Photo-induced DNA cleavage in self-assembly multilayer films

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Electrostatic self-assembly multilayer films have been successfully fabricated from a C_{60}–ethylenediamine adduct (C_{60}–EDA), and DNA. Under visible light irradiation, the DNA is cleaved and the films are destroyed. The photo-induced reaction follows a direct electron-transfer mechanism. A high oxygen concentration in the solution enhances the reaction, while separation of C_{60}–EDA and DNA retards the reaction dramatically.

Introduction

The so-called "layer-by-layer" technique has attracted more and more attention since it was first introduced by Decher in 1992.[1–3] By immersing a substrate alternately in cationic and anionic polyelectrolyte solutions, an ultrathin film can be fabricated easily with precise control of thickness. The deposition mechanism and the film structure have been studied extensively, while the resulting films find potential applications in a variety of fields, one of which is carrying out chemical reactions in the films. These reactions can be classified into two categories. In the first category, the films are used only as sites for reactions to occur in; for example, enzymes can be introduced into a film and an enzyme-catalyzed reaction allowed to occur.[4–6] In the second category, reactions between the film components can occur as the result of external stimuli. Using these reactions, the final chemical structure of the film can be adjusted. Light or heat-induced reactions are often used to improve film stability.[7–9] However, under certain conditions, the decomposition of film may also be of interest. Up to now, studies on this topic are still rather limited.[3]

Due to the phosphate esters in their backbone, ribonucleic and desoxyribonucleic acids have been used as polyanions in electrostatic self-assembly multilayer film fabrication.[10–13] Successful dye intercalation has been observed in these films.[10,11] Results concerning molecular recognition and selective pairing of single strands in these films have also been reported.[12,13] In recent years, DNA photocleavage by fullerene and its derivatives has become a very active field of research.[14–16] Although most studies have been carried out in solution, Higashi *et al.*[17] verified that DNA immobilized on a C_{60}-containing self-assembly monolayer can also be cleaved successfully. In this paper, DNA and a C_{60}–ethylenediamine adduct (C_{60}–EDA) were used as the polyanion and polycation, respectively, to fabricate self-assembly multilayer films, and photo-induced DNA cleavage in the films was studied in detail.

Experimental

Materials

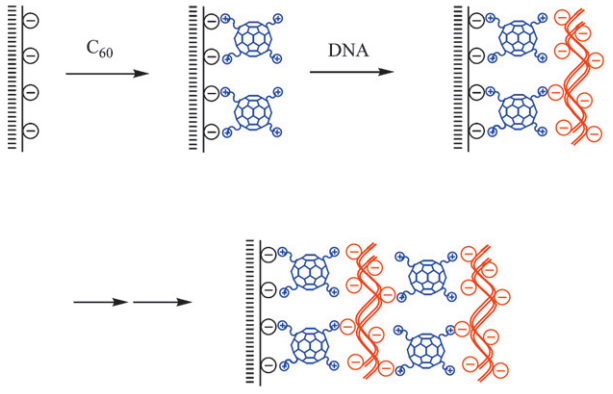
C_{60}–EDA was synthesized according to ref. 18. Its hydrochloride salt was obtained by reaction with HCl. Polyacrylic acid (PAA) was prepared at 70 °C by free radical polymerization of acrylic acid in water using isopropyl alcohol as the chain-transfer agent and potassium persulfate as the initiator. Ionene 6,6 was synthesized *via* the successive Menschutkin reaction of diamine and dibromide in DMF, according to ref. 19. Salmon DNA (Sigma D1626) was purchased from Sino-American Biotec and used without further purification.

Self-assembly film fabrication

In this study, quartz slides were used as substrates. Before use, the slides were treated in a boiling H_2O_2–H_2SO_4 mixture (3:7 v/v) for 30 min. They were thoroughly washed with water and then air-dried. In general, the slide was first immersed in the cationic solution for 4 min. After being thoroughly washed with water, it was air-dried. Then, the slide was immersed in the anionic solution for another 4 min, thoroughly washed with water and air-dried. Films were fabricated by repeating this alternate deposition process.

Photo-reaction in the film

The films were immersed in water and irradiated under visible light, using a xenon lamp as the light source. Light with a wavelength of less than 410 nm was filtered out. UV-vis spectra of the films or the aqueous solution were collected after intervals of 30 min. In order to keep the temperature constant, a double-wall reaction cell with a thermostat was used. The temperature of the system was set at 25 °C.



Scheme 1 Fabrication of self-assembly films from C_{60}–EDA and DNA.

Instruments

UV-vis spectra were collected on a Shimadzu UV 1601 PC spectrophotometer. Atom force microscopy (AFM) measurements were carried out in air at room temperature on a Digital Instruments Nanoscopy IIIA instrument in tapping mode. Commercial silicon probes (model TESP-100) with a typical resonant frequency of around 300 kHz were used to obtain the images. EPR spectra were obtained on a Bruker ESP 300 spectrometer.

Results and discussion

The self-assembly of C_{60}–EDA and DNA is shown schematically in Scheme 1. The fabrication process was followed using UV-vis spectroscopy. As shown in Fig. 1, the absorption spectra of the films show an absorption maximum at 268 nm, which is the characteristic band of DNA. The film absorption intensity increases with increasing number of dipping cycles, indicating the layer-by-layer fabrication is successful. The absorbance at both 195 and 268 nm increases linearly with the number of dipping cycles (inset of Fig. 1), indicating the film is fabricated uniformly.

The morphology of an 8-bilayer film thus fabricated on a silicon slide was studied by AFM. The image is shown in Fig. 2. The film presents a dome-like structure with a diameter of about 25 nm. The average roughness is calculated to be 5.1 nm, indicating that the film is relatively smooth.

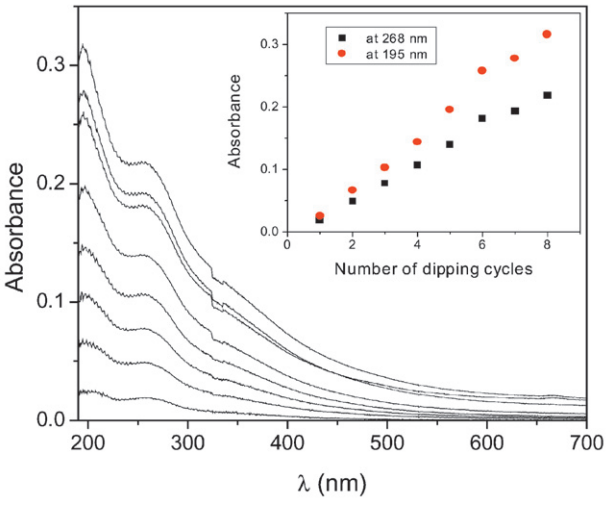


Fig. 1 The absorption spectra of the C_{60}–EDA and DNA self-assembly films with increasing numbers of dipping cycles. Number of dipping cycles (from bottom to top): 1–8. The inset shows the changes in absorbance at 195 and 268 nm as the number of dipping cycles increases.

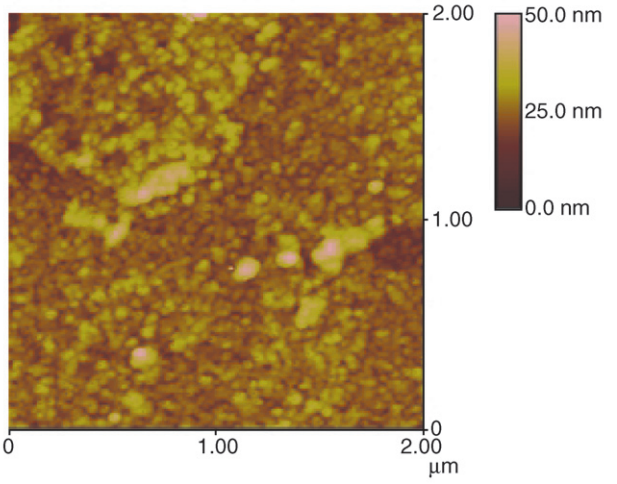


Fig. 2 AFM image of an 8-bilayer film fabricated on a silicon slide.

Under visible light irradiation, the DNA is in position to be cleaved by C_{60}–EDA in the film. The results are collected in Fig. 3. The absorption intensity of the $(DNA/C_{60}–EDA)_{10}$ film drops under visible light irradiation (line a), while it remains unchanged in dark (line b). After irradiation for 3.5 h, the absorbance of the film at 268 nm decreases by 50.5%. This result indicates that the cleavage of DNA is induced by visible light irradiation. The absorption spectrum of an aqueous solution was also measured. As shown in Fig. 4, the absorption intensity of the solution increases with increasing irradiation time. The spectra show a maximum at about 200 nm and a shoulder at about 260 nm, indicating the existence of C_{60}–EDA and DNA fragments in the solution. In contrast, no change in the absorption spectrum of the aqueous solution in the dark was observed. The results suggest that under light irradiation, DNA chains in the film are cleaved into short fragments, such as oligonucleotides and nucleotides, which are

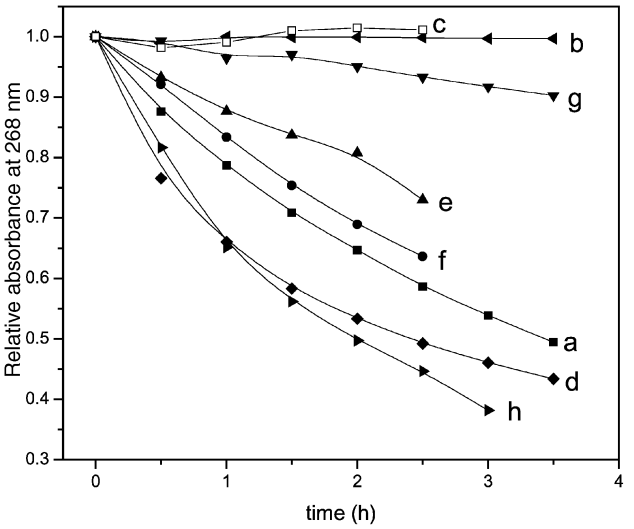


Fig. 3 Photo-induced cleavage of DNA in various films under different conditions: (a) ionene 6,6/$(C_{60}$–EDA/DNA$)_{10}$; (b) ionene 6,6/$(C_{60}$–EDA/DNA$)_{10}$, in the dark; (c) ionene 6,6/$(C_{60}$–EDA/ionene 6,6$)_{20}$; (d) ionene 6,6/$(C_{60}$–EDA/DNA$)_{10}$, O_2-saturated; (e) ionene 6,6/$(C_{60}$–EDA/DNA$)_{10}$, N_2-saturated; (f) ionene 6,6/(DNA/ionene 6,6/PAA/C_{60}–EDA/PAA/ionene 6,6$)_{10}$/DNA; (g) ionene 6,6/[DNA/(ionene 6,6/PAA$)_3$/C_{60}–EDA/(PAA/ionene 6,6$)_3]_{10}$/DNA; (h) ionene 6,6/$(C_{60}$–EDA/DNA$)_{10}$, in 0.01 M NaN_3. Irradiation intensity outside the reaction cell: 0.8 mW cm^{-2}.

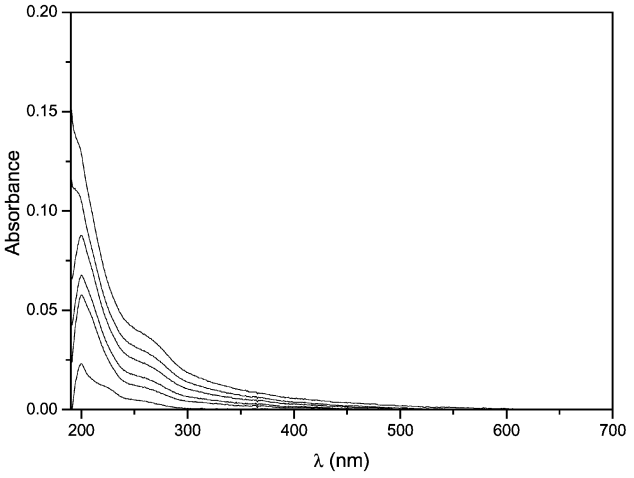


Fig. 4 UV-vis spectra of aqueous solutions in which ionene 6,6/$(C_{60}$–EDA/DNA$)_{10}$ films were immersed after exposure to visible light for various times. Irradiation time (from bottom to top): 0.5, 1, 1.5, 2, 2.5, 3 h.

able to diffuse into the solution.[17] As a control, the absorption spectrum of the ionene 6,6/$(C_{60}$–EDA/DNA$)_{10}$ film remains unchanged under visible light irradiation (line c), indicating that C_{60}–EDA acts as the sensitizer in the reaction.

The effect on the reaction of the concentration of oxygen in the solution was examined. As compared with that in the air-saturated solution (Fig. 3, line a), the reaction rate increases in the oxygen-saturated solution (line d), while it decreases in the nitrogen-saturated solution (line e). After visible light irradiation of these solutions for 2 h, the remaining relative absorbances at 268 nm are 65 (air-saturated), 53 (oxygen-saturated) and 81% (nitrogen-saturated), respectively. These results indicate that the higher the oxygen concentration in the solution is, the higher is the reaction rate.

The effect of the film structure on the reaction has also been studied. In the film, the C_{60}–EDA and DNA layers can be separated by bilayer(s) of PAA/ionene 6,6. As shown in Fig. 3, when C_{60}–EDA and DNA are separated by 1 bilayer of PAA/ionene 6,6, the reaction rate dropped slightly (Fig. 3, line f). The remaining relative absorbance at 268 nm after 2 h irradiation is 69%, slightly higher than that when C_{60}–EDA and DNA are assembled directly (65%). The reaction slows down dramatically when C_{60}–EDA and DNA are separated by 3 bilayers of PAA/ionene 6,6 (line g). After 2 h irradiation, the relative absorbance at 268 nm remains as high as 95%, suggesting only a small amount of DNA is cleaved.

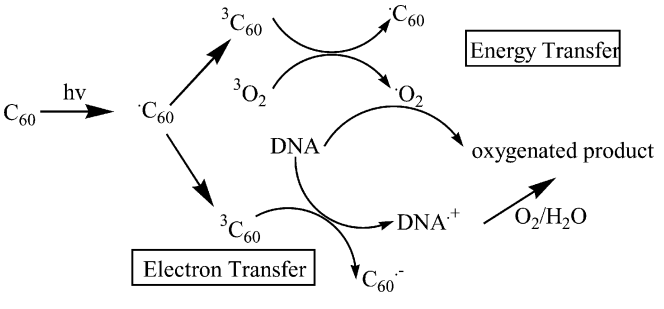
The photo-induced cleavage of DNA by C_{60} and its derivatives in solution has been investigated widely.[14,15] Two possible mechanisms have been proposed: energy transfer or electron transfer, and they are shown in Scheme 2. Under visible light irradiation, singlet oxygen is generated as a result of the interaction of photoexcited C_{60} groups and molecular oxygen, and the DNA is cleaved by the singlet oxygen.[15,16] Some authors also claim that the cleavage of DNA can be the result of direct electron transfer from DNA to photoexcited C_{60}.[14,20] To investigate the mechanism, the singlet oxygen quencher sodium azide was added into the solution and the DNA cleavage rate was measured. As shown in Fig. 3, line h, adding sodium azide does not retard the reaction, in contrast, the reaction rate increases. This result suggests that singlet oxygen is not involved in the reaction. The increase in the reaction rate is a result of the higher ionic strength of the solution. Because electrostatic self-assembly films can be etched when the ionic strength is high, especially for films composed of low molar weight molecules, addition of a low molar weight electrolyte enhances the diffusion of DNA fragments and C_{60}–EDA into solution.

Whether singlet oxygen is generated in an aqueous solution of C_{60}–EDA under visible light irradiation was also examined using the spin-trapping EPR technique. A spin trap for singlet oxygen, 2,2,6,6-tetramethylpiperdine (TEMP), was added to a C_{60}–EDA solution and then the solution exposed to light for 30 min. No change in the EPR signal was found, indicating that no singlet oxygen was generated in the solution. Therefore, the photo-induced cleavage of DNA in this case probably follows the direct electron-transfer mechanism.

The direct electron-transfer mechanism is in accordance with the photo-cleavage experiment. Oxygen is involved in both mechanisms. So it is not strange that the cleavage rate increases as the oxygen concentration in the aqueous solution increases. The effect of film structure can only be explained by the direct electron-transfer mechanism. It is known that an electrostatic self-assembly film does not consist of well-separated, distinguishable alternating layers. The adjacent layers are more or less interpenetrated and have a smeared structure along the film normal. For the film in which C_{60}–EDA and DNA are "separated" by only a single bilayer of PAA/ionene 6,6, most of the C_{60}–EDA and DNA are, in fact, in contact with each other. As a result, the cleavage rate only decreases slightly. But for the film in which C_{60}–EDA and DNA is "separated" by 3 bilayers of PAA/ionene 6,6, little of the C_{60}–EDA and DNA are in direct contact in the film, so the reaction is retarded dramatically. If the reaction followed an energy-transfer mechanism, since singlet oxygen may diffuse in the film, the separation of C_{60}–EDA and DNA would not retard the reaction so significantly.

Conclusion

Using C_{60}–EDA as the polycation and DNA as the polyanion, self-assembly multilayer films based on electrostatic interactions have been fabricated successfully. Under visible light irradiation, DNA can be cleaved and the films are destroyed. The photo-induced reaction follows the direct electron-transfer mechanism. A high oxygen concentration in the solution enhances the reaction, while separation of C_{60}–EDA and DNA retards the reaction dramatically.



Scheme 2 Two possible mechanisms for the cleavage of DNA by C_{60}.

Acknowledgements

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