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Laboratory simulation of ultraviolet irradiation from the Sun on amino acids. III. irradiation of glycine-tyrosine

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Abstract: The effects of near ultraviolet (UV) radiation on water solutions of tyrosine and glycine-tyrosine are investigated using a broadband xenon lamp in the region 200–800 nm. These experiments form a contribution in the laboratory simulation of the solar irradiation on the building blocks of life with regard to the origin of life. Results are presented showing the photodecomposition of tyrosine and glycine-tyrosine, at different concentrations, against UV doses. The analysis of the irradiated solutions is carried out by spectroscopic and analytical techniques. The findings of our laboratory simulations are used to constrain the early stages of the life emerging process.

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Introduction

One of the most enigmatic questions that still challenges science in the third millennium is how living organisms could have been generated from non-living chemical material. The method of formation of the first building blocks, such as amino acids, bases, sugars, etc., is still a matter of debate. The gap in complexity is huge when we compare a random ensemble of molecules (NH_3, H_2O, CO, CO_2, H_2S, etc.) with large bio-molecules, such as nucleic acids and proteins. Furthermore, the multiplicity of structures and functions that constitute living systems contribute additional difficulty to the understanding of the life emerging process. The number of genes in a bacterium is in the order of thousands, while in a multicellular being the gene count is in millions (Maxam & Gilbert 1977; Ward & Brownlee 2000). Then, how did the large biological molecules make their appearance on primitive Earth? Hoyle (1983) compared the probability of their spontaneous assembly to that of a whirlwind assembling a Boeing-747 from a junkyard.

At this point it seems appropriate to quote Davies' (1995) statement that there are 'three philosophical positions concerning the origin of life: (i) it was a miracle, (ii) it was a stupendously improbable accident, and (iii) it was an inevitable outworking of the laws of chemistry and physics, given the right conditions'. While the first hypothesis falls beyond scientific verification and provides no basis for discussion, the second and third ones correspond to current hypotheses.

Besides the theory proposed by Hoyle & Wickramasinghe (1979, 1981), according to which living organisms (bacteria) from space reached our planet inside meteorites, all other theories on the origin of life accept the idea that life, as we know it, started from its ingredients on our planet. The latter theories, while generally agreeing on the possibility that, under favourable conditions, life could also originate on other sites in/or outside the Solar System, they differ in the starting conditions: (i) the life building blocks (amino acids, bases, sugars, etc.) came to Earth already manufactured from space included in meteorites or cosmic dust; or (ii) the life building blocks formed on Earth from simple molecules (NH_3, H_2O, CO, CO_2, H_2S, etc.) present in the primordial environment. There could be a crucial argument to favour one theory over the other, which is chirality. In fact, the life building blocks have a definite chirality. Where does this homo-chirality come from? If it comes from space via enantiomeric selection induced by polarized light (Levine & Breslow 2008; Cecchi-Pestellini *et al.* to be published), then the extraterrestrial hypothesis is corroborated. On the other hand, if it *originated* on Earth via an *in situ* molecular selective assembling process, then the terrestrial hypothesis is supported (Bailey 1997; Sowerby *et al.* 2001).

The further steps after formation of amino acids, bases, sugars, etc. proceed along two possible scenarios. One was proposed by Gilbert (1986) with his RNA world, that is, the origin of life is ascribed to the formation of the ribonucleic acid having auto-replication and catalytic properties. The obvious difficulty in a random assembling of the units to form a nucleotide and the subsequent production of the sequence characteristic of the RNA macromolecule has motivated a different scenario. Wächteräuser (1990) formulated an

alternative idea, recently resumed by Shapiro (2006), of a metabolic cycle that is capable of evolution in the sense of Darwin's theory. The cycle consists of a network of chemical reactions starting from small molecules and ending with complex structures that are able to reproduce themselves. There is, as yet, no consensus on which of these hypotheses is correct, or even on whether they are the only hypotheses (a summary of these ideas is found in Frye (2000)).

Before concluding this section it is worth revisiting the theory of Hoyle & Wickramasinghe (1979, 1981) in light of modern cosmology. If, according to Hawking *et al.* (2002) ' … time travel through a wormhole is possible only on a microscopic scale … ', then an advanced civilization (Kardashev's III type) could have sent its own 'seeds' into our galaxy (Kaku 2005). In a wide sense 'seeds' may simply correspond to bits of 'information' on how to replicate pristine life somewhere in this or in another universe. The existence of other universes different from ours has been very recently put forward by a group of astronomers from the University of Minnesota (USA) who have discovered a gigantic hole in the cosmic background, with a diameter of about 9×10^8 light years, which might be the imprint of a parallel universe (Rudnick *et al.* 2007).

In previous papers we have investigated, in the laboratory, the effects of ultraviolet (UV) radiation on water solutions of tyrosine (Scappini *et al.* 2007a) and phenylalanine and tryptophan (Scappini *et al.* 2007b) using broadband Xe lamps between 200 and 800 nm. The results showed that in the series phenylalanine, tyrosine and tryptophan the ease of UV decomposition corresponds with the absorption coefficient ε of the aromatic band, with tryptophan being the most fragile. The latter paper outlines a discussion on the stability of dipeptides against UV irradiation, with respect to the corresponding monomers. Laboratory experiments (see references in Scappini *et al.* (2007b)) have shown that the yield in dipeptide photodecomposition is dependent on the band(s) involved, in particular that of the peptide bond. It is of interest that the rupture of the peptide bond can also take place at wavelengths longer than 190 nm, as a consequence of the excitation of different groups elsewhere.

In this work we will expose water solutions of tyrosine and glycine-tyrosine, in a range of concentrations, to different UV doses from a Xe lamp. The aim of these experiments is: (i) to investigate whether the concentration of the compound affects its own photodecomposition; and (ii) to measure the robustness of the glycine-tyrosine system against the UV irradiation as compared to that of the isolated monomers. The solutions after irradiation will be analysed with both spectroscopic and chromatographic techniques.

Experimental

L-tyrosine (Tyr), and glycine-L-tyrosine (Gly-Tyr) with purity better than 98 % from Fluka were separately dissolved in bidistilled water; air was not removed from the solutions. The irradiation experiments were performed by filling the solutions with a quartz cell to a volume of $1 \times 1 \times 1$ cm^3. A magnetic stirrer was used to ensure uniform irradiation of the sample. Tyr and Gly-Tyr have two absorption bands, due to the aliphatic chain, at around 200 nm and 220 nm and a third band, due to the benzene ring, at 275 nm with an absorption coefficient $\varepsilon = 1300\,\mathrm{l\,cm^{-1}\,mol^{-1}}$. The peptide bond absorbs at around 190 nm and glycine (Gly) has an absorption band around 200 nm.

Only the band at 275 nm was accessible to our experimental set up, see below, and it was the only one investigated both for Tyr and Gly-Tyr. A Xe lamp, PTI model 1010, was used as the radiation source. The infrared emission was cut off by a water filter at the output of the lamp. The power from 200 nm to about 800 nm was 3.5 W. The power in a 10 nm interval around 275 nm was 6×10^{-2} W. The power of the lamp decreases more than one order of magnitude near 200 nm. The beam spot size on the front side of the cuvette was 1 cm in diameter.

The samples, in concentrations from 1×10^{-3} mol l^{-1} to 1×10^{-4} mol l^{-1}, were irradiated for different time intervals. We started with 30 s corresponding to a dose (power × exposure time) of 1.8 J and ended with 480 s corresponding to 28.8 J. The samples were stored in a refrigerator at 4 °C before and after irradiation. The analyses of the transformations produced in the samples by the UV irradiation were carried out using both spectroscopy and chromatography-mass spectrometry soon after their exposition.

In the following sections we will describe the investigation procedures and the results obtained. At the end we will draw some conclusions.

Spectroscopy

Ultraviolet spectra were taken in order to determine the change in Tyr and Gly-Tyr absorbance as a function of the UV irradiation, at different concentrations of the solutions. The range of concentrations was chosen so as to meet both solubility and spectral sensitivity.

The bands below 220 nm were not considered because they were poorly affected by the lamp flux and at the limit of the spectrometer wavelength range. The spectrometer was a Jasco V-550 operated at a resolution of 5 nm. Spectra were recorded in the 200–800 nm wavelength interval. Water and cuvette material contributions to the absorbance were subtracted in a dual-beam arrangement. Care was taken to minimize the effects from sample manipulation, such as heating to room temperature, exposure to day light, contamination, etc. The estimated error in the measured absorbance is of the order of a few percent.

In order to normalize the absorbance of each irradiated sample (A) to the corresponding absorbance before irradiation (A°) the following ratio was calculated $\Delta A = [(A - A^\circ)/A^\circ] \times 100$. The results from the spectra of Tyr and Gly-Tyr in terms of ΔA, averaged over three measurements, for different concentrations and UV doses are presented in Table 1. A sample of the typical UV spectra of Gly-Tyr, at the concentration of 2×10^{-4} mol l^{-1}, irradiated from 1.8 J to 28.8 J, is shown in Fig. 1 (also see Table 1).

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Table 1. ΔA (%) *values of tyrosine and glycine-tyrosine at their aromatic absorption bands at 275 nm. The samples were irradiated at different UV doses* (*J*) *with a Xe lamp. The range of concentrations* (*mol* l^{-1}) *is shown in the first column*

	Dose (J)				
Conc (mol l^{-1})	1.8	3.6	5.4	14.4	28.8
			Tyrosine		
1×10^{-3}	10[a]	22	25	35	42
8×10^{-4}	15	27	32	39	45
4×10^{-4}	19	36	46	67	80
2×10^{-4}	20	37	48	80	93
1×10^{-4}	24	49	67	100	120
			Glycine-tyrosine		
8×10^{-4}	−4[a]	−7	−11	−28	−36
4×10^{-4}	−5	−8	−12	−28	−37
2×10^{-4}	−4	−8	−11	−25	−35
1×10^{-4}	−4	−7	−11	−25	−34

[a] Errors in the ΔA are estimated to be of the order of $\pm10\%$.

From Table 1, in the case of Tyr, it is evident that the effect of the UV irradiation is larger the more diluted the solution, while in the case of Gly-Tyr the effect of the UV irradiation is almost independent of the dilution. In Fig. 2 the ΔA of Tyr and Gly-Tyr at concentrations of 8×10^{-4} mol l^{-1} and 1×10^{-4} mol l^{-1} are plotted versus the UV doses. The different behaviour of the two species may be explained as follows.

The photodecomposition curve (Fig. 2), which is the plot of ΔA versus the UV doses, is positive in the case of Tyr, while it is negative in the case of Gly-Tyr. As already discussed in previous papers (Scappini *et al.* 2007a,b), positive values of ΔA indicate a predominance of fragments, under irradiation, with molecular weights and absorption coefficients higher than the non-irradiated species, while negative values of ΔA indicate a predominance of fragments with molecular weights and absorption coefficients lower than the non-irradiated species. According to this picture, irradiated Tyr produces more larger fragments than smaller fragments, while Gly-Tyr does the opposite. If a molecular shielding mechanism is thought to be effective during the UV irradiation, then it is reasonable to assume that Tyr becomes progressively shielded by its own fragments, as they have cross sections larger than that of Tyr itself, and that this effect increases with the concentration of the monomer. On the other hand, the cross sections of the fragments from Gly-Tyr are smaller than or similar to that of Gly-Tyr itself, and do not significantly change the course of irradiation, which as a result is almost independent of the concentration of the dipeptide.

Unfortunately, nothing can be said on how Gly compares with Gly-Tyr, due to the instrumental limitations preventing a study of Gly.

Chromatography-mass spectrometry

Four aqueous solutions of non-irradiated and irradiated Tyr (mass = 181 u) and Gly-Tyr (mass = 238 u) were analysed by high-pressure liquid chromatography-mass spectrometry

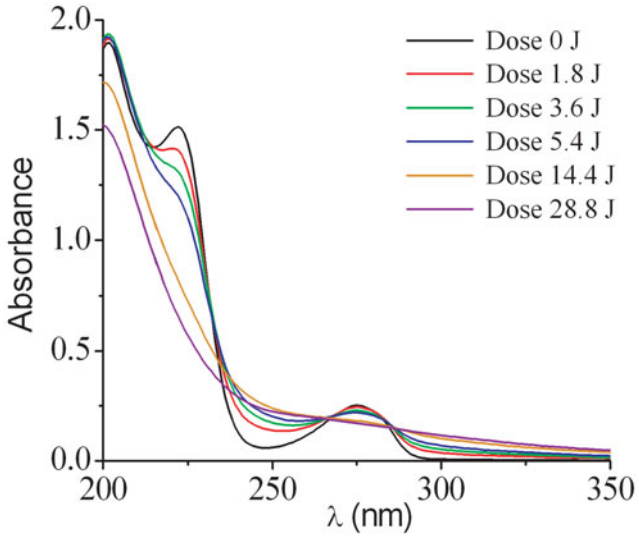

Fig. 1. UV spectra of water solutions at 2×10^{-4} mol l^{-1} of non-irradiated and irradiated Gly-Tyr versus UV doses. The features below 220 nm are not reliable (see text).

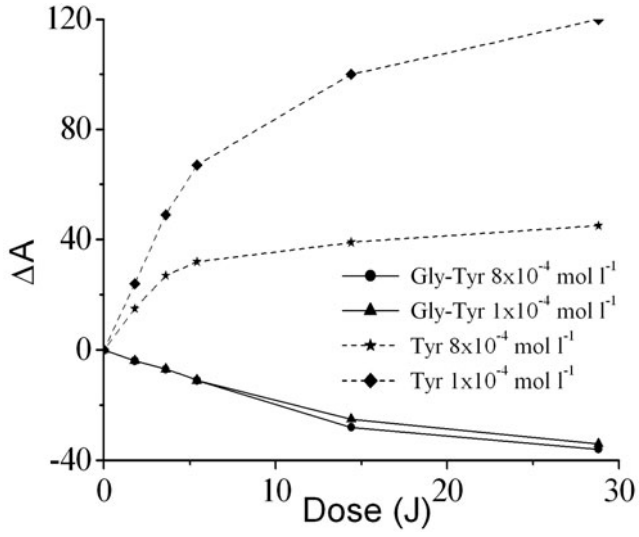

Fig. 2. Plot of $\Delta A = [(A - A^{\circ}/A^{\circ})] \times 100$ for Tyr and Gly-Tyr, at 8×10^{-4} mol l^{-1} and 1×10^{-4} mol l^{-1}, as a function of the irradiation doses (see Table 1).

(HPLC-MS) in the electron spray ionization (ESI) mode. The analytical setup consisted of an Agilent 1100 HPLC chromatograph coupled with an Esquire 3000 Plus mass spectrometer. The mass spectrometer detects positive ions resulting from species with an attached proton or Na^+ (from the ambient). For instance, Tyr with mass 181 shows peaks at 182 and 204, corresponding to $TyrH^+$ and $TyrNa^+$, respectively. The same mass spectrometer is also able to detect negative ions from a proton subtraction; thus, Tyr shows a peak at 180. Chromatographic separations were obtained using either a Zorbax Extended C-18 (4.6×1500 mm², 5 μm) or a Zorbax Eclipse XDB-C8 resin analytical column, eluting for 5 min with bi-distilled water and then with a linear

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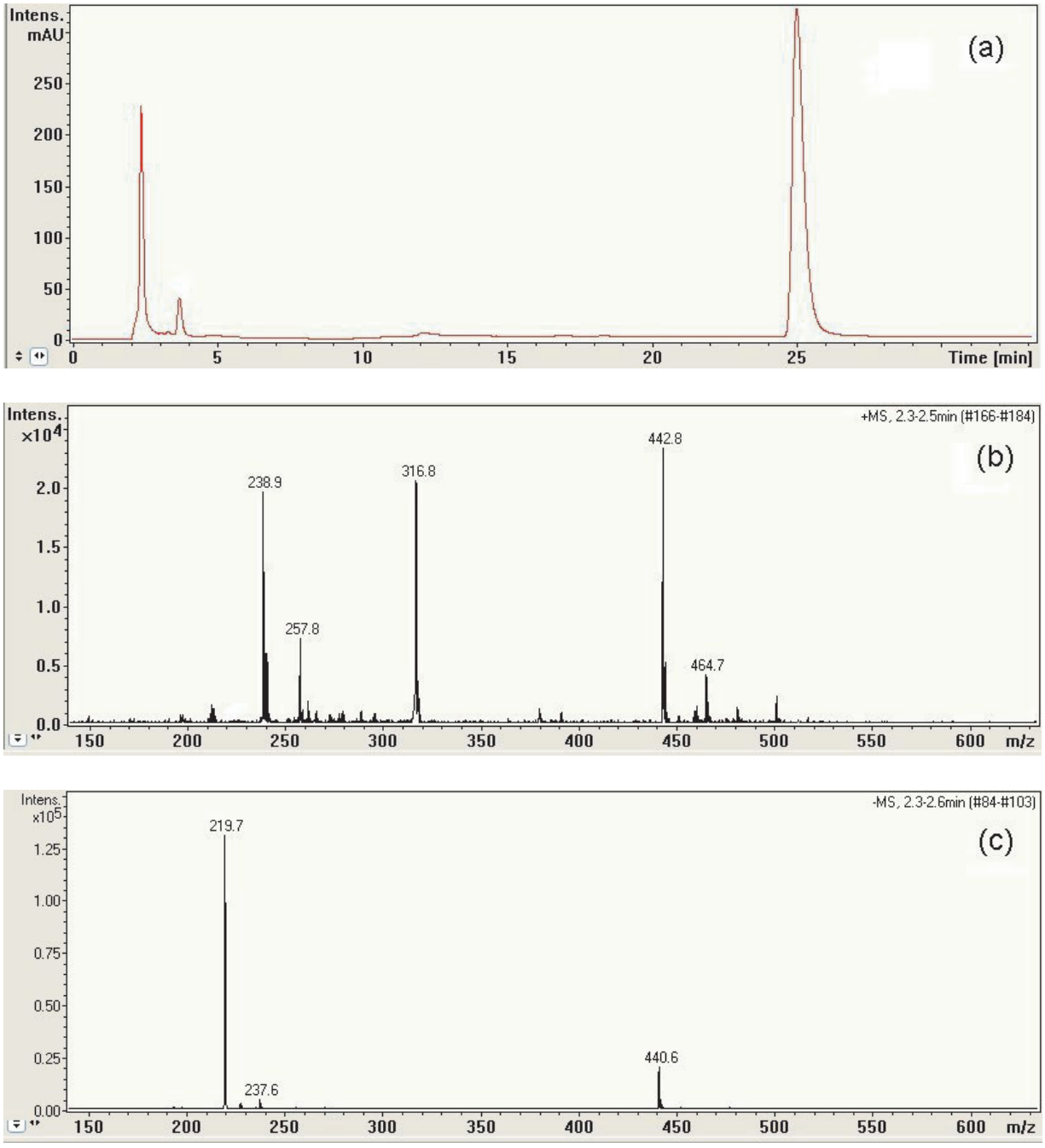

Fig. 3. (a) HPLC chromatogram at 223 nm of an aqueous Gly-Tyr sample at 8×10^{-4} mol l^{-1} irradiated for 480 s with an UV lamp from 200 to 800 nm. The main peaks correspond to degradation products (2.3–2.5 min), intact residue (3.6–3.9 min), and added standard (24.6–26.1 min). The ratio of the integrated intensity of the intact residue to that of the standard gives the percentage of the former with respect to the initial sample, as plotted in Fig. 4. (b) MS spectrum of the positive ions found under the first peak (2.3–2.5 min) of (a). The peaks at 238.9 and 316.8 are from the background. The peaks at 442.8 $[2M + H]^+$ and 464.7 $[2M + Na]^+$ correspond to a dimer of a compound [M] of mass 221. The existence of such a compound is evidenced by the spectrum in the negative mode, shown below. (c) MS spectrum of the negative ions found under the first peak (2.3–2.5 min) of (a), showing the ions at 219.7 $[M–H]^-$ and 440.6 $[2M–H]^-$, showing evidence for the existence of a compound of mass 221.

gradient of methanol 1% min^{-1} at 0.5 ml min^{-1}, following the UV absorption at 275 nm. An external standard was used in the chromatographic analysis to better constrain the quantitative determination (typical spectra are presented in Fig. 3).

By using liquid chromatography, the fractions of molecules of Tyr and Gly-Tyr not decomposed at 8×10^{-4} mol l^{-1} and 1×10^{-4} mol l^{-1} were determined in the irradiated solutions. These fractions are plotted versus UV doses in Fig. 4. The results confirm that Tyr behaves differently from Gly-Tyr

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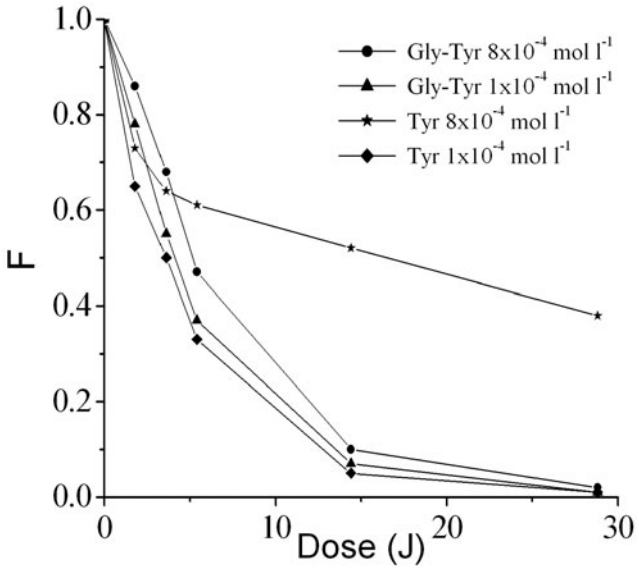

Fig. 4. Plot of the fraction of molecules not affected by irradiation (F) for Tyr and Gly-Tyr, at 8×10^{-4} mol l^{-1} and 1×10^{-4} mol l^{-1}, versus UV doses.

against UV irradiation, in accordance with Fig. 2. In fact, while Tyr decomposition shows a large increase with increasing dilution, Gly-Tyr decomposition does not. In addition, the curves for Tyr decomposition are different in slope and shape depending on the dilution, while those for Gly-Tyr are quite similar. The hypothesis of the molecular shielding mechanism, already given in the previous section, explains these phenomena. When in Tyr this shielding mechanism becomes ineffective due to dilution the curves of both species tend to coincide. Thus, the photodecomposition of Tyr and Gly-Tyr, at 1×10^{-4} mol l^{-1}, is similar. Above this concentration Tyr appears more robust than the dipeptide to UV irradiation.

A number of ions were detected in the irradiated solutions of Gly-Tyr with the HPLC-MS technique. Among them a couple of positive ions with masses of 443 and 465, corresponding to species with an attached proton or Na^+, respectively, are particularly intense. They correlate to the HPLC peak that increases with the increasing UV dose. An analysis of these ions and of the negative counterpart at 441 indicates that they are dimers of a neutral compound of mass 221, resulting from an efficient fragmentation route of Gly-Tyr. The probable structure of this compound is shown in Fig. 5. This structure is consistent with the dedicated mass spectrometry experiments and the absence in the nuclear magnetic resonance (NMR) spectra of aromatic protons in samples diluted with D_2O. The finding of the dominant ion corresponding to the neutral mass 221 and other fragments with masses smaller than Gly-Tyr explains the negative curve in the UV absorption in Fig. 2.

Discussion and conclusions

In the assumption that life originated on Earth, primordial chemical reactions and energy sources have been simulated

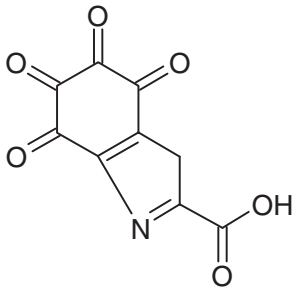

Fig. 5. Tentative structure of the compound with mass 221 found by MS spectrometry and consistent with the NMR experiments.

by many authors after the much-celebrated Miller's experiment (1953). Among the large variety of energy sources available on early Earth (Deamer 1997), solar radiation is certainly one of the most significant, being a driving factor in chemistry and largely available. The Sun was the major source of radiant energy in initiating life and is still the major source sustaining it. One crucial point in modelling plausible organic syntheses of biological components is the balance between the two effects of radiation, one stimulating the assembly and the other degrading the precursors and products.

The present above-atmosphere solar radiation flux on Earth in a 10 nm interval around 270 nm is about 5×10^{-4} J cm^{-2} s^{-1} or 6.8×10^{14} photons cm^{-2} s^{-1} (Zelik & Gregory 1998).

In our experiments, the samples were irradiated ($200 < \lambda \leqslant 800$ nm) for periods of time from 30 to 480 s, corresponding to doses from 1.8 to 28.8 J (the surface of the irradiated solutions in the cuvette being 1×1 cm^2). This corresponds to solar irradiation from 1 hour to almost 1 day, in the absence of atmosphere. However, if the UV flux of the young Sun on Earth had been stronger (5 to 100 times between 200 and 800 nm) than at present (Tehrany *et al.* 2002), the above time estimates are to be reduced.

The photochemistry of amino acids has been widely studied in the laboratory in recent decades (Bensasson *et al.* 1983; Creed 1984; Davies & Truscott 2001; Ehrenfreund *et al.* 2001; Scappini *et al.* 2007a,b), both in aqueous solutions and in solid matrices. It was found that in amino acids containing an aromatic ring the effect of the UV irradiation is reduced and in aqueous solutions some hydrogenation of the ring occurs (O'Donnell & Sangster 1970). In the present experiments we have found that the survival of Tyr to the UV irradiation is dependent on its concentration in aqueous solutions, while that of Gly-Tyr is almost independent of its concentration. At 1×10^{-4} mol l^{-1} the course of photodecomposition of Tyr and Gly-Tyr is similar. At this concentration they decompose rapidly: in fact, with a dose of 10 J only 10% of each survive. These results corroborate the hypothesis that some sort of UV protection from the ambient was necessary for the primordial peptidic syntheses to have taken place.

The non-detection in the irradiated samples of Gly-Tyr polypeptides is a consequence of the fact that an aqueous ambient does not favour condensation processes. The

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absorption of the reactants and products on mineral surfaces could have acted as to partially protect the organic material from photodecomposition and to concentrate it in the aqueous media (Bernal 1967). Alternatively, in order to form polypeptides, any other kind of catalysis could have been active. A great many catalytic materials were available and abundant in large areas. Cyanamide and dicyandiamide (Ponnamperuma & Peterson 1965; Steinman *et al*. 1965), and carbonyl sulphide (Leman *et al*. 2004), were reported as dehydration-condensing agents in the primeval polymerization reactions. Experiments in this sense would be of extreme interest, as proteins and consequently their syntheses are crucial to unfolding knowledge of the early stages of the chemical evolution toward life.

In conclusion, we have investigated the effects of the 200–800 nm UV radiation on aqueous solutions of tyrosine and glycine-tyrosine under conditions mimicking the Sun's irradiation from 1 hour to almost 1 day, in the absence of atmosphere. The effects of the irradiation turned out to be very dependent on the concentration for tyrosine, but almost independent of it for glycine-tyrosine. At diluted regimes, in the order of 1×10^{-4} mol l^{-1}, the photodecomposition of both species is similar and rather fast. It has been argued that, for the first polypeptidic syntheses to have proceeded, some UV protection and the presence of a catalytic material are to be considered necessary constraints.

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