Effect of an alkyl amino group on the binding of 1,8-naphthyridines to AP site-containing DNA duplexes

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ABSTRACT

A 1,8-naphthyridine derivative having a positively charged side-chain, N-(3-aminopropyl)-5,6,7-trimethyl-1,8-naphthyridin-2-amine (APATMND), is synthesized, and its binding to AP site-containing DNA duplexes (5’-GCA GCT CCC GXG GTC TCC TCG-3’/ 5’-CGA GGA GAC CNG GGG AGC TGC-3’, X = AP site; dSpacer, N = C, T) is examined in solutions buffered to pH 7.0 (I = 0.11 M, at 20°C). Fluorescence titration experiments reveal that, as compared to a parent ligand, 2-amin0-5,6,7-trimethyl-1,8-naphthyridine (ATMND), capable of selectively binding C over T opposite an AP site in the duplex (K_d/nM: C: 56, T: 100), APATMND shows a stronger binding affinity for T, while an affinity for C is reduced (K_d/nM: C: 135, T: 37). An examination of salt dependence of binding constants reveals that a polyelectrolyte contribution (ΔG_p) is indeed increased for C- and T-bindings of APATMND, but a loss of non-polyelectrolyte contribution (ΔG_i) is significant when binding to C. These binding properties of APATMND are discussed with a view towards further development of DNA-binding ligands suitable for gene detection.

INTRODUCTION

Studies on the chemistry of DNA-binding small ligands are of ongoing interest due to their promising functions and biological activities, including their anti-cancer properties and ability to regulate gene expression. Of particular interest to us is the development of a class of ligands suitable for gene detection, especially for single-nucleotide polymorphisms (SNP) typing.

We have recently found a series of aromatic ligands that can bind to a nucleobase opposite an abasic site (AP site) in DNA duplexes, and have proposed a new strategy of ligand-based fluorescence assay for SNP typing. In contrast to typical DNA-binding ligands capable of targeting double stranded DNAs by intercalation or groove binding, it is characteristic of our ligands to target non-Watson-Crick base-pairing sites in DNA duplexes, where the binding is promoted selectively by a pseudo-base pairing along the Watson-Crick edge of intrahelical target nucleobases. Indeed, we have successfully discovered a series of fluorescence ligands with a useful affinity and selectivity, including cytosine-selective 2-amino-7-methyl-1,8-naphthyridine (AMND), guanine-selective 2-amino-6,7-dimethyl-4-hydroxypteridine, thymine-selective amiloride, and adenine-selective alloxazine. These ligands were effectively applicable to the analysis of polymerase chain reaction (PCR) amplification products, and the SNPs genotype of samples was distinguished by combination of ligands with selectivity for respective target nucleobases.

In this work, we report on an effect of an alkyl amino group on the binding of 1,8-naphthyridines to AP site-containing DNA duplexes, with a view towards further development of this class of DNA-binding ligands. A naphthyridine derivative having a 3-aminopropyl group, N-(3-aminopropyl)-5,6,7-trimethyl-1,8-naphthyridin-2-amine (APATMND, cf. Scheme 1), is synthesized here, and its binding properties are compared to those of a parent ligand, ATMND (2-amin0-5,6,7-trimethyl-1,8-naphthyridine, cf. Scheme 1), having a binding selectivity for cytosine. Interestingly, by introducing the aminopropyl group to the mother skeleton, the binding affinity for thymine is effectively enhanced, while the affinity for cytosine is reduced. This results in a change in the binding selectivity from cytosine to thymine (K_d/nM, at 20°C, I = 0.11 M, pH 7.0: C: 135, T: 37). Such binding properties of APATMND are discussed based on the examination by fluorescence titration experiments.

RESULTS AND DISCUSSION

APATMND was synthesized in three steps from 2,6-

Scheme 1 Synthesis of APATMND.
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**Table 1 Binding parameters of naphthyridines to pyrimidine bases in AP site-containing DNA duplex**

<table>
<thead>
<tr>
<th></th>
<th>$K_{obs}$ (10^8 M⁻¹)</th>
<th>$\Delta G_{obs}$ (kcal mol⁻¹)</th>
<th>-SK</th>
<th>$\Delta G_{pe}$ (kcal mol⁻¹)</th>
<th>$\Delta G_i$ (kcal mol⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATMND - C</td>
<td>18</td>
<td>-9.6</td>
<td>-1.7</td>
<td>-1.9</td>
<td>-1.7</td>
</tr>
<tr>
<td>APATMND - C</td>
<td>27</td>
<td>-9.3</td>
<td>-2.6</td>
<td>-2.8</td>
<td>-2.8</td>
</tr>
<tr>
<td>ATMND - T</td>
<td>10</td>
<td>-9.9</td>
<td>-1.7</td>
<td>-2.0</td>
<td>-2.0</td>
</tr>
<tr>
<td>APATMND - T</td>
<td>15</td>
<td>-9.9</td>
<td>-2.9</td>
<td>-3.3</td>
<td>-3.3</td>
</tr>
</tbody>
</table>

$K_{obs}$ (M⁻¹), determined by fluorescence titration experiments (analysis at λem: ATMND: 404 nm, APATMND: 414 nm), is the 1:1 binding constant in 110 mM Na⁺ at 20°C. (NaCl] = 100 mM, [EDTA] = 1.0 mM, [sodium cacodylate] = 10 mM, pH 7.0). $\Delta G_{obs}$ is the observed binding free energy calculated from $\Delta G_{obs} = -RT \ln K_{obs}$, $SK$ is the slope of the plot of $\log K_{obs}$ versus $\log a_{eq}$. $\Delta G_{pe}$ and $\Delta G_i$ are polyelectrolyte and non-polyelectrolyte contributions to the binding free energy ($\Delta G_{pe} = (SK)RT \ln a_{eq}$, $\Delta G_{obs} = \Delta G_{pe} + \Delta G_i$) evaluated at 110 mM Na⁺. DNA duplex: 5'-GCA GCT CCC GXXG GTC TCC TCG-3' 5'-CGA GGA GAC CNG GGG AGC TGC-3', X = AP site; dSpacer, N = C or T.

diaminopyrimidine. As is shown in Scheme 1, the reaction with 3-methyl-2,4-pentanedione gave ATMND, from which 2-chloro-5,6,7-trimethyl-1,8-naphthyridine was prepared. The side-chain was then introduced by reacting with 1,3-diaminopropane. The structure of APATMND has been fully confirmed by elemental analysis, ¹H NMR, ESI-MS.

Fluorescence titration experiments were performed to examine the binding of APATMND to C or T in AP site-containing duplexes (cf. Table 1). In solutions buffered to pH 7.0 ($I = 0.11$ M, at 20°C), APATMND exhibits a significant quenching of its fluorescence upon binding to C in the duplex, as has been observed for ATMND-C binding. The resulting changes in fluorescence intensity at 414 nm can be explained by a 1:1 binding model, giving a dissociation constant, $K_{eq}$, of 135 nM. Interestingly, as compared to ATMND-C binding ($K_{eq} = 56$ nM), the binding affinity is reduced despite the presence of a positively charged amino moiety of the side-chain.

On the other hand, the introduction of the side-chain is indeed effective in increasing the binding affinity for T. APATMND shows a stronger binding affinity for T ($K_{eq} = 37$ nM) as compared to ATMND ($K_{eq} = 100$ nM). Thus, the binding selectivity of 1,8-naphthyridine is changed from C to T, by introducing the aminopropyl group to the mother skeleton.

In order to obtain more details about the effect of the side-chain on the binding events, salt dependence of binding constants was examined according to polyelectrolyte theory by Record et al.⁴, so that the observed binding free energy ($\Delta G_{obs}$) was dissected into its polyelectrolyte ($\Delta G_{pe}$) and non-polyelectrolyte ($\Delta G_i$) contributions. The parameters obtained for the ATMND or APATMND pyrimidines (C, T) interactions are summarized in Table 1. The change in the binding affinity for T ($\Delta \Delta G_{i} = +0.4$ kcal/mol), which is responsible for the decrease in the binding affinity for C ($\Delta \Delta G_{obs} = +0.4$ kcal/mol).

**CONCLUSION**

We have shown an effect of an amino alkyl group on the binding of 1,8-naphthyridines, resulting in a change in the binding selectivity from C to T. Further studies are in progress to obtain more details about APATMND-DNA interactions.

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