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ORIGINAL ARTICLE

Influence of TiO_2 on prebiotic thermal synthesis of the Gly-Gln polymer

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Abstract The role of the titanium dioxide (rutile and anatase) with and without room light on the thermal synthesis of the glycine–L-glutamine (Gly-Gln) polymer is described. The efficiency in percentage of polymerization with room light was increased in 6% in the presence of rutile and in 23% in the presence of anatase. The thermal synthesis in the molten state was carried out in the absence and presence of both oxides. In all cases, the vibrational spectra showed characteristic group frequencies corresponding to a polypeptide structure. No spectral differences were observed by room light effect on the polymer on rutile. However, the polymer obtained in the presence of anatase and room light shows spectral changes associated with the formation of shorter new abundant and conformationally different species compared with the original polymer. The SEM-EDX characterization of the solid phase involved in the thermal synthesis showed that the morphology of the polypeptide is different in the presence of rutile compared to anatase. The SDS–PAGE and GPC results suggest that smaller chains are formed in the presence of both oxides and the distribution of the size and weight of each polymer molecule is completely different when the condensation is performed in the presence of anatase or rutile. Nuclear magnetic resonance analyses confirmed the incorporation of both Gly and Gln residues in the polymers, with a prevalence of Gly. Both possible sequences *N*-GlyGln-*C* and *N*-GlnGly-*C* were also detected.

Keywords Prebiotic chemistry · Thermal synthesis · Vibrational spectroscopy · Titanium dioxide

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Introduction

The peptide bond formation under prebiotic conditions is an important process for the origin of life, as peptides may have been components of the first self-replicating systems. These theories seemed reasonable because many of the building blocks of proteins, amino acids, are easily synthesized under plausible prebiotic conditions (Miller 1955). In principle, the synthesis of peptides involves both the use of condensation agents as catalysts or other different conditions, such as high pressure and temperature (Fouche and Rohlfing 1976; Simoneit 2004) and melting processes (Fouche and Rohlfing 1976; Fox and Harada 1960; Rohlfing and MacAlhaney 1976). The aspect of the enantiomeric purity in these syntheses, and the possible role of

self-disproportion under chromatographic or sublimation conditions have been detailed to define possible scenarios for prebiotic source of enantiomerically enriched amino acids and peptides (Soloshonok 2006; Takahashi et al. 2010; Ueki et al. 2010).

Because water inhibits the formation of peptide bonds, the first step to create a peptide often involves removing water. It has been reported earlier the synthesis of oligopeptides by heating purified amino acids at 150°C for about 14 h (Fox and Harada 1960). At this temperature, water and other volatile compounds vaporize. This is important because the peptide bonding necessarily involves the water formation. These oligomers were called protenoids or thermal proteins (Fox and Harada 1960; Harada and Fox 1958; Brack 2007).

It has been widely recognized that under anhydrous conditions, the heating of a mixture of crystals of the amino acids will undergo polycondensation only in the presence of trifunctional amino acids, such as aspartic acid, glutamic acid, or lysine (Fox and Harada 1960; Mosqueira et al. 2008). Thus, the non-random sequence in the peptides obtained by thermal synthesis can be explained by the formation of diketopiperazines intermediates (Hartmann et al. 1981; Mosqueira et al. 2008).

The catalytic efficiency of minerals for amino acid polymerization has been studied in the context of salt-induced polymer formation mechanism in aqueous phase, and their importance in polymer chain growth has been mentioned in recent experimental studies (Bujdák and Rode 2001; Guo et al. 2006; Hazen 2005; Hazen and Sverjensky 2010; Kawamura et al. 2009; Lambert 2008; Meng et al. 2004; Plankensteiner et al. 2004; Rode et al. 1999; Schoonen et al. 2004; Simoneit 2004). On the other hand, the influence of a mineral surface on the thermal condensation of amino acids has not been deeply evaluated and only a few studies have been devoted to study the influence of a mineral on the thermal synthesis of peptides (Lambert 2008; Leyton et al. 2011; Napier and Yin 2006; Rohlfing and MacAlhaney 1976; Schoonen et al. 2004).

In this context, titanium oxides deserve interest as potential primary condensates detected in oxygen-rich dusty environments (Posch et al. 2003; Zeidler et al. 2011). Rutile and anatase (TiO_2) detected in cosmic dust could be considered to play an important role in dust formation in oxygen-rich stars (Hazen and Sverjensky 2010). Furthermore, due to its wide range of functions, titanium dioxide as a visible light photocatalyst is rapidly finding applications in various fields, because it requires near-ultraviolet rays with relatively long wavelengths contained in sunlight and emitted by fluorescent lamps to initiate a reaction (Amemiya 2004).

First proteins involved in the formation of living cells could have been condensed phases of the protenoids, which could have been formed from amino acids, at the surfaces of minerals similar to rutile or anatase. In this work, the reaction in the molten state between glycine and L-glutamine in the absence and presence of titanium dioxide as rutile and anatase were performed with and without room light, in the general frame of evaluating the influence of these oxides on the polymer obtained.

Materials and methods

Reagents

L-Glutamine; glycine; titanium (IV) oxide, rutile (particle size <100 nm, specific surface area 130–190 m^2/g); titanium (IV) oxide, anatase (particle size <25 nm, specific surface area 200–220 m^2/g); L-glycyl-glycine and glycyl-L-glutamine. All reagents are commercially available with >99% of purity, and were used as received.

Thermal condensation

Polymeric glycyl-L-glutamine [Gly-Gln polymer] was obtained using a modification of the Harada and Fox (1958) method. A reaction mixture composed of 0.01 mol of L-glutamine and 0.02 mol of glycine was ground together in a mortar. This mixture was heated and melted in an air open flask at 175–190°C during 1 h using a heating jacket at 1 atm and without agitation. In the experiments performed in the presence of mineral, 0.1 g of each titanium (IV) oxide was used. In the experiments conducted in the absence of room light the flask was covered with foil. When the reaction was completed a brown liquid that solidified upon cooling was obtained. For vibrational measurements, the solid residue was washed with 10 mL of deionized water and was allowed to settle down during 24 h. The solid obtained was centrifuged, dried at 50°C and weighted for yield calculation. Each experiment was repeated several times obtaining always a positive Biuret response and the same IR spectra.

Infrared and Raman spectra

Infrared (IR) spectra were measured by using a Fourier transform infrared (FT-IR) Perkin Elmer Spectrum RX spectrometer provided with a DTGS (deuterated triglycerine sulfate) detector. The spectral resolution was 4 cm^{-1} and 16 scans were obtained on each sample. KBr pellets were prepared by mixing 1.0 mg of solid sample and 200 mg of KBr.

The Raman spectra were recorded with a Renishaw Raman Microscope System RM1000 equipped with a diode laser providing a 634 nm line, a Leica microscope, an

electrically cooled CCD (charge coupled device) detector and a notch filter to eliminate the elastic scattering. The spectra were obtained by using a 100× objective. The laser power output was 2.0 mW and the spectral resolution was 2.0 cm^{-1}.

Scanning electron microscopy and energy dispersive X-ray analysis

The samples were homogenized by grinding and the powder samples were fixed on an adhesive carbon band. A scanning electron microscope (SEM) JEOL JSM6360 LV coupled to an energy dispersive X-ray (EDX) spectrometer Inca Oxford was used for the analysis. SEM photographs were taken at 1,000× and 2,000×.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis analysis

30 mg of the samples were dissolved in 1.0 mL of 50 nM sodium phosphate buffer pH 7.5. The insoluble fraction was separated by microcentrifugation 5 min at 14,000 rpm. The soluble fraction was dialyzed using a 3.5 kDa membrane molecular weight cut off against 100 volumes of sodium phosphate buffer. Then, 3.0 mg of fluorescamine were added. 15 μL of each sample were mixed with loading buffer solution and load onto the gel and electrophoresed according to Lagos et al. (2001). The polyacrylamide gel (PAG) was prepared as a discontinuous gradient of acrylamide concentration: 3, 10 and 16%. A peptide molecular weight marker from Sigma® (2–17 kDa) was used.

Gel permeation chromatography (GPC) analysis

About 10 mg of each sample was suspended in 1.0 mL of tetrahydrofuran (THF) and stirred during 15 min, thus it was allowed to rest 5 min to obtain a clear supernatant. A fraction of supernatant was collected and filtered before the analysis. The analyses were performed using a Shimadzu LC 20AT liquid chromatography equipped with two columns connected in series (Varian PL gel MIXED-D 5 μm, 1-40K and PL gel MIXED-D 5 μm, MW 500-20K) and an SPD M20A ultraviolet diode array (UV) detector set at 280 nm, using THF as eluant at a flow rate of 0.50 mL min^{-1}.

The GPC system was calibrated against polystyrene standards (molecular weight range of 890–1.86 × 10^6 g mol^{-1}).

Nuclear magnetic resonance analyses

Samples were analyzed in deuterated water (D_2O) at room temperature with a Bruker (400 MHz) spectrometer after esterification (methyl ester formation) at the end-carboxylic tails with methanol and trimethylsilyl chloride. In brief, the desired amount of sample was dissolved in methanol (5.0 mL) and added of a large excess of trimethylsilyl chloride (2.0 equivalents with respect to the substrate; the equivalents of substrate were calculated assuming the molecular weight obtained by GPC analysis). After 48 h, the excess of reagents was evaporated under reduced pressure, and the crude was analyzed without any further purification.

Theory/calculation

Theoretical infrared and Raman spectra were calculated from density functional theory (DFT). The structure and vibrational frequencies of the Gly-Gln dipeptide were determined using the Gaussian 03 W program package (Frisch et al. 2003). The Becke's three-parameter hybrid method using the Lee–Yang–Parr correlation functional (B3LYP) together with the 6-31G** basis set were used in the geometry optimization and calculations of the normal modes. At the optimized structure of the dipeptide, no imaginary frequencies were obtained, providing that a local minimum on the potential energy surface was found.

Results and discussion

The thermal polymerization was performed in the molten state. It was not possible obtaining, according to the experimental conditions used, poly-glycine and poly-L-glutamine directly from the thermal synthesis. Some authors have reported the thermal decomposition of glycine and L-glutamine at their melting point (Wesolowski and Erecinska 2005).

Vibrational assignments

The bands assignment was performed on the basis of published data of related molecules (Baran and Ratajczak 2005; Dhamelincourt and Ramirez 1993; Fischer et al. 2005; Kumar et al. 2005; La'Verne et al. 2009; Ramirez et al. 1998; Vijay and Sathyanarayana 1992), and general characteristic group frequencies (Barth 2007; Colthup et al. 1990; Pearson and Slifkin 1972; Socrates 2000). Theoretical infrared and Raman frequencies are in good agreement with the experimental corresponding data. Moreover, the results allowed completing the experimental band assignment and distinguishing overlapped components in broad bands.

The infrared and Raman spectra of glycine and L-glutamine aminoacids and of the product obtained from the thermal synthesis in the absence and in the presence of titanium oxides and room light are shown in Figs. 1 and 2, respectively. The spectra for all the products obtained are

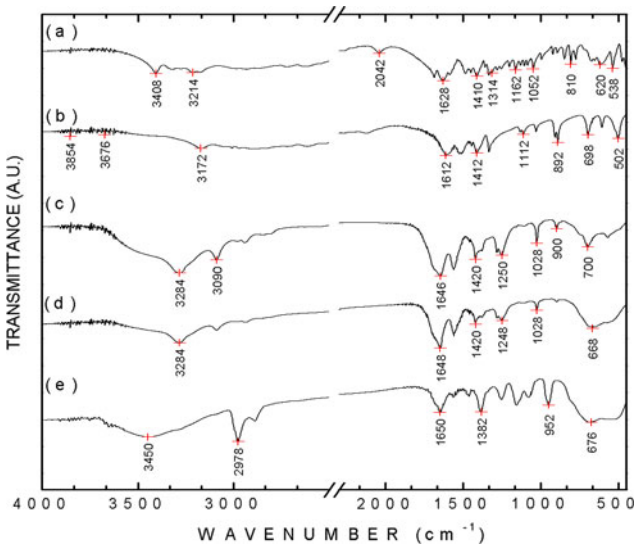

Fig. 1 Infrared spectra in KBr pellet obtained for **a** L-glutamine (Gln), **b** glycine (Gly), **c** polymer obtained by thermal synthesis in absence of titanium oxides, **d** polymer obtained by thermal synthesis in the presence of rutile and room light, **e** polymer obtained by thermal synthesis in the presence of anatase and room light

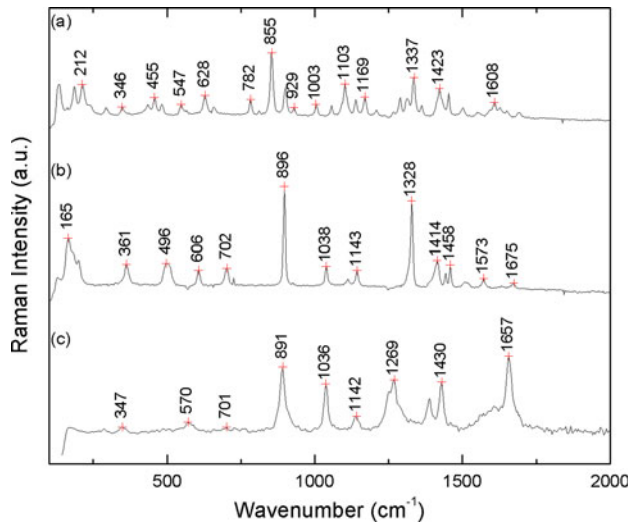

Fig. 2 Raman experimental spectra obtained for **a** L-glutamine (Gln), **b** glycine (Gly), **c** polymer obtained by thermal synthesis in absence of titanium oxides

very similar and clearly showed the appearance of strong bands at 1,646 cm^{-1} in the infrared spectrum and at 1,657 cm^{-1} in the Raman spectrum, it was assigned to amide I and arises mainly from the C=O stretching vibration, with minor contributions from the out of phase CN stretching vibrations, CCN deformation and NH in-plane bending. The band at 1,561 cm^{-1} in the infrared spectrum corresponds to the amide II mode (δ(NH) + ν(CN)). The bands located at 1,284 cm^{-1} in IR and 1,269 cm^{-1} in the Raman spectrum correspond to the amide III mode. This mode resulted from the in-phase combination of NH bending and CN stretching vibrations, with small contributions from the δCO and νCC modes. These bands appeared at characteristic wavenumbers usually assigned to a polypeptide with the α form (Socrates 2000). However, the expected strong intensity band associated with the skeletal mode in the 900–960 cm^{-1} region in the Raman spectra of the polypeptide obtained in the absence of titanium oxides is not observed, suggesting that the polypeptide displays a random coil structure (Carey and Salares 1980). Circular dichroism results revealed that the proteinoids obtained under these experimental conditions do not have optical activity, confirming the random polypeptide structure suggested by vibrational analysis (data not shown). In addition, the absorptions located at 3,284 and 3,090 cm^{-1} were assigned to ν_a(N–H) and ν_s(N–H) modes, respectively. On the other hand, the infrared spectra of the polymer obtained in the presence of anatase and room light (Fig. 1e) shows additional bands as compared to the IR spectra in the absence of titanium oxides and in the presence of rutile and room light (Fig. 1c, d). Table 1 lists the observed infrared and Raman frequencies and the proposed band assignments for the polymers obtained from the synthesis in the absence and presence of TiO_2 and light.

In the presence of anatase and room light several spectral changes were observed. Several bands ascribed to the CH, amine and skeletal modes increased in intensity 2,978, 1,382, 1,155, 1,075 and 952 cm^{-1}; in addition, the carboxylate stretching band at 1,420 cm^{-1} and the amide II band 1,560 cm^{-1} decreased in intensity. These spectral behaviors suggest that new polymeric species with a different conformation, coexisting with the original random structure poly Gly-Gln are present.

In Fig. 3, the experimental Raman spectra obtained for the standard dipeptides glycyl-glycine and glycyl-L-glutamine and the calculated spectra obtained by using B3LYP/6-31G** basis set for glycyl-L-glutamine are shown. A comparison of these spectra with the Raman spectra obtained in the absence of titanium oxides, allows proposing that the predominant sequence in the polymer is Gly-Gln.

This preferential sequence can be produced through diketopiperazine (DKP) rings between glycine and L-glutamine indicating that the formation of the peptide bonds proceeds through the nucleophilic attack of an amino group of the amino acids on the DKP accompanied by the ring opening (Basiuk et al. 1992; Kawamura et al. 2009; Mosqueira et al. 2008; Parrish and Mathias 2002). For this reason, there was not observed bands associated with DKP ring breathing vibrations that usually appear at 914 and 809 cm^{-1} in the infrared spectra of the product obtained (Cheam and Krimm 1984a, b). Previous studies reporting the dimerization of glycine by pyrolysis do not describe the

Table 1 Experimental data from the vibrational spectra of the polymers obtained by thermal synthesis

Polymer		Polymer/rutile		Polymer/anatase		Vibrational assignment	Comments
IR (cm^{-1})	Raman (cm^{-1})	Without room light IR (cm^{-1})	With room light IR (cm^{-1})	Without room light IR (cm^{-1})	With room light IR (cm^{-1})		
n.d.	n.d.		n.d.	n.d.	3,450 s, br	ν(OH)	
3,284 s	3,291 m	3,287 s	3,284 s	3,289 s	3,286, w	$\nu_a(NH_2)$	
3,090 m	n.d.	3,089 m	3,089 m	3,088 m	3,086, w	$\nu_s(NH_2)$	
2,979 vw	2,978 m	2,972 vw	n.d.	2,972 w	2,978 s	$\nu_a(CH_2)$	
2,941 w	2,948 s	2,934 w	2,937 w	2,932 vw	n.d.	$\nu_s(CH_2)$	
1,646 vs	1,657 s	1,649 vs	1,648 vs	1,649 vs	1,650 s	ν(C = O) + ν(CN) + δ(NH)	Amide I
1,561 s	1,562 w	1,560 s	1,559 s	1,560 s	1,560 w	δ(NH) + ν(CN)	Amide II
1,464 vw	n.d.	1,462 vw	1,461 vw	1,463 vw	1,461 m	$\delta(CH_2)$	
1,420 s	1,430 m	1,420 m	1,420 s	1,420 m	1,423 w,sh	ν_s(COOH)	
1,380 m,sh	1,387 m	1,379 sh	1,378 sh	1,379 sh	1,382 s	$\omega(CH_2)$	
1,284 sh	1,269 m	1,281 sh	1,283 sh	1,280 sh	n.d.	ν(CN) + δ(NH) + ν(CO) + δ(O = CN)	Amide III
1,250 m	1,249 sh	1,249 m	1,248 m	1,249 m	1,250 m	$\rho(NH_2)$	
1,154 vw	1,142 w	1,155 vw	1,154 vw	1,157 vw	1,155 s	$\rho(NH_2)$ + $\tau(NH_2)$	
n.d.	n.d.	n.d.	n.d.	n.d.	1,075 m	ν(C–N)	
1,028 m	1,036 m	1,028 m	1,028 m	1,028 m	1,027 vw	ν(C–N) + ν(C–C)	
n.d.	n.d.	n.d.	n.d.	953 w	952 s	γ (OH)	
900 w	891 s	900 w	899 vw	900 w	n.d.	ν_s(CNC)	
700 s	701 vw	696 sh	695 sh	697 sh	698 sh	δ(NC = O)	Amide IV
n.d.	n.d.	670 m, br	668 m, br	676 m, br	676 m, br	ν(TiO)	Rutile/anatase
567 w	570 w	566 sh	565 sh	566 sh	562 sh	δ(C = O)	Amide VI
n.i.	347 w	n.i.	n.i.	n.i.	n.i.	δ(skel)	

v stretching, *as* antisymmetric, *s* symmetric, δ bending, ρ rocking, ω wagging, τ torsion, γ out of the plane bending, *vw* very weak, *w* weak, *m* medium, *br* broad, *sh* shoulder, *s* strong, *vs* very strong, *n.d.* non detected, *n.i.* non-investigated

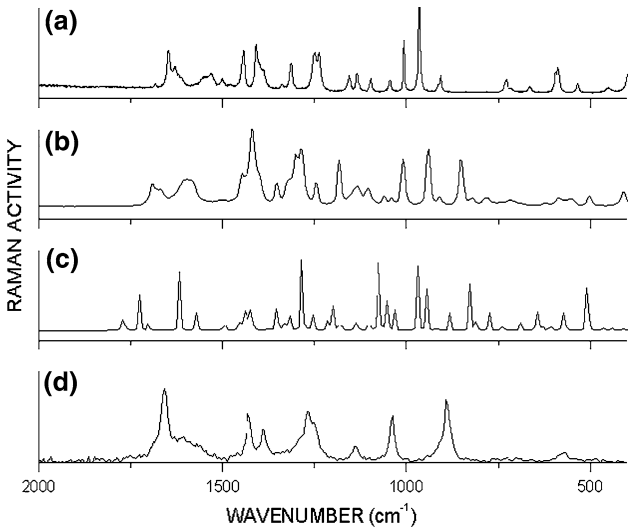

Fig. 3 Raman spectra of **a** L-glycyl-glycine dipeptide p.a.; **b** glycyl-L-glutamine p.a.; **c** glycyl-L-glutamine dipeptide calculated spectra by using B3LYP/6-311G** and **d** polymer obtained by thermal synthesis in the absence of titanium oxides

formation of L-glutamine DKP type compounds (Choi and Ko 2010). In this context, the synthesis of lineal peptides instead of diketopiperazines when two or more amino acids are heated has been reported (Fouche and Rohlfing 1976; Fox and Harada 1960; Harada and Fox 1958; Hartmann et al. 1981; Meng et al. 2004; Rohlfing and MacAlhaney 1976).

Concerning to the mechanism, vibrational data suggest that the interaction between the amino acids and the TiO_2 surface is rather physical: neither frequencies shift nor intensity modifications were observed in the vibrational spectra as compared to the polymer obtained in the absence of titanium oxides. Probably, irrespective of the phase used in the experiments (rutile vs. anatase), the acid Lewis sites present on the TiO_2 surface can catalyze the formation of the reactive diketopiperazine intermediates, recently it has been reported the acidic properties of TiO_2 (Akizuki et al. 2011). In accordance to this hypothesis, examples on the role of acidic catalysis in the formation of diketopiperazines have been recently reported in the literature (Yamazaki et al. 2009).

Scanning electron microscopy: energy dispersive X-ray analysis

Scanning electron microscopy pictures of the sample of the polymer obtained in the presence of titanium oxides and room light are shown in Fig. 4. The elemental mapping analysis made on the samples (data available on request) revealed that white particles have signals of Ti and O while gray particles correspond to C, N and O atoms. Taking into consideration, the elemental analysis, the microphotographies obtained at 1,000× and 2,000× show small particles of rutile (white particles) (Fig. 4a, b) dispersed between globular particles of polypeptide (gray particles) of about 5–10 μm. This result shows that, after thermal treatment, all particles of the Gly-Gln polymer on rutile have similar size and morphology. On the other hand, the microphotographies obtained at the same magnifications show that polymer particles obtained on anatase (Fig. 4c, d), are smaller and do not show any particular morphology at the same magnification. In this case is noted that small white particles of titanium dioxide are dispersed also between the polypeptide particles.

SEM-EDX characterization of the solid phase involved in the thermal synthesis showed that in both cases, titanium dioxide particles are dispersed between polypeptide particles. Moreover, the polymer obtained in the presence of rutile and room light has globular morphology, which was not observed in the presence of anatase and room light. The polymerization percentage was increased in 6–23% in the presence of room light and oxides, respectively. In the absence of light, the polymerization percentage increased just in 5% approximately in the presence of both oxides (data not shown). These results suggest that, during the thermal treatment, titanium dioxide particles act as nucleation site for the Gly-Gln polymer.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

In Fig. 5, the results obtained by SDS-PAGE are shown. As can be seen, all samples have a population of peptides located between a wide range of molecular weight between 23.5 and 6.5 kDa with an average of 15 kDa, and another signal below 2.51 kDa. Samples obtained in the absence of light have slightly higher molecular weights. The samples are characterized by the presence of a small band of very low molecular weight which probably corresponds to a small peptide that could not be removed during dialysis.

GPC analysis

Figure 6 shows the GPC analyses of the polymers obtained in the absence and in the presence of titanium oxides. The weight average molecular weight of novel polymers (Table 2) appears to be higher in the absence of titanium dioxide both in the rutile and anatase phases ($\overline{M_W} = 4.2$ kDa). On the other hand, irrespective of the experimental conditions, polymerizations performed in the presence of titanium dioxide were

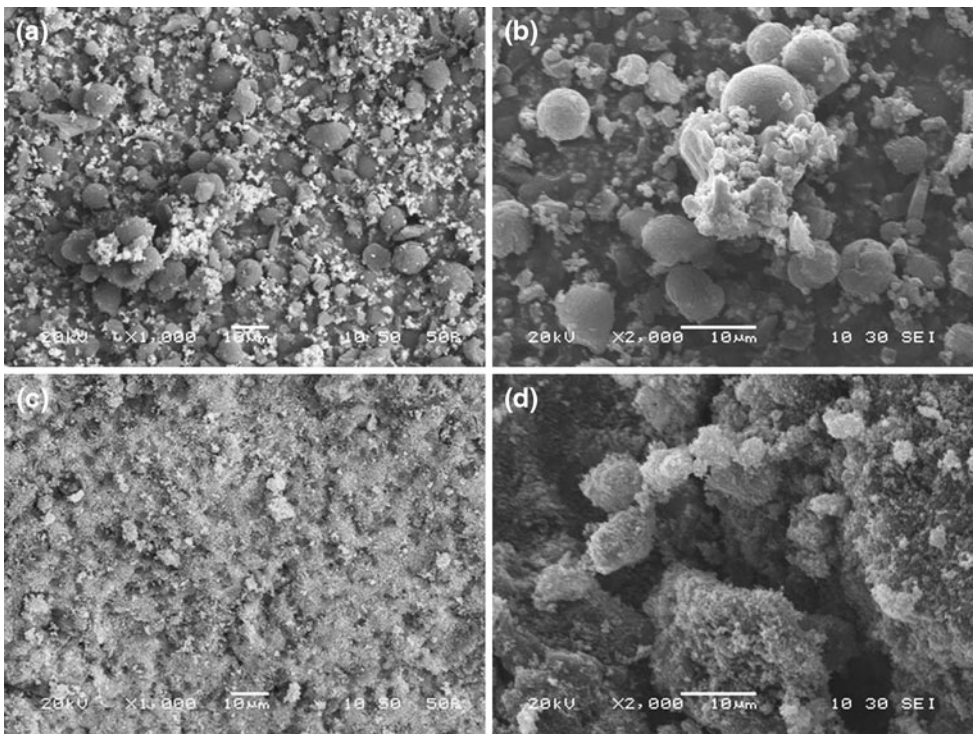

Fig. 4 SEM microphotography at 2,000× on the polypeptide obtained by thermal synthesis in the presence of room light and rutile: **a** 1,000×, **b** 2,000× and in the presence of room light and anatase, **c** 1,000×, **d** 2,000×

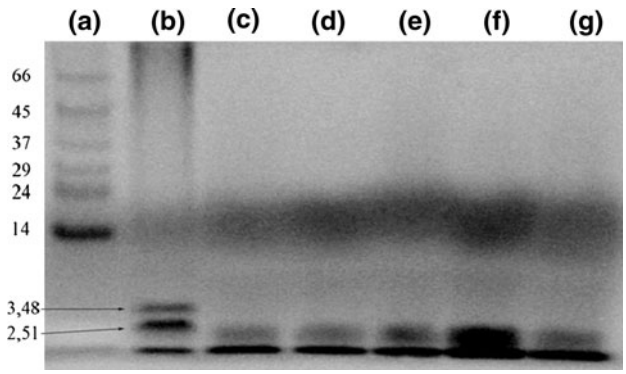

Fig. 5 SDS-PAGE results for **a** and **b** molecular weight markers, **c** polymer obtained by thermal synthesis in the absence of titanium oxides and room light, **d** polymer obtained by thermal synthesis in presence of anatase and room light, **e** polymer obtained by thermal synthesis in the presence of anatase and absence of room light, **f** polymer obtained by thermal synthesis in the presence of rutile and room light, **g** polymer obtained by thermal synthesis in the presence of rutile and absence of room light

more selective, affording smaller oligopeptides ($\overline{M_W} = 0.6 - 1.0$ kDa). About the effect of the light, the values of number average molecular weight ($\overline{M_N}$) increased in the presence of light for both titanium dioxide phases. Instead, a different reaction pattern was observed for anatase and rutile phases in the case of the value of $\overline{M_W}$. In fact $\overline{M_W}$ increased in the presence of rutile but decreased in the presence of anatase (this is reflected also in the polydispersity index).

The SDS-PAGE and GPC results showed an increase in the peptide chain length in the absence of titanium dioxide; allowing for the postulation that a greater number of polymer chains of shorter length were formed in the presence of rutile and anatase compared with the reference. Furthermore, the polydispersity index is lower in the presence of titanium oxides.

On the other hand, irrespective of the experimental conditions, polymerizations performed in the presence of titanium dioxide were more selective, affording smaller oligopeptides. In principle, this reaction pattern might be due to a selective degradation of the initially produced bigger polymer, even if a specific effect of the titanium dioxide surface on the polymerization process cannot be completely ruled out. The same effect was found in the presence of another oxide (Leyton et al. 2011). Therefore, the distribution of the size and weight of each polymer molecule in the family of compounds analyzed is completely different when the condensation was performed in the presence of anatase or rutile. The surface area and particle size of rutile and anatase used in this study can explain these results; on the anatase larger surface area, a greater number of smaller chains were obtained.

If it is considered that the predominant sequence in the polypeptide obtained is Gly-Gln (molecular weight

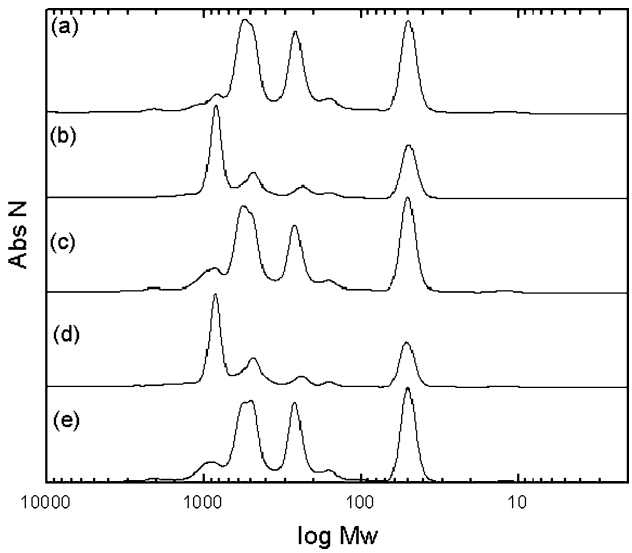

Fig. 6 Gel permeation chromatography of **a** polymer obtained by thermal synthesis in absence of titanium oxides and room light, **b** polymer obtained by thermal synthesis in the presence of anatase and room light, **c** polymer obtained by thermal synthesis in presence of anatase and absence of room light, **d** polymer obtained by thermal synthesis in the presence of rutile and room light, **e** polymer obtained by thermal synthesis in the presence of rutile and absence of room light

Table 2 Weight average ($\overline{M_W}$) and number average ($\overline{M_N}$) molecular weights and polydispersity index ($\overline{M_W}/\overline{M_N}$) of polymer samples obtained from thermal synthesis

Sample	$\overline{M_W}$	$\overline{M_N}$	$\overline{M_W}/\overline{M_N}$
Polymer	4,160.89	207,330.7	49.82
Polymer/anatase/room light	888.46	9,423.18	10.60
Polymer/anatase	707.58	10,236.75	14.46
Polymer/rutile/room light	978.03	12,548.20	12.83
Polymer/rutile	585.48	6,220.34	10.62

221.2 g/mol), then from the $\overline{M_W}$ data are possible to calculate the approximate number of dipeptide units in each sample. According to these calculations, it can be concluded that in the synthesis of Gly-Gln polymer in the absence of the titanium oxides, there was obtained linear chains of about 19 Gly-Gln units, while in the presence of rutile or anatase and room light, the linear chains contain around four dipeptide units.

Proton nuclear magnetic resonance studies

^{1}H-NMR analyses were performed in deuterated water (D_2O) at room temperature with a Bruker (400 MHz) spectrometer after esterification (methyl ester formation) at the end-carboxylic tails with methanol and trimethylsilyl

chloride. The spectrum of the sample obtained in the presence of rutile without irradiation (Fig. 7a) showed the presence of all the expected signals for the glycine and L-glutamine residues. In particular, the CH_2-β and CH_2-γ proton signals of the Gln residues appeared as multiple signals in the range of 2.1–2.7 ppm, while the CH_2-α proton signal of Gly residues was a broad singlet centered at 4.1 ppm. The ratio of the integrals of these signals, normalized on the basis of the number of the respective adsorbing nuclei, furnished an estimated value of the ratio Gly:Gln = 2.85:1.0 (that is a total amount of Gly c.a. threefold than Gln). Moreover, two signals were detected for the CH-α proton signal in Gln, at 3.4 and 4.3 ppm, respectively. These data are coherent with the presence of two possible sequences, *N*-GlyGln-*C* and *N*-GlnGly-*C*, in the polymer. On the basis of the ratio of the integrals of these signals and according to NMR model simulations (ACD NMR simulation software) was possible to tentatively assign a prevalence for the *N*-GlyGln-*C* sequence (*N*-GlyGln-*C*:*N*-GlnGly-*C* = 1.0:0.3). A different pattern was observed for the rutile sample after irradiation (Fig. 7b). In this latter case, the Gly:Gln ratio was 1.8:1.0 (that is an amount of Gly only twice than Gln) suggesting a major incorporation of Gln in the polymers. About the prevalent sequence motif, a reversed pathway was detect, and the *N*-GlnGly-*C* sequence appeared to be the most frequently represented in the polymeric chains (*N*-GlyGln-*C*:*N*-GlnGly-*C* = 1.0:3.9). In the case of the sample produced with anatase without irradiation, the CH_2-β and CH_2-γ proton signals of the Gln residues (1.9–2.45 ppm), as well as the CH_2-α proton signal of Gly residues (broad singlet centered at 3.85 ppm) were detected in regions of the NMR spectra similar to that previously observed for samples from rutile. The Gly:Gln ratio was 3.0:1.0 with a prevalence of the *N*-GlnGly-*C* sequence (*N*-GlyGlu-*C*:*N*-GluGly-*C* = 2.0:7.0). Similar results were obtained for the anatase sample after irradiation which showed a Gly:Gln ratio value of c.a. 3.4:1.0, the *N*-GlnGly-*C* sequence being again the most abundant in the polymeric chains.

The NMR data have been also used to calculate the polymerization degree of peptides, on the basis of the signals referred to the methyl ester groups selectively introduced on the terminal carboxylic moieties of oligomers (they appeared as well defined sharp singlets in the range of 3.80–3.85 ppm depending on the nature of the sample). The polymerization degree was obtained calculating the ratio between the sum of the integrals of the signals referring to Gln and Gly units and that of the methyl ester groups, all of the data being normalized on the basis of the number of the respective adsorbing nuclei. The data obtained confirmed the general pattern previously observed by GPC analysis. In particular, peptides produced without irradiation showed a higher polymerization degree (c.a. 7.0 and 9.0, for rutile and anatase, respectively) than that in the presence of irradiation (c.a. 5.0 and 7.0, for rutile and anatase, respectively). Moreover, some differences observed in the absolute value of the polymerization degree calculated by GPC or NMR analyses can be explained by the presence of possible aggregation processes operative during the GPC analysis.

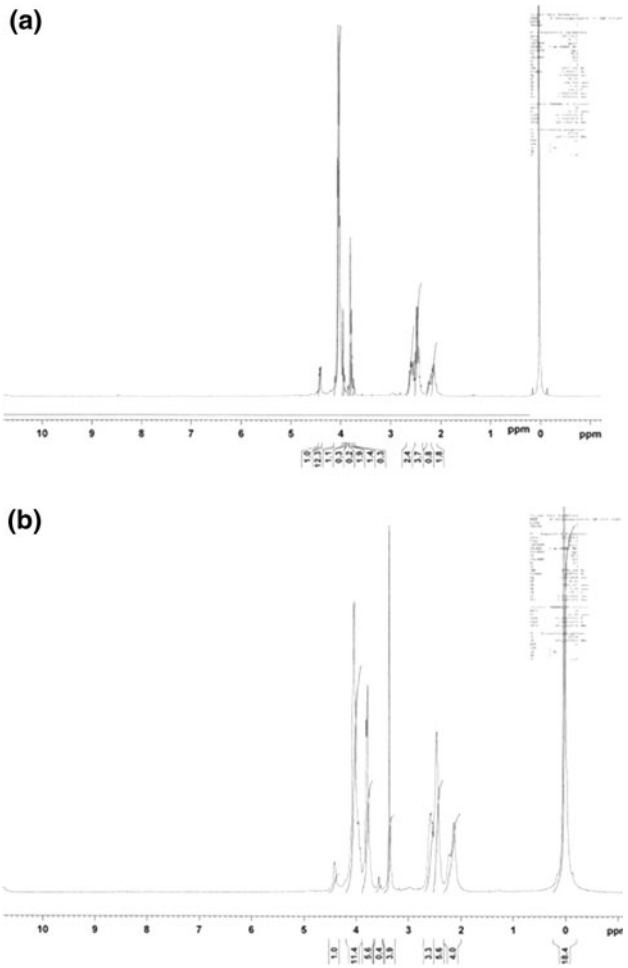

Fig. 7 ^{1}H-NMR spectrum of the polymer obtained by thermal synthesis in the presence of rutile and **a** room light and **b** absence of room light

Conclusions

The thermal polymerization was performed in the molten state. In this case, the reaction products were mainly determined based on the temperature of the melting process. The results suggest that the polymerization of glycine and L-glutamine amino acids occurs also in the absence of mineral surfaces. The efficiency in percentage of polymerization was increased in 6% in the presence of rutile and 23% in the presence of anatase. The thermal synthesis in the molten state was carried out in the absence and

presence of the both oxides. In all cases, the vibrational spectra showed characteristic group frequencies corresponding to a polypeptide structure with random coil. No spectral differences were observed by room light effect on the polymer on rutile. In the presence of anatase and room light several spectral changes were observed suggesting that new polymeric species with a different conformation coexisting with the original random structure poly Gly-Gln are present. The observed spectral bandwidth increasing in the synthetic polypeptide is in agreement with a polymeric structure. The theoretical spectral data support the experimental proposed Gly-Gln main sequence for the polymer. The SEM-EDX characterization of the solid phase involved in the thermal synthesis showed that the morphology of the polypeptide is different in the presence of rutile as compared to those obtained in the presence of anatase. The polydispersity index is lower when the condensation is performed in the presence of titanium oxides. Nuclear magnetic resonance analyses confirmed the incorporation of both Gly and Gln residues in the polymers, with a prevalence of Gly (the amount of which depending on the experimental conditions used). Both possible sequences *N*-GlyGln-*C* and *N*-GlnGly-*C* were also detected.

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References

Akizuki M, Tomita K, Oshima Y (2011) Kinetics of solid acid catalyzed 1-octene reactions with TiO_2 in sub- and supercritical water. J Supercritical Fluids 56(1):14–20

Amemiya S (2004) Titanium-oxide photocatalyst. Three Bond Tech News 62:1–8

Baran J, Ratajczak H (2005) Polarised IR and Raman spectra of the γ-glycine single crystal. Spectrochim Acta Part A 61:1611–1626

Barth A (2007) Infrared spectroscopy of proteins. Biochim Biophys Acta 1767:1073–1101

Basiuk VA, Gromovoy TY, Chuiko AA, Soloshonok VA, Kukhar VP (1992) A novel approach to the synthesis of symmetric optically active 2, 5-dioxopiperazines. Synthesis 1992(05):449–451

Brack A (2007) From interstellar amino acids to prebiotic catalytic peptides: a review. Chem Biodiversity 4:665–679

Bujdák J, Rode BM (2001) Activated alumina as an energy source for peptide bond formation: consequences for mineral-mediated prebiotic processes. Amino Acids 21:281–291

Carey PR, Salares VR (1980) Raman and resonance Raman studies of biological systems. In: Clark RJH, Hester RE (eds) Advances in infrared and Raman spectroscopy, vol 7. Heyden & Sons Ltd., London, pp 1–45

Cheam TC, Krimm S (1984a) Vibrational analysis of crystalline diketopiperazine-I. Raman and i.r. spectra. Spectrochim Acta Part A Mol Spectroscopy 40(6):481–501

Cheam TC, Krimm S (1984b) Vibrational analysis of crystalline diketopiperazine-II. Normal mode calculations. Spectrochim Acta Part A Mol Spectroscopy 40(6):503–517

Choi S-S, Ko J-E (2010) Dimerization reactions of amino acids by pyrolysis. J Anal Appl Pyrol 89:74–86

Colthup NB, Daly LH, Wiberley SE (1990) Introduction to infrared and Raman spectroscopy, 3rd edn. Academic Press, London

Dhamelincourt P, Ramirez FJ (1993) Polarized micro-Raman and FT-IR spectra of L-glutamine. Appl Spectroscopy 47(4):446–451

Fischer G, Cao X, Cox N, Francis M (2005) The FT-IR spectra of glycine and glycylglycine zwitterions isolated in alkali halide matrices. Chem Phys 313:39–49

Fouche CE Jr, Rohlfing DL (1976) Thermal polymerization of amino acids under various atmosphere or at low pressures. Biosystems 8:57–65

Fox SW, Harada K (1960) The thermal copolymerization of amino acids common to protein. J Am Chem Soc 82(14):3745–3751

Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Montgomery JA Jr, Vreven T, Kudin KN, Burant JC, Millam JM, Iyengar SS, Tomasi J, Barone V, Mennucci B, Cossi M, Scalmani G, Rega N, Petersson GA, Nakatsuji H, Hada M, Ehara M, Toyota K, Fukuda R, Hasegawa J, Ishida M, Nakajima T, Honda Y, Kitao O, Nakai H, Klene M, Li X, Knox JE, Hratchian HP, Cross JB, Adamo C, Jaramillo J, Gomperts R, Stratmann RE, Yazyev O, Austin AJ, Cammi R, Pomelli C, Ochterski JW, Ayala PY, Morokuma K, Voth GA, Salvador P, Dannenberg JJ, Zakrzewski VG, Dapprich S, Daniels AD, Strain MC, Farkas O, Malick DK, Rabuck AD, Raghavachari K, Foresman JB, Ortiz JV, Cui Q, Baboul AG, Clifford S, Cioslowski J, Stefanov BB, Liu G, Liashenko A, Piskorz P, Komaromi I, Martin RL, Fox DJ, Keith T, Al-Laham MA, Peng CY, Nanayakkara A, Challacombe M, Gill PMW, Johnson B, Chen W, Wong MW, Gonzalez C, Pople JA (2003) Gaussian03. Revision B.0, 4 edn. Gaussian Inc., Pittsburg

Guo L, Meng M, Zha Y (2006) Adsorption and thermal condensation of L-α-glutamic acid on Al_2O_3. Cuihua Xuebao 27:189–194

Harada K, Fox SW (1958) The thermal condensation of glutamic acid and glycine to linear peptides. J Am Chem Soc 80(11): 2694–2697

Hartmann J, Christel Brand M, Dose K (1981) Formation of specific amino acid sequences during thermal polymerization of amino acids. Biosystems 13:141–147

Hazen RM (2005) Genesis: rocks, minerals, and the geochemical origin of life. Elements 1:135–137

Hazen RM, Sverjensky DA (2010) Mineral surfaces, geochemical complexities, and the origins of life. Cold Spring Harbor Perspect Biol 2(5):a002162

Kawamura K, Takeya H, Kushibe T (2009) Effect of condensation agents and minerals for oligopeptide formation under mild and hydrothermal conditions in related to chemical evolution of proteins. Adv Space Res 44:267–275

Kumar S, Rai AK, Singh VB, Rai SB (2005) Vibrational spectrum of glycine molecule. Spectrochim Acta Part A 61:2741–2746

La'Verne SJ, Srivastava S, Srivastava S, Gupta VD (2009) Vibrational dynamics of poly(L-glutamine). J Appl Polym Sci 113:1406–1414

Lagos R, Baeza M, Corsini G, Hetz C, Strahsburger E, Castillo JA, Vergara C, Monasterio O (2001) Structure, organization and characterization of the gene cluster involved in the production of microcin E492, a channel-forming bacteriocin. Mol Microbiol 42(1):229–243

Lambert JF (2008) Adsorption and polymerization of amino acids on mineral surfaces: a review. Orig Life Evol Biosph 38(3):211–242

Leyton P, Zárate RA, Fuentes S, Paipa C, Gómez-Jeria JS, Leyton Y (2011) Influence of aluminum oxide on the prebiotic thermal synthesis of Gly-Glu-$(Gly\text{-}Glu)_n$ polymer. Biosystems 104(2–3):118–126

Meng M, Stievano L, Lambert JF (2004) Adsorption and thermal condensation mechanisms of amino acids on oxide supports. 1. Glycine on silica. Langmuir 20:914–923

Miller SL (1955) Production of some organic compounds under possible primitive earth conditions. J Am Chem Soc 77:2351–2361

Mosqueira FG, Ramos-Bernal S, Negrón-Mendoza A (2008) Prebiotic thermal polymerization of crystals of amino acids via the diketopiperazine reaction. BioSystems 91:195–200

Napier J, Yin J (2006) Formation of peptides in the dry state. Peptides 27:607–610

Parrish DA, Mathias LJ (2002) Five- and six-membered ring opening of pyroglutamic diketopiperazine. J Org Chem 67:1820–1826

Pearson JF, Slifkin MA (1972) The infrared spectra of amino acids and dipeptides. Spectrochim Acta Part A Mol Spectroscopy 28(12):2403–2417

Plankensteiner K, Righi A, Rode BM, Gargallo R, Jaumot J, Tauler R (2004) Indications towards a stereoselectivity of the salt-induced peptide formation reaction. Inorg Chim Acta 357:649–656

Posch T, Kerschbaum F, Fabian D, Mutschke H, Dorschner J, Tamanai A, Henning T (2003) Infrared properties of solid titanium oxides: exploring potential primary dust condensates. Astrophys J Suppl Ser 149:437–445

Ramirez FJ, Tunon I, Silla E (1998) Amino acid chemistry in solution: structural study and vibrational dynamics of glutamine in solution: an ab initio reaction field model. J Phys Chem B 102:6290–6298

Rode BMS HL, Suwannachot Y, Bujdak J (1999) The combination of salt induced peptide formation reaction and clay catalysis: A way to higher peptides under primitive earth conditions. Orig Life Evol Biosph 29:273–286

Rohlfing DL, MacAlhaney WW (1976) The thermal polymerization of amino acids in the presence of sand. Biosystems 8:139–145

Schoonen M, Smirnov A, Cohn C (2004) A perspective on the role of minerals in prebiotic synthesis. Ambio 33(8):539–551

Simoneit BRT (2004) Prebiotic organic synthesis under hydrothermal conditions: an overview. Adv Space Res 33:88–94

Socrates G (2000) Infrared and Raman characteristic group frequencies. Tables and charts, 3rd edn. Wiley, Chichester

Soloshonok VA (2006) Remarkable amplification of the self-disproportionation of enantiomers on achiral-phase chromatography columns. Angew Chem Int Ed 45:766–769

Takahashi M, Tanabe H, Nakamura T, Kuribara D, Yamazaki T, Kitagawa O (2010) Atropisomeric lactam chemistry: catalytic enantioselective synthesis, application to asymmetric enolate chemistry and synthesis of key intermediates for NET inhibitors. Tetrahedron 66(1):288–296

Ueki H, Yasumoto M, Soloshonok VA (2010) Rational application of self-disproportionation of enantiomers via sublimation—a novel methodological dimension for enantiomeric purifications. Tetrahedron Asymmetr 21(11–12):1396–1400

Vijay A, Sathyanarayana DN (1992) Theoretical study of the ground-state vibrations of nonionized glycine. J Phys Chem 96:10735–10739

Wesolowski M, Erecinska J (2005) Relation between chemical structure of amino acids and their thermal decomposition. Analysis of the data by principal component analysis. J Therm Anal Calorim 82:307–313

Yamazaki Y, Mori Y, Oda A, Okuno Y, Kiso Y, Hayashi Y (2009) Acid catalyzed monodehydro-2, 5-diketopiperazine formation from N-α-ketoacyl amino acid amides. Tetrahedron 65(18): 3688–3694

Zeidler S, Posch T, Mutschke H, Richter H, Wehrhan O (2011) Near-infrared absorption properties of oxygen-rich stardust analogs. The influence of coloring metal ions. Astron Astrophys 526:A68