Electron Resonance Decay into a Biological Function: Decrease in Viability of *E. coli* Transformed by Plasmid DNA Irradiated with 0.5–18 eV Electrons

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Abstract

Transient negative ions (TNIs) are ubiquitous in electron-molecule scattering at low electron impact energies (0–20 eV) and are particularly effective in damaging large biomolecules. Because ionizing radiation generates mostly 0–20 eV electrons, TNIs are expected to play important roles in cell mutagenesis and death during radiotherapeutic cancer treatment, although this hypothesis has never been directly verified. Here, we measure the efficiency of transforming *E. coli* bacteria by inserting into the cells, pGEM-3ZfL(–) plasmid DNA that confers resistance to the antibiotic ampicillin. Before transformation, plasmids are irradiated with electrons of specific energies between 0.5 and 18 eV. The loss of transformation efficiency plotted as a function of irradiation energy reveals TNIs at 5.5 and 9.5 eV, corresponding to similar states observed in the yields of DNA double strand breaks. We show that TNIs are detectable in the electron-energy dependence of a biological process and can decrease cell viability.

Graphical Abstract

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Supporting Information

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A more complete description of experimental methods is given. (PDF)

Enlarged schematic of assembly used to measure incident electron beam energy and current. (PDF)

The concept of resonance states was first introduced to neutron–nuclear scattering by Bethe in the 1930s¹ and has subsequently been applied across a broad range of fundamental problems in the physical sciences.^{2–5} Such compound states occur when an incident particle or a photon combines with a target for a period exceeding the usual scattering time. The compound states formed in electron–molecule collisions are transient negative ions (TNIs), which can decay by electron emission, dissociative electron attachment (DEA), or electron stabilization on the molecule.^{5,6} In each of these processes, energy can be transferred to the molecule, its fragments or the surrounding medium.^{6,7} Experimentally, TNIs can be detected by observing well-defined features or maxima in the incident-electron-energy dependence of specific decay channels (i.e., *yield functions*), such as vibrational and electronic excitation or DEA.^{5–7}

Soon after their discovery in helium by Schulz,⁸ electron resonances were found in small, isolated molecules⁵ and about two decades later, observed in atomic and molecular solids and molecules adsorbed on surfaces.^{9–11} However, it was not until the year 2000 that they were observed to decay into dissociative channels of large biomolecules, causing biologically significant damage.¹² They were identified as pronounced maxima at 0.8, 2.2, 4.5, and 10 eV^{13,14} and at 6 and 10 eV^{12,14} in the yield functions of DNA single and double strand breaks (SSBs and DSBs), respectively, obtained from irradiation of thin DNA films with low-energy electrons (LEEs). The first two peaks are single-particle shape resonances, whereas the others were interpreted as core-excited resonances.¹⁵ The TNIs are usually first formed on the bases; breaking of the backbone (i.e., SSBs and DSBs) is due to electron transfer from a base to the phosphate group, where the C–O bond is ruptured via DEA.¹⁵ Because most secondary electrons generated by high-energy radiation have energies lying below 20 eV, such results, and related theoretical calculations,^{16,17} modified the established paradigm of radiobiological damage. They indicated that, largely due to electron resonances, most secondary electrons could efficiently damage the genome via electron capture by DNA subunits.

Here, we report the first observation of electron-molecule resonances in a *biological function*; that is, in decay channels that destroy the functionality of DNA in living cells. *E. coli* bacterial cells are incubated in an antibiotic-rich (i.e., ampicillin) environment, which would normally kill them. Using previously described methods,^{18,19} we introduce plasmids into the cells by temporarily rendering their membranes permeable. The plasmids consist of double-stranded DNA containing a gene that encodes an enzyme. The enzyme can inactivate ampicillin and thus keep the bacteria alive. The “*transformed*” bacteria replicate the plasmid along with their own DNA. In our experiment, before this “*transformation*”²⁰ the plasmids are irradiated by electrons of controlled fluence and energy (in the range 0.5 and 18 eV). Subsequently, we determine the *transformation efficiency* (TE) (i.e., the fraction of the bacteria that are resistant to the antibiotic) by recording the number of colonies that grow after incubation in the ampicillin-rich environment.

The electron-energy dependence of the number of resistant bacteria is found to exhibit two strong minima at 5.5 and 9.5 eV, which coincide with the energy of previously recorded core-excited resonances in the yield function of DSBs.¹⁴ The TE function shows that

electrons from 3 to 18 eV can reduce the viability of DNA and cell replication. Furthermore, this reduction is considerably enhanced at the resonance energies.

Figure 1 shows the variation of the TE of JM109 bacterial cells as a function of electron fluence at 10 eV from the data of Kouass Sahbani et al.,¹⁹ who additionally recorded at each fluence the percentage yields of the different DNA conformers; that is, the supercoiled (S), circular (C), and linear (L) forms, that correspond to undamaged DNA, SSBs, and DSBs, respectively. Digestion with repair enzymes also revealed base damage, as well as locally multiply damaged sites (LMDS), consisting of combinations of lesions in close proximity (i.e., within a distance of about ten base pairs). The variation of TE with fluence “ φ ” at an irradiation energy “ E_i ” can be expressed as¹⁹

$$TE(\varphi, E_i) \propto [\rho_S \cdot S(\varphi, E_i) + \rho_C \cdot C(\varphi, E_i) + \rho_L \cdot L(\varphi, E_i)] \cdot \left[e^{-\beta_R(E_i)\varphi} \sum_{k=0}^{n-1} \frac{(\beta_R(E_i)\varphi)^k}{k!} \right] \quad (1)$$

Here ρ_S , ρ_C , and ρ_L represent the probabilities that the supercoiled, circular, or linear forms traverse into the bacteria. β_R is an effective cross section that represents the yield of lethal damage, so that the product $\beta_R \cdot \varphi$ corresponds to the mean number of toxic lesions induced per plasmid. β_R is independent of DNA conformation. The integer n is the number of toxic lesions needed to inactivate the plasmid. Previously, ρ_C/ρ_S was measured to be 3 and ρ_L close to zero.¹⁹ Thus, for a fixed fluence, $TE(E_i)$ depends on the variation with electron energy of two independent terms: an *entry function* that models the transfer of plasmids into the *E. coli* and a *survival function*, which relates the functionality of the plasmid to the number of potentially lethal lesions it receives. The initial increase in TE seen in Figure 1 derives from the entry function and the conversion of S- to C-form DNA that enters the bacteria more easily (i.e., $\rho_C > \rho_S$).¹⁹ The decrease at higher fluence is controlled by the survival function and the accumulation of lethal damage.

Kouass Sahbani et al.,¹⁹ identified this damage as due to LMDS and possibly to the formation of cross-links from the intercalation of 1,3-diaminopropane (DAP) molecules with DNA to stabilize the film.²¹ However, a fraction of the damage remained unassigned, probably due to limitations of the repair enzymes used to reveal LMDS, by converting base damage and apurinic/apyrimidinic sites into DSBs.¹⁸ Indeed, detection efficiency is expected to decrease when lesions are formed close together (i.e., within one or two base pairs), which is a likely outcome with short-range 3–18 eV electrons.²² We expect the LMDS identified by Kouass Sahbani et al., to be present essentially above 5 eV, because this is the minimum energy required for breaking bonds on two distant DNA subunits with one electron.¹⁵ Nevertheless, multiple-bond ruptures are potentially possible on the sugar at lower energies.^{23,24}

The electron-energy dependence of the loss of TE recorded at a fluence of 27×10^{13} electrons/cm² is shown in Figure 2a. Here, TE is expressed as a fraction of the value obtained with unirradiated samples subjected to all other experimental procedures and is plotted against an inverted y scale to represent the loss of TE. Each of the points in Figure 2a

is the average of TE from three different DNA samples and the error bars represent the standard deviations of these points. Curve 2a exhibits two strong peaks at 5.5 and 9.5 eV with a saturating maximum above 15 eV. In interpreting these results it is important to note that only the survival function can decrease TE below values obtained for unirradiated samples; that is, TE minima (or TE loss maxima) relate to the accumulation of lethal damage in the plasmid.

The electron-energy dependence of the yields of SSBs, DSBs, and DNA cross-links in different types of DNA has been intensively investigated during the past decade.^{15,17} Luo et al.¹⁴ compared the results of ten different experiments and concluded that between 3 to 15 eV, the yields of SSBs and DSBs are controlled by at least two electron resonances observed at 4.6 and 9.6 eV and 6.1 and 9.6 eV, in the respective yield functions. The most recent results are shown in Figure 2; they represent, from (b) to (d), the yield functions for SSBs, DSBs, and interduplex cross-links, respectively.

The strong peak at 9.5 eV in the loss of TE function can easily be correlated with the maximum at 9.6 eV in the yield function of SSBs and DSBs. The one at 5.5 eV correlates more precisely with the maximum at 6.1 eV in the yield function of DSBs, which was recorded in a linear fluence-response regime¹⁴ and hence results from single electron-DNA interactions.

Because SSBs are easily repaired and hence not expected to be lethal to cells, it is not surprising to find better agreement between curves a and c in Figure 2. It is probably for the same reasons that the other resonances, observed by Martin et al.,¹³ at 0.8 and 2.2 eV in the yield functions of SSBs, are not readily observed in Figure 2a. Any loss of TE below 3 eV would therefore have to be attributed to other lesions such as a base damage. However, the loss of TE below 3 eV is very small and the structure lies within experimental errors. In any case, the comparison of the curves a to c clearly shows that electron resonances control the loss of TE in *E. coli* bacterial cells below 13 eV.

Detailed analysis of the products induced by LEE impact on thin films of small oligonucleotides of varying length and of different types of DNA has shown that within the 4–15 eV range, strand breaks occur predominantly via electron capture by the positive electron affinity of an electronically excited state of a base (i.e., a core-excited resonance), followed by electron transfer to the phosphate group.^{15,25} Thus, a likely process to create a LMDS with a single electron is the decay of the initial core-excited resonance of the base into a dissociative electronically excited state, accompanied by electron transfer onto the phosphate group of the opposite strand, which could also rupture via DEA, producing base damage and a strand break. This pathway has recently been observed experimentally in single-stranded DNA.²⁶ Other scenarios are possible, such as damage to two adjacent bases. *The yield functions of all LMDS pathways initiated by electron capture by a base would exhibit peaks at the same energies corresponding to those of the initial TNIs.* Hence, despite the minor role of DSBs in reducing TE at the resonance energies,¹⁹ the above arguments could explain the energy coincidence between the peaks of (a) and (c) in Figure 2.

From our previous experiment with 10 eV electrons,¹⁹ it has been proposed that LEE-induced radicals on either DNA or the DAP molecules²¹ could cause cross-linking between these molecules. Such cross-linking could contribute to the loss of TE. In our films, the DNA layers are held together by the electrostatic interaction between the ammonium cations at each end of the DAP molecule and negative oxygen atoms of the phosphate group in DNA.²¹ Hence, cross-linking is expected to result from the creation of a radical arising from a dissociative state at, or near, the ammoniumcation/oxygen site. If the dissociative state does not arise from resonance decay, no peak should be observed in the yield function of cross-links. The yield function for formation of cross-links in intercalated DAP–DNA films has not yet been measured, but we show in Figure 2d the result¹⁴ for cross-linking between circular and supercoiled DNA in a pure DNA film. From 3 to 18 eV, these yields are fairly constant within experimental errors. Thus, radicals responsible for cross-linking are either not formed via electron resonances or (rather implausibly) occur from the decay of a large number of overlapping transient anion states of similar magnitude. Assuming a similar behavior for the formation of cross-links between DAP and DNA, we conjecture that below 13 eV, cross-links do not play a major role in the loss of TE. Above 13 eV, strand breaks yields are not resonant (Figure 2b and c) and this characteristic is reflected in the loss of TE, which remains fairly constant from 14 to 18 eV. Such a behavior is more characteristic of nonresonant cross-linking processes.

We have shown that the TE of *E. coli* bacterial cells can be reduced, by damaging with LEEs, the genome responsible for their survival and proliferation. The loss of TE strongly depends on electron energy in the 3 to 15 eV range, where peaks of large amplitude appear at 5.5 and 9.5 eV. These peaks are due to core-excited resonances formed on the DNA bases, which amplify the damage. This result demonstrates the ability of TNIs formed in DNA to modify cell growth and survival and shows that electron resonances can be observed in a biological function. This discovery may open a new area of investigation, where instead of measuring the immediate effects of LEE-induced phenomena at the molecular level, one observes the biological consequences of the LEE-biomolecule interactions. There exists a plethora of biomolecules in cells that affect specific biological functions and it may thus become possible to investigate the effects of LEEs and resonances on a specific cellular activity. In addition to their obvious importance for Radiobiology, such LEE impact experiments may provide another approach to probe the mechanisms of cell survival.

EXPERIMENTAL METHODS

More detailed descriptions of the procedures are given in the Supporting Information. In brief, plasmid DNA [pGEM-3Zf(–), 3197 base pairs, ca. 1 968 966 amu per plasmid], prepared as previously described,^{27,28} was deposited via the soft absorption method of Boulanouar et al.,²¹ onto a graphite substrate. This method uses protonated DAP to link together the plasmids and so form soluble DNA-Dap films of uniform and controllable thickness (11 nm or 5 monolayers (ML)). Such films are less prone to charging than lyophilized samples,²⁹ allowing a larger fraction to be irradiated with LEEs and affording higher yields of damaged DNA. Prepared samples were transferred into a previously described electron irradiator²⁷ and exposed to an electron beam of variable energy (0.5–18 eV) and a current density of 1.5×10^{11} electrons $\text{s}^{-1} \text{cm}^{-2}$, for periods ranging from a few

seconds to several tens of minutes. After irradiation, the samples were removed from the UHV chamber and recovered into solution.

The TE of controls and irradiated plasmids was determined using the method of Kouass Sabahni et al.¹⁸ Following their transformation,²⁰ the cells were spread on LB agar plates containing ampicillin at a concentration of 50 $\mu\text{g}/\mu\text{L}$,¹⁸ to select transformants (i.e., colonies, which can grow in the presence of ampicillin). After incubation at 37 °C overnight, the transformants were counted. The efficiency of the transformation is reflected by the number of transformants per micrograms of plasmid DNA. All transformation experiments were repeated three times for a total of 113 cell survival measurements (including 14 additional controls).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Variation of transformation efficiency of JM109 *E. coli* cells by pGEM 3Zf(–) plasmids, with incident electron fluence at 10 eV. The data is taken from ref 19. Each point is the average of three identical measurements. At each fluence, the error bars represent the standard deviation. The solid line is a fit to the data using the parametric model described in ref 19.

Figure 2.

(a) Variation of transformation efficiency of JM109 *E. coli* cells by pGEM 3Zf(–) plasmids irradiated by 0.5 to 18 eV electrons at a fluence of 27×10^{13} electrons/cm 2 . The y axis is inverted. Effective yield functions for (b) single strand breaks (SSBs), (c) double strand breaks (DSBs) and (d) DNA cross-links, from ref 14.