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Protective effect of clay minerals on adsorbed nucleic acid against UV radiation: possible role in the origin of life

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Abstract: The effect of UV radiation on solutions of free and clay-adsorbed DNA has been investigated. It turns out that clay (montmorillonite/kaolinite) adsorbed nucleic acid undergoes less radiation damage than free nucleic acid. Our laboratory experiments have an astronomical counterpart in terms of solar irradiance on the Earth. An origin of life scenario is proposed where ubiquitous clay minerals lead the surface chemistry of the molecules relevant to the biological evolution and at the same time protect them from the deadly rainfall of UV photons.

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Introduction

The identification of very many molecules, most of them carbon containing, in molecular clouds, comets and meteorites is evidence for the hypothesis that the starting material for the origin of life on our planet came from space (Anders 1989; Ehrenfreund 1999). However, the evolution of life required that certain conditions were met, such as available energy, temperature level, concentration of precursor molecules, preservation of the large organic structures produced by chemical reactions, etc. Because organic chemistry needs water and life needs water it was proposed by Haldane (1929) and Oparin (1938) that, together with the above conditions, an aqueous ambient was also necessary. However, if water is a prerequisite it is at the same time detrimental, since it favours hydrolysis instead of polymerization (Pace 1991). A brilliant solution to the problem was proposed by Bernal (1951) who suggested from thermodynamic and kinetic arguments that mineral surfaces, in particular clay minerals, could have acted as to concentrate the organic material present in a primordial ocean, protect the material (reagents and products) from radiation damage, i.e. UV light and X-rays, and catalyze polymerization. Many laboratory experiments have shown that, in fact, biochemical reactions can easily proceed in the presence of clay minerals leading to the formation of biopolymers and/or self-replicating structures (Ertem & Ferris 1996; Ferris *et al*. 1996; Franchi *et al*. 1999; Franchi & Gallori 2003). On the other hand, studies on the persistence/degradation of DNA/RNA in natural environments have proved that the genetic material retains its integrity and functionality for a long period upon adsorption and binding on clay minerals (Lorenz & Wackernagel 1994; Stotzky *et al*. 1996; Gallori *et al*. 1998; Luther *et al*. 1998). The last results assess the resistance of clay-adsorbed DNA/RNA to biotic and abiotic degradation.

With no ozone layer the *primordial* Earth was a hostile place to live. The UV radiation four billion years ago was at least one order of magnitude larger than today's level and able to break up molecular bonds easily. Thus, it was crucial to the formation and survival of biological molecules, such as DNA/RNA, that they were protected from radiation damage. It is known, in fact, that UV radiation, among other effects, induces the formation of thymine dimmers, which affect negatively the DNA replication (Friedberg *et al*. 1995). The ubiquitous presence of clay minerals might have played the important role, at the dawn of biological complexity, of favouring chemical reactions and protecting the accumulated reagents/products from degradation.

Molecules which store genetic information, i.e. DNA and RNA, are central to all life forms on the Earth and this is also likely to be true on the other planets. In this work we undertake a systematic approach to the problem of the effects of the UV radiation on free DNA and clay-adsorbed DNA. If life originated on the surface of the Earth, as opposed to originating in submarine hydrothermal systems, the present experiments propose an explanation of how biological molecules were able to survive the high levels of UV radiation hitting the newly formed planet.

Table 1. *Transformation frequencies of free DNA not irradiated, f_T°, and irradiated, f_T, for different time intervals (s) and with different laser energy (J) settings. A 2 Hz pulsed Nd: YAG laser at 266 nm (4th harmonic) was used as irradiation source. The width of the pulses was 20 ns*

Energy (J)	Irradiation time (s)						
	0	10	18	90	**180**	360	540
0	3.1×10^{-4}						
2.0×10^{-6}		3.6×10^{-5}	3.3×10^{-5}	2.8×10^{-5}	3.6×10^{-5}		
$\mathbf{5.1 \times 10^{-5}}$			1.9×10^{-5}	8.8×10^{-6}	$\mathbf{1.7 \times 10^{-6}}$		
5.1×10^{-4}			1.3×10^{-5}	4.1×10^{-6}	6.1×10^{-7}	1.3×10^{-7}	1.6×10^{-7}
2.5×10^{-3}					1.8×10^{-7}	4.5×10^{-7}	
5.1×10^{-2}					2.4×10^{-7}	4.7×10^{-8}	1.0×10^{-8}

Note. Donor: *Bacillus subtilis* BD170 (*trp*C2, *thr*-5); Recipient: *Bacillus subtilis* BD1512 (*his*A1, *met*B5, *leu*A8, Cm^r); Selection: His^+.

Table 2. *Transformation frequencies of clay-DNA complexes not irradiated, f_T°, and irradiated, f_T, in the experimental configuration of Table 1*

Energy (J)	Irradiation time (s)				
	0	10	18	90	**180**
		Montmorillonite-DNA			
0	3.7×10^{-5}				
2.0×10^{-6}		1.9×10^{-6}	1.8×10^{-6}	1.6×10^{-6}	6.2×10^{-7}
$\mathbf{5.1 \times 10^{-5}}$			3.6×10^{-6}	2.1×10^{-6}	$\mathbf{1.5 \times 10^{-6}}$
5.1×10^{-4}			3.4×10^{-6}		5.4×10^{-7}
		Kaolinite-DNA			
0	2.7×10^{-5}				
$\mathbf{5.1 \times 10^{-5}}$				1.3×10^{-6}	$\mathbf{7.9 \times 10^{-7}}$

Note. See Table 1.

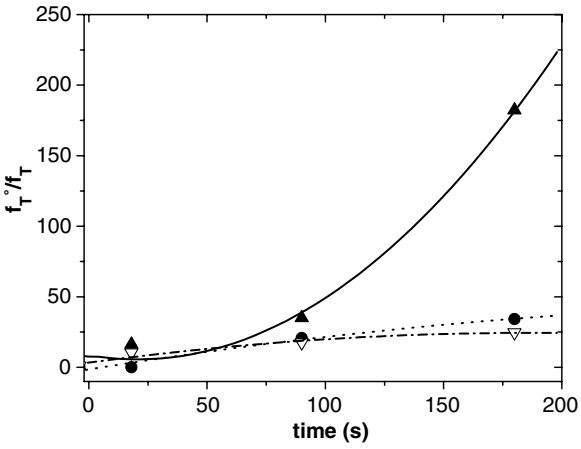

Fig. 1. Plot of the UV radiation damage, f_T°/f_T, for free DNA (solid line), M-DNA (dot-dashed line) and K-DNA (dotted line), respectively, against the irradiation time at a constant laser power of 5.1×10^{-5} J/impulse.

Experimental part

Chromosomal DNA from *Bacillus subtilis* BD170 was prepared as described by Kanna & Stotzky (1992). Its weight ranged from 15 to 25 Mu (1 Mu = 1.66043×10^{-18} g) and its length from 10 to 15 μm. The quantity and purity of DNA were determined by the method of Sambrook *et al.* (1989). DNA was dissolved in water and the solutions consisted of 20 μg of DNA in 2 ml of deionized distilled water (dd H_2O).

For the clay nucleic acid complexes two minerals were used, montmorillonite (M) and kaolinite (K), on which DNA is known to adsorb (Franchi & Gallori 2004). The complexes were prepared by starting with a solution of DNA and clay mineral in dd H_2O. The solutions were gently shaken (40 rev/min) for 120 min and centrifuged for 20 min at room temperature. The quantities of the components and the procedures were such that the final solutions contained 20 μg of DNA adsorbed on 2 mg of clay in 2 ml of dd H_2O.

The solutions of free DNA and clay-adsorbed DNA, respectively, were placed in a 10×10 mm² quartz-made cell. They were stirred with a magnetic stirrer in order to ensure a uniform distribution of the irradiation. A 2 Hz pulsed Nd: YAG laser at 266 nm (4th harmonic) was used as radiation source. The width of the pulses was 20 ns.

Most of the samples were irradiated for different time intervals and with different laser power settings. This was necessary when we started the experiments on free DNA and on the M-DNA complex, since we did not know what irradiation time and laser power produced significant damage without totally destroying the nucleic acid. We started with 10 s and 2.0×10^{-6} J/impulse and ended with 540 s and 5.1×10^{-2} J/impulse. The analysis of the damage produced after UV irradiation on both DNA and clay-adsorbed DNA was conducted by evaluating the efficiency of the irradiated samples to transform bacterial competent cells (Streips 1991). By this method, the transformation frequency f_T = (No of transformed bacterial cells)/(No of total bacterial cells) is obtained, which is inversely proportional to the number of damaged DNA molecules in the sample.

The results are presented in Tables 1 and 2. In Table 1 the transformation frequencies of free DNA are reported at different time intervals and at different laser power settings. In addition, the transformation frequency of DNA not irradiated, f_T°, is given for comparison. In Table 2 the transformation frequencies of the clay–DNA complexes are reported with the same criteria as in Table 1. The errors on the transformation frequencies are of the order of 20%. The best results, in terms of reproducibility and signal-to-noise ratio,

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refer to the following conditions: 180 s and 5.1×10^{-5} J/impulse, called 'standard' from now on. In the tables the standard conditions are indicated in bold characters.

Discussion and conclusions

By comparing the transformation frequency of free DNA not irradiated with that of DNA after UV irradiation in the standard conditions, the latter appears to be reduced 180 times.

The same comparison in the case of clay–DNA complexes yields a factor of 25 for M and 34 for K. These results show that clay-adsorbed DNA is less damaged than free DNA. The protective effect of clay against UV radiation is evidenced in Fig. 1, where the ratio f_T°/f_T is plotted for different exposure times at the standard laser power of 5.1×10^{-5} J/impulse. The above ratio, illustrative of the UV radiation damage, is shown for free DNA and clay-adsorbed DNA.

The amount of scattering of the radiation by the walls of the cell, water, DNA and clay has been determined by measuring the laser radiation intensity at the input and output of the cell. It turns out that the scattering of the clay does not affect the transformation frequencies beyond the 20% error already mentioned.

The astronomical connection of the present laboratory experiments is established as follows. The solar flux on the Earth in a 10 nm band around 270 nm is about 10^{-4} J cm^{-2} s^{-1}, today. In our experiments the samples were irradiated (standard conditions) with an energy dose of 1.84×10^{-2} J. This is equivalent to a solar irradiation (the surface of the cell being about 1 cm^2) of 3 minutes or hours/days, assuming a 100% atmospheric transmission or less, respectively. As said, the UV flux of the young Sun on the Earth was at least one order of magnitude larger than at present and this reduces, consequently, the above time estimate.

The origin of life scenario that is advocated in this contribution is that the Earth at the time of its formation was inseminated with the raw material (prebiotic molecules) that under favourable conditions evolved toward biological structures. The chemistry which governed this process took place in aqueous ambients on the surface of ubiquitous clay minerals. Here reactants could accumulate and, together with their products, find protection against the deadly rainfall of UV photons.

It has been shown that the adsorption of the nucleic acid takes place only on the surface of the clay mineral (Franchi *et al*. 1999). Thus, the protective effect of clay against UV radiation is not a shield effect. It is determined by morphological and chemical factors. Adsorbed DNA is, in fact, likely to change its configuration from B to A, the latter being more compact, and at the same time bind to the substrate by electrostatic and/or hydrogen bonds.

The possible involvement of mineral (clay) surfaces in the evolution of life on the Earth, as the present experiments suggest, has the consequence of smoothing out the distinction between the role played by inorganic and organic matter. During its evolutionary endeavour, life relied on both of them.

The results of experiments on the same samples irradiated with X-rays will be presented in a forthcoming paper (Ciaravella *et al*., submitted).

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