Measurement of DNA Mutations Caused by Seconds-period UV-irradiation

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(Received November 6, 2006; CL-061299; E-mail: koichi@fms.saitama-u.ac.jp)

UV is a potent mutagen threatening human health. A genome analysis technology, genome profiling, was found to be used to measure UV-irradiated DNA mutations quantitatively within the range of 500 J/m^2 of UVA. An intriguing phenomenon was also observed for the UV-irradiation to in vivo DNA that the apparent amount of mutations oscillated responding to an increasing amount of UV-dosage. This method is advantageous in measuring the alteration of DNA directly, eliminating the necessity of complicated interpretation required for bioassays.

UV is the most common mutagen threatening human health. This well-recognized mutagen leaves still much to be studied. Recently, it has become clear that the skin cancer is caused by UVA (around 360 nm) through cyclobutane pyrimidine dimers which had been thought to be induced exclusively by UVB (around 265 nm).[1,2] The degree of DNA damage is usually measured by monitoring the viability of cells or the SOS response except some cases that will do DNA sequencing.

The genome profiling (GP) method has been shown to be effective in identifying[3] and classifying species.[4] And recently, it was proved to be applicable to measure the strength of mutagenic chemical reagents such as AF2 and ethidium bromide.[5] In this paper, it is shown that GP can measure the effect of UV, a kind of physical mutagen, also. At the same time, the amount of mutation accumulated on DNA after a short period of UV irradiation was proportional to its dosage, indicating the ability of GP to measure mutations quantitatively. The short period UV-irradiation was performed using an apparatus fabricated in our laboratory (Figure 1), which was made to facilitate controlling short period irradiation and uniformality of irradiation. Both in vitro and in vivo DNAs were subjected to UV-irradiation (UVA generated by 4W UVGL-25 lamp (UVP, U.S.A.) from the distance of 10, 20, and 40 cm) in concentrations of 200 ng/mL or 2×10^9 cells/mL (*Escherichia coli* (S26) cultivated to the late log phase), respectively, which are equivalent in the effective molar concentration of DNA. The DNAs and cells were dissolved in 1 × PBS(−) buffer (pH 7.2, GIBCO) or Davis medium contained in a vessel (MMV; multi-micro vessel made of acrylamide gel with 100 wells (2 μL volume) in 1 inch^2), respectively, and were UV-irradiated for 3–960 s. The DNAs or cells were directly applied to random PCR.[6] Random PCR is nearly equivalent to pick up major DNA fragments that are generated by more stable hybridization of the primer DNA. The random PCR solution (50 μL) contained 200 μM dNTPs (N = G, A, T, C), 0.5 μM primer, 10 mM Tris-HCl (pH 8.8), 50 mM KCl, 2.5 mM $MgCl_2$, 0.02 unit/μL Taq DNA polymerase (Biotech International, U.S.A.) and template DNA (arbitrary amount). Random PCR was carried out with 30 cycles of denaturation (94 °C, 30 s), annealing (28 °C, 2 min) and extension (47 °C, 2 min) using a PTC-200 PCR machine (MJ

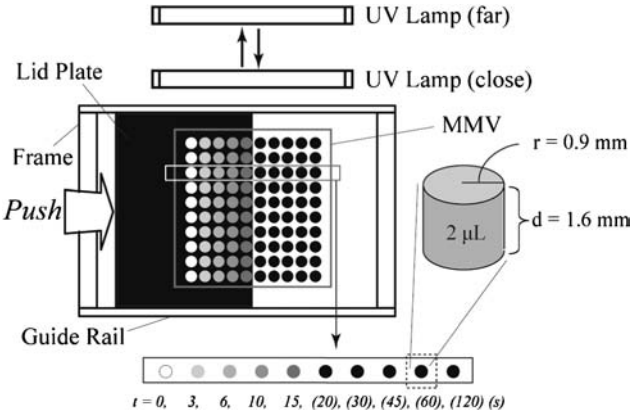

Figure 1. Experimental setup for UV-irradiation to 200 μL of samples. DNAs or cells (*Escherichia coli*) (65 amole DNA molecules or an equivalent amount of cells were dissolved/suspended in Davis media depleted with glucose) were loaded on a tiny plate (2.5 × 2.5 cm^2) with 100 wells of 2 μL volume each made of acrylamide gel (MMV) so as to facilitate parallel and uniform irradiation and rapid chilling of samples. To regulate the irradiation time, the black lid plate were slid to shield the sample.

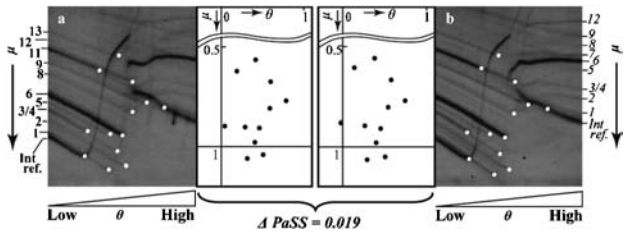

Figure 2. How to measure ΔPaSS value. GP (genome profiling) method, which analyzes DNAs (obtained by random PCR) using microTGGE (temperature gradient gel electrophoresis), was employed to obtain the featuring points (the white dots in Panels a) and b) and the computer-processed *spiddos* (species identification dots) in each corresponding center panel. The pattern similarity score (PaSS) was calculated between two genome profiles (UV-irradiated in vitro DNA for 0 s (left) and 60 s (right)) and by comparing the coordinates of corresponding *spiddos*.[8] Here, ΔPaSS is defined to be 1 − PaSS ($0 \leq$ PaSS ≤ 1). The directions of migration of DNA (μ) and temperature gradient (θ) are shown beside. The internal reference DNA bands for normalization of mobility and temperature are shown with Int. ref. and the other DNA bands are designated with a number. Bold letters (Panel a, left) and Italic letters (Panel b, right) represent double stranded and single stranded DNAs, respectively.

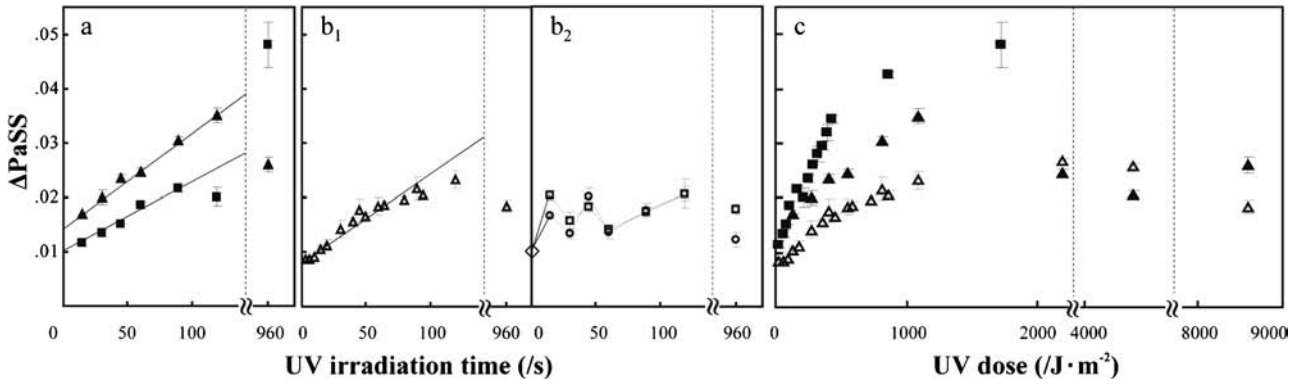

Figure 3. Dependence of ΔPaSS on UV-irradiation distance/time/dosage. DNAs irradiated by UVA were in vitro state (symbols are shown in filled symbols) (Panel a) or in vivo state (shown in blank) (Panels b_1 and b_2). Throughout this figure, the same symbols; triangle (10 cm), circle (20 cm), and square (40 cm) were used to represent the distance of UV lamp from the samples. Panel c represents the dependence of ΔPaSS on UV dosage for both of in vitro and in vivo DNAs (converted from Panels a and b_1 with omission of b_2 for the sake of clarity). a. DNAs in solution (10 and 40 cm apart). b_1. DNA in cells (10 cm). b_2. DNA in cells (20 and 40 cm), as above mentioned. Duplicate or more experiments were performed for each data. The diamond at time 0 in Panel b_2 represents the estimated bias of ΔPaSS.

Research, Inc., U.S.A.).

Random PCR products were subjected to μTGGE (micro temperature gradient gel electrophoresis),[7] generating specific band patterns (see Figure 2). The featuring point of each band was processed to provide *spiddos* (species identification dots) used for measuring the pattern similarity score (PaSS).[8] Here, ΔPaSS is defined to be 1 − PaSS where the PaSS is the score measured between two sets of *spiddos* obtained from cells before UV-exposure and cells after UV-exposure for particular seconds (Figure 2). The PaSS value thus obtained ($0 \leq \text{PaSS} \leq 1$) is empirically known to be strongly correlated with the degree of similarity between two genomes.[3–6,8,9] This work, which, first, examined the effect of UV-irradiation on DNA mutations using GP, showed the linear relationship between the UV dosage and ΔPaSS value in the range of relatively small amount of UV-dosage, i.e. within 500 J/m^2 (Figures 3a and 3b_1). Since the degree of DNA damage can be regarded to be proportional to the amount of UV-dosage, the result here obtained supports that GP can quantitatively measure the difference between two genomes so far as the difference is not so great. It is intriguing that even the same UV dosage resulted in a different outcome depending on the strength of light; longer irradiation of weaker light (that of 40 cm apart in Figure 3c; ■) caused more mutations than shorter irradiation of stronger light (that of 10 cm apart in Figure 3c; ▲). This seems to be also the case with in vivo DNA (that of *E. coli* cell) (Figure 3b_1), although the effect of irradiation with weaker UV light (that of 20 or 40 cm apart) was unexpectedly an oscillating one (Figure 3b_2). For this phenomenon, presently we do not have any reasonable interpretations but for that it might be related to a competitive phenomenon of DNA repairing system including SOS response which acts to reduce the lesions induced by UV-irradiation. That is, a competition between increasing lesions by UV and decreasing them by repair enzymes may be hard to balance and thus oscillating in weaker UV light (Figure 3b_2). Noteworthily, the level of mutations of in vivo DNA seems to be lower than that of in vitro DNA (Figure 3c) probably due to the repairing activities. The non-zero value at the time 0 can be reduced to the statistical fluctuation due to the random PCR (Taq DNA polymerase-derived replication errors (10^{-4}/base/replication) cannot be ruled out nor directed (thus, empirically around 0.01 of bias generated in PaSS value).[8] The deviation of ΔPaSS value from the linear relationship in the range of the larger amount of UV-irradiation (Figure 3) indicates that some complex mechanisms are working there where DNA lesions are much propagated. Although the elucidation of this requires further investigation, the experimentally reproducible nature of these phenomena will enable us to reveal unknown mechanisms. So far as we know, this is the first study to quantitate mutations directly using the genomic DNAs without sequencing (which requires cloning and a lot of efforts to detect a low frequency of mutations such as that of less than 10^{-3}/strand) and thus greatly enhance studies in this field. Evidently, one useful application of this technology is sensitive UV-dosimetry like a UV film badge made of DNA.

We thank Mr. Takahiro Tayama for technical assistance.

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Published on the web (Advance View) January 30, 2007; doi:10.1246/cl.2007.358