Immobilization and cleavage of DNA at cationic, self-assembled monolayers containing C_{60} on gold

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A cationic, self-assembled monolayer on gold substrate can immobilize DNA without impairing its native structure; the site-specific photocleavage of the DNA is achieved by incorporation of [60]fullerene into the monolayer.

The immobilization of DNA on a two-dimensional solid surface is of interest both in studies of DNA itself and in various applications. For instance, in atomic force microscopy, the DNA must be firmly affixed to a surface to minimize the effects of tip interactions during scanning.[1] Immobilization of DNA on an electrode is also used to produce biosensors.[2,3] The strategy employed in this study was to prepare, on a gold surface, a well-ordered monolayer assembly that contained cationic groups, such as quaternary ammonium salts, that would interact electrostatically with the phosphate groups of DNA. Monolayer assemblies of this type have been described previously.[4–6] Here we report immobilization of DNA on a self-assembled monolayer (SAM) of **1** and cleavage of the DNA, which is important from biological and clinical viewpoints. Recently, [60]fullerene (C_{60}) was found to have a G (guanine base)-selective DNA cleaving ability in the presence of visible light.[7] If C_{60} could be successfully incorporated in the SAMs of **1** (Fig. 1), the immobilized DNA might be expected to cleave site-specifically.

The preparation of **1** and its adsorption property on gold substrate have been described elsewhere.[4,6] The monolayers were first formed by soaking gold mirror plates (prepared by evaporation of gold onto one side of a glass plate, 2 × 2 cm) in solutions of **1** in EtOH for 1 h. Calf thymus DNA was immobilized on the surface by soaking the SAM film in a buffer solution (50 mmol dm^{-3} tris buffer, pH 7.4) of DNA (1 mg ml^{-1}) for 2 h. The film was then rinsed several times with the tris buffer to remove free DNA, and dried at room temperature. The reflection–absorption spectrum of this film on gold, which was measured using a multi-channel photodetector (Otsuka Electronics, MCPD–100, Japan), gave an absorption maximum at 263 nm assigned to the π–π* transition of nucleic acid bases of DNA, suggesting the binding of DNA onto the monolayer surface through electrostatic interaction. To estimate the stoichiometry of such an ion complex, the XPS spectra were measured before and after DNA binding. As a result, the phosphorous (phosphate anion in DNA) : nitrogen (ammonium group and amide bond of **1**) ratio was determined to be 2 : 1.7 (±0.3); the ammonium cation : phosphate anion ratio is thus calculated to be 1 : 1.7. This result implies that about half of the total phosphate anions of DNA contributed to the complexation with the cationic surface of **1**-SAM; in other words, half of the anions remained free.

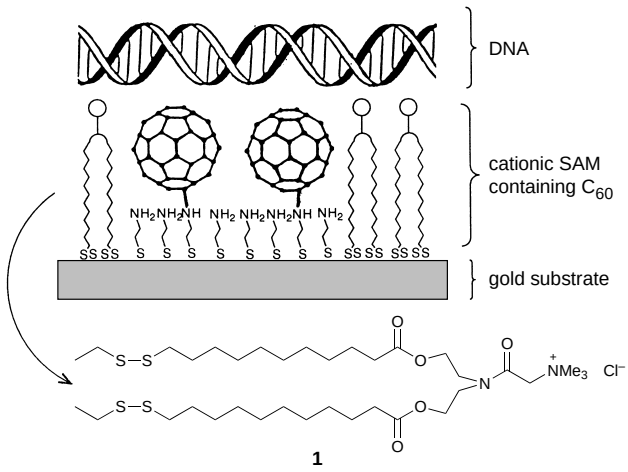

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Fig. 1 Schematic illustration for immobilization of DNA on the cationic SAM containing C_{60} on gold

Subsequently, the intercalation of Methylene Blue was examined by spectroscopic means. In aqueous solution, intercalation of Methylene Blue into DNA base pairs is known to cause both hypochromism and red shifting of the absorption maximum.[8] These observations are explained by stacking interactions of intercalated dye with base pairs in DNA and electrostatic interactions with phosphate anions of DNA, respectively.[9] In the case of the monolayer-bound DNA, the spectrum of Methylene Blue showed a 6 nm red shift of the absorption maximum. This spectral feature suggests that Methylene Blue was trapped in the monolayer-bound DNA through intercalation, although we were not able to obtain information regarding hypochromism because of difficulty in comparing the absolute absorbance of Methylene Blue before and after intercalation.

To locate C_{60} close to the DNA immobilized at the monolayer surface, mixed monolayers of **1** and 2-mercaptoethylamine (cysteamine) were prepared on gold substrates. Cysteamine, which can form a SAM on gold, was employed since Caldwell *et al.*[10] described that C_{60} could be bound covalently onto cysteamine-modified gold substrates. The mixed monolayer (mole fraction of **1** in the mixture, f_1 = 0.2) was prepared by soaking the gold plate in a 1 mmol dm^{-3} EtOH solution containing **1** and cysteamine for 24 h. The monolayer-modified gold plate thus obtained was then soaked in a 1 mmol dm^{-3} benzene solution of C_{60} for 24 h at room temperature. The resulting substrates were thoroughly rinsed in benzene to remove residual physisorbed C_{60}. Incorporation of C_{60} in the monolayers was confirmed by measuring the contact angle of water.‡ When the same procedure was performed on a pure **1** monolayer (f_1 = 1)-modified gold plate, no binding of C_{60} was observed, as expected. Immobilization of DNA on the mixed monolayer-modified gold plates was carried out in the same manner as described above. To reveal the site-specificity of photocleavage, several types of DNA {salmon sperm DNA, calf thymus DNA, poly[$(dGdC)_2$] and poly[$(dAdT)_2$]} which were different in base pair composition were used. Irradiation experiments were performed under the same condition for all samples. If cleavage of DNA took place, the resultant short fragments, such as oligonucleotides and nucleotides, readily diffused into the bulk phase and were then detectable by spectroscopy.§ Fig. 2 shows UV spectra of the bulk aqueous phases from which poly[$(dGdC)_2$]-immobilized monolayer plates (f_1 = 0.2 and added 1) were withdrawn after 3 h irradiation. For comparison, the UV spectrum of an aqueous solution of poly[$(dGdC)_2$] itself at a concentration of 6 × 10^2

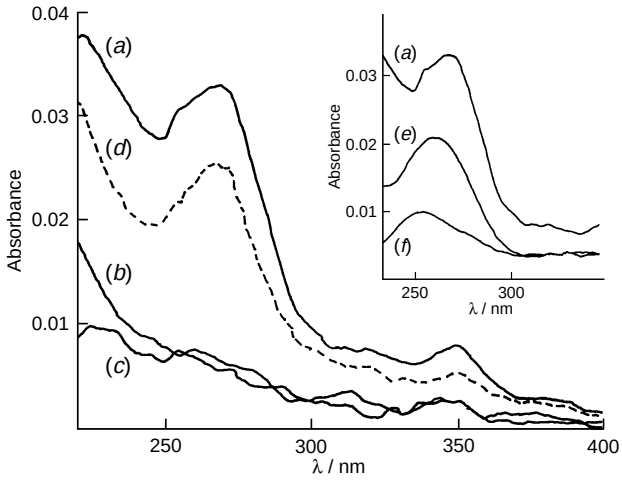

Fig. 2 UV absorption spectra of aqueous solutions resulting from irradiation of poly[(dGdC)$_2$] monolayers under standard conditions; (*a*) f_1 = 0.2 monolayer; (*b*) f_1 = 1 monolayer; (*c*) f_1 = 0.2 monolayer shielded from the visible light; (*d*) aqueous solution of poly[(dGdC)$_2$] itself at a concentration of 6×10^2 ng ml^{-1}. Inset: spectra of aqueous solutions of DNA monolayers; (*e*) salmon sperm DNA; (*f*) calf thymus DNA, compared with (*a*) treated under the same conditions.

ng ml^{-1} is included in the figure. In the case of f_1 = 1 without C_{60}, no peak was observed at around 260 nm based on the π–π* transition of nucleic acid bases. In contrast, the mixed monolayer (f_1 = 0.2)-bound poly[(dGdC)$_2$] gave an absorption peak at 260 nm due to cleaving although the same poly[(dGdC)$_2$] shielded from the visible light did not. These results strongly suggest that the DNA cleavage was caused by singlet oxygen[11] generated by interaction of the photoexcited C_{60} group with molecular oxygen. For the mixed monolayer (f_1 = 0.2)-bound poly[(dAdT)$_2$], light irradiation led to no cleavage (data not shown here). Fig. 2 (inset) displays a comparison of UV spectra for different DNA samples. The absorbance at around 260 nm was found to decrease in the following order: ply[(dGdC)$_2$] > salmon sperm DNA > calf thymus DNA, which implies G-selective cleavage of DNA because this order corresponds well to the guanine content in these DNA samples.[12]

In summary, we have demonstrated that cationic SAMs can immobilize DNA without disrupting its intrinsic higher-order structure, and that site-specific cleavage of the DNA is successfully achieved by incorporation of C_{60} into the SAM. A more detailed examination of the cleaved fragments is now in progress.

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Footnotes and References

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‡ The mixed monolayer (f_1 = 0.2) gave a hydrophilic surface (θ_{H_2O} = 49°) before adsorption of C_{60}. After soaking this mixed monolayer plate in the C_{60} solution, the increased hydrophobicity of the resulting surface (θ_{H_2O} = 62°) was confirmed *via* contact angle measurements.

§ Immobilization of the DNA samples on the mixed monolayer-modified gold plates (2 × 2 cm) was first carried out, and then the plates were immersed in 2 ml tris buffer solutions (pH 7.4). After 3 h irradiation with visible light (λ > 500 nm), UV spectra of the aqueous phases (2 ml) were measured.

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