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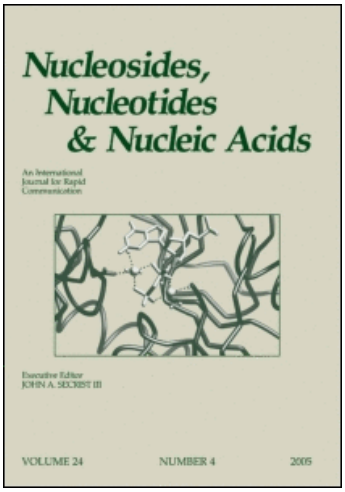

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SELECTIVE FLUORESCENCE DETECTION OF 8-OXOGUANOSINE WITH 8-oxoG-CLAMP

Osamu Nakagawa, Sayaka Ono, Akira Tsujimoto, Zhichun Li, and Shigeki Sasaki □ *Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka, Japan*

□ *8-oxoguanosine, which is derived from the oxidation of guanosine (dG), is known to induce transversion mutations (G:C→ T:A) in DNA. The compounds with a small molecular weight for recognizing 8-oxoG were designed on the basis of the structure of the G-clamp, which is reported to have selective affinity toward guanosine. The G-clamp derivatives with the additional binding units toward 8-oxoG were effectively synthesized and named "8-oxoG-clamps." The 8-oxoG-clamp completely discriminated 8-oxoG from other nucleosides by fluorescence quenching.*

Keywords 8-Oxoguanosine; g-clamp; 8-oxoG-clamp; fluorescence quenching

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INTRODUCTION

DNA in living organisms suffers from oxidative damage by reactive oxygen species. 8-oxoguanosine (8-oxoG) is derived by the oxidation of guanosine (dG) and is known to induce G:C to T:A transversion mutations in DNA.[1] The level of 8-oxoG is regarded as an index of the oxidative stress of cells, and a variety of analytical methods have been studied such as with HPLC-EC, GC-MS, and so on.[2,3] Unfortunately, artificial oxidation during sample preparation and analysis has been reported in instrumental measurements. Therefore, a method for direct detection of 8-oxoG in cells will be useful to evaluate intracellular oxidative stress. Although antibodies were developed, small molecular recognition molecules for 8-oxoG should be more useful for intracellular use. Herein, we would like to describe "8-oxoG-clamp" as the first example of a specific fluorescence receptor for 8-oxoG.

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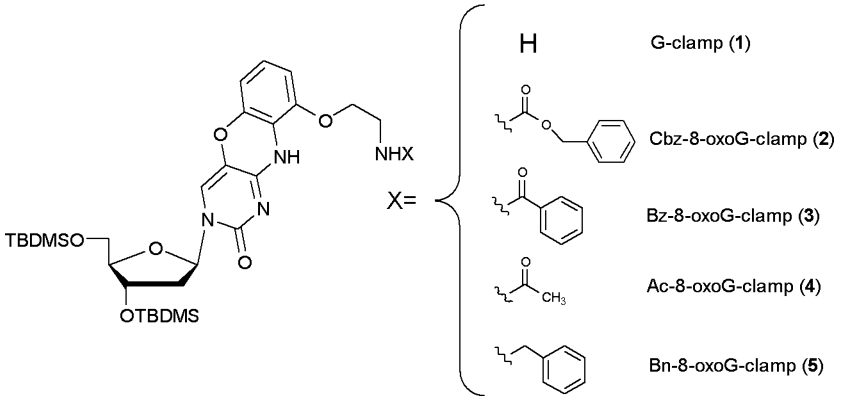

FIGURE 1 Structures of G-clamp (**1**) and 8-oxoG-clamps (**2–5**).

RESULTS AND DISCUSSION

The only difference between 8-oxoG and dG is the reversal of the acceptor and donor of the proton of N7, and the oxygen atom in the 8-position of 8-oxoG. Therefore, it is important to strictly recognize these slight differences. We focused on G-clamp (**1**),[4–6] which has been known to have selective affinity toward dG in DNA. G-clamp (**1**) is composed of the phenoxazine skeleton with the aminoethoxy unit at the end (Figure 1). In our approach to recognition molecules for 8-oxoG based on G-clamp, introduction of an additional functional group at the aminoethoxy terminal was designed so that it would be interactive with 8-oxoG and repulsive toward guanosine (Figure 2). The derivatives with benzyloxycarbonyl (**2**, Cbz), benzoyl (**3**, Bz), acetyl (**4**, Ac), and benzyl (**5**, Bn) were designed and were named "8-oxoG-clamps" (Figure 1). Based on molecular modelling, the additional carbonyl

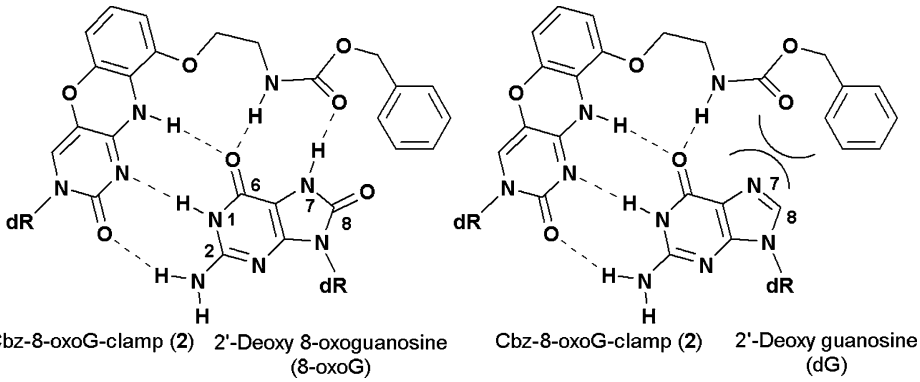

FIGURE 2 Design concept and proposed complexation of 8-oxoG-clamp with 8-oxoG and dG.

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group in **2–4** was expected to form hydrogen bonding with H-N7 of 8-oxoG, and to be repulsive to N7 of G (Figure 2).

8-OxoG-clamps with the functional unit (**2–5**) were efficiently synthesised from G-clamp or its intermediates. The introduction of the Cbz unit (**2**) was achieved according to the report of Matteucci et al.[4] And the functional groups of Bz (**3**) and Ac (**4**) were introduced with acid chlorides under the basic conditions. The derivatives **2–5** were protected with TBDMS groups at both 3′-*O* and 5′-*O* positions of the sugar moiety to enhance solubility in organic solvents. The binding affinities between 8-oxoG-clamps (**2–5**) and 2′-deoxy-8-oxoguanosine (8-oxoG) were measured by fluorescence quenching of 8-oxoG-clamps by the ability to quench 8-oxoG.[7,8] Fluorescence titration profiles were measured by adding target nucleosides (8-oxoG, dG, dA, dC, and dT) to 8-oxoG-clamps in a chloroform solution buffered with an organic base and acid (10 mM triethylamine-2.7 mM acetic acid). Figure 3 and Table 1 summarize the results with G-clamp (**1**) and 8-oxoG-clamps (**2–5**) toward 8-oxoG and other nucleosides. Surprisingly, Cbz-8-oxoG-clamp (**2**) showed pronounced fluorescence quenching with the addition of 8-oxoG. In Job plot analysis, the complexation was identified to be formed in a 1:1 ratio (data not shown). The calculated binding constant of $K_s = 2.3 \times 10^6\ M^{-1}$ is as high as that of a natural G:C base pair. Surprisingly, the fluorescence quenching of Cbz-8-oxoG-clamp (**2**) did not take place with dG, dA, dC, and dT, indicating that the Cbz-8-oxoG-clamp (**2**) is

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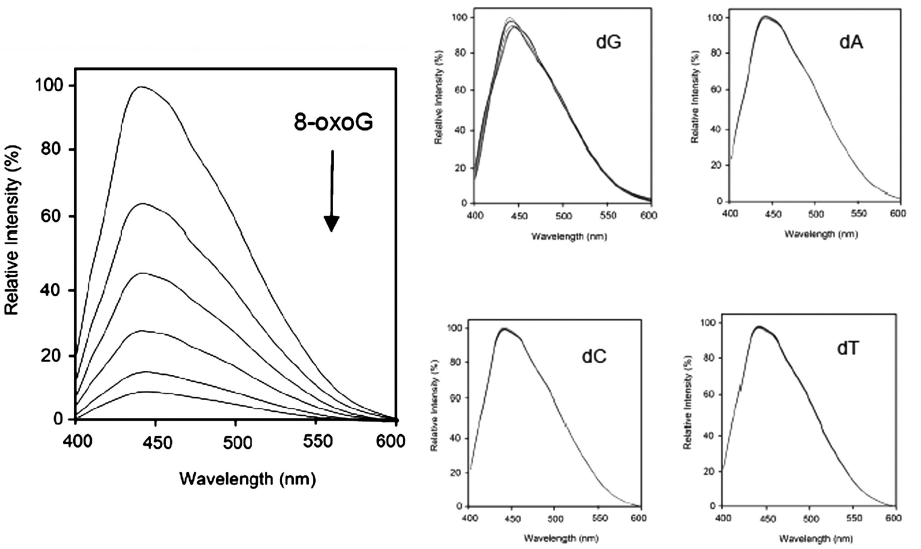

FIGURE 3 Fluorescence titration spectra of Cbz-8-oxoG-clamp (**2**) toward 8-oxoG, dG, dA, dC and dT. Conditions: $CHCl_3$ buffered with 10 mM triethylamine-2.7 mM acetic acid, 0–10 μM target nucleosides, 1 μM Cbz-8-oxoG-clamp (**2**) at 25°C, excitation 365 nm. **2** and target nucleosides (8-oxoG, dG, dA, dC and dT): 3′*O*,5′*O*-di-*tert*-butyldimethylsilyl-2′-deoxynucleoside.

TABLE 1 Binding constants of G-clamp (1) and 8-oxoG-clamps (2–5) toward 8-oxoG and dG in CHCls buffered at pH 7[a]

		K_s (IVT[1])			
G-clamp (X=)	8-oxoG	dG	dA	dC	dT
1 (H)	7.3×10^5	7.1×10^5	nq	nq	nq
2 (Cbz)	2.3×10^6	nq	nq	nq	nq
3 (Bz)	2.9×10^5	nq	nq	nq	nq
4 (Ac)	3.0×10^5	nq	nq	nq	nq
5 (Bn)	3.9×10^5	nq	nq	nq	nq

Conditions: $CHCl_3$ buffered with 10 mM triethylamine-2.7 mM acetic acid, 0–10 jLiM 8-oxoG or dG, IjiM G-clamp (1) or 8-oxoG-clamps (2–5) at 25°C, excitation 365 nm. nq: no fluorescence quenching.

highly specific to 8-oxoG (Table 1). In a remarkable contrast, the original G-clamp (**1**) showed nonspecific fluorescence quenching for both 8-oxoG ($K_s = 7.3 \times 10^5\ M^{-1}$) and dG ($K_s = 7.1 \times 10^5\ M^{-1}$). The binding constants of G-clamp (**1**), 8-oxo-G-clamp (**2**, **3**, **4**, and **5**) were obtained by fluorescence quenching and are summarized in Table 1. It is clearly shown that the nature of nonquenching by dG is due to substitution at the amino group of **1**. The binding constant of **1** with 8-oxoG was decreased to some extent by the introduction of the benzoyl group (**3**: $K_s = 2.9 \times 10^5\ M^{-1}$), the acetyl group (**4**: $K_s = 3.0 \times 10^5\ M^{-1}$) or the benzyl group (**5**: $K_s = 3.9 \times 10^5\ M^{-1}$), whereas the benzyloxycarbonyl group of **2** increased binding affinity approximately 3 times more than that of G-clamp (**1**). These results suggest that the functional group at the amino terminal of G-clamp is responsible for selective fluorescence quenching to 8-oxoG.

We have developed 8-oxoG-clamps as the first example of a specific fluorescence receptor for 8-oxoG. The 8-oxoG-clamps could completely discriminate 8-oxoG from other nucleosides by fluorescence quenching. It is expected that the 8-oxoG-clamp may be applicable to various detection methods such as the intracellular direct detection of 8-oxoG and so on. Further studies on the structure of the complex between 8-oxoG-clamp derivatives and 8-oxoG, and its application, are now in progress.

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