Role of Clays in Protecting Adsorbed DNA against X-ray Radiation

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Abstract

We studied the effects of soft X-rays radiation on free and clay (montmorillonite, kaolinite) adsorbed DNA. The DNA samples were exposed to X-rays of 1.49, 4.51 and 8.04 keV for exposure times ranging from 2 min up to 16 h. The biological transformation technique was used to estimate the damage of the DNA molecules. Free and clay adsorbed DNA are differently affected by X-rays. The former is damaged by X-rays and the level of damage depends on the energy dose rather than the hardness of the radiation. The clay adsorbed DNA is not damaged by X-rays for energy doses up to 5.8×10^4 erg. Clays materials could have protected the building blocks of life on the primordial Earth when the solar X-ray emission was much stronger than today.

Keywords: X-ray irradiation of DNA, clay minerals, clay nucleic acid complexes, surface mediated origin of genetic material, origin of life

Introduction

One of the main, so far unanswered, question of exobiology is the source of the organic material needed for the starting of life on the Earth. The building blocks of life could have been produced in situ (Oparin 1938) or brought from extraplanetary objects. In the latter hypothesis comets and asteroids are likely to be the vehicles as they heavily bombarded the early Earth providing a large supply of organic molecules and water (Oró 1961; Greenberg 1993; Brack 1999). Water is an essential ingredient for the emergence of life (Lang 1986, Horneck and Brack 1992) while clays may play an indispensable role in

the process of assembling complex structures as biological polymers (Bernal 1951, Bonner et al 1985, Wachtershauser 1990, 1994).

Whether biological molecules were spontaneously generated on the Earth or brought to the Earth from space, any credible theory of the origin of life must answer the question: how and under which conditions life was created? Belongs to the second question the problem of the surviving of life building blocks in the presence of solar irradiation that at the primordial Earth was much more extreme than today. It is known that UV and X-ray emissions fade as a star ages. In particular, 4.5×10^9 year ago, X-ray radiation from the Sun was two orders of magnitude higher than today and, moreover, the Earth atmosphere was most probably unable to shield the solar radiation at these wavelengths. It is interesting to investigate the existence of survival conditions for biological molecules under harsh irradiation such as from a young Sun.

In this framework we studied the effects of X-ray radiation on free and clay adsorbed DNA. For the clay nucleic acid complexes, two minerals are used: montmorillonite (M) and kaolinite (K) on which DNA is known to adsorb (Franchi and Gallori 2003). In particular, we investigate how the level of DNA damage depends on the X-ray energy dose and under which conditions DNA molecules survive in the presence of X-ray radiation.

X-ray irradiation

The X-ray irradiation have been performed at the X-ray Astronomy Calibration and Testing (XACT) facility of the INAF-Osservatorio Astronomico di Palermo G.S. Vaiana (Collura et al. 1994, Barbera et al. 2000). The XACT facility used in development and calibration programs for X-ray astronomical instruments includes various X-ray, UV, and visible sources, detectors and monochromators covering the energy range $\sim$ 2 - 20000 eV (wavelength range $\sim$ $0.6 - 7000$ Å).

We irradiated the DNA samples with monochromatic X-rays of 1.49, 4.51 and 8.04 keV produced by an electron impact X-ray source, selecting anodes of high purity Al, Ti and Cu, respectively. Filters of the same materials as the anodes were used to cut the bremsstrahlung continuum above the absorption edge, namely 10 μm of Al, 20 μm of Ti and 20 μm of Cu. The spectra emitted by the source have been measured using a gas flow proportional counter detector filled with P10 gas (10% Ar, 90% CH_4). The detector was located inside the test chamber of the vacuum beam line at a distance of 1613 cm from the X ray source. Figure 1 shows as an example the spectrum of the Al K α line measured by the proportional counter.

The photon flux (F_{det}) at the detector is derived from the measured rate (R_{det}) as:

$$F_{det} = \frac{R_{det}}{A_{win}} \times \frac{1}{QE} \qquad [\text{cts cm}^{-2}\text{sec}^{-1}] \tag{1}$$

where $A_{win} = 0.2$ cm^2 is the area of the detector entrance window, and QE is the quantum efficiency of the detector at the energy of interest:

$$QE = T_{win} \times T_{mesh} \times (1 - T_{gas}) \quad (2)$$

Here T_{win}, and T_{gas} are the transmissions of the detector entrance window, and detector filling gas at the energy of interest. $T_{mesh} = 0.67$ is the energy independent transmission of the gold plated tungsten wire mesh that supports the detector entrance window against the internal gas pressure. The adopted values of QE at 1.49, 4.51, and 8.04 keV energies are 0.60, 0.51, and 0.17 respectively.

Since we used soft X-rays, the irradiation was performed placing the container with the DNA solution in vacuum. For this reason we designed and built a stainless steel vacuum tight container provided with a transparent entrance window. The container is a cylinder of 54 mm outer diameter and 9.5 mm height with an inner cell of 25 mm diameter and 2.5 mm depth ($\sim$ 1.2 cc volume) available for the sample solution. The container is closed by a top cover with a thin entrance window of 25 mm diameter allowing full illumination of the sample cell. The window is made of a 1 μm polipropylene film supported by a gold plated tungsten wire mesh like the one used in the proportional counter detector. A rubber gasket outside the sample cell guarantees the vacuum tightness.

The photon flux at the DNA sample solutions is given by

$$F_{sample} = (D/d)^2 \times F_{det} \times T_{mesh} \quad (3)$$

where D = 1631 cm and d = 30 cm are the distances of the proportional counter and the DNA sample from the X-ray source, respectively. The DNA sample container was located inside a small vacuum chamber close to the X-ray source. A gate valve can isolate this chamber from the X-ray source to allow access to the sample without the need to turn off and vent the X-ray source chamber.

Table 1 reports the energies and the fluxes at the DNA sample solutions used during the experiments. We estimated an uncertainty of 10% on such fluxes, mainly due to the uncertainty in the quantum efficiency of the proportional counter detector.

We irradiated free DNA and two different clay (montmorillonite and kaolinite) adsorbed DNA in 1400 μl water solution. The DNA from Bacillus subtilis BD170 was obtained as described by Kanna and Stotzky (1992). The DNA weight is 15 - 20 Mu (1Mu = 1.66043×10^{-18} g) and length is 10 - 15 μm. The quantity of DNA in each solution was of 10μg. The quantity of clay in the mixture was 2.0 mg. In order to find the radiation dose able to determine a significant DNA damage, we initially used X-rays of 8.04 keV for a wide range of exposure times, from 2 min up to 16 h. Subsequently, we explored the effects of 4.51 and 1.49 keV X-rays. The exposure times at 4.51 and 1.49 keV were selected to give to the DNA samples approximately the same energy dose as at 8.04 keV. The energy dose in the Cu line was initially estimated using the total counts at the detector corrected

for the quantum efficiency of the detector. However, during the analysis of the data a more accurate study of the Cu spectrum revealed that a significant fraction of photons were not absorbed by the gas but in the stainless steel case of the counter. these photons do not have to be counted in the estimate of the incident flux. This circumstance implies an energy dose at Cu wavelength smaller than initially estimated. A similar behavior was observed in the Ti spectrum. As result the energy doses at Cu, Ti and Al wavelengths resulted slightly different.

The DNA Damage Evaluation

The integrity of DNA molecules after X-ray irradiation was estimated by transformation experiments (Smith and Danner 1981). The technique provides the efficiency of the irradiated DNA to transform competent cells in a bacterial culture (Streips 1991). The frequency of transformation (f_T) was calculated as:

$$f_T = \frac{\text{Number of transformed bacterial cells}}{\text{Total number of bacterial cells}} \tag{4}$$

The integrity of DNA samples was evaluated by comparing the f_T of irradiated and untreated DNA. Table 2 reports the results of the transformation experiments for the DNA samples irradiated at 8.04 keV. Tables 3 and 4 show those for the DNA samples irradiated at 4.51 and 1.49 keV. Typical errors on the transformation frequencies are 20%.

The results of Table 2, 3 and 4 are summarized in Figure 2. Here we plot the ratio of transformation frequencies of the irradiated (f_T) to non irradiated ($f_T{}^0$) DNA as a function of the given energy dose, for free (asterisks), kaolinite adsorbed (diamonds) and montmorillonite adsorbed (squares) DNA irradiated at 8.04 (blue symbols), 4.51 (red symbols) and 1.49 keV (green symbols). The figure highlights two important aspects: (*i*) the transformation frequency is almost constant for the clay adsorbed DNA and, within the quoted uncertainties, equal to the non irradiated DNA (see Tables 2, 3 and 4). Clays shield DNA against X-ray radiation while free DNA is severely damaged. The transformation frequency for free DNA is reduced by three orders of magnitude after 9h of 8.04 keV X-ray (energy dose of 3.2×10^4 erg) irradiation and becomes undetectable after 16h (energy dose of approximately 5.8×10^4 erg, blue asterisk with arrow in Figure 2). (*ii*) In the explored range of energy and energy dose, the DNA damage is a function of the total amount of X-ray energy rather than the hardness of the radiation itself.

Discussion and Conclusions

We studied the effect of X-ray radiation on free and clay adsorbed DNA. X-rays of 8.04, 4.51 and 1.49 keV, and energy doses between 100 and 5.8×10^4 erg, were used to irradiate

the samples and the DNA damage was estimated by transformation experiments on Bacillus subtilis. Free DNA can be severely damaged by X-rays and the level of damage depends on the energy dose rather than the hardness of the radiation. A significant decrease in the efficiency of transformation of free DNA is detected for energy doses higher than 10^3 erg. At the energy dose of 5.8×10^4 erg the efficiency of transformation is negligible and 100 % of the free DNA is destroyed. On the other hand, clay adsorbed DNA is not affected by X-ray over the whole range of adopted energy doses: within the experimental uncertainties the transformation efficiency is the same as the non irradiated clay adsorbed DNA.

It is interesting to compare the X-ray fluxes used in our experiments with those emitted by astrophysical sources. X-ray emission is a common feature of almost all stars across the HR diagram, and the level of X-ray activity can be very different among the stars with similar optical characteristics (Vaiana et al 1981). The Sun, among stars of similar spectral type, is a very modest X-ray emitter. Its average X-ray luminosity, in the 0.1 - 3 keV range, is 10^{27} erg sec^{-1} and becomes as low as 2.7×10^{26} erg sec^{-1} at the minimum of solar activity and as high as 4.7×10^{27} erg sec^{-1} at the solar maximum (Peres et al 2000). The X-ray spectrum peaks around 0.1 keV and 0.2 keV at solar minimum and maximum, respectively. During flares a second peak at 1 keV (10 MK) appears. Flares, although transient, can temporarily rise the X-ray luminosity of the Sun by one order of magnitude. Typical solar fluxes above the Earth atmosphere (1 AU)[1] in different regions of the spectrum are listed in Table 5. Thus, X-ray fluxes used in the present work are higher than those due to the present Sun in the 1.5 - 12.4 keV band. With the values of Table 5, in the band 1.5 - 12.4 keV, an energy dose of 10^3 erg to the DNA sample would require exposure times of $\sim$ 8 months, $\sim 19^h$ and $\sim 34^m$ for minimum, maximum and flaring solar fluxes, respectively.

More intense X-ray luminosities are expected in younger stellar sources. Studies of the evolution of stellar X-ray spectra show, in fact, that X-ray activity changes during the life of a star becoming weaker and softer as the star ages. In particular, Micela (2002) shows that in the band 1-10 keV the stellar luminosity decreases by more than two orders of magnitude in 5×10^9yr. Thus the Sun today is 10^2 times weaker in the 1-10 keV band than it was at earlier times, when our planet system formed. In our experiments the adopted X-ray fluxes somehow mimic the young Sun.

Scappini et al (2004) investigated the effects of UV radiation on free and clay adsorbed DNA and found that clay adsorbed nucleic acid undergoes less radiation damage than free nucleic acid. Using comparable energy doses in the soft X-ray range we observe a qualitatively similar behaviour in the DNA response. A more quantitative comparison among X and UV experiments requires further investigation.

Are X-rays directly responsible for the DNA damage and which is the role of clay material in protecting the DNA against X-rays ? To answer these questions we need to consider that low energy X-rays are strongly absorbed by water. The X-ray attenuation path length in water is about 1, 0.2 and 0.01 mm at 8.04, 4.51 and 1.49 keV, respectively.

Since the thickness of the DNA samples inside the container is 2.5 mm the incoming X-rays are mainly absorbed in the outer layers. However, the local interaction of the X-rays with the water of the solution induces a fluorescent UV cascade which is likely responsible for the DNA damage.

The diluted clay materials, in the sample solution, are much more transparent to X-rays radiation than water, e.g. the kaolinite attenuation path length is 210.0, 40.0, 5.9 mm at 8.04, 4.51 and 1.49 keV respectively. Thus, clays do not directly shield DNA against X-rays. Besides DNA molecules are adsorbed only on the surface of clays (Franchi et al 1999). As suggested by Scappini et al (2004), the more compact morphological configuration of DNA when adsorbed on clays and the binding to the substrate by electrostatic and/or hydrogen bonds can play a protective role against the impinging radiation. Clays, thought as essential in assembling polymers (Bonner et al 1985, Wachtershauser 1990, 1994), are also important in protecting DNA from solar/stellar radiation.

The results of the present experiment might help to understand how much harsh environmental conditions affected the evolution of prebiotic molecules toward complex systems. The X-ray young Sun, at the epoch when life appeared on the Earth, was certainly brighter and harder than today. As a consequence, in the hypothesis that life started on the surface of our planet, a basic requirement was either the existence of an atmosphere able to protect the biogenic compounds against the highly penetrating X and UV solar emission (Owen, 1985) or some kind of ground protection. While the former conditions ae unlikely before the appearance of living organisms, the present experiments, together with earlier experiments by Scappini et al 2004, have shown that biological molecules, such as DNA, adsorbed on the ubiquitous clay materials, do survive X-rays and UV photodegradation.

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Table 1: X-ray Energies and Fluxes

Line	Energy (keV)	Flux (10^7 cts $s^{-1}cm^{-2}$)	Flux (erg $s^{-1}cm^{-2}$)
Al	1.49	6.4	0.15
Ti	4.51	2.4	0.17
Cu	8.04	1.6	0.20

Table 2: Transformation Frequencies after Irradiation at Cu 8.04 keV

Irrad. Time	Energy Dose (erg)	DNA F[1]	DNA K	DNA M
0	0	9.3×10^{-4}	7.3×10^{-6}	7.5×10^{-6}
2^m	120	9.7×10^{-4}	5.4×10^{-6}	7.3×10^{-6}
10^m	600	5.8×10^{-4}	6.4×10^{-6}	8.4×10^{-6}
30^m	1800	9.4×10^{-5}	5.8×10^{-6}	4.9×10^{-6}
1^h	3600	5.5×10^{-5}	8.1×10^{-6}	6.4×10^{-6}
3^h	11000	8.4×10^{-6}	7.5×10^{-6}	6.2×10^{-6}
9^h	32000	8.4×10^{-7}	6.6×10^{-6}	5.9×10^{-6}
16^h	58000	nd[2]	-	4.8×10^{-6}

[1] Free DNA.
[2] The transformation frequency is below detactability.

Table 3: Transformation Frequencies after Irradiation at Ti 4.51 keV

Irrad. Time	Energy Dose (erg)	DNA F	DNA K	DNA M
0	0	9.3×10^{-4}	7.3×10^{-6}	7.5×10^{-6}
1^h 45^m	5200	7.1×10^{-5}	4.4×10^{-6}	6.4×10^{-6}
5^{h} 10^{m}	15600	3.9×10^{-6}	3.2×10^{-6}	6.2×10^{-6}
15^{h} 30^{m}	47000	2.9×10^{-7}	5.1×10^{-6}	5.9×10^{-6}

Table 4: Transformation Frequencies after Irradiation at Al 1.49 keV

Irrad. Time	Energy Dose (erg)	DNA F	DNA K	DNA M
0	0	9.3×10^{-4}	7.3×10^{-6}	7.5×10^{-6}
2^{h} 25^{m}	6500	6.5×10^{-5}	5.6×10^{-6}	8.7×10^{-6}
7^{h} 15^{m}	19500	2.8×10^{-6}	6.0×10^{-6}	9.5×10^{-6}

Table 5: Solar Fluxes at 1AU (erg cm^{-2} sec^{-1})[1]

	1-8Å 12.4 -1.5 keV	8-20Å 1.5-0.6 keV	20-200Å 0.6-0.06 keV
Minimum	10^{-5}	10^{-4}	10^{-1}
Maximum	3×10^{-3}	2×10^{-2}	1
Large Flares	10^{-1}	5×10^{-1}	10

[1] Zombeck, 1990

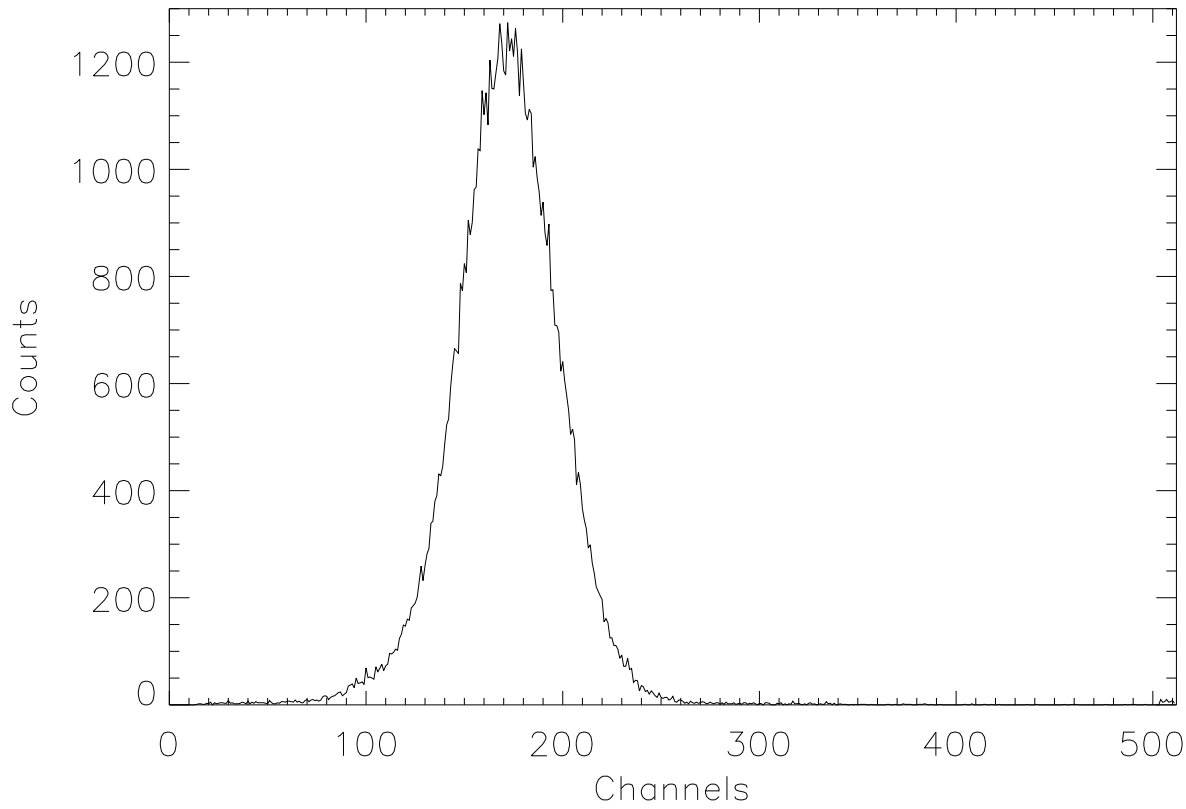

Figure 1: The Al 1.49 keV spectrum as measured in 101 sec by the proportional counter detector.

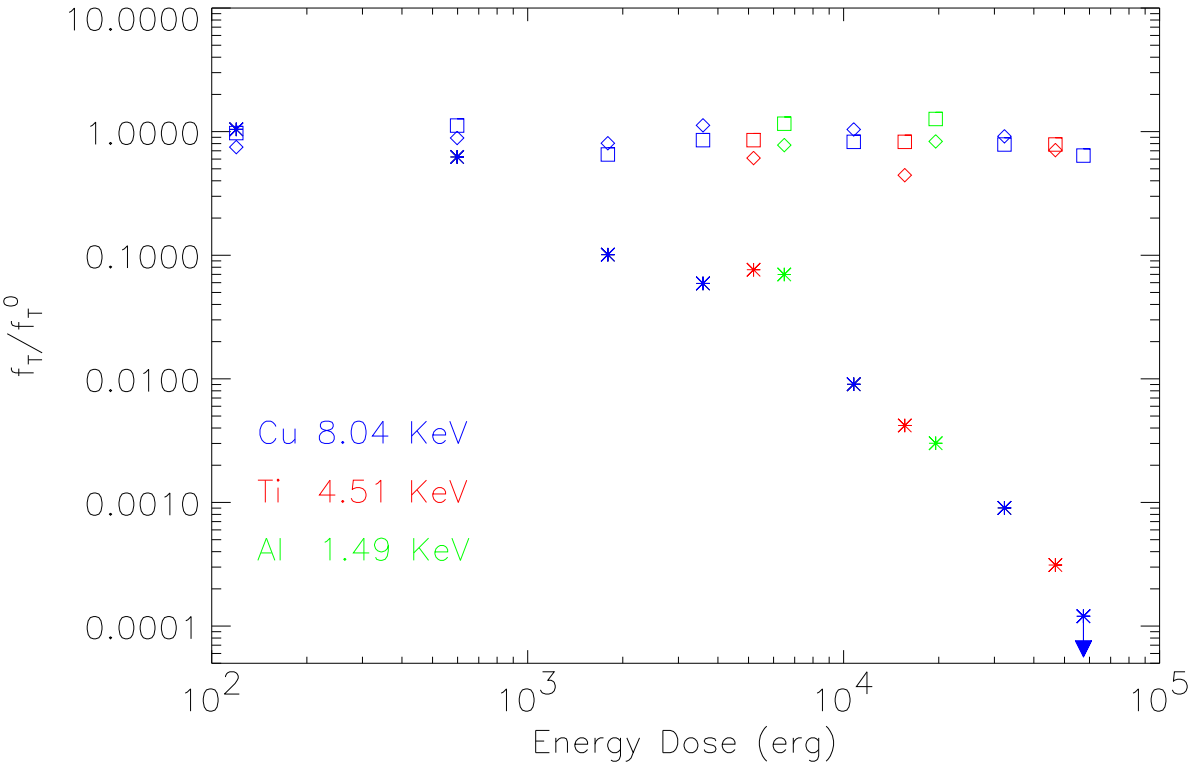

Figure 2: Ratio of the transformation frequencies of the irradiated (f_T) to the non irradiated ($f_T{}^0$) DNA as a function of the energy dose. Asterisks are for free DNA, diamonds for kaolinite adsorbed DNA and squares for montmorillonite absorbed DNA. The blue, red and green colors are for X-rays of 8.04, 4.51 and 1.49 keV respectively. The transformation frequency for free DNA becomes zero at the energy dose of 5.8×10^4 erg, blue asterisk with arrow.