Scanning tunneling microscope observation of plasmid DNA under electron irradiation at 8–40 eV

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The structural changes in plasmid DNA adsorbed onto graphite following low-energy electron irradiation were investigated. Using a scanning tunneling microscope (STM), we observed networks or islands of DNA consisting of entangled molecules and compared the shapes of the DNA before and after electron irradiation at 8–40 eV field emitted from the tip of the STM. The shape of the DNA changed depending on the electron energy. Electrons with very low energy, such as 8 or 13 eV, extended the area of a DNA island, while the electrons at 18 or 38 eV degraded it. Both types of changes tend to saturate as the electron dose increases. We also discuss the above results in terms of the chemical reactions, such as strand breaks or molecular dissociation, induced by low-energy electrons.

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I. INTRODUCTION

The interaction between high-energy radiation and living cells does not in general directly lead to DNA strand breaks. The primary interaction removes electrons from various molecules in the cells. As a result of charge transfer and energy dissipation processes, chemical bonds can be ruptured, generating neutral or ionic radicals as secondary species. On such occasions, electrons with kinetic energies below 20 eV are generated as the most abundant secondary species.[1]

It was previously unclear whether such low-energy electrons are able to induce damage in DNA. Sanche and coworkers have intensively studied the effects of irradiation with free ballistic electrons (3–20 eV) on DNA.[2–4] In these studies, it was demonstrated that DNA strand breaks were initiated by the formation and dissociation of transient anions localized on the various DNA components (base, phosphate, deoxyribose, and water). Resonance in the yield curves of DNA strand breaks was observed at around 10 eV, which corresponds to the maxima in the electron energy-dependent desorption yield of fragmented negative ions such as H^-, as measured in condensed films of DNA components. The mechanism of strand breaks was confirmed by embarking on resonantly dissociative electron attachment. Hanel *et al.* demonstrated that electrons at energies below the threshold for electronic excitation (<3 eV) effectively decompose gas-phase uracil, generating a mobile hydrogen radical and the corresponding uracil fragment anion.[5] They concluded that the reaction is energetically driven by the large electron affinity of the fragment radical. These results have significant importance for the molecular picture of radiation damage of DNA due to secondary electrons following high-energy irradiation.

In order to further clarify the mechanism of electron-induced reactions, we have attempted to directly observe the structural changes that occur in DNA molecules following electron irradiation. We developed a technique for irradiating a sample surface with field-emission (FE) electrons from the tip of a scanning tunneling microscope (STM).[6,7] This technique enables us to observe the molecular structure of an FE-irradiated area using the same STM tip as a probe. We have observed the structural changes in DNA adsorbed onto a graphite surface under electron irradiation at 8–40 eV using this technique.

II. EXPERIMENT

We carried out the experiments with an STM (remodeled USM-1100S, Unisoku Ltd.) mounted in an ultrahigh-vacuum chamber with a base pressure of about 3×10^{-11} torr. The STM tips were fabricated from 0.3-mm-wide tungsten wire that was electrochemically etched in a 1.0-N KOH solution. Prior to use, the tips were bombarded with an electron beam in a vacuum to remove any oxide.

The plasmid DNA sample (pUC18, 2686 base pairs) was supplied by Takara Bio Co. The stock DNA concentration was 0.5 μg/μl in buffer [10 mM Tris-HCl 1 mM EDTA (ethylenediaminetetraacetic acid)]. The stock solution was diluted to a concentration of about 5 ng/μl with distilled water containing 10 mM magnesium chloride ($MgCl_2$) and 40 mM ammonium acetate (CH_3COONH_4). These two additives were used to facilitate the adhesion of DNA to the graphite surface.[8] The sample solution (200 μl) was deposited on freshly cleaved, highly oriented pyrolytic graphite (HOPG) at room temperature. After being allowed to stand for 10 min, the sample solution was washed away with distilled water and nitrogen gas.

The sample surface was exposed to FE electrons from the STM tip at room temperature. First, a position over the STM image was selected for irradiation. The tip was then positioned over this location and the feedback loop was activated to move the sample away from the tip. The tip-to-sample distance was varied between 10 and 180 nm by applying a driving voltage to a Z-piezo device. A voltage between 0 and +100 V was applied to extract FE electrons, and an FE current was detected.

We checked the difference in the resolution of a tip before and after irradiation. When very large current (μA level) happened to flow from the tip, the resolution became highly degraded. Such degradation could be due to a damaged tip. If the field-emission current was kept below 100 nA, however,

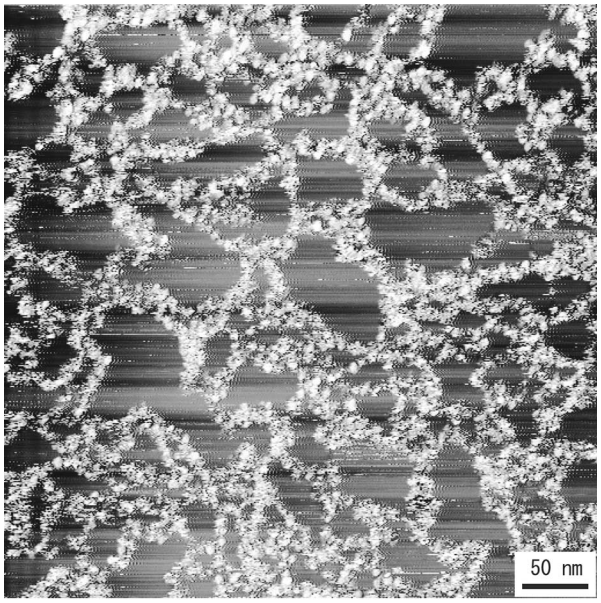


FIG. 1. STM image of plasmid DNA adsorbed onto graphite. Sample bias and tunneling current were +2.5 V and 50 pA, respectively.

tip damage was negligible. We also compared the STM images of a freshly cleaved surface of graphite before and after irradiation with field-emitted electrons, and confirmed that the surface remained virtually unchanged by the irradiation (data not shown).

The relative exposure dose for the FE electrons is defined by multiplying the FE current (nA) by the irradiation time (s) divided by the square of the tip-to-sample distance (nm^2). The exposed electron density in this study was on the order of 10^{23} electrons/cm^2. We confirmed the uniformity of the electron dose density as follows. When the FE electron beam irradiates a surface fully adsorbed with halogen, the irradiated area becomes visible in the STM image due to desorption of adsorbed molecules.[6,7] Under the irradiation conditions we used, the irradiated area was largely round and uniformity in the topograph was within ±10%. Consequently, the electron dose was considered sufficiently uniform to carry out this study.

The kinetic energy of FE electrons must be corrected for the contact potential difference between the emitting and receiving surfaces. If we assume that most electrons are emitted at the Fermi level of the tip surface and that the work function for a dispersedly DNA-adsorbed graphite surface is similar to that for a clean graphite surface (~4.5 eV), the kinetic energy of an FE electron, E_k, is given by $E_k = E_x - 4.5$ (eV), where E_x is the extraction voltage applied to the sample (V).

III. RESULTS AND DISCUSSION

The STM image of the DNA molecules depended on the sample bias. A network structure for the DNA was stably observed at sample biases of 1.5–2.5 V and tunneling currents of 50–100 pA, as shown in Fig. 1. The STM image became noisier at narrower gaps between the tip and sample. This could be due to the enhanced contact between the tip and sample. The DNA molecules at a sample bias of more than 3.0 V looks noticeably brighter than at a sample bias of 2.5 V. Furthermore, the image of the area, once it has been scanned at 3.0 V, is irreversibly altered from that previously observed at a lower sample bias; e.g., after scanning at 3.0 eV, one cannot return to the image observed initially at, say, 2.5 V, even if the area is observed again at 2.5 V. These results indicate that the DNA molecular structure may be changed when the tip scans the area at a sample bias of more than 3.0 V. The electric field generated in the gap between the tip and the cleaved graphite surface is considered to be on the order of ~1 V/nm. This value is one or two orders of magnitudes lower than the field required for the evaporation of atoms. The DNA-adsorbed surface is, however, rather bumpy on a nanometer scale, so the field being applied on DNA molecules may be able to overcome the threshold for evaporation of atoms. Martin *et al.* reported that single-strand breaks of DNA could occur at electron energies of 0–4 eV.[9] Hanel *et al.* also reported that gas-phase uracil could be decomposed by electrons at energies below the threshold for electronic excitation (<3 eV).[5] Taking this and our observations of sample bias effects into account, we decided to use sample biases of 1.5–2.5 V for our subsequent experiments. We repeated each experimental run 2 or 3 times under the same conditions and confirmed that there were no significant differences among the observations for a set of runs.

The apparent minimum width of the lines of the networks shown in Fig. 1 was approximately 5 nm, which we consider to be due to a deformation of the actual width of the DNA, caused by the finite size of the tip.[10] The structure of the DNA is entangled, either intermolecularly or intramolecularly. Densely entangled areas looked like islands, as described below. Furthermore, we also confirmed that a film of DNA was formed when we used solutions with higher DNA concentrations (data not shown).

STM images of DNA before and after electron irradiation at different energies are shown in Figs. 2–5. We repeated the irradiation and observation several times at each electron energy by altering the targeted area on the sample. Representative images are shown. Figure 2 shows STM images of DNA before and after irradiation with FE electrons at 8 eV. The DNA area appears to be extended at many sites by irradiation after the first exposure dose of 0.15 nA s nm^{-2}. However, little change was seen with additional irradiation at the same dose. The height profile obtained by scanning over the extended area is shown in Fig. 3. The results indicate that the thickness of DNA is thinned by the extensions. The extension of the DNA area was also observed with electron irradiation at 13 eV. In contrast, electron irradiation at 18 eV degraded the DNA area, but scarcely extended it, as shown in Fig. 4. In this case, DNA degradation appeared to saturate at an electron dose density of 0.15–0.3 nA s nm^{-2}. Greater degradation was seen with irradiation at 38 eV, as shown in Fig. 5. In addition to degradation, extension was observed in other areas with irradiation at 38 eV. Thus, plasmid DNA in the entangled phase changes its structure depending on electron energy.

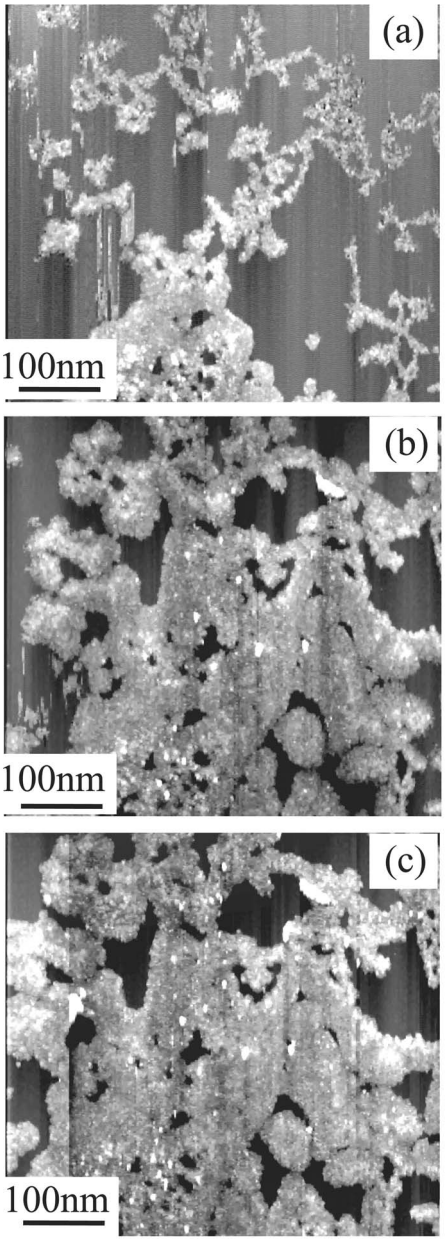


FIG. 2. STM images of plasmid DNA adsorbed onto graphite, (a) before electron irradiation, (b) after electron irradiation with 0.15 nA s nm^{-2} (current, 5 nA; irradiation time, 10 s; tip-sample distance, 18 nm) at 8 eV, (c) after electron irradiation with 0.3 nA s nm^{-2} (5 nA, 20 s, 18 nm) at 8 eV.

Sanche and co-workers used agarose-gel electrophoresis to study the type and yield of strand breaks induced in plasmid DNA by electrons at 3–100 eV.[2,3] They classified the types of the strand breaks induced in plasmid DNA by electron energy. Their results are summarized, together with the present results, in Table I. Low-energy electrons at 3–15 eV induce single (SSB's) and double (DSB's) strand breaks via the formation and decay of molecular resonances involving DNA components. Above 15 eV, SSB's and DSB's occur via nonresonant mechanisms related to excitation, ionization, and dissociation.

Sanche and co-workers also measured the yield of multiple double strand breaks (MDSB's) induced by electrons

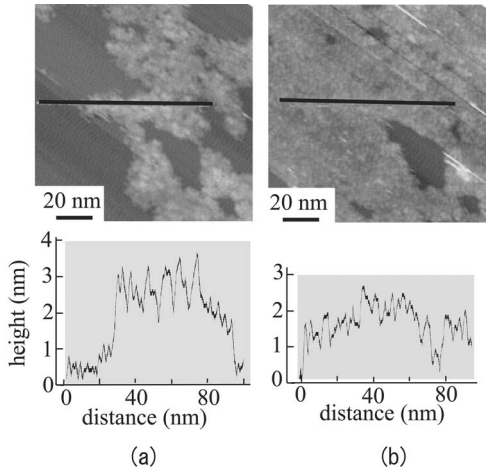


FIG. 3. Magnified STM images and height profiles (a) before and (b) after electron irradiation with 0.15 nA s nm^{-2} (current, 5 nA; irradiation time, 10 s; tip-sample distance, 18 nm) at 8 eV.

with energies below 100 eV. MDSB's are defined as the occurrence of DSB's at two or more sites in the same DNA molecule. MDSB yields showed a strong monotonic increase above 30 eV and were caused nonresonantly by multiple successive interactions with a single electron in the same molecule. On the other hand, MDSBs occur resonantly near 20–30 eV, although the yield is low. Furthermore, the group investigated electron-stimulated desorption of anions from thin films of plasmid DNA at 3–20 eV. Resonant structures in the curves of the desorption yield of H^- and O^- were observed with a maximum yield at 9–9.5 eV, which is similar to the maximum yields of SSB's and DSB's. Electrons with energies in this range are capable of dissociating hydrogen bonds between the bases. They concluded that most of the SSBs and DSBs below 15 eV are initiated by resonant electron attachment to the components of DNA, such as the bases or phosphate groups.

In the light of the results of Sanche and co-workers, we speculate here on the likely mechanisms that led to the observed damage in our experiment. A plasmid with one DSB may become linear with full length. Similarly, a plasmid with a SSB will take on a relaxed circular conformation. The dissociation of hydrogen bonds between the bases readily leads to an unraveling of the double helix. These transformations in a DNA molecule in the entangled phase presumably lead to the extended areas seen under the limited resolution of the STM. On the other hand, MDSB's occurring at energies over 30 eV dissociate the DNA molecule into smaller fragments, which are likely desorbed by subsequent electron irradiation. Such fragmentation and desorption result in the degradation of DNA area, as was seen with irradiation at 38 eV. The extension behavior observed at 38 eV could be due to inelastic scattering and/or ionization. The electrons may bounce back from the substrate with energies between 5 and 15 eV and resonantly interact with DNA. A similar explanation applies to the electrons emitted from ionization of DNA. As mentioned above, irradiation at 18 eV degraded the DNA area, but scarcely extended it. The yield of MDSB's increases up to around 20 eV, after which MDSB's occur with

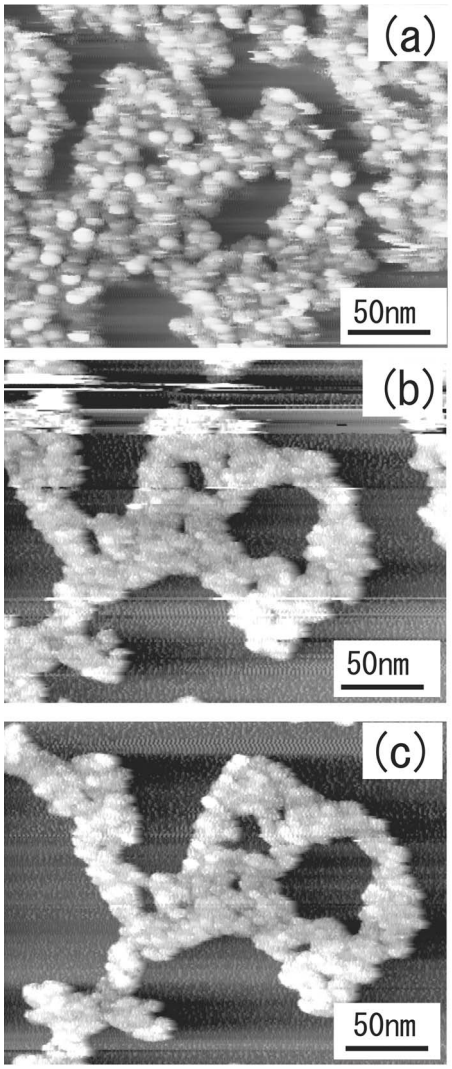


FIG. 4. STM images of plasmid DNA adsorbed onto graphite, (a) before electron irradiation, (b) after electron irradiation with 0.15 nA s nm^{-2} (current, 7 nA; irradiation time, 7 s; tip-sample distance, 18 nm) at 18 eV, and (c) after electron irradiation with 0.3 nA s nm^{-2} (7 nA, 14 s, 18 nm) at 18 eV.

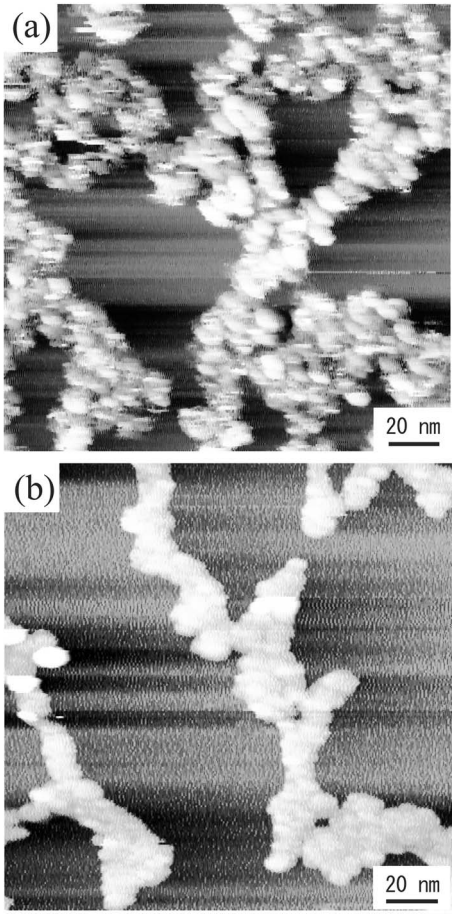


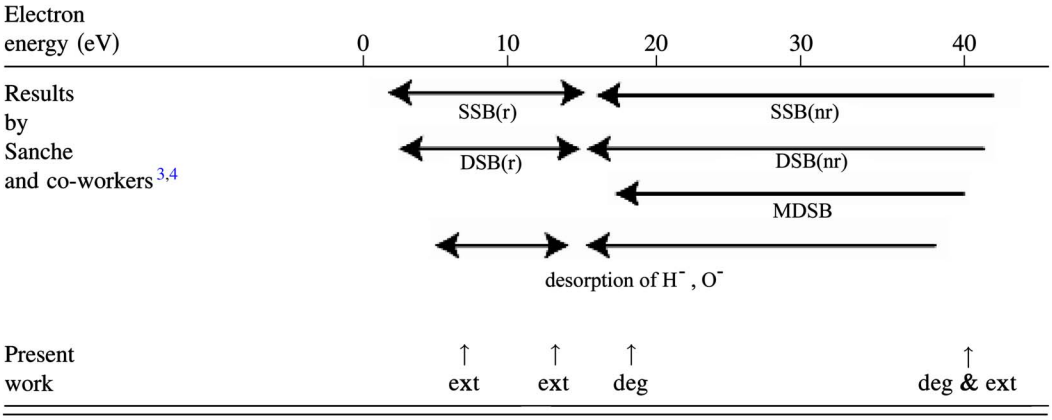
FIG. 5. STM images of plasmid DNA adsorbed onto graphite, (a) before and (b) after electron irradiation with 0.1 nA s nm^{-2} (current, 4 nA; irradiation time, 8 s; tip-sample distance, 18 nm) at 38 eV.

successive electrons. Such MDSB's may contribute to the degradation of DNA area, although the yield is low. However, it is uncertain why only a slight extension of the DNA is observed, despite the fact that the yield of DSB's and SSB's at 18 eV is on the same level as that at 8 eV. According to the measurements of Huels *et al.* the linear regime (i.e., the dose range within which the observed damage caused by a single electron) lies between zero and 900 nA s mm^{-2}.[3] The dose of 0.15 nA s nm^{-2} used in our experiments is two or three orders of magnitudes higher than the limiting dose for the linear regime. Consequently, highly multiple fragmentation of DNA might be caused by this condition, leading to desorption of small fragments and modification of clustering. Furthermore, polymers also can undergo intramolecular or intermolecular cross-linking at high doses of any radiation, even if they are easily decomposed at low doses. Washino and Schnabel reported that cross-linking of DNA occurs via reactions involving hydrated electrons.[11] It is likely that DNA molecules in the entanglement phase undergo cross-linking at such high electron doses. Strand breaks or molecular dissociations may compete with the cross-linking, and the balance among them depends on the dose, in addition to the energy of the electrons. It seems probable that changes like extension or degradation do not readily occur in densely cross-linked DNA when compared with DNA that is not cross-linked.

IV. CONCLUSION

We investigated the structural changes in plasmid DNA adsorbed on graphite after irradiation with the low-energy electrons field emitted from an STM tip. The manner of structural changes depends on electron energy. At electron energies of 8 eV or 13 eV, the dominant change is the extension of the DNA island, which is most likely caused by the relaxation of DNA molecules via strand breaks or unraveling of the double helix. The higher-energy electrons of 18 eV or 38 eV degrade the area of DNA islands due to the electron-induced desorption of fragmented pieces. As electron dose increases, strand breaks and desorption compete with the cross-linking reaction. As a result, the two types of

TABLE I. Incident electron energy ranges where damage is observed in plasmid DNA. SSB, single strand breaks; DSB, double strand breaks; MDSB, multiple double strand breaks; ext, extension; deg, degradation; r, resonant; nr, nonresonant.



change, extension and degradation, become saturated at high electron doses. In the present study, the DNA sample was in a moderately entangled state and the obtained results correspond fairly well to the results of measurements by electrophoresis or mass spectroscopy of solid-state samples. In order to clarify the structural changes at the molecular or atomic scale, experiments involving electron irradiation of singly adsorbed DNA molecules are in progress.

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