Supplementary information for

Photoinduced conformational changes in DNA by poly(vinyl alcohol) carrying a malachite green moiety for protecting DNA against attack by nuclease

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Protection ability of malachite green oxalate against DNase

The results of the DNase I protection experiment in the system of malachite green oxalate are shown in Figure S1. The experimental details are the same as that for PVAMGs excluding irradiation. The concentration of ungraded DNA was similar to that for the naked DNA sample even at high ratio of $[MG^+]/[DNA]$. Then it is concluded that the monomeric malachite green oxalate is ineffective for the protection against DNase.

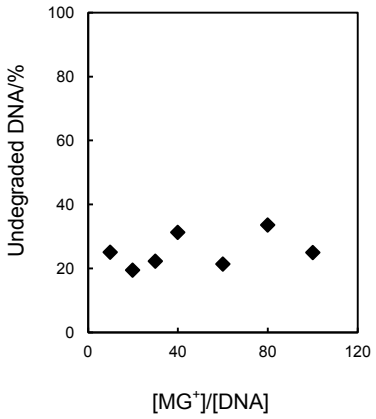

Figure S1. Ability of malachite green oxalate to protect DNA against DNase. The initial concentration of DNA was fixed at 1.9 μM. The details of treatment are as described in the text.

Effect of DNA condensation on determination of DNA concentration

The effect of DNA condensation on the measurement of Quant-iT dsDNA HS assay kit was investigated by using cetyltrimethylammonium chloride (CTAC). Cationic surfactant is known to condense DNA and the compaction of T4 DNA was reported by using cetyltrimethylammonium bromide (CTAB).[1] The determination of DNA concentration in this assay is based on the fluorescence of Quant-iT dsDNA HS reagent (λ_{ex} = ~ 480 nm and λ_{em} = 530 nm). Though the fluorescence signal is linear with DNA concentration, the coexisting CTAC decreases its intensity. Figure S2 shows the influence of CTAC on the fluorescence for the detection of calf thymus DNA (0.94 μM).

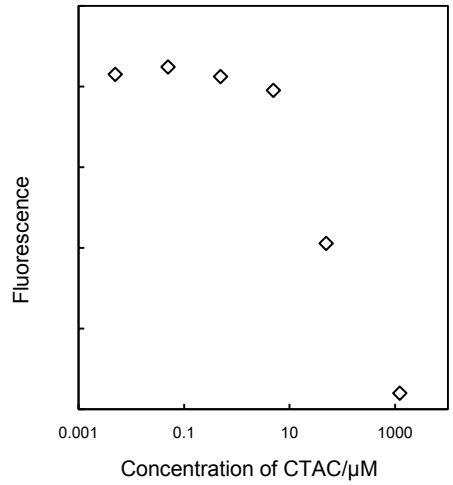

Figure S2. Influence of CTAC on fluorescence of Quant-iT dsDNA HS reagent. [DNA] =0.94 μM.

The fluorescence intensity was almost unchanged up to 5 μM of the coexisting CTAC, above which that it decreased abruptly. Yoshikawa and colleagues have reported that the transition of T4 DNA from coil to globule was induced by CTAB > 10^{-5}M (DNA concentration was 0.6 μM).[2] The concentration of CTAB shows a good agreement with the concentration of the abrupt decrease in fluorescence intensity

observed in Figure S2. These results therefore indicate that DNA compaction caused the decrease in fluorescence intensity of Quant-iT dsDNA HS reagent.

(1) Mel'nikov, S. M.; Sergeyev, V. G.; Yoshikawa, K. *J. Am. Chem. Soc.* **1995**, 117, 2401-2408.

Fluorescence emission spectra of PVAMG

Figure S3 shows fluorescence emission spectra of PVAMG. Ionized malachite green moiety of PVAMG was excited at 590 nm. The concentration of PVAMG was 0.071 gL^{-1} ($[MG^{+}]$ = 1.5 - 3.2 μM). Figure S3 indicates that emission peak around 657 nm is considerably small in the sample excluding DNA and that any significant difference among PVAMGs were not observed. Therefore, it is clear that the dependence of fluorescence intensity on the molecular weight of PVAMG shown in Figure 4 is due to the PVAMG complexation with DNA.

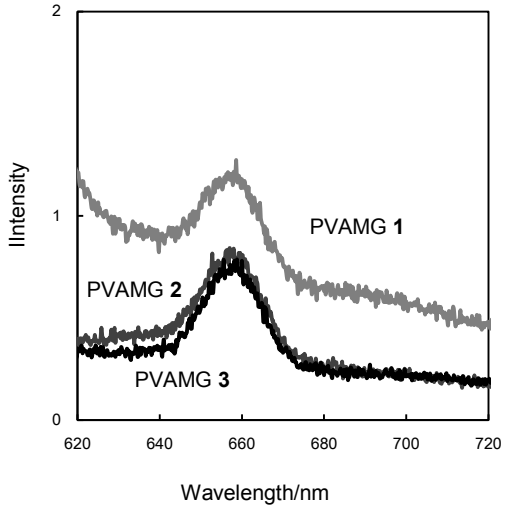

Figure S3. Fluorescence emission spectra of PVAMGs after UV irradiation. The concentration of PVAMG was 0.071 gL^{-1}. The fluorescence intensity was calibrated with the fluorescence of 3.0 μM malachite green oxalate in buffer solution containing 1.0 gL^{-1} of poly(vinyl alcohol).

Benesi-Hildebrand plot and Scatchard plot

Figure S4 shows the fluorescence intensity of malachite green oxalate in the solution containing various amount of DNA. The concentration of malachite green oxalate was maintained at a constant of 15 μM. The fluorescence intensity of the completely bound MG^+ is estimated from the change in the fluorescence using Benesi-Hildebrand equation.[1]

$$1/\Delta F=1/(K_a \cdot \Delta F_0)[DNA]+1/\Delta F_0$$

where ΔF is florescence intensity change, K_a is the binding constant, and ΔF_0 is maximum florescence intensity change. We have plotted $1/\Delta F$ vs 1/[DNA] in the DNA concentration range from 0.038 to 0.38 mM in Figure S5. The double reciprocal plot is linear and then the extrapolation to the ordinate gives $1/\Delta F_0$. F_0 was found to be 1.32×10^2.

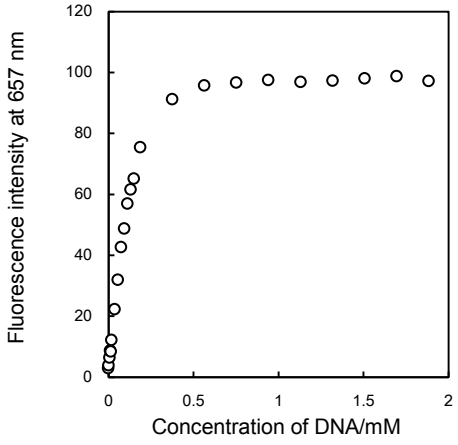

Figure S4. Dependence of fluorescence intensity of malachite green oxalate on the concentration of DNA. λ_{ex} = 590 nm. The concentration of malachite green oxalate was 15 μM. The fluorescence intensity was calibrated with the fluorescence of 3.0 μM malachite green oxalate in buffer solution containing 1.0 gL^{-1} of poly(vinyl alcohol).

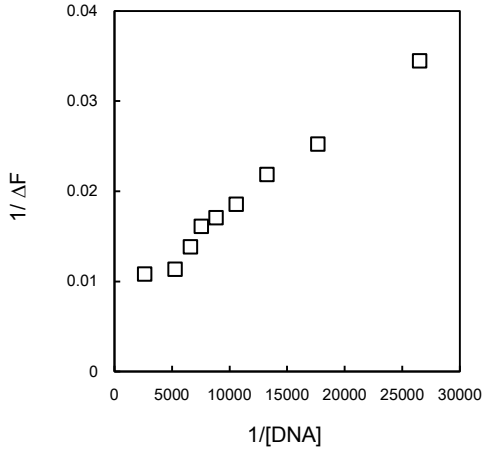

Figure S5. Benesi-Hildebrand plot of malachite green oxalate (15 μM) and DNA. The extrapolation of the ordinate corresponds to $1/\Delta F_0$.

The fluorescence data in Figure 4 were cast into the form of a Scatchard plot.[2] The concentration of the bound compound was calculated using the extinction coefficient of

the completely bound drug determined from the intercept of a Benesi–Hildebrand plot. Figure S6 shows Scatchard plots for PVAMGs binding to DNA. The Scatchard plots are hyperbolic with a concave upward shape, indicating that PVAMG binding to DNA contains two types of binding sites differing in affinity, or that they show negative cooperativity, where ligand binding adversely influences the binding of subsequent ligands. Waring and colleagues have also examined crystal violet, triphenylmethyl cation, binding to DNA and also observed upward-curved Scatchard plots.[3] They concluded that neither simple Scatchard analysis nor the excluded site model of McGhee and von Hipple[4] were amenable. They therefore estimated the intrinsic binding constant by extrapolating the ordinate axis of the Scatchard plots. We also calculated the intrinsic binding constant by the same manner. The plots at r < 0.013 (PVAMG **1**), 0.027 (PVAMG **2**), and 0.050 (PVAMG **3**) were applied to the extrapolation.

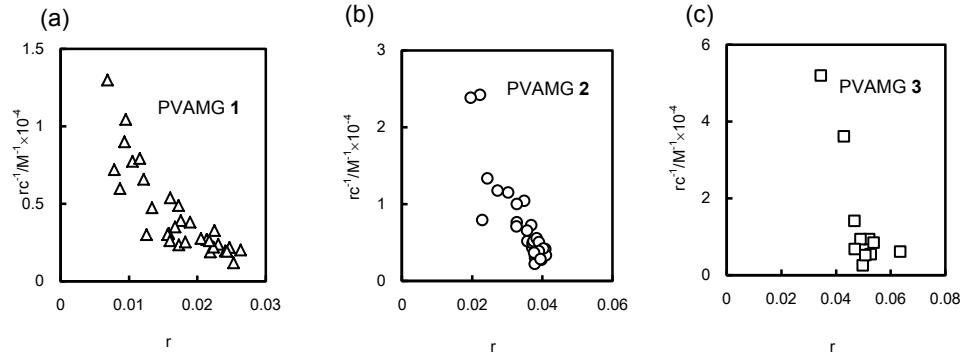

Figure S6. Scatchard plots for the binding of PVAMG (a) PVAMG **1** in the ratio of $[MG^+]/[DNA] > 0.0096$. (b) PVAMG **2** in the ratio of $[MG^+]/[DNA] > 0.016$. (c) PVAMG **3** in the ratio of $[MG^+]/[DNA] > 0.027$. r is the PVAMG bound per DNA and c is the concentration of free PVAMG.

(1) Benesi, H.A.; Hildebrand, J.H. *J. Am. Chem. Soc.* **1949**, *71*, 2703-2707

(2) Scatchard, G. Ann. N. Y. Acad. Sci. **1949**, *51*, 660-672

(3) Wakelin, L.P.G.; Adams, A.; Hunter, C.; Waring, M.J. *Biochemistry*, **1981**, *20*, 5779-5787

(4) McGhee, J.D.; Von Hippel, P.H. *J. Mol. Bio.* **1974**, *86*, 469-489

Polyacrylamide gel electrophoresis

Polyacrylamide gel electrophoresis gives information about the molecular weight of calf thymus DNA. Figure S7 shows that calf thymus DNA was composed of 3000-4000 bp.

Figure S7. Polyacrylamide gel electrophoresis results for calf thymus DNA. (Lane 1) DNA ladder. (Lane 2) Calf thymus DNA. The experimental details were described previously.[1]

(1) Uda, R. M.; Ohshita, M. *Biomacromolecules*, **2012**, *13*, 1510-1514.