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Construction of Highly Reactive Probes for Abasic Site Detection by Introduction of an Aromatic and a Guanidine Residue into an Aminooxy Group

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Detection and quantification of DNA lesions plays an important role in studies on causal relationships between DNA lesions and mutagenesis.[1,2] Chemical probes specific for DNA lesions are powerful tools for detection[3−5] because probe-mediating detection is convenient and does not require derivatization or digestion of DNA.[6,7] Apurinic/apyrimidinic (AP) sites are a prevalent type of DNA lesion, arising from hydrolysis of glycosidic bonds of nucleotides or excision repair of bases modified by radiation and alkylating agents.[8] Oxidized AP lesions, 2-deoxyribonolactone, C4-AP, or DOB are also produced by the reactive species of antitumor agent bleomycin[9] or by the diradicals of neocarzinostatin chromophore.[10] These oxidized AP lesions, as well as normal AP sites, not only are mutagenic but also cause strand scission of DNA.[8,11]

Since AP sites exist at equilibrium between cyclic hemiacetal and open-chain aldehyde forms, an aldehyde-reactive probe (ARP, Figure 1) is frequently used for detection of AP sites.[3,12] Oxidized AP lesions can also be specifically detected with smartly designed molecules such as cystein[13] and a Tris derivative.[14] These probes make it possible to detect AP sites using a biotin−avidin complex system after conjugation with the DNA lesion.[15,16] Recently, AP sites are also used as reactive intermediates, provided by deoxyuridine and uracil DNA glycosylase (UDG), to obtain labeled DNA for gene expression profiling.[17]

Although ARP has a simple structure, it is not necessarily designed for high reactivity toward AP sites.[3] We therefore constructed highly reactive AP site-detecting probes by assembling a hydrophobic and a hydrophilic residue into an aminooxy group and report here the construction and the structure−function relationships.

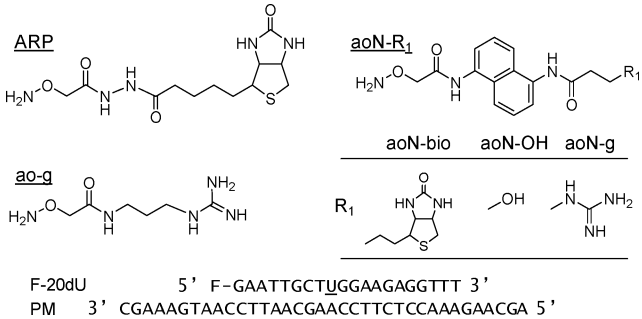

Figure 1. Structures of aminooxy derivatives and sequences of double-stranded oligonucleotides (F and underlined U indicate fluoresceine and deoxyuridine residue, respectively).

AP sites in DNA are flanked by bases (or base pairs) at their 5′- and 3′-sides, and some aromatic hydrocarbons interact with DNA by stacking interactions.[18] To take advantage of the stacking effects with the adjacent bases, we synthesized naphthalene-containing aminooxy derivatives (aoN-bio and aoN-OH in Figure 1; *N* denotes naphthalene). The structure of aoN-bio is just like a naphthalene-inserted construct at the hydrazine of ARP, and aoN-OH bears a hydroxyl group in place of a biotin residue (Figure 1). Since an amido bond linking to an aromatic ring is planar,[19] we expected that this arrangement of an aminooxy group close to bis-amido-naphthalene would act effectively in both conjugation and stacking in an AP site pocket. We also synthesized aoN-g and ao-g having a guanidine residue (*g* denotes guanidino group) to enhance electrostatic interaction or hydrogen bonding with backbone phosphate groups of nucleic acids, since a guanidine has a positive charge at neutral pH ($pK_a \approx 12.5$).

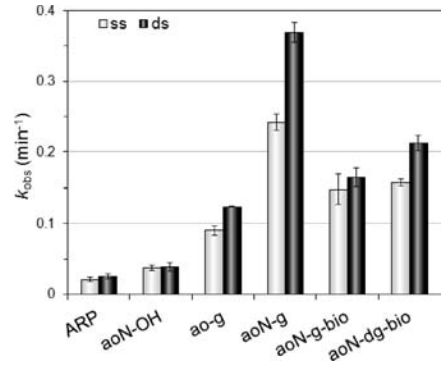

Figure 2. Observed rate constants for each probe for single-stranded (F-20dU) and double-stranded (F-20dU/PM) ONTs. White bar: F-20dU; black bar: F-20dU/PM.

In contrast to ARP, aoN-bio was insoluble in aqueous solution due to the hydrophobic naphthalene residue. Other probes without a biotin were dissolved, and in particular, aoN-g and ao-g could be easily dissolved in aqueous solution due to the guanidine residues. Thus, aoN-OH, aoN-g, and ao-g were subjected to labeling reactions with AP sites generated by UDG treatment of single-stranded (ss) (F-20dU) or double-stranded (ds) (F-20dU/PM; Figure 1) oligonucleotides (ONTs) containing a deoxyuridine. Observed rate constants (k_{obs}) were determined in the presence of excess amounts of these probes. Rates were slightly higher for aoN-OH than for ARP, and those for the guanidine-containing probes (aoN-g, ao-g) were significantly higher than those for aoN-OH (Figure 2 and Figure S2 in Supporting Information (SI)). In particular, aoN-g showed the fastest reaction rates among all probes, and the rate for ds-ONT was 9.5-fold higher than that of aoN-OH without a guanidine.

These differences in reaction rate are closely related with probe structures. First, the guanidino group has the capability to increase the conjugation efficiencies of aminooxy derivatives. The guanidino group is thought to promote association of these compounds with ONTs by hydrogen bonding or electrostatic interactions, as previously reported.[20−22] Next, the naphthalene residue also contributes

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to fast conjugation, as shown by the different rates for aoN-g and ao-g. The effect of the naphthalene residue is due to stacking interactions with bases or base pairs adjacent to the AP site, as supported by the fact that aoN-g decreased reaction rates for ds-ONTs containing mismatched base pairs adjacent to the AP site (Figure S2 in SI). Interestingly, these results indicate that the high reactivity of aoN-g is due to synergetic effects of guanidine and aromatic residues and that the hydrophobic interaction of naphthalene might work effectively when the probes are accessible to the target ONTs with the help of the guanidino group. Although aoN-g showed high reactivity to AP site, it did not react to 2-deoxyribonolactone (SI).[13,23] However, aoN-g should be active for C4-AP, similar to other aminooxy derivatives.[23]

aoN-g containing both naphthalene and guanidine is a promising candidate for a new labeling reagent. Hence, we synthesized its biotin conjugate by linking a biotin to the guanidine with an ethyleneglycol linker (aoN-g-bio; Figure 3A). For a cognate probe, we prepared aoN-dg-bio, which has a guanidino group directly connected to the naphthalene ring (Figure 3A). Both of the naphthalene-guanidine-containing probes were still soluble in aqueous solution, in contrast to aoN-bio, due to the presence of the guanidino groups. Observed rate constants for these biotin-tagged probes were obtained from reaction with ONTs (Figure 2). The rates for aoN-g-bio and aoN-dg-bio were approximately 6- to 8-fold faster than that for ARP. Interestingly, aoN-dg-bio showed slightly faster reaction rates than did aoN-g-bio in reaction with either ss- or ds-ONT. Compared with aoN-g, both aoN-g-bio and aoN-dg-bio decreased the reaction rates with ss- and ds-ONTs, and the decrease was significant in the reaction with ds-ONT. This decrease is apparently derived from the biotin attachment, and it is thought that the biotin insertion obstructed the guanidine-effects exerted in aoN-g.

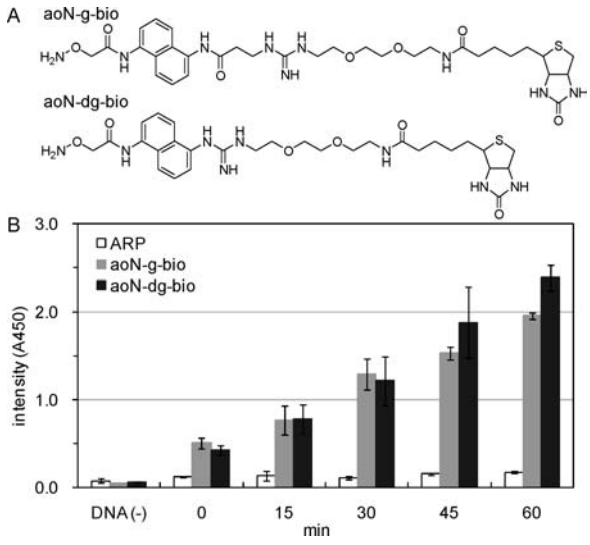

Figure 3. (A) Structures of biotinylated aminooxy probes. (B) Analysis of AP sites in calf thymus DNAs by ARP (white bar), aoN-g-bio (gray bars), and aoN-dg-bio (black bars). All probes were reacted in each well at concentrations of 0.5 mM.

Finally, three biotin conjugates—aoN-g-bio, aoN-dg-bio, and ARP—were used to measure AP sites in genomic DNA. After calf thymus DNA samples were incubated in acidic solution for various times to hydrolyze glycosidic bonds,[3] labeling reactions were carried out in a microwell plate under the same buffer conditions used for the ONT reactions (SI). AP sites were detected colorimetrically by an ELISA-like assay using a biotin−streptavidin complex formation system. For all probes, signal intensity depended on incubation time (Figure 3B). Both aoN-g-bio and aoN-dg-bio showed higher signal intensities than did ARP, and aoN-dg-bio showed slightly higher signal intensity than did aoN-g-bio. These results are consistent with those obtained for the ONT reactions and also prove the superiority of the naphthalene-guanidine-containing aminooxy probes.

In summary, we synthesized aminooxy derivatives containing either a naphthalene or a guanidine residue and found that probes with both functional groups can react very efficiently with aldehyde groups in DNA. Their biotin conjugates enable sensitive detection of AP sites, compared with conventional detecting probes. We think that these probes can be useful tools for the detection of DNA damage or in various genetic assays using AP sites.

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Supporting Information Available: Experimental procedures for the synthesis and characterizations of all the compounds, and other experimental and information. This material is available free of charge via the Internet at http://pubs.acs.org.

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