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Biological Ultraviolet Dosimetry in Low Earth's Orbit

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Abstract

The biological dosimetry of the solar UV radiation functioned correctly on the Earth's surface. The aim of the present studies was to extend the dosimetry to the extraterrestrial solar radiation in LEO. Similar to the Earth's surface bacteriophage T7 and polycrystalline uracil thin layers were used as detectors and exposed to the simulated and to the real space environmental parameters aiming to perform the *in situ* biological UV dosimetry in the space, more exactly on the external pallet of the ISS. The UV detectors have been used in specific cases in thin layer form. In contrast to the Earth's surface the extraterrestrial solar UV radiations contains wavelength components (λ ~ 190-200 nm), which cause photolesions (photoproducts) in the nucleic acids/their components similar to the UV-B photons. However, these wavelengths cause not only photolesions but with a wavelength-dependent efficiency the reversion of some photolesion, too. Our biological detectors measured either in simulation or in situ conditions the resultant of both reactions induced by the extraterrestrial UV radiation. From this aspect the role of the photoreversion in the extension of the biological UV dosimetry and in the survival of the living systems in the space are discussed.

Keywords: Photoreversion; Extraterrestrial UV climate; UV dosimetry in the space

Abbreviations: CPD: Cyclobutane Pyrimidine Diner; DLR: German Aerospace Center; ds-break: Double Strand Break; EST: Experiment Sequence Test; EVT: Experiment Verification Test; ISS: International Space Station; IWF: Institut für Weltraumforschung; LEO: Low Earth's Orbit; OD: Optical density [log(I0/I)]; PCR: Polymerase Chain Reaction; PLC: Payload Computer; RGB: Research Group for Biophysics; ss-break: Single Strand Break; UV: Ultraviolet

Introduction

The two main problems of exo/astrobiology are connected to the beginning and to the exclusiveness of the life on the Earth, which can be completed by the problems of forward and backward contamination of the other planets and the Earth respectively. The ultraviolet (UV) light is the driving force in the solar system for the synthesis of organic compounds and for the life. Therefore the quantification of the biologically effective UV radiation is essential to understand the problems of astrobiology. Theoretical and experimental astrobiology has been developed. The theoretical one constructs mathematical or physical model which supports the life or analyzes the physical, chemical parameters of the planets in terms of the evolution or the interplanetary transport of life [1-3]. The experimental astrobiology uses possible terrestrial model of specific planets, like deserts, permafrost, simulation chambers etc [4-9]. These provide environmental parameters similar to the space and to the specific planet respectively, while the *in situ* measurements allow studying the life conditions directly in the specific environment [10-12].

At the end of 1996 an announcement of opportunity was issued by ESA for the utilization of the International Space Station (ISS) in externally mounted payloads to answer–among others–specific questions of exobiology/radiation biology. The proposal of the Research Group for Biophysics of the Hungarian Academy of Sciences (RGB) focused on the ultraviolet (UV) climate of the space.

The UV radiation in Earth's orbit is produced by the Sun. In comparison to the terrestrial UV the extraterrestrial solar spectrum contains short wavelength components (λ<280 nm), too. For *test systems* (detectors) bacteriophage T7 and a pyrimidine basis Uracil, a constituent of the RNA have been used. RGB joined the scientific consortium ROSE (Response of Organisms to the Space Environment) with the experiment Phage and Uracil Response, PUR [5,13].

The measurement of the biologically weighted UV radiation (biological UV dosimetry) is based on the damage of the nucleic acid bases induced by UV photons: as a consequence of the UV effect pyrimidine dimers can be formed [14] either inside the nucleic acid of the phage particle or in the polycrystalline uracil film (thin layer). Bacteriophage T7 consists of a small DNA molecule with about 40 thousand base pairs. More than 90% of the DNA is occupied by essential genes [15] and it was demonstrated that in solution one single dimer can cause the loss of viability of phage T7 [16,17]. In the uracil thin films under the influence of UV photons dimerization takes place too, and this effect can be detected and quantified by the change of the characteristic absorption spectrum of the uracil [18]. Both of our *test systems* proved to be convenient for biological UV dosimetry in the conditions of the Earth's surface [19-22].

Experimental Section

Bacteriophage T7 (ATTCC 11303-B7) and polycrystalline uracil (Sigma-Aldrich Co. U 0750) were used as detectors of the dosimetry systems. Both detectors served as flying samples in the form of homogeneous thin films/layers on the silica/Fluoride plates of 16 mm diameter according to the requirements of the space technology [23,24]. The uracil and phage T7 thin layer samples were accommodated in EXPOSE facility[1] in a sandwich like structure inside the specific small holders manufactured for exposure purposes. The scheme of the holders

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is shown in Figure 1. The samples are deposited in the form of thin films on the inner side of a calcium-fluoride window of 2mm thickness, which is the upper window of the small vacuum-tightly closed steel case. Irradiation both in Ground Based Experiments and in the space was performed on the samples in the holders. The transmission of the window is a constant value (90%) for the wavelengths between 200 nm and 7 μm, which was taken into account by the evaluation of the physical UV dose. (See: Para Experiment on EXPOSE-R). The samples with the detector (Phage T7, Uracil) molecules were located both in the simulation chamber and on the external pallet of ISS in the EXPOSE facility [5,25].

The final accommodation of the samples of PUR experiment in EXPOSE-R facility took place in a separate compartment which contained 2×16 places into which the sample holders were fitted in two lines. Each sample holder contained inert gas and was closed vacuum tightly. The lower line of the samples served as dark control of the flight samples, while covered by Mg F_2 (neutral density) filters the upper line was exposed. The attenuation power of the neutral density filters was the following: the surface of the compartment to be irradiated was divided into four quarters, with neutral density filters of different transparency. The first filter had 100 %, the second, third and fourth 1% and 0.01% and 0.0001% transmissions for the UV light respectively.

In the simulation (EVT, EST) experiments the selected space parameters (temperature, variation of temperature, UV radiation etc.) were tested partly in the laboratory of RGB or in the Institute of Space Research, Graz and partly in Planetary and Space Simulation Facilities (PSI), operating in DLR, Cologne.

The effects caused by the selected space parameters in the bacteriophage T7 and uracil thin film respectively, have been investigated with several methods: counting the survivor phage particles on infected *Escherichia coli* B host cells, determination of the UV absorption spectrum and the quantitative characteristics of the spectra [26], quantitative PCR (QPCR), enzymatic digestion, electrophoretic pattern [27,28], in addition, thin layer chromatography (TCL), mass spectroscopy of the photoproducts.

Figure 1: Components of the sample holder constructed and manufactured in RGB for EXPOSE facility. From left to right the parts of the sample holder are: Sample case of stainless steel, – quartz or Calcium Fluoride window depending on the type of the experiment, – uracil/bacteriophage T7 thin layer, – Viton ring, – quartz window, – closing part of the sample case of stainless steel.

[1]Designed and constructed by Comp. Kayser-Threde.
[2]Tungsram 15W, main emission line 254 nm „UV-C".
[3]SOL 2000, DLR; the spectrum contains short wavelength components down to 200 nm.
[4]Oriel, 300 W, polychromatic continuous spectrum 200-400 nm.
[5]developed in cooperation with IWF, Graz [29].

The UV radiation on the Earth's surface and on the Earth's orbit is emitted by the Sun. The spectral composition of the solar radiation at different distance from the Earth is different due to the filter effect of the various thickness of the atmosphere. During the flight in LEO the effect of the short wavelength components can have of specific interest.

In the simulation irradiation experiments various UV sources were applied (low pressure Mercury lamp[2], Solar simulator[3], Deuterium lamp[4], "Mars Lamp[5]") [29]. The spectral irradiances of the different artificial UV sources were measured directly in the wavelength range 200-400 nm by an OL-754 spectroradiometer. The spectrum of SOL 2000 corresponded most of all to the solar spectrum.

For studying selectively the "monochromatic" effect of the unusual short wavelengths in the range 200-400 nm a specific interference filter system was constructed.

Results

On the ISS the samples have a radiation environment which contains both ultraviolet photons and charged particles of the cosmic radiation, thus the samples on the EXPOSE-R facility were exposed to both types of radiation, while in ground based experiments the effect of the two types of radiation can be studied separate. On the ISS the separation of the effects is possible through the "dark control" samples accommodated under the UV irradiated ones, having chance to receive cosmic and no UV radiation.

Ground based simulation

The UV radiation (SOL2000) was applied separately. The dose—effect relations were determined, where the effect is the formation of photoproducts both in phage T7 and in the polycrystalline uracil (i.e. UV damaging of phage DNA and uracil respectively). The irradiation with solar simulator resulted in an exponential dose—effect relation from the aspect of the average number of UV lesions per phage particles. The results of the detailed molecular biological analysis of the lesions in phage DNA are summarized in Figure 2, where the columns indicate the average number pro phage of the various photoproducts (CPD, ds-breaks, ss-breaks, apurinic sites) induced by the radiation. From the graphics it is clear that in our experimental conditions the CPD is the leading photoproduct in the phage particle.

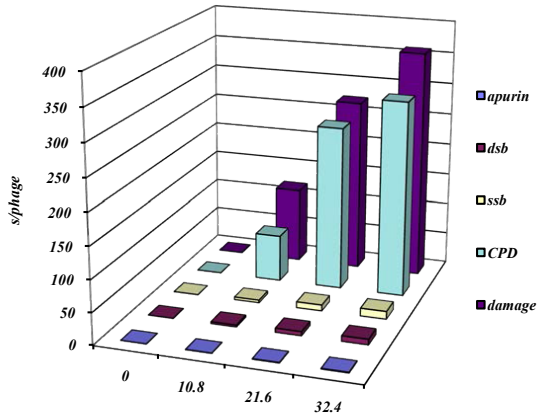

Figure 2: Molecular biologic analysis of the photoproducts in bacteriophage T7 induced by SOL 2000 lamp.The average number of lesions per particle (s/phage) is given in dependence on the UV dose in kJ/m^2 units.

The UV damage of the uracil molecules was measured with spectrophotometer using the specific absorption properties of the thin layer. The term ΔOD denotes the change in the absorbance (Optical Density, OD=lg (I_0/I)) of the uracil at λ=288 nm due to the dimer formation in the sample. Figure 3 presents the dose-effect relation, where the quotient $\Delta OD/OD_0$ indicates the change of the uracil absorbance induced by irradiation in relation to the starting value (OD_0 denotes the OD value of the layer before irradiation). The studied dose range covers 6-7 orders of magnitude. With increasing administered radiation the number of photoproducts, i.e. the value of $\Delta OD/OD_0$ quotient increases.

The spectral composition of the extraterrestrial solar UV radiation differs from the terrestrial one; it contains short wavelength components down to the range of X-Rays. The reversion of the pyrimidine dimers induced by the wavelength shorter than 260 nm has been detected earlier [14,30-32]. However, the quantification of the wavelength dependence of the reversion process in the UV-C range remained uninvestigated probably because of the lack of biological relevance of the short wavelengths on the Earth's surface.

In the simulation experiments the dimerization and reversion efficiencies of the selected[6] short wavelength radiation were determined on uracil thin layers. In Figure 4 the effect induced by two selected wavelengths (290 nm and 300 nm) is demonstrated in dependence of the dose. The effect of UV exposure is presented as the change of the OD value at the wavelength 288 nm. If we had have only dimerization the dose-effect curve would be a straight line, however, the function deviates from the exponential type because of the presence of the reversion process.

For quantification of the dimerization κ value was defined and determined from the starting part of the dose-effect curve in the wavelength range 200-280 nm. The value of κ expresses the UV dose in J/m^2 which reduces the OD of the thin layer to the e-th proportion of the starting value. The highest dimerization efficiency was found for wavelengths 260-280 nm (10-60 J/m^2); while outside of this region both towards the shorter and the longer wavelengths about 100–1000 times higher UV doses were found [33].

The presence of the monomerization reaction is indicated by the specific feature of the dose-effect relation: the slope of the curve deviates from the exponential shape, i.e. it tends to a saturation level. This saturation can be observed in Figure 4. The monomerization (photoreversion) efficiency (σ) was determined in the wavelength range 200-240 nm where the value for the parameter σ was found at about the same order of magnitude than the dimerization efficiency. The irradiation at wavelengths 250, 260, 270 nm did not cause any significant change in the saturation level indicating the equilibrium of the dimerization and the monomerization processes [33].

Experiment on EXPOSE-R

In the irradiation experiment on the ISS the UV source was the Sun. During the flight (approximately 2 years) the equivalent exposure duration took about 2687 hours which corresponds to a total UV dose at the PUR experiment about 1100 MJ/m^2 according to the model calculation of RedShift BVBA[7]. The UV dose values both for the uracil and T7 samples were taken into account according to the attenuation of the solar radiation by the neutral density filters in 6 orders of magnitude. The UV effect was estimated at every dose as the average of the $\Delta OD/OD_0$ values of the uracil/phage T7 spectrum at λ=288 nm. The results are presented in Figure 5 in the form of two dose-effect functions. Based on the rises of the two curves uracil is more sensitive than phage T7 for the extraterrestrial UV radiation.

Discussion

1. In the simulation experiments (EVT, EST) the separation of the effect of UV photons and charged particles was simple: for irradiation only UV source (SOL 2000) was used. In the *in situ* experiment on the ISS 16 samples of "dark control" (8 uracil and 8 phage T7) were accommodated in the second line of the samples under the UV exposed ones. For the dark control samples the characteristic (200-400 nm) absorption spectra (and their second derivatives) were determined before and after the flight. For comparison the spectra were analyzed from the aspect of destruction induced by the charged particles or by other space parameters. In the spectra measured before and after the flight no difference was found. On the EXPOSE-E facility during the1.5 years flight time at the samples from cosmic radiation a dose of 180 mGy was measured [34,35] Assuming a very similar cosmic radiation at the EXPOSE-R facility one can estimate for

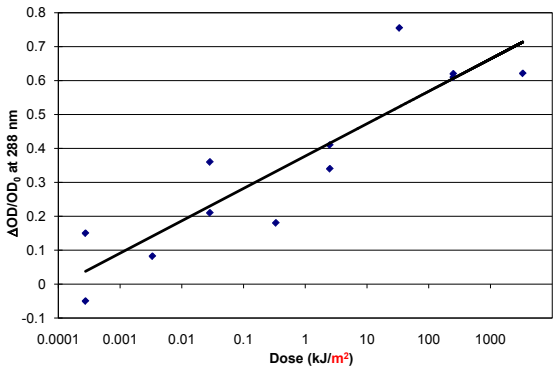

Figure 3: Dose-effect relation of uracil dimerization irradiated in simulation experiment (SOL 2000). The measurement was performed with spectrophotometer at 288 nm, the quotient ΔOD/ OD0 indicates the change of the uracil optical density related to the starting value. The trendline equation is y = 0,0414•ln(x) + 0,3775; r^2 = 0,8111.

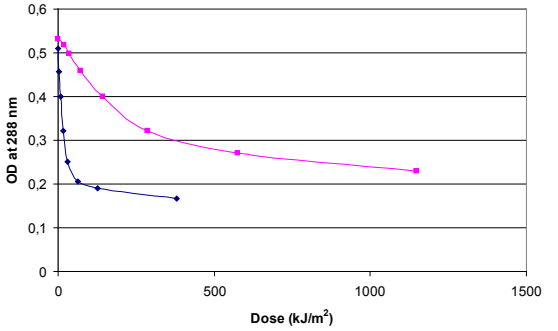

Figure 4: Uracil dimerization kinetic curves under the influence of wavelengths 290 nm (♦) and 300 nm (■). The maximal dose until saturation is less than 400 kJ/m^2 for 290 nm, while for 300 nm 1200 kJ/m^2, thus the dimerization efficiency of the wavelength 290 nm is higher, while the wavelength 300 nm is less efficient [33].

[6]The selection was by interference band pass filters: 220BP10, 230BP10, 260BP10, 270BP10, 280BP10, 290BP10, 300BP10, 310BP10, 320BP10, where the first number denotes the wavelength of maximal transparency and the last one the band width.

[7]RedShift BVBA,23/12/20110.

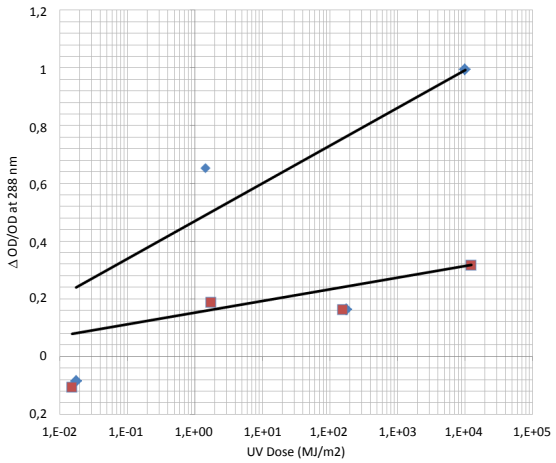

Figure 5: The effect of the extraterrestrial solar UV on bacteriophage T7 (■) and uracil (♦) films.The dose values are indicated according the RedShift model calculation taking into account the attenuation effect of the filters. Linear regression equations for T7 $y = 0,0276 \cdot \ln(x) + 0,0651$; $r^2 = 0,8249$ and for uracil $y = 0,0596 \cdot \ln(x) + 0,2701$; $r^2 = 0,5073$.

our case an exposure of 200-220 mGy and probably in the uracil and phage T7 samples no dramatic structural damage has been induced either by the cosmic particles or other space parameters.

2. UV (particularly UV-C, far UV) radiation is a characteristic space parameter, thus several simulation results are available. The nucleic acid is the target for UV photons in the living systems and their biological effect can be important for the survival in the space. For example *B. subtilis* spores were irradiated with UV-C lamp. The killing effect of the UV was detected; the dose-effect curves and the killing efficiency were measured [36]. The detected photoproducts, induced by the wavelengths 200-300 nm, were identified as spore photoproducts containing thymine bases [37]. A similar result was obtained by [38]: spore photoproducts and pyrimidine dimers were found in the irradiated spores down to wavelength 206 nm. The nature of the products induced in nucleic acid or in di/oligo-nucleotides by the UV radiation of various wavelengths has been thoroughly investigated in ground based studies. Hieda (1994) could demonstrate in the samples three types of pyrimidine photoproducts (cis-syn, trans-syn dimers and (6-4) dipyrimidine adducts) in addition, by synchrotron radiation at wavelengths lower than 206 nm photodestruction products were induced. At the wavelength 130 nm photodestruction products became dominant, while the single- and double-strand breaks were found in plasmid DNA at wavelength under 130 nm (8.3 eV).

   Outside the Earth's atmosphere e.g. in the Earth's orbit or on another planet (e.g. on Mars) the spectrum of the solar UV can contain either the whole solar UV range (in the space) or only a specific part of the shortest UV components e.g. wavelengths down to 200 nm (on the Mars). From the aspect of the human space activity, in particular astrobiology, the biological relevance of the shorter UV wavelengths became more important. The survival of microorganisms in the space could be an indicator of the biological relevance [1].

   The role of the short wavelengths (<280 nm) of the extraterrestrial solar UV radiation is partly the inactivation and partly protection and/or preservation, too. The microorganisms, containing nucleic acid can be damaged by solar UV radiation but the damage can be reverted with a certain probability by the same/similar UV photons. The photoreversion can be added to the protective effect of water ice and various minerals surrounding the microorganisms [39,40] participating in their interplanetary transport. For the protective effect of short UV a further argument is [27]: found in the inactivation action spectrum of *B. subtilis* HA 101 spores (in the wavelength range 210 nm-290 nm) a decrease of the killing efficiency of the UV radiation at about 230 nm. This effect coincides with our results concerning the photoreversion. Namely the highest reversion efficiency of the photoproducts was found at the wavelengths 220 nm-240 nm.

3. The measurement and evaluation of solar radiation's effect on the EXPOSE-R platform raised several problems.

   The platform was supplied with radiometer which was able to measure the UV-A, UV-B, UV-C and PAR irradiances. The original concept of the PUR experiment was to compare the results of the physical dosimetry with those of the biologically weighted phage T7 and uracil dosimeters. Unfortunately, the data collection by the PLC did not function from June, 2009 until the end of December, 2009 because of the computer was broken down. Thus a half-year environmental data (temperature, radiation etc.) are missing and the physical dose values of the whole exposure could be only roughly estimated. This is the reason while the dose-effect curves can be presented in dependence of the calculated doses only (Figure 5).

The physical radiometers were accommodated in a definite compartment of the EXPOSE-R platform. However, at regular function of the radiometers the distribution of the solar irradiance on the platform can be uneven because of the shadowing which makes difficult the comparison of the physical radiometric data with the biological UV dosimetry. The shadowing effect was taken into account in the model calculation of RedShift.

In the present form the uracil based biological UV dosimeter measures two quantities: the OD values of the uracil thin layer before and after the flight of the uracil and provides information on the UV dose accumulated during the whole flight. In this respect the passive uracil dosimeter offers the advantage of automatic function without electric connection. However, the time-course of the dose accumulation cannot be followed. For the future space research the *in situ* measurements combined with telemetry are promising [12] either in Earth's Orbit (e.g. on microsatellites) or on any other planet. Comparison of the dose–effect functions to the detectors in thin layer form the uracil dosimeter is more sensitive than phage T7.

Conclusion

The biological UV dosimetry could play an important role in understanding the problems of the history and future of the living systems on the Earth, in the space or in the Universe. For this reason further detailed information on the UV climate, on the distribution of the UV radiation in the space are needed. On the early Earth and in the Universe the protective effect of the ozone layer is/was absent. In the evolution and distribution of the living systems the higher photoreversion efficiency of the short wavelength solar UV radiation can have an impact on the protective effect. In addition, the effect of photoreversion can have an importance in the *planetary safety*

enhancing the contamination with living systems, which can survive the transport through the space.

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Disclosure statement

No competing financial interests exist.

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