The young hard active Sun: soft X-ray irradiation of tryptophan in water solutions

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Abstract: The X-ray emission of the young Sun was much harder and intense than today and might have played a significant role in the evolution of complex organics in protoplanetary environments. We investigate the effects of soft X-rays on tryptophan molecules in aqueous solutions at room temperature. As results of the irradiation experiments we detect several light species indicative of fragmentation, together with large molecular structures such as tryptophan dipeptide and tripeptide. Complexification is more evident in H_2O solution than in D_2O, probably due to isotopic effects. The abundances of peptides depend on the irradiation dose and decrease with increasing energy deposition. Radicals such as D, OD, H and OH, induced by the X-ray interaction with solvents, play a major role in determining the final products.

Introduction

There is an increasing body of observational evidence for the existence of large molecules in the interstellar medium and in interplanetary space. Observations reflect a very interesting chemistry, in which of particular interest are large partly hydrogen-saturated molecules (Snyder 2006), many of them being of prebiotic nature (e.g. the isomers glycolaldehyde, methyl formate and acetic acid). Such molecules share several chemical properties with amino acids. Except for the controversial detection of glycine (Kuan *et al.* 2003; Snyder *et al.* 2005), amino acids have not been detected in interstellar space. However, amino acids are commonly found in comets and incorporated in minor bodies of the Solar System (Chyba & Hand 2005). This is consistent with laboratory studies (e.g. Ehrenfreund *et al.* 2001) suggesting that amino acid in the gas phase would be easily destroyed by ultraviolet (UV) radiation. Thus, amino acid must have been shielded from UV radiation prior to its delivery to early planets. If efficiently formed (e.g. Bernstein *et al.* 2002; Muñoz Caro *et al.* 2002), amino acids may induce the synthesis of larger, more complex biomolecules in space (Wincel *et al.* 2000).

During the very early stages of life of a solar-type star, spectral components different from the UV contribute significantly to the stellar flux. From the study of hundreds of young solar-type stars, it appears that the Sun must have been much more active than today and it emitted copious amounts of energetic radiation, both in the X-ray and extreme UV ranges, especially in the form of intense daily or weekly flares (Feigelson & Montmerle 1999; Favata *et al.* 2005; Feigelson *et al.* 2005). According to observations of solar-type stars in the Pleiades cluster, it can be inferred that when the Sun was only 100 Myr old X-ray emission was more than three orders of magnitude higher than today (Micela 2002). Only recently has the importance been recognized of such energetic emission in the formation and evolution of stars and planetary systems, as well as in life emerging and its initial evolution on our planet (Bergin *et al.* 2007). Synthesis and evolution of complex organic molecules must thus have proceeded under conditions that are quite different from today, and might have been accelerated or even made possible by the high-energy radiation ubiquitous in solar-type protostars.

The subject of the effects of ionizing radiation on amino acids has also received renewed attention in relation to the astrobiological search for biomarkers, such as evidence of past or present life on Mars (Parro *et al.* 2005). The exposure of amino acids and other organic compounds on Mars, or in protoplanetary environments, to ionizing radiation from space and to the decay of radionuclides might induce a severe reduction of their concentrations, as well as an increase in the
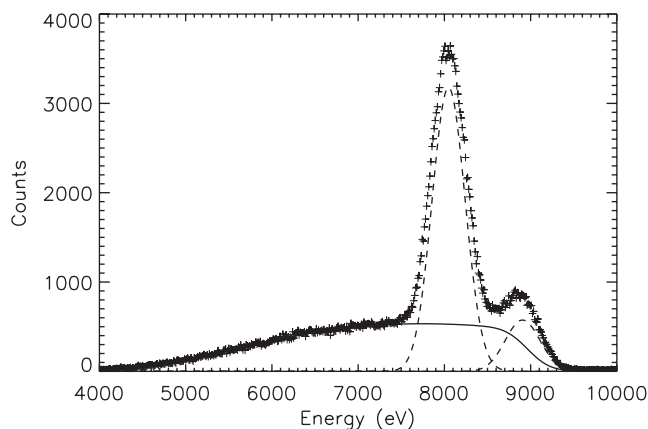
Fig. 1. X-ray spectrum (crosses) obtained in 1000 s. The spectrum is the result of three components: the continuum (solid line), the Cu Kα 8047 eV line and the Kβ 8905 eV spectral line (dashed lines).

Fig. 2. Tryptophan molecular structure.

nuclear magnetic resonance (NMR) techniques are briefly described in Appendix.

X-ray irradiation

Experimental set-up

X-ray experiments were performed at the X-ray Astronomy Calibration and Testing (XACT) facility of the INAF–Osservatorio Astronomico di Palermo 'G.S. Vaiana'. The facility, designed for development and calibration of X-ray astronomical instruments, includes several sources, mono-chromators and detectors covering the wavelength range 0.6–7000 Å (see Collura *et al.* 1994 and Barbera *et al.* 2006 for a detailed description). The samples were irradiated using the emission from an electron impact X-ray source, with a high-purity Cu anode. A filter of 20 μm of Cu was used to cut the bremsstrahlung continuum above the absorption edge. The X-ray spectra emitted by the source was measured using a solid-state Amptek XR100-CR detector before and after the irradiation to monitor the stability of the source. The X-ray spectrum reported in Fig. 1 includes three components, the Cu Kα 8047 eV and Kβ 8905 eV lines, and the continuum. Among the possible choices of soft X-ray energies available we selected the Cu spectrum because, while it is entirely absorbed within the 2.5 mm thick sample, it was also a reasonable choice to simulate the X-ray emission of a young solar-like star dominated by frequent very energetic flaring activity (Favata *et al.* 2005). The flux at the sample (~ 1 erg s^{-1} cm^{-2}) used in the experiments is about one order of magnitude larger than that hitting the Earth atmosphere today, in the band 1.5–12.4 keV, during large Solar flares.

The irradiation with soft X-ray requires the sample to be placed in a vacuum. Thus, a stainless steel vacuum tight container with an X-ray transparent entrance window (of 25 mm diameter) was used. The container had a cylindrical inner cell for the sample of 25 mm diameter and 2.5 mm height. To prevent sedimentation and/or not uniform irradiation of samples, the container was assembled on a rotating device that provided a slow and continuous mixing of the solution during the entire irradiation time. The temperature inside the irradiation chamber was also monitored during the experiments.

Tryptophan irradiation

Of the 20 biogenic amino acids, tryptophan ($C_{11}H_{12}N_2O_2$) is among the biggest and it contains both aspects of the molecular structure of the amino acids, i.e. aromatic moiety (indole) and aliphatic alanine-like chain; see Fig. 2. Thus,

degree of local complexity (e.g. it might induce the formation of oligopeptides; Simakov *et al.* (1997)). The aim of the present study is the investigation of photostability of amino acids and their derivatives, in a radiation scenario characteristic of the early evolutionary stages of a solar-type star, as a part of a more comprehensive project devoted to the study of the effects of such radiation on the synthesis and evolution of complex organics at the dawn of prebiotic chemistry.

In this work we present the results of soft X-ray irradiation of tryptophan in aqueous solutions with water (H_2O) and deuterated water (D_2O) at room temperature (planetary conditions). Meteoritic materials show the significant presence of β-and γ-amino acids (e.g. β-alanine; Sephton 2002). Nevertheless, tryptophan is a reasonable choice in light of the existing large body of literature, and because aromatic amino acids play a major role in the photochemistry of proteins. Since the experiments of Stein & Weiss (1948) on deamination of amino acids in aqueous solutions by X-rays, which followed the theory proposed by Weiss (1944), several works on the subject have been put forward, most of them generally aimed at amino acid irradiation by very high energetic photons (e.g. 50 keV; Moan 1974; Moan & Kaalhus 1974), and/or exploiting very powerful sources (e.g. Zubavichus *et al.* 2004). Moreover, in those experiments amino acids are frequently in the solid phase, in the form of thin films or pressed powders.

Energy and flux are selected to somehow 'resemble' the emission of a young active flaring solar-like star. In addition, the irradiation experiments have been performed with a 'gentle' energy deposition in order to reproduce more realistic energy injection. Due to the resulting low irradiation doses, we detected moderate effects. Although difficult to analyse, the products highlight major reaction channels.

In the following section we present the experiments and in the third section the results of the analysis of the samples. The fourth section contains a discussion of the results. In the final section we summarize our results. High-performance liquid chromatography–mass spectrometry (HPLC/MS) and
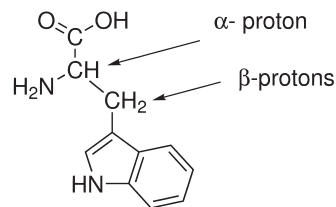
Fig. 4. HPLC/MS chromatogram of irradiated tryptophan in H_2O. The deposited X-ray energy is 1.3×10^6 erg.

information on trace amounts of compounds. In the attempt to corroborate the mass spectroscopy analysis, the structural modifications were also investigated by NMR. For this purpose we used a 300 MHz spectrometer.

Mass spectroscopy

A brief description of the HPLC/MS techniques and the specific procedure used in the analysis is given in Appendix. A background subtraction of the total ion chromatogram (TIC) of tryptophan from the TIC traces of the irradiated samples enhanced the differences, showing the chemical modifications of the tryptophan under X-ray irradiation. Figure 3 shows TICs for the non-irradiated (top panel, hereafter called the reference sample) and the irradiated samples in D_2O (middle and bottom panels). The deposited X-ray energies were 1.3×10^6 and 7.8×10^6 erg for the samples shown in the middle and bottom panels, respectively. The effects of the exposure to X-rays of tryptophan in H_2O is shown in Fig. 4. The total X-ray energy deposited is 1.3×10^6 erg, as in the middle panel of Fig. 3.

TIC analysis can be summarized as follows:
- the chromatograms of the irradiated samples (see Figs 3 and 4) showed several peaks over the entire range of retention time (R_t) that did not exist or were negligible in the reference sample;
- the profiles of the chromatograms at R_t greater than 14 min suggested on-going radiation-induced formation of complex molecular structures;
- transient species formed: peaks arising with low irradiation times disappeared as deposited X-ray energy increased, the most evident example being the peak at $R_t = 8.97$ min (the middle panel of Fig. 3), which was missing in the chromatogram of the sample irradiated with a dose an order of magnitude larger (see the bottom panel of Fig. 3);
- X-ray irradiation effects were different for tryptophan in D_2O and H_2O;
- the most intense peaks in Figs 3 and 4 were at $R_t = 8.97$ and 2.36 min obtained in the D_2O and in the H_2O solutions, respectively.

In Table 1 we list R_t and the corresponding protoned molecular masses MH^+ of the peaks in the chromatograms of Figs 3 and 4, where the intensities of the extracted ions were higher than 10^5 counts s^{-1}, i.e. higher than 1% of the peak of the unaffected tryptophan molecule. The chromatograms of the reference samples were checked to ensure that
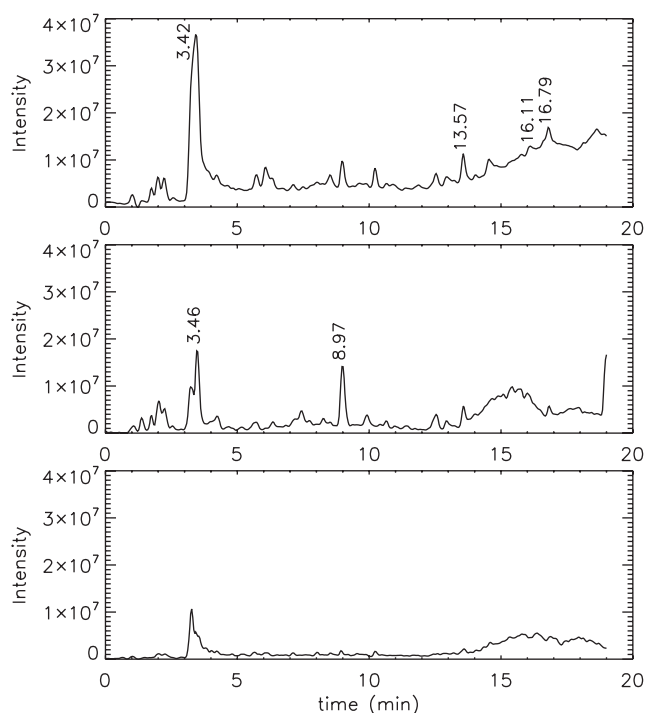
Fig. 3. HPLC/MS chromatograms of tryptophan in D_2O solution: non-irradiated (top panel), irradiated with a total X-ray energy of 1.3×10^6 erg (middle panel); irradiated with 7.8×10^6 erg (bottom panel). The chromatogram of the non-irradiated sample has been subtracted from the irradiated ones.

tryptophan provides the possibility of investigating the radiation effects on the aliphatic chain as well as on the more stable aromatic ring. Furthermore, studies of the effects of hard X-rays on tryptophan molecules present in the literature provide the opportunity for a comparison. The irradiation was performed at room temperature in solution with unbuffered D_2O and for comparison in H_2O as well. L-($-$)-tryptophan from Alpha-Aesar (Italy) of 99% purity and D_2O and H_2O from Sigma/Aldrich (Italy) of 99.9% purity were used. We used solutions with high tryptophan concentrations of 9 mg ml^{-1} corresponding to 4.41×10^{-2} M at 20 °C, in order to achieve the highest signal in the NMR diagnostic analysis. Moreover samples in solution with D_2O can directly be analysed by NMR technique reducing the possible contamination effects in the process of the sample preparation. The concentration used implies a number of water molecules approximately 1300 times larger than the number of tryptophan molecules, while the number of atoms of the solvent is 150 times higher than the number of tryptophan molecules. The high penetration of X-ray photons and the continuous mixing of the sample during irradiation prevent screening effects due to high concentrations. Total X-ray energies of 1.3 and 7.8×10^6 erg, equivalent to doses of about 11 and 60 kRad, were deposited on both samples in the D_2O and H_2O solutions.

Sample analysis and results

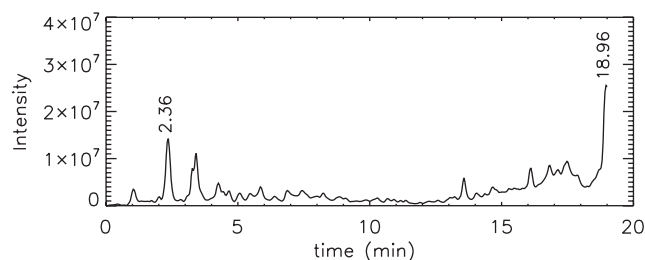We analysed the irradiated samples by means of HPLC/MS, which is the most sensitive and capable technique able to give

Table 1. *Retention times and corresponding protonated molecular masses*

Trp + D_2O 1.3×10^6 erg		Trp + D_2O 7.8×10^6 erg		Trp + H_2O 1.3×10^6 erg	
R_t	$[M+H]^+$	R_t	$[M+H]^+$	R_t	$[M+H]^+$
1.33	165				
2.21	211(D₂)[†*]	2.28	211(D₂)[†]	2.21	209[†*]
				2.36	239[†*]
3.04	131				
4.22	147(D)[†]	4.22	147(D)[†]	4.26	146[†]
				4.45	271*
4.49	423(D)	4.49	424(D₂)	4.49	422
				4.68	238[†]
				4.75	162[†]
5.10	174[†*]			5.10	174[†*]
				5.44	180
				5.85	226
				6.88	426*
7.18	191[†]	7.14	193(D₂)[†]/194(D₃)[†]	7.15	191[†]
7.41	147(D)[†]			7.41	146[†]
7.72	345[†]				
		8.06	273		
8.21	174(D)	8.21	174(D)	8.21	173
		8.55	183		
8.97	316	8.97	316		
9.50	480				
9.92	294*			9.80	294
				10.68	291
				10.91	249
11.44	274				
12.43	297				
		15.28	541		
15.43	552				
		15.80	566		
16.07	391[†]			16.07	391[†*]
				16.98	526
				17.44	448
				17.90	395
		18.00	566		
18.4	577[†]				
				18.96	552

The number of deuterium atoms in the deuterated species is shown in parentheses.
† Masses with proposed molecular structures, see Fig. 6.
* Adducts with Na species are also detected.

the peaks of Table 1 were not present or are at least one order of magnitude less intense than in the irradiated samples. As an example, we report in Fig. 5 the chromatograms of the extracted ions for the peaks of the irradiated sample in H_2O related to the molecular structures in Fig. 6 (see the Discussion Section). Many of the new derivatives present in the sample irradiated in D_2O are often present in their not deuterated form in the sample irradiated in H_2O. Table 1 lists several peaks corresponding to a mass higher than that of tryptophan indicating an increase in the molecular complexity of the solution, such as polymerization in response to the irradiation. Several reactions involving polymerizations seem indeed to take place in our experiments, for example ions at $m/z = 448,526$ and 552 (see Table 1). The peak at $R_t = 16.07$ min, corresponding to $m/z = 391$, suggests the possible formation of the tryptophan dipeptide molecules. The most probable molecular structures for the ions marked with '†' in Table 1 are reported in Fig. 6. These structures are

hypothesized on the basis of peroxyl radical oxidation reactions, which have previously been reported to involve both side chain and aromatic moieties of aminoacids (Domingues *et al.* 2003). Moreover, as shown in Table 1, several cationated species are present that further confirm the identified quasi-molecular ($[M+H]^+$) ion masses.

NMR

Although the effects detected in the NMR spectra are often negligible when small amounts of chemical changes are involved, we obtain from such a diagnostic technique additional information which is consistent with the HPLC/MS analysis and the literature. The ¹H NMR spectra of the sample in D_2O solution irradiated with a total energy of 1.3×10^6 (middle panels) and 7.8×10^6 erg (bottom panels) along with the reference (top panels) are shown in Fig. 7. The sample irradiated with 1.3×10^6 erg shows very small variations as compared to the reference spectrum (top panels), testifying to
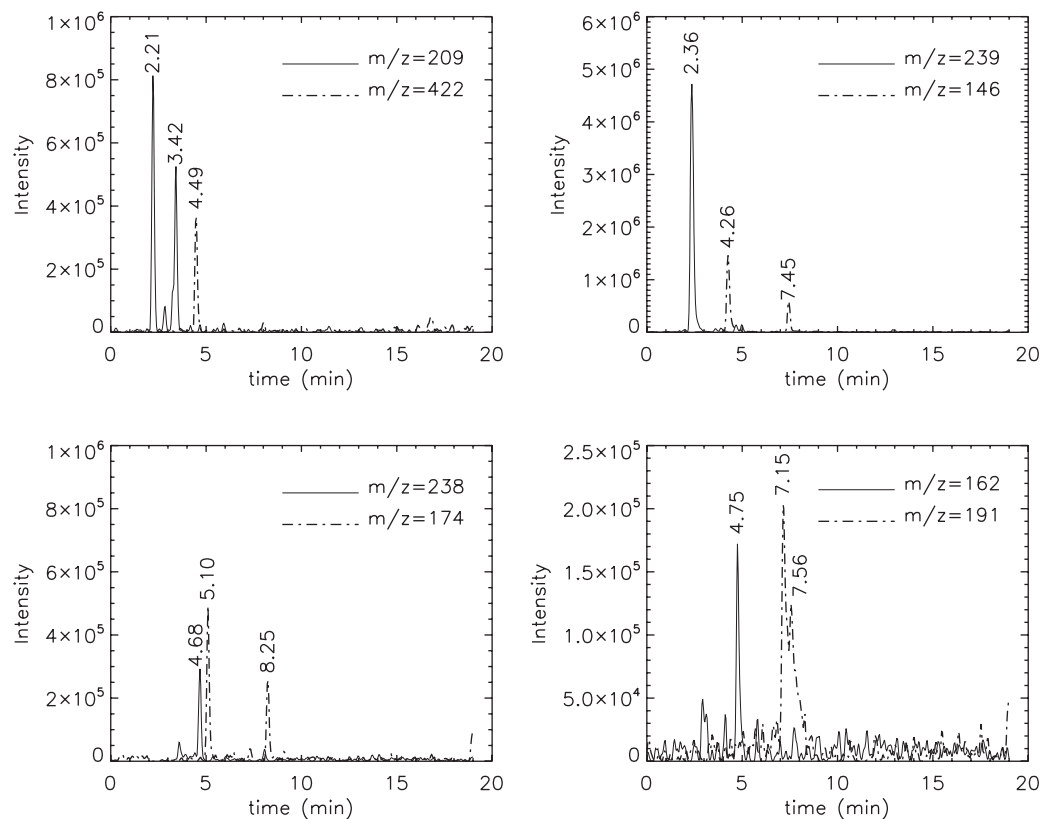
Fig. 5. HPLC/MS chromatograms of some extracted ions for the sample irradiated in H_2O (Fig. 4). The deposited X-ray energy is 1.3×10^6 erg.

the slight extent of the changes in the chemical composition. Under these experimental conditions, the spectrum shows only a well detectable signal appearing as a doublet at $\delta_H = 1.19$ ppm (peak *b*) consistent with the presence of CH_3- or $R-CH_2-$ protons both coupling with an adjacent $-C-H-$. Such a peak could account for an alanine-like chain, probably detached from the aromatic indole ring by reaction with species such as D, OD or OOD radicals originating from the D_2O interaction with X-ray photons, as mentioned in Beenakker *et al.* (1974) and Ajello (1984). Given the high concentration of the tryptophan in the sample, the direct formation of radical fragments from the solute could also contribute to this effect. In other experiments dealing with radical reactions, a chemical shift range similar to that observed in our samples has been attributed to paraldehyde species (Matsushita *et al.* 2007).

In addition, a weak multiplet signal is detectable at $\delta_H = 4.05$ ppm (peak *a* in Figure 7), which is slightly shifted upfield of 0.2 ppm with respect to the α-parent proton peak. This moderate shielding effect suggests that some modifications occurred onto the β-hydrogens. Probably, the former (the less hindered and randomly more favored) could be attacked and substituted by D radicals producing an alanine-like coupling system. The analysis of the peak area of the proton signals highlights a moderate increase of the doublet at $\delta_H = 1.19$ ppm at the expense of those at $\delta_H = 3.25-3.55$ ppm. Such a pattern could be consistent with

substitutions occurred onto β-protons by radical species or with a total detachment of an alanine-type chain.

At higher irradiation dose (the bottom panel in Fig. 7), a number of significant changes are evident in the spectrum, both in the chemical shifts and in the intensities of the resonances. Noteworthy among these there are the progressive changes observed mainly in the aliphatic regions, i.e. the peaks in the range $\delta_H = 1.14-3.50$ ppm. In this range, we can observe, other than the unmodified peaks such as for the α and β protons, signals indicating the formation of further products of degradation of the supposed detached alanine-like chain. Consistently, the peak *a* disappears, while the doublet (peak *b*) splits into a multiplet at $\delta_H = 1.14-1.19$ ppm (peak *g*, bottom panels in Figure 7). Taking into account their related deuterated forms which influence the chemical shift, this signal falls in the range of small molecules such as alkyl amines, 2-propanol and other species. Analogously, the weak singlet at $\delta_H = 2.07$ ppm (peak *f*) is compatible with other products of degradation such as acetic acid, acetaldehyde and acetonitrile (Gottlieb *et al.* 1997). Some of these molecules have also been detected in harsher irradiative conditions (Matsushita *et al.* 2006). The peak at $\delta_H = 3.35$ ppm (peak *e*) is attributable to the methanol signal, which is a typical product of degradation of the aliphatic chain. At $\delta_H = 3.50$ ppm (peak *d*), a new multiplet signal, slightly shifted downfield, may suggest modifications of the α-hydrogen, but remains difficult to attribute. Thus, as the energy furnished to
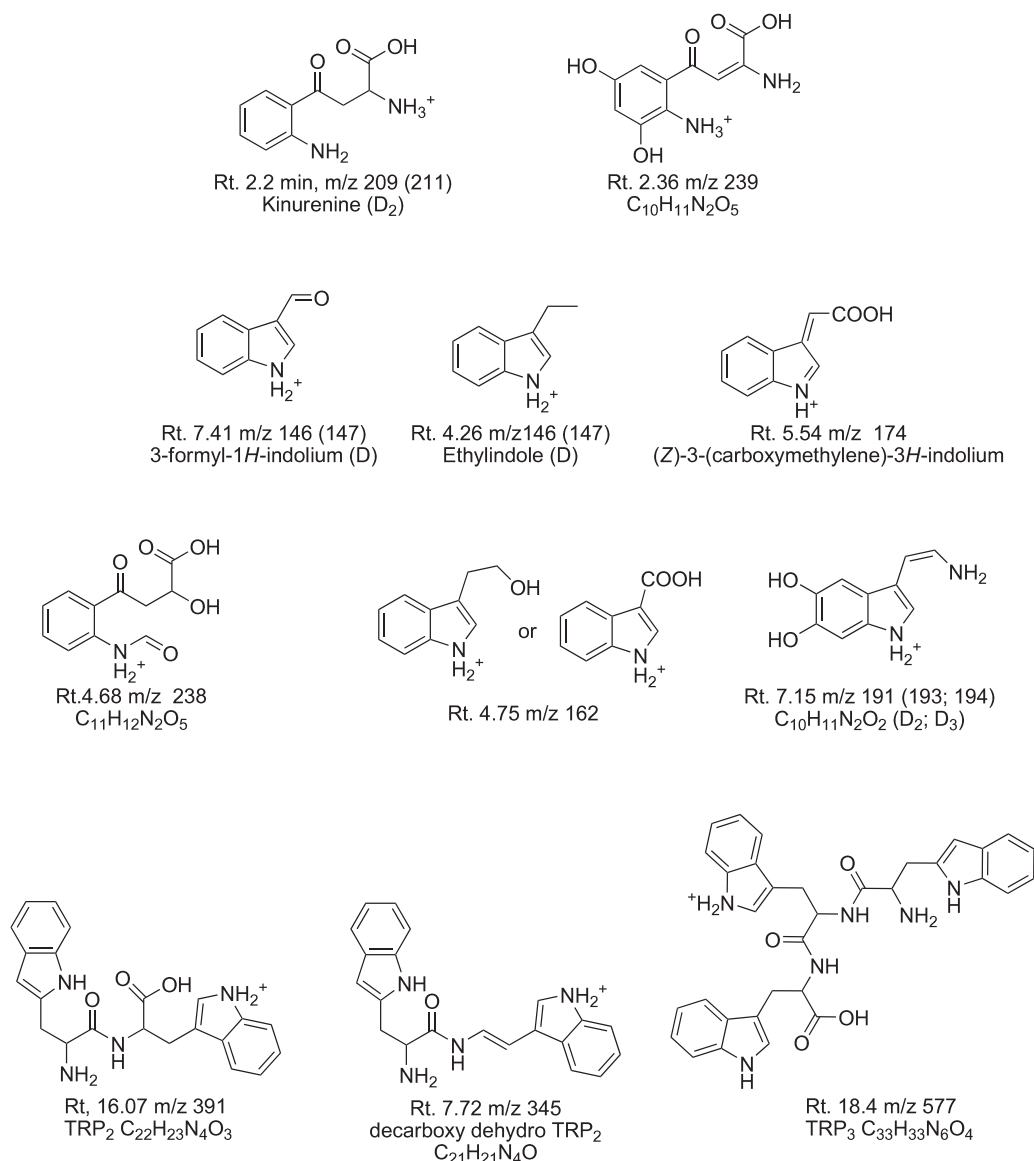
Fig. 6. Suggested structures for the ions marked with † in Table 1. The values of *m/z* of the deuterated species are shown in parentheses. Note that the protonation site in all of the indole structures has been arbitrarily attributed to the indole nitrogen.

the sample increases, the alanine-like compounds behave as transient species. Another main characteristic feature of the high irradiation dose is the appearance of new signals at high δ_H values (10.2 ppm, peak *c*), the typical range of aldehyde protons. Under such conditions (100 % D_2O), and with reference to standard chemical shifts, the resonance originating at $\delta_H = 10.2$ ppm can be reliably assigned to an aldehyde proton. The high exchange rate of the labile indole ring NH proton with D_2O does not permit the appearance of mobile protons in the same field range. In support of the aldehyde-containing molecule, in the HPLC/MS analysis we detected a peak at $m/z = 147$ associated to a formyl-tryptophan-derived compound (see Fig. 6 for related proposed structures). Finally, given the low sensitivity of the NMR diagnostic technique, the very weak signals observed in the aromatic range are difficult to interpret reliably.

The NMR data presented above mainly provide some support for the degradation products of tryptophan in D_2O solution. However, the formation of aldehyde species associated with radicalic tryptophan fragments provides favourable conditions for the formation of more complex molecules as detected in the HPLC/MS analysis.

Discussion

The irradiation of tryptophan molecules in solution with D_2O and H_2O at room temperature with X-rays of 8 keV leads to the formation of light species indicative of fragmentation of tryptophan, as well as to large molecular structures. Given their high energy, X-ray photons interact with single atoms in a molecule rather than directly affecting the molecular bonds. The subsequent ionization injects high-energy
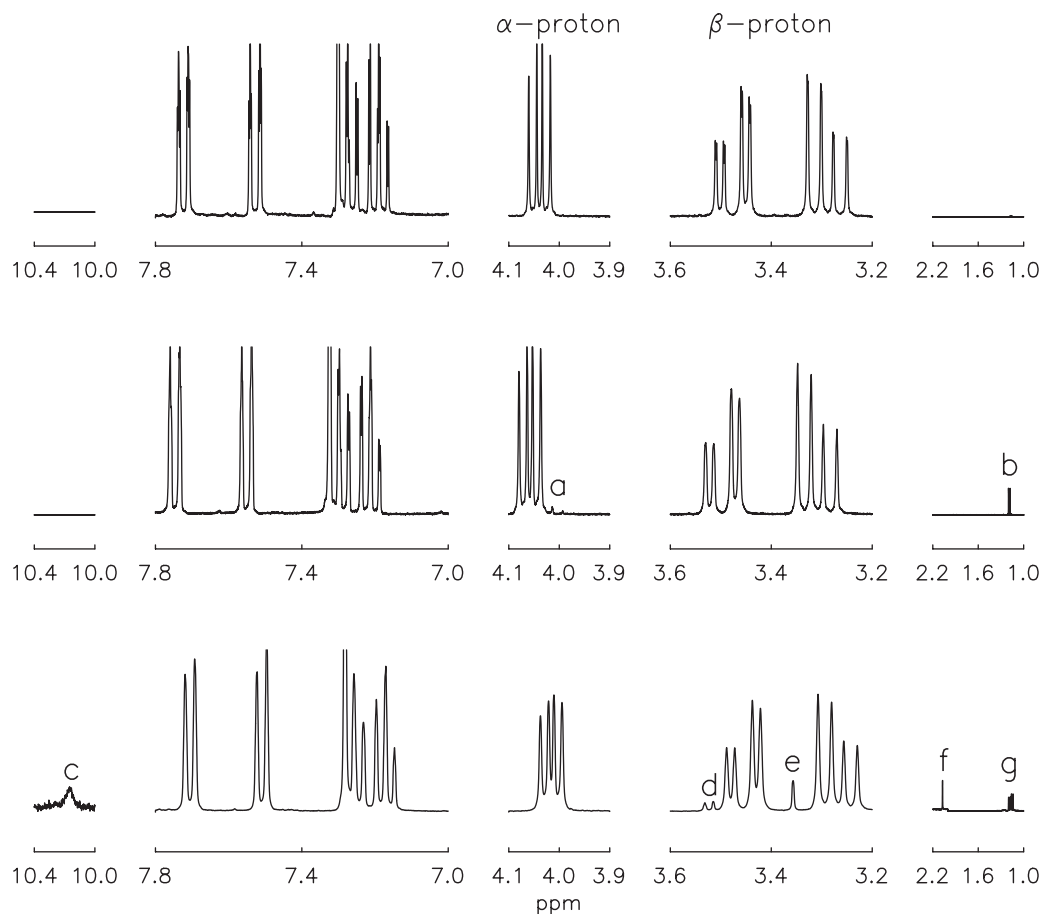
Fig. 7. ^{1}H NMR spectra of tryptophan in D_2O solution: the reference (top row); sample irradiated with 1.3×10^6 erg (middle row); with 7.8×10^6 erg (bottom row). The peak of the solvent is calibrated at 4.80 ppm. The chemical shift is expressed in ppm. The spectra intensities are amplified differently in order to highlight weaker peaks. The first two columns from the left represent the portion of the aromatic structure while the others refer to the aliphatic chain.

photo-electrons within the solution, which in turn loose their energies by ionizing, exciting and dissociating molecular species. The amount of energy deposited onto the samples was 1.3 and 7.8×10^6 erg, corresponding to a total of $\approx 1–6 \times 10^{14}$ X-ray photons. The ratio of atoms in the samples to the total number of X-ray photons deposited into the samples is greater than 10^8. Furthermore, the number of molecules and atoms in the solvent was much larger than in tryptophan, i.e. 1300 and 150 times, respectively. Therefore, direct photo-destruction channels are unlikely, and chemical effects must arise mainly through exchange with the solvent. X-ray irradiation of water leads to the production of H (D) and O atoms, OH (OD) radicals (as recognized more than 50 years ago by Weiss (1944)) as well as ions producing secondary emission over a broad range of energies, dominated by H I Ly-α and Ly-β lines (Beenakker *et al.* 1974; Ajello 1984). Among all the secondary products caused by the X-ray radiation, both H (or D) atoms and OH (or OD) radicals are very reactive, the first being a reducing agent while the latter a strong oxidizing reactant. Thus, the main destruction channels of tryptophan are most likely radical reactions, as suggested by the HPLC/MS and NMR analysis, although some contribution from photodestruction by secondary UV

radiation, in the range 200–300 nm cannot be excluded. H I Ly-α radiation contributes additional OH (OD) radicals from water dissociation. Therefore, solvents play a major role in determining the final products (see Figs 3 and 4), as D and OD, and H and OH forming in D_2O and H_2O solutions have different reactive effects.

Evidence for polymerization is suggested by several high mass derivatives listed in Table 1. The chemistry leading to the polymerization products is not easily attributable, being formed through aspecific radical mechanisms. The peak at 16.07 min corresponding to $m/z = 391$ that is present in both D_2O and H_2O samples is in agreement with the formation of a dipeptidic bound; see Fig. 6. The presence of tryptophan di-petide is more evident in H_2O than in D_2O, probably due to H and D isotopic effects, and it appears to be dependent on the irradiation dose, decreasing with the increase of deposited energy.

We also observed ions at $m/z = 345$ and 577, which along with $m/z = 391$ could arise from condensation reactions of tryptophan. In particular, $m/z = 577$ is reasonably attributable to tryptophan tripeptide, while ions at $m/z = 345$ could be due to the de-carboxy de-hydro tryptophan dimer.

Summary and conclusions

In this work we have presented experiments in which trypto-
phan molecules in water solutions, at room temperatures, are
irradiated with soft X-ray radiation of 8 keV and flux one
order of magnitude larger than the flux reaching the Earth
atmosphere today, in the band 1.5–12.4 keV, during large
solar flares. The irradiation was carried out at a low energy
rate, in order to reproduce a realistic radiation environment.
Analysis of the irradiated sample showed that X-rays lead to
both fragmentation of the tryptophan molecules and to the
formation of larger species of increasing complexity. Among
the largest molecular structures tryptophan dipetide and tri-
peptide species were detected.

Our experimental conditions imply that X-rays cannot be
directly responsible for the effects observed on tryptophan
molecules. We detected second generation products mainly
induced by radicals originating from the interaction of the
X-rays with solvents. The results of this study strengthen the
role of 'primordial' radiation field conditions in the under-
standing of pre-biotic chemical evolution, and open out to the
more general problem of chemical evolution in space. Since
amino acids possess a limited photostability to UV light
(Ehrenfreund *et al.* 2001) the evolution to the next level of
complexity in peptidic chains might occur either in protected
environments or under substantially different radiative con-
ditions. Soft X-ray induced chemistry may be a promising
candidate. This suggestion is also supported, for example, by
the work of Kaneko *et al.* (2005). who demonstrated glycine
dimer formation, via soft X-ray irradiation of thin films of
glycine, and found evidence for the activation of hetero-
geneous pathways on the surface of solid amino acids.

In this work liquid solutions have been used. In space
conditions most material is frozen into water icy mantles ac-
creted onto the surface of dust grains (e.g. Gibb *et al.* 2004).
The present investigation bears out our interest in exploring a
more extensive set of experimental conditions. It would be of
interest to compare energy yields in the solid and liquid pha-
ses. The recent discovery of a mixture of water ice and organic
materials, directly detected on the surface of an asteroid
(Campins *et al.* 2010; Rivkin & Emery 2010), supports the
importance of such studies.

Diagnostic methods

As mentioned in the results section, the HPLC/MS exper-
iment is among other analytical techniques one of the most
sensitive and is capable of giving information on trace
amounts of compounds. The approach used implied the
analysis of the same amount of pure and irradiated trypto-
phan in the same experimental conditions. In particular,
through HPLC/MS analysis, a separation among tryptophan
and its derivatives was achieved, which were shown in
the chromatograms as peaks at certain R_t. In the case of
HPLC/MS, the signal is constituted by mass spectra that are
recorded during the run at a rate of one spectrum every se-
cond. Ion intensities are generally summed to give a TIC trace

Table 2. *Parameters of the HPLC/MS analysis*

Time (min)	Water %	Methanol %	Flow (μm/min)
0	85	15	250
10	30	70	250
12	0	100	250
15	0	100	250
20	85	15	250

(as in Fig. 3). The mass spectrum itself is a bi-dimensional
plot, where the m/z ratio of each ion is reported versus its
intensity (in counts s^{-1}). This implies that a HPLC/MS trace
is thus a three-dimensional array, where, for each specific R_t,
information on the mass of the species can also be deduced
from the corresponding mass spectrum. On the other hand,
given the m/z ratio of a selected ion its R_t can be easily
identified. This allows a direct detection of trace components
that cannot be easily distinguished on the basis of the TIC
alone (Fig. 5). It must also be pointed out that the HPLC/MS
technique, once the experimental conditions are definitely
established, is very reproducible providing the opportunity to
compare the chromatograms of different samples. This also
renders the background subtraction an available option in
order to evidence minor or trace amounts of compounds, as
in the middle and bottom panels of Fig. 3 and in Fig. 4. In our
case just a simple background subtraction of the TIC of
tryptophan from the TIC traces of the irradiated samples
shows peculiar changes that give evidence for the fact that
chemical modification of the starting substance has taken
place as in Figs 3 and 4.

HPLC/MS measures were performed on an Thermo TSQ
Quantum Access triple Quadrupole instrument coupled with
a Thermo Accela HPLC/MS system and equipped with an
Heated Electrospray (H-ESI) source. The chromatographic
conditions were as follows: Column Thermo Hypersyl Gold,
2.1 mm inner diameter, length 10 cm; the volume injected was
10 μl. The analysis was carried out using a gradient combin-
ing a mobile phase (A, water 0.1% formic acid) and a mobile
phase (B, methanol) as reported in Table 2. The mass spec-
trometry experiments were performed under the following
experimental conditions: spray voltage 4100 V, skimmer off-
set −10 V, vapourizer temperature 80 °C, sheath gas pressure
20 (a.u.), ion sweep gas pressure 2.0 (a.u.), aux gas pressure 35
(a.u.), capillary temperature 270 °C, capillary offset 35 (a.u.).

^{1}H NMR spectra were recorded at 298 K with a Bruker
Avance 300 MHz spectrometer. Routinely, one-dimensional
spectra of tryptophan in unbuffered 100% D_2O were ac-
quired with spectral widths of 14 ppm at 298 K. Chemical
shifts (ppm) are calibrated with respect to the sharp peak of
D_2O at $\delta_H = 3.80$ ppm.

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