Synthesis of adamantyl naphthalene diimide and its interaction with double stranded DNA

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ABSTRACT
An adamantyl naphthalene diimide derivative (AND) was synthesized as a highly selective double stranded DNA binding reagent. The binding studies with sonicated calf thymus DNA revealed that AND can bind to double stranded DNA by the threading mode, where the two adamantyl moieties are located in the major and minor grooves of DNA duplex separately. In the presence of β-cyclodextrin (β-CD), the complex of AND with DNA duplex was stabilized by capping of the adamantyl moieties of AND by β-CD. In other words, β-CD serves as a stabilizer for the complex of AND with DNA duplex to result in an enhanced selectivity of AND for double stranded DNA over single stranded DNA.

INTRODUCTION
DNA detecting reagents having very high discrimination ability between single stranded DNA (ssDNA) and double stranded DNA (dsDNA), can be used in DNA sensing because DNA sensing is based on the discrimination of dsDNA (hybrid of target DNA with probe DNA) from ssDNA (unreacted DNA probe and sample DNA). We have been developing a DNA sensing system based on ferrocenyl naphthalene diimide (FND), which has high selectivity for dsDNA. FND acts as a threading intercalator and its ferrocenyl substituents are projecting over the major and minor grooves of DNA resulting in the slow dissociation of FND from dsDNA.

Herein, we synthesized adamantyl naphthalene diimide (AND, Scheme 1) aiming at a ligand with an even higher selectivity for dsDNA. β-Cyclodextrin (β-CD) has a cavity large enough to accommodate adamantane. When β-CD interacts with the adamantyl moieties of AND bound to dsDNA, the dissociation of AND from dsDNA may be retarded. This notion was tested in this paper by studying the binding behavior of AND with dsDNA in the absence and presence of β-CD.

EXPERIMENTAL
Synthesis of AND
AND was synthesized by the condensation reaction of the terminal amino moieties of naphthalene diimide precursor with adamantaneacetic acid. The naphthalene diimide precursor was synthesized by the reaction of 1,4,5,8-naphthalenetetraacarboxylic acid and N, N-bis-(3-aminopropyl)methylamine.

Binding study
Binding constant (K) and binding site size (n) were calculated by fitting the data to the MacGhee and von Hippel equation: $v/L=K(1-nv)((1-nv)/(1-(n-1)v))^{n-1}$, where $v$ and $L$ refer to the saturation rate and the concentration of unbound DNA, respectively.

Kinetic analysis
Dissociation rate constants for the interaction of AND with DNA in the absence or presence of β-CD were determined on a double mixing stopped-flow system equipped with a
temperature controller. The dissociation rate constant ($k_d$) was obtained from the sodium dodecyl sulfate (SDS)-driven dissociation process. Thus, when the DNA-AND complex was mixed with an SDS solution, free AND was incorporated into the SDS micelle. Since this process is diffusion-controlled, the entire absorption change represents the $k_d$-dependent process and, therefore, the fitting of the kinetic trace provided the $k_d$ value. Both $k_d$ values from the intercept and the SDS-driven kinetics were in good agreement within error. The two-exponential equation rather than the single-exponential one was needed to obtain good fitting of the curve. The exact reason for this is not known, but the binding of the indicator from the major and minor grooves may not be equivalent.

RESULT AND DISCUSSION
An absorption maximum was observed at 383 nm for AND in 10 mM MES and 1 mM EDTA (pH 6.4) containing 0.1 M NaCl (Figure 1). Upon addition of sonicated calf thymus DNA, this absorption maximum shifted to longer wavelength with a decrease in absorbance (hypochromic and red shifts). Since this behavior is similar to that of analogous naphthalene dimide derivatives, AND seems to bind to dsDNA by threading-type intercalation. Similar spectral change was observed in the spectrophotometric titration experiments in the presence of β-CD. The data obtained in the absence and presence of β-CD were analyzed by Scatchard plots and fitted with the theoretical curves generated by the binding equation to give a binding constant of $(8.7±0.3) \times 10^4$ and $(1.4±0.0) \times 10^5$ M$^{-1}$, and site size of 3.2±0.1 and 2.7±0.1, respectively. In other words, β-CD improves the binding of AND to DNA about 1.6 times and the complex is stabilized presumably by the capping of the adamantane parts located in the major and minor grooves by β-CD.

Kinetic experiments were also carried out at 25 °C in the same medium. The dissociation rate constants ($k_ds$) thus obtained for the binding of AND with calf thymus DNA were 1.18, 0.84, and 0.65 s$^{-1}$ with 0, 0.5, and 0.25 of [AND]/[β-CD], respectively. These values compare well with those of related FND$^2$ and were reasonable for AND as a threading intercalator and β-CD could stabilize the complex of AND with dsDNA.

The results obtained here present a novel supramolecular complex consisting of dsDNA, AND, and β-CD and give a clue to the design of a hybridization indicator having higher preference for dsDNA.

![Figure 1. Spectrophotometric titration of 5μM AND with 0-0.2 mM sonicated calf thymus DNA in 10 mM MES and 1 mM EDTA (pH 6.4) containing 0.1 M NaCl.](https://academic.oup.com/nass/article-abstract/2/1/213/1058590)

![Figure 2. Scatchard plots of 5μM AND with sonicated calf thymus DNA in the absence (o) or presence of 30μM β-CD (●) in 10 mM MES and 1 mM EDTA (pH 6.4) containing 0.1 M NaCl.](https://academic.oup.com/nass/article-abstract/2/1/213/1058590)

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