Strong binding of naphthyridine derivatives to cytosine in an AP site-containing DNA duplex and their application to fluorescence detection of single nucleotide polymorphisms

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ABSTRACT

Here we report on a significant enhancement of the binding affinity of naphthyridine-based fluorescence ligands that can selectively bind to cytosine opposite an AP site in a DNA duplex (5'-TCC AGX GCA AC-3'/3'-AGG TCC CGT TG -5', X = AP site, C = target). We have previously reported that 2-amino-7-methyl-1,8-naphthyridine (AMND) binds to cytosine with a dissociation constant of 370 nM (pH 7.0, I = 0.11 M, at 20 °C). In this work, AMND is modified simply by introducing two methyl groups to the naphthyridine ring. The present ligand, 2-amino-5,6,7-trimethyl-1,8-naphthyridine (ATMND), shows a stronger binding affinity for cytosine, and a dissociation constant reaches 56 nM. ATMND is effectively applicable to the analysis of cytosine-related mutation of PCR products. These promising abilities of ATMND are presented based on the examination by melting temperature (Tm) and fluorescence measurements.

INTRODUCTION

The need for the rapid detection of single nucleotide polymorphisms (SNPs) has led to a number of available methods including high-density arrays, primer extension methods, real time PCR, Invader assay, and Sniper assay.1 These methods, however, require several time-consuming steps, use of fluorophore-labelled oligonucleotides, and/or special enzymes. A quick, simple and cost-effective method is highly desirable for the routine detection of SNPs, which would allow for the implementation of individualized medical treatment based on genetic variations.

For this purpose, we have recently proposed a new strategy of ligand-based fluorescence assay for SNPs typing, in combination with abasic site (AP site)—containing probe DNAs.2,3 In contrast to current methods based on hybridization or enzymatic assays, our method is characterized by the construction of the AP site in DNA duplexes, which provides microenvironments for small ligands to bind target nucleotides with fluorescence signalling. 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),4 guanine-selective 2-amino-6,7-dimethyl-4-hydroxypteridine,5 and thymine-selective amiloride.6 These ligands were applicable to the analysis of PCR (polymerase chain reaction) amplification products, where a complexation-induced fluorescence quenching of these ligands were utilized to detect the single nucleotide mutation. However, the binding affinities of these ligands are in the order of sub-micromolar range, so that our assay requires considerable amounts of PCR amplification products. In order to develop an assay more suitable for practical use, further efforts are therefore necessary to improve the binding affinities of this class of ligands. This would also offer a valuable insight into the molecular basis of interactions for the development of various kinds of DNA-binding molecules.

In this work, we report on a significant improvement in the binding affinity of cytosine-selective AMND (Fig. 2), for which two methyl groups are attached additionally to the mother naphthyridine ring. Despite such simple modification, the binding affinity of the present ligand, 2-amino-5,6,7-trimethyl-1,8-naphthyridine (ATMND, cf. Fig. 2), is indeed one order magnitude higher than that of parent AMND, and the dissociation constant reaches 56 nM when binding to C in an AP site-containing DNA duplex (at 20 °C, I = 0.11 M, pH 7.0). Such a promising ability of ATMND is presented based on the examination by melting temperature (Tm) measurements and fluorescence titration studies.

RESULTS AND DISCUSSION

First, we examined the interaction between ATMND and cytosine in a 11-meric AP site-containing DNA duplex (5'-TCC AGX GCA AC-3'/3'-AGG TCC CGT TG-5', X = AP site, C = target) by melting temperature (Tm) measurements. For comparison, the binding of AMND was also examined. As is shown in Fig. 1, all melting curves of the duplex give a sigmoidal shape typical for the thermal denaturation of DNA duplexes. In the absence of ligands (curve a), the Tm of the duplex is determined as 30.5 °C from the first derivative of the melting curve. In the presence of ligands (AMND: curve b, ATMND: curve c), an increase in Tm is clearly observed, indicating that the ligand is incorporated
into the AP site by the binding to cytosine, which results in an increase in the thermal stability of the DNA duplex. The stabilization by ATMDN is much significant as compared to AMND, where the Tm increases by as much as 20.6°C (curve e). It is therefore highly likely that the binding affinity of ATMDN is much stronger than that of AMND.

The ligand-cytosine interaction was then examined by fluorescence titration experiments. As is shown in Fig. 2, ATMDN exhibits the significant quenching of its fluorescence upon addition of DNA duplexes containing cytosine opposite the AP site. The resulting titration curve can be analyzed by a 1:1 binding isotherm, giving a dissociation constant Kd of 56 nM (pH 7.0, f = 0.11 M, at 20°C). Under the identical experimental condition, AMND shows a Kd of 370 nM.3 Thus, the attachment of methyl groups to the naphthryl ring is indeed effective for the increase in the binding affinity, and ATMDN has a striking ability to bind cytosine in AP site-containing DNA duplexes.

Preliminary experiments reveal that ATMDN is effectively applicable to the analysis of cytosine-related mutation present in 107-mer DNAs (K-ras gene,4 codon 12, anti-sense strand). After asymmetric PCR amplification, the products were analyzed at 5°C in a buffer solution (pH 7.0, 100 mM sodium cacodylate) containing 1.6 mM EDTA, 50 nM ATMDN and 1.0 μM AP site-containing 20-mer probe DNA. Fluorescence quenching of ATMDN (403 nm) is observed for the cytosine-containing wild type sequence (ACC: 41%), while the quenching efficiencies are only moderate for the mutation sequences (ATC: 10%, AAC: 2%, AGC: 2%). The analysis requires no time-consuming steps such as purification of PCR products and careful control of temperature and the result is readily obtained after PCR.

**CONCLUSION**

In summary, we have successfully developed a cytosine-selective fluorescence ligand with a nanomolar range of binding affinity. The results presented here clearly indicate that the introduction of methyl groups to the mother ring is strikingly sensitive to the binding of this class of ligands.

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**REFERENCES**