAs and dsDNA. Compound **1** bound strongly to G-quadruplexes DNAs ($10^6\ M^{-1}$ order) with diminished binding to dsDNA ($10^4\ M^{-1}$ order) in 100 mM AcOH-AcOK buffer (pH 5.5) containing 100 mM KCl. Interestingly, **1** showed an approximately 50-fold higher selectivity to mixed hybrid-type telomeric G-quadruplex DNA ($K = 3.4 \times 10^6\ M^{-1}$ and a 2:1 stoichiometry) than dsDNA ($K = 7.5 \times 10^4\ M^{-1}$) did. Furthermore, **1** showed higher thermal stability to G-quadruplex DNAs than it did to dsDNA with a preference for *c-kit* and *c-myc* G-quadruplex DNAs over telomeric and thrombin binding aptamers. Additionally, **1** exhibited telomerase inhibitory activity with a half-maximal inhibitory concentration (IC_{50}) of 0.4 μM. Compound **2** showed a preference for G-quadruplex; however, the binding affinity magnitude and preference were improved in **1** because the former had a cyclic structure.

G-quadruplex-forming sequences occur at the ends of eukaryotic chromosomes and are known as telomeres, and they also exist in other vital regions of the genome as oncogene promoters, introns, 5′-UTR regions, or aptamers.[1–3] The G-quadruplex is a four-stranded nucleic acid structure produced by guanine-rich repeating sequences such as TTAGGG, which are composed of π-π-stacked on guanine tetrads (G-tetrads) by Hoogsteen-bonds.[2] The structure, stability, and topology of G-quadruplex DNAs depend on numerous factors such as the sequence, length of the loops, and mainly the presenting metal ions.[3] For instance, the human telomeric DNA can fold into a mixture of G-quadruplex topologies (parallel, antiparallel, and hybrid) in vitro under physiological conditions, compared to duplex DNAs.[4] The formation of G-quadruplex structures can regulate the transcriptional and translational activity of telomerase, expression of oncogenes, or both in the body. The telomere contains the length of the telomere DNA and is involved in approximately 85% of all cancers.

Molecules that stabilize G-quadruplex structures can inhibit telomerase or oncogene activities, or both and, thereby, inhibit intermittent cancer progression.[5] These molecules are considered potential anticancer therapeutic agents.[6–9] Notably, G-quadruplex-forming activity is very low in human somatic cells. Therefore, one of the major challenges in this field is to design a suitable new molecule as a ligand that selectively binds to G-quadruplex over dsDNA and exhibits ligand specificity toward a particular G-quadruplex topology.

Numerous ligands that preferentially bind G-quadruplex DNAs over duplex DNA have been reported over the past few decades. These ligands are well studied as G-quadruplex stabilizers both in vitro and in vivo that exhibit anticancer activity.[6–8] However, their binding affinities are still nonselective, as they show affinity to duplexes and other structures. Previously, we reported the synthesis and interaction studies of numerous cyclic naphthalene diimide (NDI) derivatives with G-quadruplex DNAs and dsDNA.[10–13] Furthermore, other macrocyclic NDI derivatives have been synthesized and studied by Marchetti et al.[14] to investigate their interaction with DNAs. These cyclic ligands showed versatile binding characteristics to G-quadruplex DNAs and dsDNA. We had numerous questions about the selectivity of these ligands to G-quadruplex DNAs over dsDNA including cyclic ligands with substituents or chain variations. These cyclic ligands were synthesized by cyclization with benzene or cyclohexane ring with two types of connecting chains (Fig. 1).

We hypothesized that these rings might have some beneficial or harmful effects on their binding with G-quadruplex DNAs or dsDNA, respectively. Particularly, the cyclohexane fused ring structure is more flexible during the binding with dsDNA than it

* Corresponding author at: Department of Applied Chemistry, Kyushu Institute of Technology, Kitakyushu 804-8550, Japan.
E-mail address: shige@che.kyutech.ac.jp (S. Takenaka).

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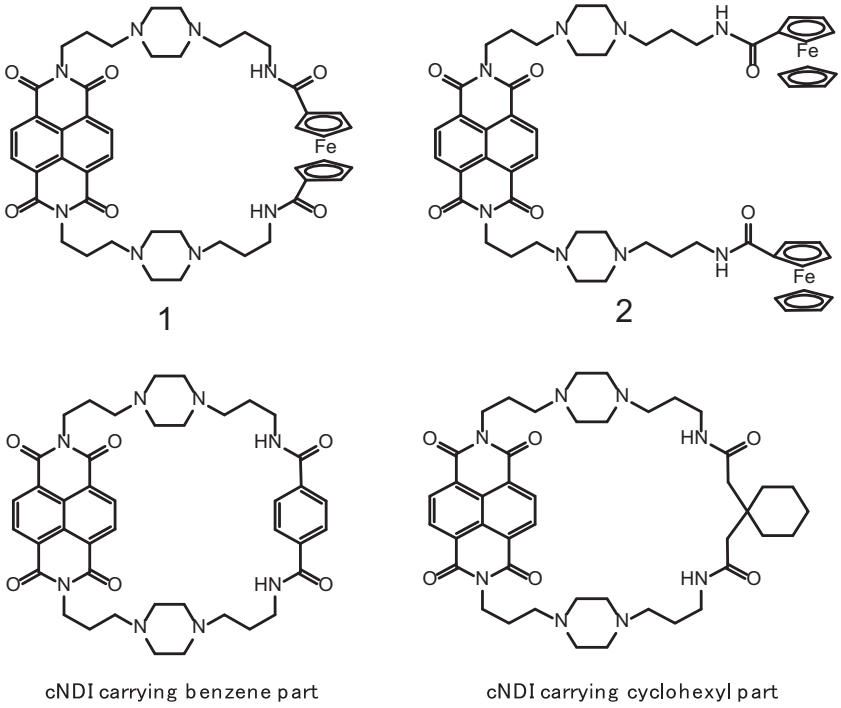

Fig. 1. Chemical structures of **1** and **2** with cNDI carrying benzene[9] or cyclohexyl[10] parts.

is with other DNAs. It cannot contribute to the intercalation with dsDNA, but it may be partially stacked on dsDNA, which consequently reduces binding with dsDNA. However, the cyclohexane rings in cyclic ligands do not have beneficial effects on their binding with G-quadruplex DNAs because the interaction between the cyclohexane ring and the loop part of the G-quadruplex DNAs has no positive effect. On the other hand, the benzene ring in cyclic ligands may have a suitable effect on their binding with G-quadruplex DNAs because of the π-π overlapping between the benzene ring and nucleic base in the loop part of the G-quadruplex DNA. However, the benzene ring of cyclic ligands can go through the dsDNA base pair, resulting in intercalation such as bis-intercalation or catenation and, thus, the selectivity of cyclic ligands to G-quadruplex DNAs over dsDNA is reduced.

To address the above challenges and discover suitable cyclic ligands that selectively bind to G-quadruplex DNAs over dsDNA, we designed and synthesized a new cyclic ligand by cyclization using thick and rigid substituent such as ferrocene. We predicted that the ferrocene in the cyclic ligand might facilitate its binding with G-quadruplex loop through π-π overlapping and at the same time, the ferrocene moiety would prevent the intercalation or catenation binding with dsDNA. On the other hand, the thickness and rigidity of the ferrocene moiety should strengthen the cyclic structure and decrease the flexibility, and thereby reduce the binding to dsDNA. With this aim in mind, we synthesized cyclic ferrocenylnaphthalene diimide (cFND, **1**) by connecting NDI and ferrocene through an alkyl chain, piperazine, and amide group. The amide part of the linker chain of **1** may reduce the binding affinity to dsDNA because one face of the intercalator (NDI moiety) covered the hindered alkyl chain and, consequently, reduced intercalation to dsDNA. Furthermore, these new types of cyclic molecules may facilitate end stacking onto the G-quartet plane using a single face of the NDI moiety or both faces of NDI or ferrocene moiety. In this study, we also investigated compound **2**, which was reported to be a dsDNA binder that exhibits threading intercalation[15] with different types of G-quadruplex DNAs and dsDNAs, as a negative control to strengthen the evidence supporting the macrocyclization approach. We attempted to differentiate the binding properties of cyclic **1** and non-cyclic **2** with the DNAs and determine their selectivity towards the G-quadruplex DNA.

We aimed to determine the selectivity of **1** and **2** towards G-quadruplex DNAs and dsDNA, by examining the interaction of these ligands with G-quadruplex DNA carrying a human telomeric region (a-core and a-coreTT), promoter region (*c-myc* and *c-kit*), or thrombin-binding aptamer (TBA) and dsDNA. These different conformations of G-quadruplex DNAs were confirmed using circular dichroism spectra. A detailed evaluation of the selective binding of compound **1** and **2** with the G-quadruplex DNAs over dsDNA was carried out using ultraviolet–visible (UV–vis) titration, circular dichroism (CD) titration, CD melting, and UV–vis melting, as well as fluorescence resonance energy transfer (FRET) melting, topoisomerase I isomerase, and telomeric repeat amplification protocol (TRAP) assays.

To investigate the binding properties of **1** and **2** with various G-quadruplex DNAs and dsDNA, spectrophotometric titrations were carried out. In particular, we focused our attention on differentiating the binding properties between G-quadruplex DNAs and dsDNA, as well as the telomeric and non-telomeric G-quadruplex DNA. Fig. 2A depicts an example of the interaction between **1** and the human telomere G-quadruplex DNA (a-core) in K^+ ion, which revealed that the highest absorption was at 384 nm. After adding the pre-annealed a-core DNA to **1**, large hyperchromicities (45–60%) and small redshifts (3–8 nm) were observed, which indicate the strong stacking interaction and specific mode of binding with G-quadruplex DNAs.[10] On the other hand, the interaction of **1** with dsDNA showed hypochromic (25–30%) and red (2–4 nm) shifts that were smaller than those observed with the G-quadruplex DNAs. This result suggests that this compound is not a good dsDNA binder (Fig. 2B). The Scatchard plot (Fig. 2C) of the G-quadruplex DNA was analyzed using the McGhee–von Hippel Scatchard equation to obtain a binding constant (K) and the ratio of **1** per DNA n value.[16] Furthermore, the nK values of the dsDNA were estimated using the Benesi–Hildebrand method because the binding saturation of **1** with dsDNA was not observed.[17]