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Laboratory simulation of UV irradiation from the Sun on amino acids. I: irradiation of tyrosine

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Abstract: The effects of ultraviolet (UV) irradiation on water solutions of tyrosine ($HO—C_6H_4—CH_2—CHNH_2—COOH$) have been investigated using a Xe lamp in the region 200–800 nm. This is a step in laboratory simulation towards reproducing the action of the Solar radiation on the building blocks of life, specifically α-amino acids, in the primitive Earth anoxic conditions. Results are presented showing the photostability of tyrosine against different UV doses. Degradation products partly maintain life building capability and partly do not. A tendency towards structure complexification was observed. The analysis of the irradiated tyrosine solutions was conducted using various spectroscopic and analytic techniques. The laboratory results are discussed in the light of a primordial life-emerging scenario.
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Key words: amino acids, astrobiology, chemical evolution, origin of life, UV irradiation of amino acids.

Introduction

The fundamental questions 'what is life' and 'what is its origin' have always stimulated the scientific community for an answer. To the first question, biology has helped with distinguishing criteria between living organisms (tree, fish, animal, etc.) and inorganic entities (rock, water, crystal, etc.). To the second question, geology and astronomy have essentially contributed two answers: a terrestrial one and an extraterrestrial one. The terrestrial hypothesis is based either on the 'primordial soup', where molecules from the Earth's atmosphere could have aggregated into life-building biopolymers, or on mineral surfaces, where the same molecules could have assembled to generate life precursors. The extraterrestrial hypothesis is based on the existence of interstellar molecular clouds where the presence of molecules in large abundance favours their complexification on the surface and/or in the interior of interstellar dust grains (Duley & Williams 1984; Cecchi-Pestellini *et al*. 2004). These complex molecules, from the pre-Solar nebula, inseminated the Earth during the process of planet formation and later by the heavy bombardment from comets and meteorites. Depending on whether the Earth primordial atmosphere was reducing (CH_2, CH_4, NH_3, H_2O) or oxidizing (CO, CO_2, N_2, H_2O) either theory has more credibility. In fact, under reducing conditions, molecules important for life, such as amino acids, form easily (Miller 1953). Conversely, under oxidizing conditions they do not form at all or do so only in small amounts in hydrothermal vents or in localized hydrogen-rich regions. Thus, in the most likely oxidizing conditions (Walker 1977; Chyba 2005), the extraterrestrial hypothesis appears quite reasonable: the organic molecules are first manufactured in space and then delivered to the Earth by a different means.

After about 500 million years from its formation the Earth's surface cooled enough for water vapour to condense into liquid and form the first oceans. Then, the organic material from space could find its way into the shallow waters near the continents and give rise to a great many chemical reactions. Water, in fact, provided a convenient environment for chemistry to proceed and at the same time controlled the Solar ultraviolet (UV) radiation effects on reactants and products, as the atmosphere in the absence of oxygen was transparent to it. It was the balance between radiation-stimulated formation reactions and radiation-induced destruction reactions that determined the fate of the final products.

In previous papers we outlined laboratory experiments investigating the effect of UV and X-ray radiation on DNA in different environmental conditions (Ciaravella *et al*. 2004; Scappini *et al*. 2004). In this work we aim to explore the effect of UV radiation on much simpler species in the life evolutionary scenario: the α-amino acids. In fact, the α-amino acids are important components of life as we know it: they are easy to form, water soluble and capable of producing polypeptide chains leading to proteins.

Muñoz Caro *et al*. (2002) produced a large number of amino acids, of which six were proteinaceous, by the UV irradiation of interstellar ice analogues with a microwave-stimulated hydrogen flow discharge lamp.

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Ehrenfreund *et al.* (2001) investigated the effect of UV radiation from a hydrogen flow lamp on some aliphatic amino acids, including the two proteinaceous glycine and L-alanine, in Ar, N_2 and H_2O ices. The authors' conclusion is that the studied amino acids are highly susceptible to UV photodestruction and their survival in extraterrestrial regions requires that they be shielded from the UV field in protected environments. It is of interest that the rate of racemization was found to be much lower than that of destruction, and was almost insignificant under the experimental conditions.

Protein amino acids were also tested against degradation and racemization in Earth orbit experiments. Depending on the thickness and composition of the deposited films different degrees of decomposition were shown by the samples, but again no racemization was detected in the limit of 0.1% (Barbier *et al.* 1998, 2002; Boillot *et al.* 2002).

In this work, solutions of an aromatic α-amino acid, namely tyrosine, will be irradiated with different energy doses from a Xe lamp and investigated with various spectroscopic and analytic techniques. The aim of the present study (and subsequent studies) is to check to what extent α-amino acids are degraded by a UV flux similar to that of the Sun on the early Earth, when no free atmospheric oxygen existed to block the shortest wavelengths. The survival of the amino acids, as well as the survival of the other biological molecules, to the Solar UV radiation was, in fact, a pivotal condition for the emergence of life on the Earth.

The results of the experiments will be discussed in the light of their importance in understanding the origin of life.

Experimental part

L-tyrosine with a purity of about 99% from Aldrich, Germany, was dissolved in bi-distilled water to a concentration of 5×10^{-3} mol l^{-1}. The irradiation experiments were performed with the solution filling a quartz cell to a volume of 1 cm^3. Air was not removed from the solutions. A magnetic stirrer was used to ensure uniform irradiation of the sample. Besides the main UV absorption band at 223 nm, tyrosine has a band due to the benzene ring ($^1B_{2u} - {}^1A_{1g}$) at 275 nm with an absorption coefficient $\varepsilon = 1300$ l cm^{-1} mol^{-1}. An Oriel 6258-300 W–Xe lamp was used as radiation source. The infrared emission was cut off by a water filter at the output of the lamp. The power in the band from about 200 to 800 nm was 1.5 W. The power in the 10 nm band around 275 nm was 2.5×10^{-2} W, and the power in the band around 223 nm was one order of magnitude lower. The beam spot size on the front side of the cuvette was 1 cm in diameter.

The samples were irradiated for different time intervals. We started with 80 s corresponding to a dose of 2 J and ended with 7 h corresponding to 630 J. The samples were stored, before and after irradiation, in a refrigerator at 4 °C to ensure chemical stability. The analyses of the transformations produced on tyrosine by the UV radiation were carried out using both spectroscopy and chromatography-mass spectrometry, soon after irradiation.

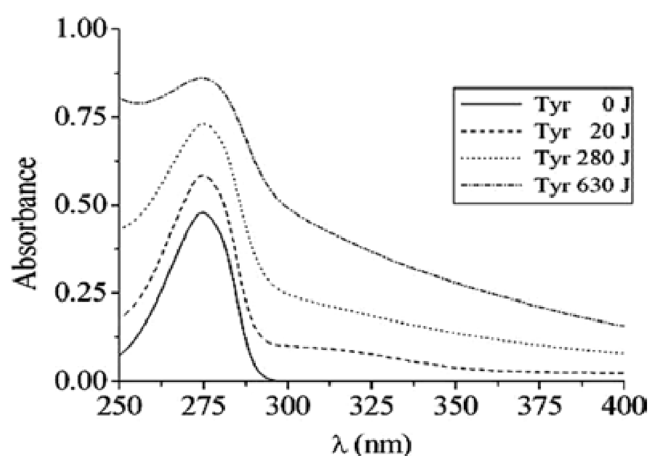

Fig. 1. UV spectra of water solutions of non-irradiated and irradiated tyrosine at different doses (J) with a Xe lamp. Spectra were taken at 1×10^{-3} mol l^{-1}, while irradiation was performed at 5×10^{-3} mol l^{-1}.

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

Spectroscopy

UV spectra were taken in order to monitor the change in tyrosine absorbance around 275 nm as a consequence of the UV irradiation. The band at 223 nm was not taken into consideration because it was poorly affected by the lamp flux and at the limit of the spectrometer range. The spectrometer used was a Jasco V-550 (Jasco, Japan) operated at a resolution of 5 nm. Spectra were recorded in the wavelength region 200–400 nm. Water and cuvette material contributions to the spectra were subtracted in a dual-beam arrangement.

Care was taken to minimize effects on the absorbance from sample manipulation, such as those resulting from heating to room temperature, exposure to daylight, contamination etc. The estimated error on the measured absorbance is of the order of a few percent. Irradiation was conducted on samples at a concentration of 5×10^{-3} mol l^{-1} and spectra were taken at the concentration of 1×10^{-3} mol l^{-1}. A sample of typical UV spectra is presented in Fig. 1.

In order to normalize the absorbance of each irradiated sample (A) to the corresponding absorbance before irradiation (A°) the following ratio was calculated: $\Delta A \equiv [(A - A^\circ)/A^\circ] \times 100$. The results from the UV spectra in terms of ΔA, averaged over three measurements, for different doses (power multiplied by exposure time) are given in Table 1.

The energy of a photon at a wavelength of 275 nm is $E = 7.2 \times 10^{-19}$ J (1.7×10^{-19} cal), equivalent to a molar value of 4.3×10^5 J mol^{-1} (103 kcal mol^{-1}). This energy is enough to break most of the single molecular bonds. The irradiation of tyrosine molecules leads to one of four possible outcomes: (i) re-emit the absorbed photon at the same or at a different frequency; (ii) undergo collisions with other molecules, mostly water molecules, and transfer their energy to them; (iii) collide reactively with other molecules; or (iv) react

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Table 1. *ΔA values, at 275 nm, of tyrosine solutions irradiated to different doses (J) with a Xe lamp. Spectra were taken at 1 × 10⁻³ mol l⁻¹, while irradiation was performed at 5 × 10⁻³ mol l⁻¹*

Dose	0	2	10	20	30	50	90	180	280	450	630
ΔA[a]	0	5.5	6.5	9.5	16	22	34	43	56	69	76

[a] Errors in the ΔA values are estimated to be of the order of $\pm 5\%$.

intramolecularly, either by transferring the excitation energy to a breakable bond or by ejecting electrons. As a consequence of outcomes (iii) and (iv) radicals and/or ions are formed. Thus, following UV radiation absorption, a number of fast ($10^{-3}-10^{-9}$ s) reactions take place, leading to the formation of different products.

From the spectra compilation of Fig. 1, and from Table 1, it appears that the absorbance curves of the irradiated samples are distributed above the absorbance curve of the non-irradiated tyrosine, and that the wavelengths of the peaks do not move significantly from 275 nm. The values of absorbance higher than that of non-irradiated tyrosine can be explained by the transformation of tyrosine into products, some of which have a larger absorption coefficient than tyrosine. It is known that the absorption coefficient increases as the complexity of amino acids increases. In the series phenylalanine, tyrosine and tryptophan the absorption coefficients are 200, 1300 and 5000 l cm^{-1} mol^{-1}, respectively. Therefore, the products with absorption coefficients larger than tyrosine should also have larger molecular weights.

Figure 2 shows a plot of the data from Table 1. The curve represents the course of the 275 nm absorption changes upon tyrosine irradiation from a broad-band UV source. It shows that the absorbance of the solution increases with the radiation dose. While this indicates that products with absorption coefficients greater than that of tyrosine are formed, it does not exclude the presence of products with smaller absorption coefficients. It is, in fact, the contribution of all the absorption coefficients together with the concentration of the species that determines the measured ΔA. The almost asymptotic behaviour of the curve is due to the decrease in the concentration of tyrosine within the dose and the consequently reduced formation rate of more complex products.

Before starting UV spectroscopy, we took infrared spectra with a Bruker113 spectrometer (Bruker, Germany) at a resolution of 2 cm^{-1}, in the solid phase. Even if results were not conclusive a general decrease in the absorption bands of irradiated tyrosine as a result of its decomposition was observed.

In the next section we will discuss again the nature of the products resulting from the UV irradiation of tyrosine by the use of chromatography-mass spectrometry.

Chromatography-mass spectrometry

Aqueous solutions of non-irradiated and irradiated tyrosine, see Table 1, were analysed by high-pressure liquid chromatography-mass spectrometry (HPLC-MS) in the electron spray

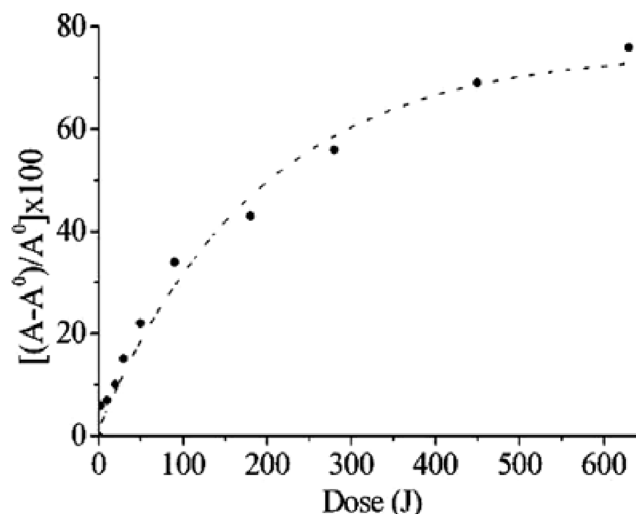

Fig. 2. Plot of $\Delta A \equiv [(A - A^{\circ})/A^{\circ}] \times 100$, see Table 1, as a function of the irradiation dose (J).

ionization (ESI) mode. The analytical set-up consisted of an Agilent 1100 HPLC chromatograph (Agilent, Germany) coupled to an Esquire 3000 Plus mass spectrometer (Bruker, Germany). The mass spectrometer detects molecular ions complexed with a proton or a Na^{+} (from the ambient). For instance, tyrosine with mass 181 shows peaks at 182 and 204 corresponding to $TyrH^{+}$ and $TyrNa^{+}$, respectively. Chromatographic separations were obtained using a Zorbax Extended C-18 column (4.6×150 mm^2; Agilent, Germany) eluting for 5 min with bi-distilled water and then with a linear gradient of Methanol 1% min^{-1} at 0.5 ml min^{-1}, following the UV absorption peaks at 223 and 275 nm. Both analyses show the same qualitative pattern, but the chromatograms at 275 nm are more sensitive to differences among samples irradiated at different doses.

Figure 3 correlates the UV chromatogram at 275 nm of a sample irradiated to a dose of 280 J, either detected with (a) chromatograms of the extracted ions or (b)–(d) detected during the MS part of the analysis. Trace (b) correlates peaks 1 and 2 ($R_t = 2.2$–3.1 min) with compounds of mass 197. Formally, this mass can be attributed to molecules derived from the addition of a hydroxyl group to tyrosine, eventually followed by a cyclization reaction to give an indole derivative. Peak 2 was identified as DOPA (3,4-dihydroxyphenylalanine) after comparison with a commercial sample.

Trace (c) shows a good correlation between peaks 2 and 4 ($R_t = 2.7$–3.8 min) and compounds of mass 193. These can be tentatively assigned to molecules formed from the addition of a hydroxyl group to the tyrosine ring followed by a cyclization reaction to yield amino-coumarines and/or dehydrogenated indole derivatives. Unfortunately, none of these compounds are available for HPLC identification.

Trace (d) shows the presence of ions corresponding to compounds with mass 165 that are hardly visible in the UV chromatogram. The ion eluting at $R_t = 5.8$ min corresponds to that produced by a sample of pure phenylalanine.

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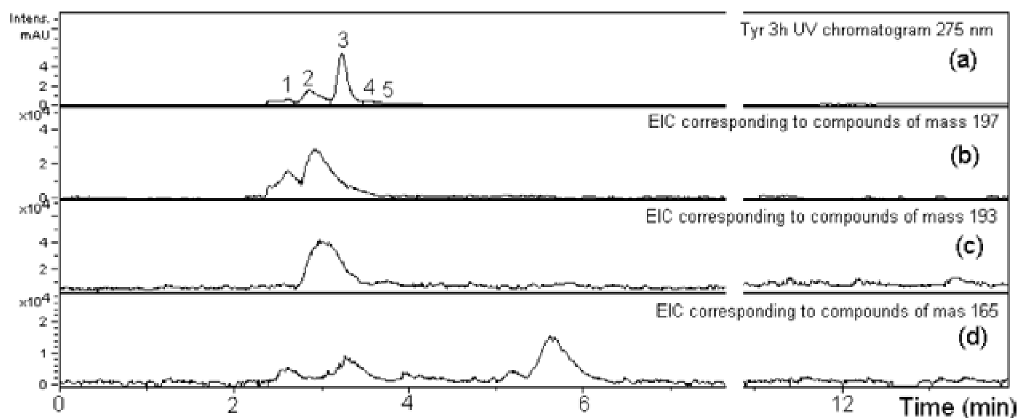
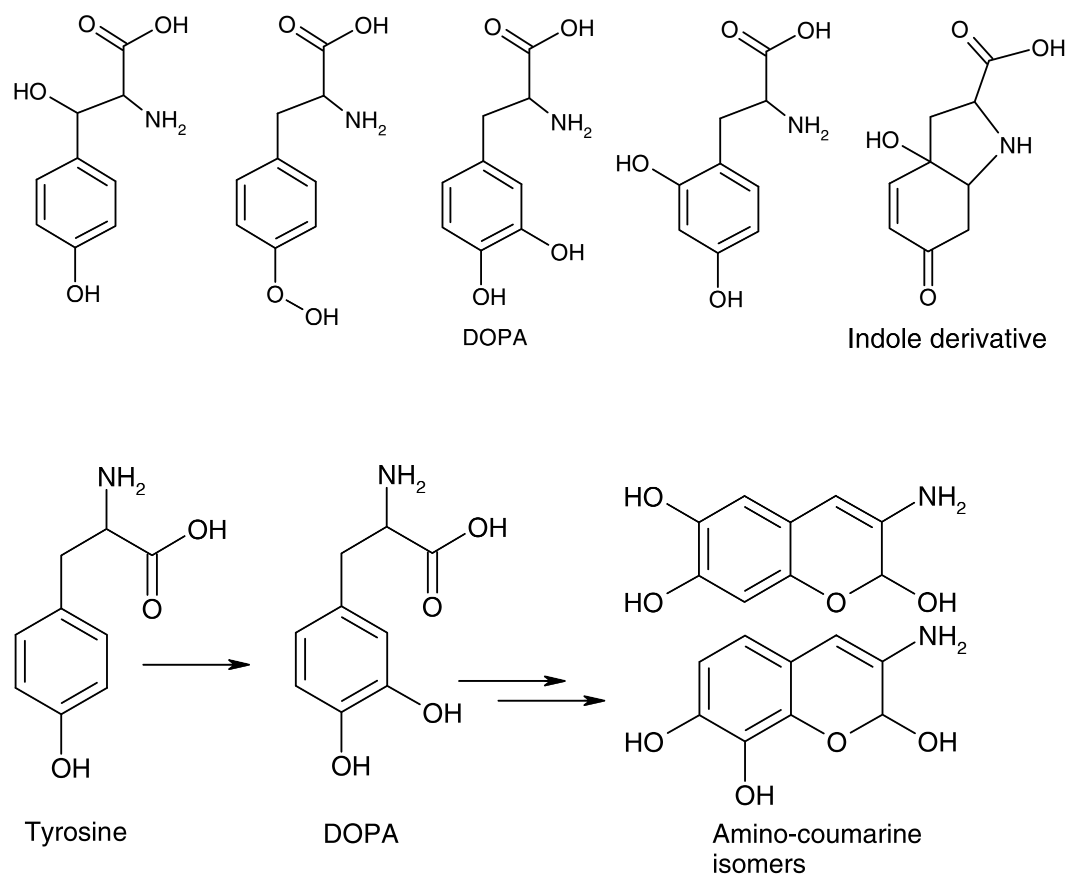


Fig. 3. Correlation between (a) the UV chromatogram at 275 nm of a water solution of tyrosine irradiated to a dose of 280 J with a Xe lamp and the extracted ion chromatograms for the following peaks: (b) on mass 198, (c) on mass 194 and (d) on mass 166. The peak 3 (R_t = 3.2 min) shown in (a) is due to tyrosine.



The pH of a solution (5×10^{-3} mol l^{-1}) of irradiated tyrosine to a dose of 280 J was found to be 5.8 compared with 5.2 for the non-irradiated sample. This result motivated a chromatographic search for ammonia. At the same time, the irradiated sample was also examined for the occurrence of CO and CO_2 (inorganic carbon). While the content of inorganic carbon was found to be similar in both irradiated and non-irradiated samples, the concentration of free ammonium ions was found to be 15 times higher in the irradiated sample (12 ppm versus 0.8 ppm). Thus, in the irradiated sample ammonia accounts for about 12% of the initial amount of tyrosine. The presence of ammonia is an indication that, in addition to the degradation products discussed above, a significant amount of low molecular weight products are to be expected, which may be below the mass detection limit.

From the MS analysis the ratio between the integrated signals corresponding to masses 193 and 197 and the signal

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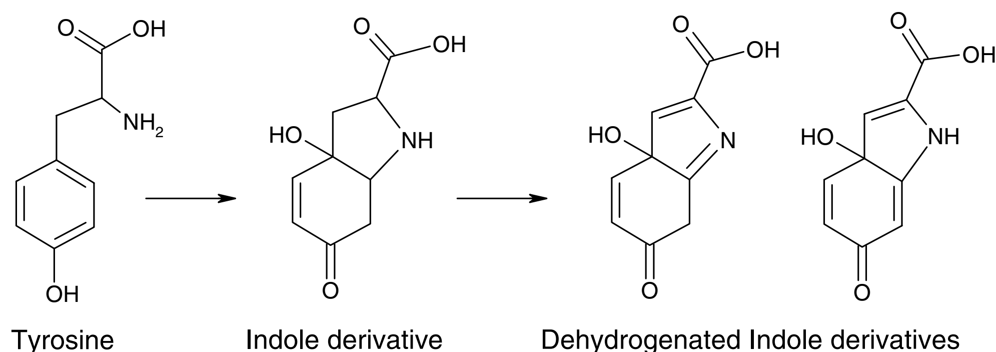

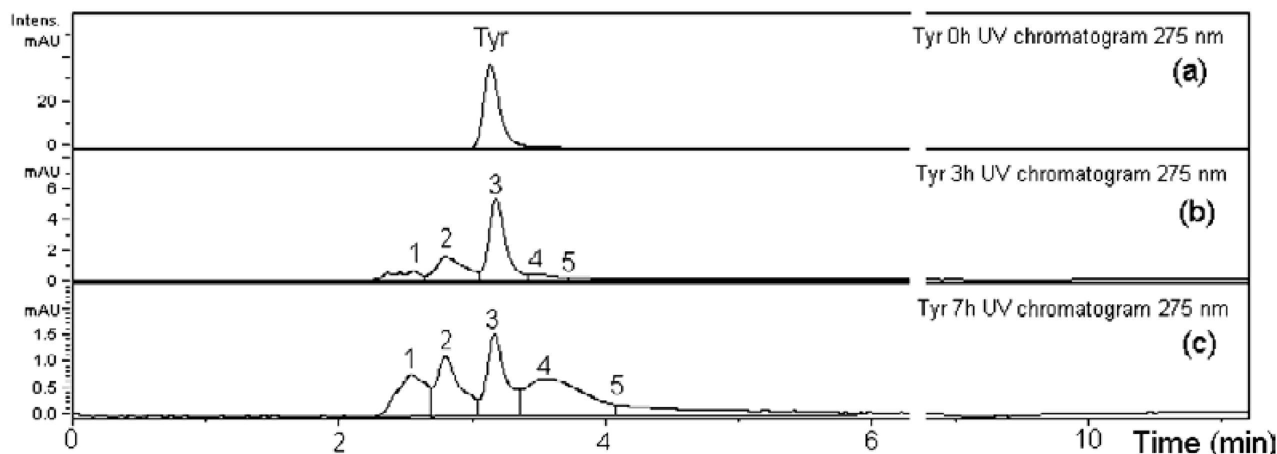

Fig. 4. (a) HPLC profile at 275 nm of a water solution of non-irradiated tyrosine. The peak appears at $R_t = 3.2$ min. (b) HPLC profile at 275 nm of a water solution of tyrosine irradiated to a dose of 280 J with a Xe lamp. (c) Same as (b) but to a dose of 630 J. The sequence (a)–(c) shows the decrease of tyrosine due to UV irradiation: 100%→50%→20%.

corresponding to mass 165 is 2.5 at a dose of 280 J, while it rises to 4.2 at a dose of 630 J. On the other hand, from the HPLC analysis the integrated area of peaks 1, 2, 4 and 5 increases with the UV dose (see Fig. 4). This leads to the conclusion that compounds with molecular weights higher, rather than lower, than tyrosine become dominant as the irradiation progresses. The findings are consistent with the behaviour of the absorbance curve of Fig. 2.

Discussion and conclusions

Amino acids are the basic components of proteins and therefore are essential constituents of all organisms. The survival of amino acids, as well as all other biogenic molecules, on the surface of the early Earth was dependent on many factors. Among these factors their photostability to UV Solar radiation is crucial to constrain the environmental conditions for the emergence of life on Earth.

Our experiments have been conducted in water solutions because water is a necessary biological condition. All biochemical reactions occur in water as the participating organic compounds are water-soluble. In fact, the volatile compounds disperse in the atmosphere and are degraded by radiant energy, while the insoluble compounds sink to the bottom and are removed by further interactions. This leaves soluble organic compounds in aqueous solution.

Solar radiant energy (and all other energy sources) determined the early fate of all biological systems, bringing about chemical transformations by its interplay with matter. Many experiments were devised to simulate these primordial chemical reactions and the driving energy sources, which eventually yielded the important constituents of proteins and nucleic acids. These experiments have shown that amino acids in water solutions undergo dehydration–condensation (polymerization) reactions in the presence of UV light and condensing agents such as cyanamid or clay minerals (Ponnamperuma 1972). Thus, protein molecules could have been formed in the absence of life, contrary to what is constantly performed in living organisms by way of enzymes. However, owing to the continuous input of Solar radiation (and radiations from all other sources) not only did the primordial syntheses of organic compounds proceed but their degradation also occurred. This led to a steady-state cycling between precursors, amino acids and degradation products, which was driven by the energy input.

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The Solar radiation flux on the Earth in a 10 nm band around 275 nm is currently about 5×10^{-4} J cm^{-2} s^{-1} or 6.8×10^{14} photons cm^{-2} s^{-1}, measured outside our atmosphere (Zeilik & Gregory 1998). In our experiments the samples were irradiated for 80 s to 7 h, corresponding to doses of 2 J to 630 J. This is equivalent to a Solar irradiation from 1 h to 2 weeks. In these calculations a 100% atmospheric transmission is assumed. A lower value, owing to environmental conditions, implies longer Solar irradiation periods. According to the standard Solar models the Solar flux of the young Sun was lower than at present, but more recent Solar evolutionary models provide a value significantly higher than the present value (Sackman & Boothroyd 2003; Lammer *et al.* 2004 and references therein). Furthermore, observational evidence on Solar-like stars at different evolutionary stages indicates that the early Sun should have experienced a highly active phase in particle and radiation emission from 4.5 to 3.5 Gyr ago (Tehrany *et al.* 2002), i.e during the time span relevant to the emergence of life on Earth. In particular, far ultraviolet (FUV) and UV radiations, in the region 100–400 nm, of the young Sun are expected to have been 5–10 times stronger than at present. This higher energy input would reduce the above time estimates.

The UV irradiation of tyrosine has been studied since the 1960s. Several compounds were reported to form upon irradiation at 254 nm or with a broad-band Hg lamp (Creed 1984; Jin *et al.* 1995). However, the irradiation conditions chosen in the present work to simulate the action of the young Sun on the primordial Earth have led to new results. While the formation of DOPA is confirmed, that of phenylalanine is discovered for the first time. Other compounds with masses 193 and 197 are also observed and their structures hypothesized (see the 'Chromatography-mass spectrometry' section). Among these latter compounds some were already found in previous experiments (Jin *et al.* 1995), while others could represent new findings.

From the spectra in Fig. 1 it can be seen that the peaks of the different absorbance curves do not change appreciably from that of non-irradiated tyrosine at 275 nm, but exhibit broader peaks. This indicates the presence, in the irradiated solutions, of degradation products with absorbance peaks around 275 nm and others with peaks at shorter and longer wavelengths. The broad shape of the curve corresponding to 280 J and the even broader one at 630 J can be accounted for by the presence of DOPA, (coumarine)/indole derivatives, phenylalanine etc, as shown by chromatography-mass spectrometry (see the Chromatography-mass spectrometry section). The absorption coefficient of DOPA is 6200 l mol^{-1} cm^{-1} and its absorbance peak is at 280 nm.

In conclusion, the irradiation of water solutions of tyrosine from 200 to 800 nm, under conditions mimicking the irradiation of the Sun over periods from about 1 h to 2 weeks, has shown that the main degradation products are DOPA, (coumarine)/indole derivatives, phenylalanine, etc. In particular, the concentration of tyrosine decreases to approximately 50% after 3 h (280 J) and to approximately 80% after 7 h (630 J), see Fig. 4, corresponding to an exposure to the Sun of 6 days and 2 weeks, respectively. The degradation products partly maintain life-building capability, as phenylalanine still belongs to the protein amino acid set, and partly do not. By increasing the dose above 600 J most of the tyrosine is transformed into degradation products. From the findings a tendency to complexification is manifest.

In our experiments water was the dominant foreign collision partner of tyrosine, while in the primordial bath there would have been many more. This favoured a larger number of chemical reactions than were included in our simplified model.

The time required by the amino acids to form polypeptide chains leading eventually to proteins was dependent on many factors, such as the concentrations of reactants, the presence of catalysts, chemical conditions, temperature etc. This time had to be compatible with the survival of the amino acids as monomers and/or oligomers against the hostile UV flux from the young Sun. Owing to the wide variety of chemical–physical conditions from one location to another, the life-building process did not occur in a uniform manner. Thus, protected/protective environments such as hydrothermal vents/clay minerals might have been means to extend, when necessary, the survival time of the precursors until the construction of the first proteins were completed.

Similar experiments to those performed on tyrosine are under way on other protein amino acids (Scappini *et al.* 2007).

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References

Barbier, B., Chabin, A., Chaput, D. & Brack, A. (1998). *Planet. Space Sci.* **46**, 391–398.

Barbier, B., Henin, O., Boillot, F., Chabin, A., Chaput, D. & Brack, A. (2002). *Planet. Space Sci.* **50**, 353–359.

Boillot, F., Chabin, A., Buré, C., Venet, M., Belsky, A., Bertrand-Urbaniak, M., Delmas, A., Brack, A. & Barbier, B. (2002). *Origins Life Evol. Biosph.* **32**, 359–385.

Cecchi-Pestellini, C., Scappini, F., Saija, R., Iati, M.A., Giusto, A., Aiello, S., Borghese, F. & Denti, F. (2004). *Int. J. Astrobiol.* **3(4)**, 287–293.

Ciaravella, A., Scappini, F., Franchi, M., Cecchi-Pestellini, C., Barbera, M., Candia, R., Gallori, E. & Micela, G. (2004). *Int. J. Astrobiol.* **3(1)**, 31–35.

Chyba, C.F. (2005). *Science* **308**, 962–963.

Creed, D. (1984). *Photochem. Photobiol.* **39**, 563–575.

Duley, W.W. & Williams, D.A. (1984). *Interstellar Chemistry*. Academic Press, London.

Ehrenfreund, P., Bernstein, M.P., Dworkin, J.P., Sandford, S.A. & Allamandola, L.J. (2001). *Astrophys. J.* **550**, L95–L99.

https://doi.org/10.1017/S1473550407003588 Published online by Cambridge University Press

Jin, F., Leitlich, J. & von Sonntag, C. (1995). *J. Photochem. Photobiol. A: Chemistry* **92**, 147–153.

Lammer, H., Ribas, I., Griessmeier, J.M., Penz, J., Hansmeier, A. & Biernat, H.K. (2004). *Harvard Obs. Bull.* **23**, 139–155.

Miller, S.L. (1953). *Science* **117**, 528–529.

Muñoz Caro, G.M., Melerhenric, U.J., Shutte, W.A., Barbier, B., Arcones Segovia, A., Rosenbauer, H., Tiemann, W.H.-P., Brack, A. & Greenberg, J.M. (2002). *Nature* **416**, 403–406.

Ponnanperuma, C. (1972). *The Origins of Life*. Thames and Hudson Ltd., London.

Sackmann, I.-J. & Boothroyd, A.I. (2003). *Astrophys. J.* **583**, 1024–1039.

Scappini, F., Casadei, F., Zamboni, R., Franchi, M., Gallori, E. & Monti, S. (2004). *Int. J. Astrobiol.* **3**(**1**), 17–19.

Scappini *et al.* (2007). *Int. J. Astrobiol.* (sent for publication).

Tehrany, M.G., Lammer, H., Selsis, F., Ribas, I., Guinan, E.F. & Hansmeier, A. (2002). The particle and radiation environment of the early sun. In *Proc. of the 10th European Solar Physics Meeting, ESA SP-506*, ed. A. Wilson. Noordwijk: ESA Publication Division, pp. 209–212.

Walker, J.C.G. (1977). *Evolution of the Atmosphere*. Macmillan, New York.

Zeilik, M. & Gregory, S.A. (1998). *Astronomy and Astrophysics*. Sounders College Publishing, New York.

https://doi.org/10.1017/S1473550407003588 Published online by Cambridge University Press