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Selective fluorescence quenching of the 8-oxoG-clamp by 8-oxodeoxyguanosine in ODN

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ABSTRACT

The 8-oxoG-clamp, a specific fluorescent probe for 8-oxo-deoxyguanosine (8-oxo-dG), was incorporated into the oligodeoxynucleotide (ODN) within or at the 3′-end of the purine and the pyrimidine sequences. Based on the UV-melting temperature, the 8-oxoG-clamp showed slightly lower stabilizing effects on the duplexes containing 8-oxo-dG at the complementary site than that with dG. On the other hand, 8-oxo-dG in DNA was selectively detected by fluorescence quenching of the 8-oxoG-clamp.

8-Oxo-deoxyguanosine (8-oxo-dG) is a major oxidatively damaged metabolite of deoxyguanosine (dG) by active oxygen species (Fig. 1), and causes G/C to T/A transversion mutations in DNA[1,2] The 8-oxo-dG level is regarded as an index of the oxidative stress of cells[3] and is believed to have relevance to some diseases[4] and aging.[5] Accordingly, analysis of 8-oxo-dG is of great significance, and a variety of methods have been developed using HPLC-EC[6], HPLC/GC-MS,[7] antibodies,[8] etc.[9]

It is also of great importance to detect 8-oxo-dG in DNA, and such a method using a sequence of reactions was reported.[10] Fluorescent nucleobases have been widely used for the detection and in the structural study of nucleic acids.[11] However a fluorescent probe for the detection of 8-oxo-dG in DNA is lacking. Recently, we reported the 8-oxoG-clamp as the specific fluorescent probe for 8-oxo-dG with a high discrimination ability from other nucleosides (Fig. 2).[12] The structural analysis by ^{1}H NMR has shown that the selectivity of the 8-oxoG-clamp is due to the complex formed involving multiple hydrogen bonds with 8-oxo-dG including the 7N-H of 8-oxo-dG (Fig. 2, (a)).[13] We now report that fluorescent property of the 8-oxoG-clamp and its selective quenching by 8-oxo-dG are retained in the ODN probes.

The diol derivative of the 8-oxoG-clamp (**1**) was transformed to the corresponding 5′-dimethoxytrityl (DMTr)-protected 3′-phosphoramidite precursor (**2**) by a conventional method, which was incorporated into the oligodeoxynucleotides (DNA**3-5**) by the DNA automated synthesizer (Scheme 1).[14] The recognition property of the 8-oxoG-clamp was first evaluated by measuring the melting temperature (T_m) using DNA **3** and **4** incorporating the 8-oxoG-clamp in the middle position of the sequence (Table 1).

As already reported, 8-oxo-dG has a comparable effect on duplex stabilization with dC and dA (T_m, °C: 53, 52 or 53, 50).[15] The 8-oxoG-clamp showed a slightly lower T_m value to 8-oxo-dG than to dG (T_m, °C: 53 vs 51 or 51 vs 48). These results are inconsistent with our expectation based on the monomer experiments, in which the 8-oxoG-clamp showed a higher affinity to 8-oxo-dG than to dG (K_s M^{-1}: 2.3×10^6 vs 2.1×10^5).[12] It was reported that the parent G-clamp without the *N*-carbobenzyloxy (CBZ) group increased the T_m value toward dG (ΔT_m + 18 °C) compared to the dG and 5-methyl-dC base pair.[16] Unlike the parent G-clamp, the CBZ group of the 8-oxoG-clamp might not form a hydrogen bond with 7N-H probably due to steric repulsion between the benzene ring of

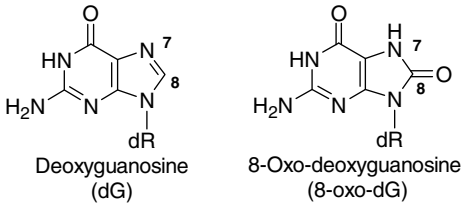

Figure 1. Structures of deoxyguanosine and 8-oxo-deoxyguanosine. dR: β-D-2′-deoxyribofuranosyl.

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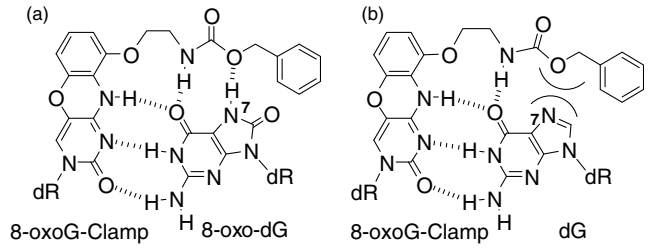
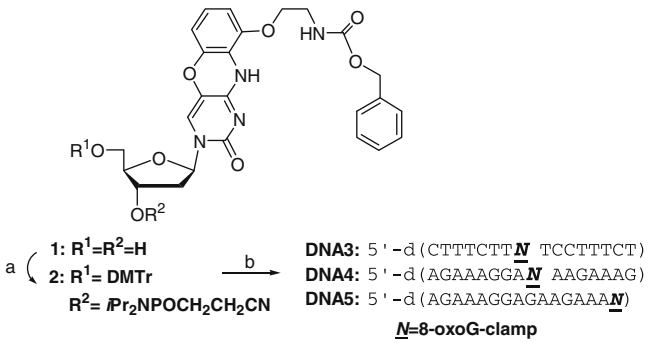


Figure 2. Complex structure of 8-oxoG-clamp with 8-oxo-dG determined by ^{1}H NMR (a). Unfavorable repulsion postulated toward dG (b).[12]



Scheme 1. Synthesis of 8-oxoG-clamp modified oligonucletotides (**3**–**5**). Reagents and conditions: (a) 1—DMTrCl, pyridine, 2—iPr$_2$NP(Cl)OCH$_2$CH$_2$CN, iPr$_2$NEt, CH$_2$Cl$_2$, 0 °C, 1 h (82%); (b) DNA synthesizer, HPLC purification.

the CBZ group and the sugar-phosphate backbone of the complementary strand. Considering such a steric requirement, it was expected that the 8-oxoG-clamp might form a complex with 8-oxo-dG in a sterically non-crowded site at the end of the duplex. However, DNA**5** incorporating the 8-oxoG-clamp at the 3′-end formed duplexes with the same T_m values toward both 8-oxo-dG and dG (Table 2). In this case, the hydrogen bonds of the 8-oxoG-clamp to 7N-H of 8-oxo-dG might be so weakened in an aqueous environment that 8-oxo-dG and dG did not form stable base pairs to affect the thermal stability of the duplex.

Table 1
T_m values of 8-oxoG-clamp modified oligonucleotides (**3** and **4**)[a]

DNA**3**: 5′-d(CTTTCTT-***N***-TCCTTTCT)-3′
DNA**4**: 3′-d(GAAAGAA-***N***-AGGAAAGA)-5′

DNA**3**, ***N***=	DNA**4**, ***N***=					
	8-oxoG-clamp	8-oxo-dG	G	C	A	T
8-oxoG-clamp	47	**51**	**53**	48	48	48
8-oxo-dG	**48**	45	45	**53**	**52**	45
G	**51**	46	47	**56**	45	48
C	45	**53**	**54**	40	42	42
A	46	**50**	48	43	44	**53**
T	45	45	47	43	**54**	44

[a] UV-melting profiles measured using 2 μM each of the DNA strand in 10 mM sodium phosphate buffer (pH 7.0) containing 100 mM NaCl and 10 mM $MgCl_2$ at the scan rate of 0.5 °C min^{-1} at 260 nm.

Table 2
T_m values of 8-oxoG-clamp modified oligonucleotides (**5** and **6**)[a]

DNA**6**: 5′-d(***N*** -TTTCTTCTCCTTTCT)-3′
DNA**5**: 3′-d(***N′***-AAAGAAGAGGAAAGA)-5′

Sequences ***N***, ***N′***	T_m
DNA**6** (*N* = 8-oxo-dG)	56
DNA**5** (*N′* = 8-oxoG-clamp)	
DNA**6** (*N* = dG)	56
DNA**5** (*N′* = 8-oxoG-clamp)	

[a] UV-melting profiles measured using 2 μM each of the DNA strand in 10 mM sodium phosphate buffer (pH 7.0) containing 100 mM NaCl and 10 mM $MgCl_2$ at the scan rate of 0.5 °C min^{-1} at 260 nm.

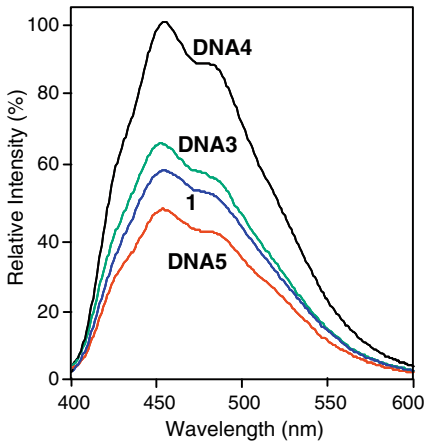


Figure 3. Fluorescent spectra of DNA (**3**, **4**, **5**) incorporating the 8-oxoG-clamp and the 8-oxoG-clamp monomer (**1**). Fluorescent spectra were measured using 1 μM of the probe DNA (**3**, **4**, **5**) or the monomer **1** in a buffer containing 100 mM NaCl, 10 mM $MgCl_2$, and 10 mM phosphate at pH 7.0, 25 °C, with excitation at 365 nm.

We next investigated the fluorescence quenching properties of the 8-oxoG-clamp using DNA**3**–**5**. The fluorescence intensity of the compounds is sometimes decreased when they are incorporated into the ODN.[11] The fluorescent spectra of the DNA probes incorporating the 8-oxoG-clamp showed a similar or somewhat higher intensity compared to the monomer 8-oxoG-clamp (Fig. 3). When the DNA**3** incorporating 8-oxoG-clamp in the homopurine strand was titrated in a buffer containing 100 mM NaCl, and 10 mM $MgCl_2$, 10 mM sodium phosphate, fluorescence quenching was observed in the order of 8-oxo-dG > dA > dG > dC and dT, although the selectivity was not very high. Interestingly, when the titration was performed in a low-salt buffer containing 10 mM NaCl and 10 mM sodium phosphate in the absence of $MgCl_2$, selective quenching was observed for 8-oxo-dG (Fig. 4A).

On the other hand, the 8-oxoG-clamp in the homopyrimidine strand in DNA**4** showed less selective quenching to 8-oxo-dG in the homopurine DNA**3** in a buffer containing 100 mM NaCl, 10 mM $MgCl_2$, and 10 mM sodium phosphate (Fig. 4B). The DNA**5** incorporating 8-oxoG-clamp at the 3′-end showed selective quenching to 8-oxo-dG in DNA**6** (Fig. 4C). In these cases, the titration experiments in the low-salt buffer produced similar quenching profiles. These results have indicated that the 8-oxoG-clamp is useful for the detection of 8-oxo-dG in DNA.

It is reported that photoinduced electron transfer and proton-coupled electron transfer play an important role for the mechanism of fluorescence quenching of dyes by nucleobases,[17] and quenching efficiency between a fluorescent dye and a nucleobase depends on their distance.[11,18] In the case when nucleobases are oxidized, the quenching efficiency is in the order of 8-oxoguanine > guanine > adenine > thymine > uracil.[19] This model for quenching mechanism may rationalize our previous results that the fluorescence of the 8-oxoG-clamp was most effectively quenched with 8-oxo-dG by forming a selective complex with multiple hydrogen bonds.[12] The original G-clamp in DNA**3** showed higher T_m value to dG in DNA**4** (48 °C) than to 8-oxo-dG (40 °C) under the low-salt buffer conditions, and displayed less selective quenching to dG and 8-oxo-dG in the order of their intrinsic quenching efficiency (Fig. 5). It is clear form the comparison of

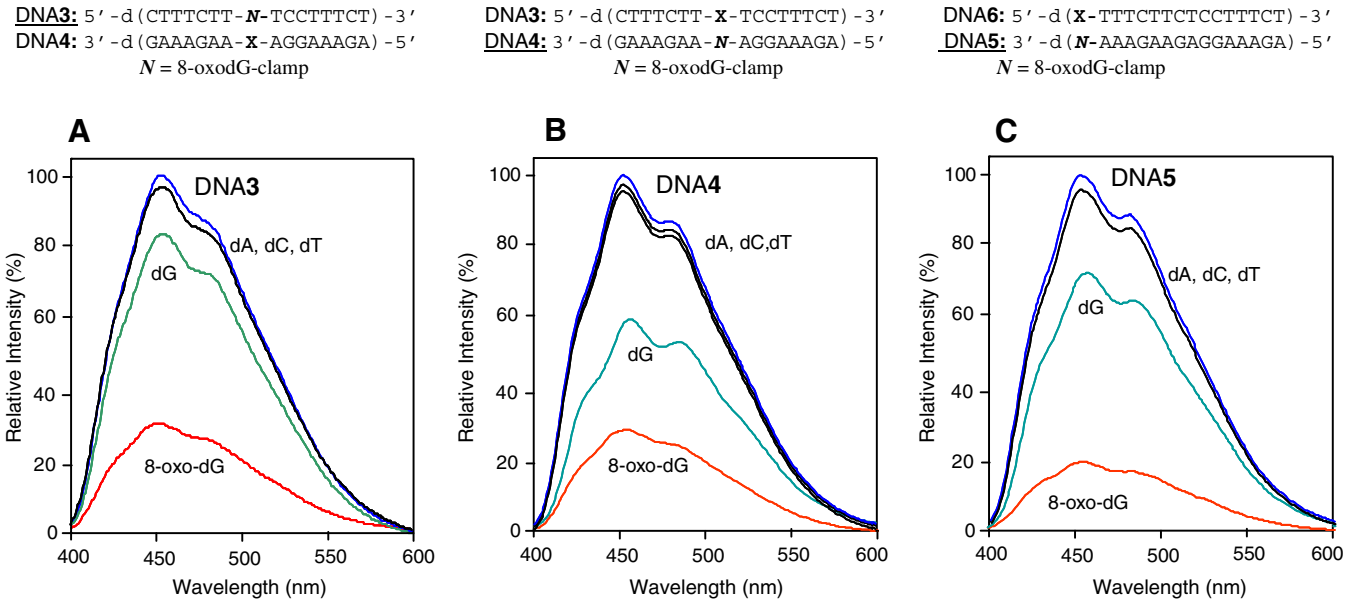


Figure 4. Fluorescence quenching of the DNA probes (DNA**3**, DNA**4** and DNA**5**) containing the 8-oxoG-clamp. The fluorescence spectra were measured using 1 μM each of the probe DNA and the target strand at pH 7.0, and 25 °C, with excitation at 365 nm. Each curve shows the spectra of the probe DNA alone (blue), the duplex with the target strand with *X* = dA, dC, dT (black), dG (green) and 8-oxo-dG (red). The underlined strands represent the fluorescent probe ODN. DNA**3** was titrated in a buffer containing 10 mM NaCl, and 10 mM phosphate (A), DNA**4** and DNA**5** were titrated in a buffer containing 100 mM NaCl, 10 mM $MgCl_2$, and 10 mM phosphate (B and C).

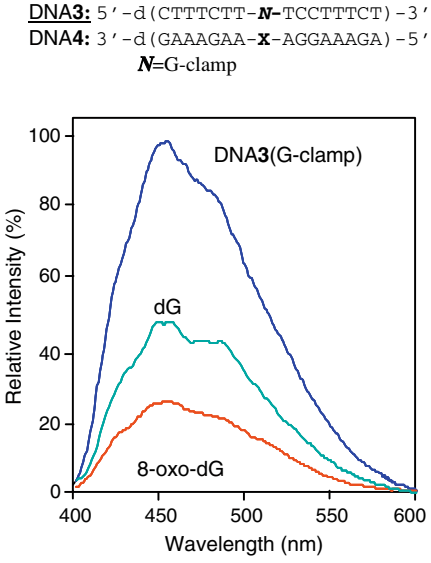


Figure 5. Fluorescence quenching of DNA**3** containing G-clamp. The conditions are the same as described in the footnote to Figure 4A.

Figures 4A and 5 that the benzyloxycarbonyl group of 8-oxoG-clamp inhibits effective quenching of G-clamp by dG, although it is not clear whether interactive hydrogen bonds or a repulsive interaction (Fig. 2) are involved or not.

Compared to the high selectivity of the 8-oxoG-clamp for 8-oxo-dG in organic solvents,[12] its selectivity in ODN was lower in the sense that quenching was observed to some extent also with dG. Steric hindrance of the benzyloxycarbonyl group might disturb the complex formation for effective fluorescence quenching of the 8-oxoG-clamp incorporated in ODN, and new 8-oxoG-clamp derivatives with a variety of *N*-substituted groups are currently under investigation to solve this problem.

In conclusion, we have shown that selective fluorescence quenching of 8-oxoG-clamp by 8-oxo-dG is retained in the ODN probe. Although selectivity for 8-oxo-dG over dG is not enough, these results indicate the potential of the 8-oxoG-clamp as a lead compound for new analytical systems to determine 8-oxo-dG in DNA. For the more selective and sensitive detection of 8-oxo-dG in DNA, the development of new 8-oxoG-clamp derivatives is now in progress.

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13. Subsequent study with the use of 8-oxoG-clamp derivatives with various recognition unit showed that the sp^3 oxygen atom was involved for the formation of the hydrogen bond to N^7H of 8-oxo-dG as shown in Figure 2(a), which will be reported elsewhere.
14. MALDI TOF-MS: DNA**3** $[M-H]^-$ 4994.29 (calcd 4995.78), DNA**4** $[M-H]^-$ 5284.33 (calcd 5285.85), DNA**5** $[M-H]^-$ 5284.57 (calcd 5285.85).
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