Poster

ATOMIC FORCE MICROSCOPY CAPTURES UVA DAMAGE TO INDIVIDUAL DNA MOLECULES

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There is increasing evidence that UVA radiation, which makes up ~95% of the solar UV light reaching the Earth's surface and is commonly used for cosmetic purposes, is genotoxic(*1-3*). However, in contrast to UVC and UVB, the mechanisms by which UVA produces various DNA lesions are still unclear(*1-6*). In addition, relative amounts of various types of UVA damages and their mutuagenic significance are also a subject of debate. Here, we exploit atomic force microscopy imaging of individual DNA molecules, alone and in complexes with a suite of DNA repair enzymes and antibodies to directly detect UVA damage and to re-examine its basic mechanisms. By combining the activity of endonuclease IV and T4 endonuclease V on highly purified and UVA-irradiated pUC18 plasmids we show, by direct AFM imaging that UVA produces a significant amount of abasic sites and pyrimidine dimers (CPD) (Fig. 1). Importantly, we find that only half of the T4 endonuclease V-sensitive sites, which are commonly counted as CPDs, are true CPDs, the other half being actually abasic sites. In addition, we find that separation by gel electrophoresis of UVA-irradiated and enzyme treated supercoiled DNA may produce an incorrect ratio of intact and damaged plasmids, erroneously suggesting that CPDs have not been produced by UVA. Most importantly, our results obtained by AFM imaging of native and synthetic DNA, using T4 Endonuclease V, photolyase and anti CPD antibodies, strongly suggest that pyrimidine dimers are produced by UVA *directly*. Thus, our observations contradict the present view that as yet unidentified photosensitizers are required to transfer the energy of UVA to DNA in order to produce CPDs. Taken together our results clarify some of the controversial observations about UVA damage to DNA and suggest that more fundamental studies on the interaction between UVA and DNA are warranted.

References:

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Figures:

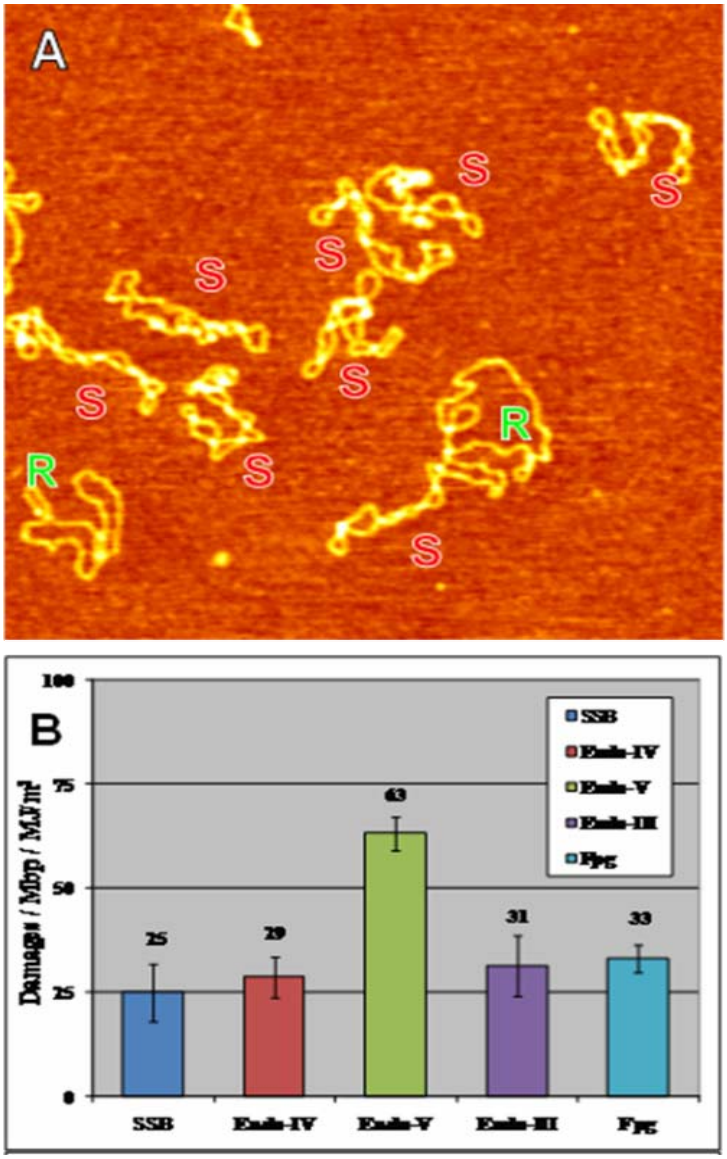

Figure 1. An AFM image on mica of different supercoiled pUC18 DNAs that were subjected to 1.3 MJ/m^2 UVA radiation and different enzyme treatments prior to imaging in air. DNA was dialyzed in 10 mM Tris-HCl, 1 mM EDTA and 100 mM NaCl buffer and irradiated in the same solution by UVA. After that the sample was diluted back to the suitable buffer for different enzyme incubation: (A) T4 endonuclease V. Scan size is 1 x 1 μm^2. Code: supercoiled DNA (S); relaxed circular plasmids (R); linear DNA (L). The error bars in the figures represent the standard deviation. (B) Histogram summarizes the number of different damages per million base pairs per MJ/m^2 after UVA irradiation and specific enzyme treatments. The values shown in the histogram represent averages from 2-5 separate experiments.