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Formation and self-assembly of long prebiotic oligomers produced by the condensation of unactivated amino acids on oxide surfaces**

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Dedication: This work is dedicated to Luca Daveggio, talented student who decided to devote his Master Thesis to the extension of this research, but the fate decided differently for his young life

Abstract: Here, we report *in-situ* IR and mass spectrometry evidence for the catalytic formation on SiO₂ and TiO₂ surface of glycine oligomers (poly-Gly) long up to 16 terms by successive feedings with monomers from the vapor phase. Parallel experiments carried out using another material, namely hydroxyapatite, resulted in an unreactive adsorption of Gly, indicating that the oligomerization observed for the two oxides was not dependent on the adsorption method adopted.

Furthermore, poly-Gly moved on the surface when contacted with H₂O vapor, forming self-assembled aggregates containing both helical and β -sheet-like structural motifs. Hence, our results indicated that polypeptide formed by condensation of amino acids adsorbed on a mineral surface can evolve in structured supramolecular assembly.

The polymerization of amino acids under abiotic conditions is among the topics addressed when investigating the origin of life.^[1] Since the seminal Bernal's hypothesis, the possibility of mineral surfaces acting as adsorbents for amino acids and as catalysts for their condensation to form peptides has been considered.^[2] Accordingly, understanding of the molecular events involved in the condensation reaction between amino acids, both activated and unactivated, at the surface of various solids has been significantly advanced.^[3, 4] However, the following issues remain unresolved: i) the limited extent of the catalytic oligomerization (up to 6 monomers) under non-thermal conditions when unactivated amino acids are considered^[5] and ii) the mobility of the oligomers on the catalytic surface to form organized structures.^[3]

Furthermore, the catalytic formation of long polypeptides from unactivated amino acids could be of interest for new synthetic strategies aimed at avoiding poor atom economy reagents, in addition to well-established solid-phase peptide synthesis methods.^[6]

In a previous study, we revealed the role of the (101) and (001) facets of anatase TiO₂ nanoparticles in activating adsorbed carboxylate moieties toward a nucleophilic attack of amines to form amides.^[7] In the present study, we extend the investigation to the adsorption of glycine (Gly) vapors (with molecules in the nonionic HOOC-CH₂-NH₂ form) by adopting the experimental procedure established for adsorbing Gly onto hydroxyapatite (HA) nanoparticles.^[8] The adsorption from aqueous solution is the process most relevant to prebiotic chemistry; however, results obtained by adsorbing amino acids from the gas phase can be significant, either for elucidating the features of particular adsorption modes or confirming the reaction pathway of the adsorbed amino acids when the water activity is decreased via drying (e.g., under prebiotic conditions, by exposure to the sun).^[3] Gly was among the products of the Miller experiment, which examined the production of amino acids under possible primitive Earth conditions,^[9] and it has been considered a reference molecule in a large number of experimental and theoretical investigations regarding the adsorption of amino acids on mineral matter.^[3, 10, 11] Titania was considered a model substrate in several studies that were primarily concerned with the adsorption of amino acids onto mineral oxides from both the aqueous and the gas phases.^[3] Moreover, TiO₂ may have actually been present on prebiotic Earth.^[12] However, we studied also amorphous SiO₂ (see Materials in the SI) due to its importance in the abiotic polymerization of amino acids.^[3, 13] The primary results for the admission of Gly vapors onto HA (for comparison), SiO₂ and TiO₂ are summarized in Figure 1.

For HA (curve a), the spectral profile resulting from the adsorbed Gly arose from the monomeric amino acid molecules in the zwitterionic form.^[8] In contrast, the spectra collected for both the SiO₂ and TiO₂ (curves b and c, respectively) after contact with glycine exhibited components in the 1700–1650 and 1560–1530 cm⁻¹ ranges, which can be identified as the amide I and amide II bands typical of peptide species.^[14] Moreover, for Gly/SiO₂ and Gly/TiO₂, one could observe that these signals are accompanied by a partner at 3310/3307 cm⁻¹ (Figure 1, inset), which can be assigned to the N-H stretching in polyglycine (poly-Gly) species.^[15-17] Finally, a weak component appeared at 1760 cm⁻¹ in the Gly/SiO₂ spectrum and was assigned to the ν C=O mode of the grafted glycine $\equiv\text{Si}_{\text{surf}}\text{-O-C(=O)-CH}_2\text{NH}_2$ species (the so-called “surface mixed anhydride”).^[18, 19]

The exposure of the HA and TiO₂ to the Gly vapor had to be limited to ensure that less than a monolayer of amino acid molecules was adsorbed (Figure S1 in the SI), in order to observe only the results of Gly molecules-surface interaction. Conversely, SiO₂ was significantly less active in retaining the Gly molecules and the spectrum recorded corresponded to the maximum amount of Gly retained by the silica surface. After Gly adsorption, the

samples were removed from the IR cell and washed with ultrapure water (extraction yield: ~60% see SI, Figure S2). The resulting solution was analyzed via high-resolution mass spectrometry (HR-MS) (see Methods in the SI). As expected, only Gly molecules in their monomeric form ($m/z = 76$; the additional unit with respect the actual m/z of Gly is due to the protonation of the molecules as required by the experimental method, see the SI) were present in the extract from the Gly/HA system (Figure 2, top), whereas peptides containing 3 ($m/z = 190$) to 11 ($m/z = 646$) monomers and 2 ($m/z = 133$) to 16 ($m/z = 931$) monomers were detected in the aqueous phases resulting from washing the Gly/SiO₂ and Gly/TiO₂, respectively (Figure 2, center and bottom). The signal at $m/z = 115$ present in the Gly/TiO₂ system is due to the cyclic Gly-Gly dimers, diketopiperazine.

The absence of reactivity in the Gly/HA indicated that the temperature reached by the system during the Gly adsorption (403 K) was insufficient to allow a thermal polymerization of the amino acids,^[20, 21] which would be expected in the presence of trifunctional amino acids.^[22]

Because the same experimental conditions were adopted to adsorb Gly onto the other two samples, the formation of peptides on SiO₂ and TiO₂ clearly indicates that these materials exhibit catalytic activity toward the formation of poly-Gly. The catalytic activity of TiO₂ in the formation of long polypeptides was observed by Leyton et al.,^[21] whose work operated in a thermal polymerization regime (448–463 K) with the amino acids in the molten state.

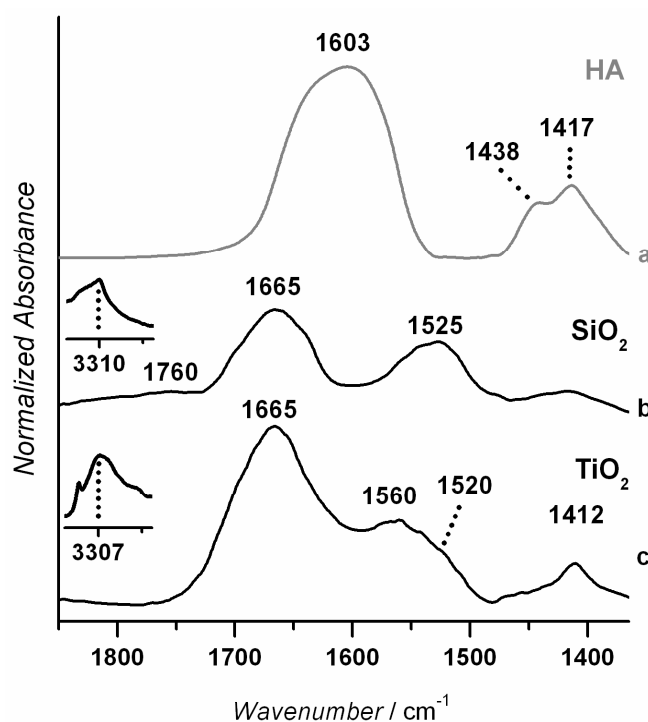


Figure 1. IR spectra resulting from the adsorption of Gly from the vapor phase on HA, SiO₂ and TiO₂. Inset: 3500-3000 cm⁻¹ range of the Gly/TiO₂ spectrum. The intensity of the spectra has been normalized with respect the specific surface area of the materials and the optical thickness (mg·cm⁻²) of the self-supporting pellets used for the measurements. In the case of SiO₂, the spectrum reported corresponds to the maximum amount of Gly that was possible to adsorb on the material. For the estimation of surface coverage by Gly on HA and TiO₂ see Figure S1 in the SI. The spectra are reported in Absorbance having subtracted the spectra of the materials obtained before Gly adsorption as background.

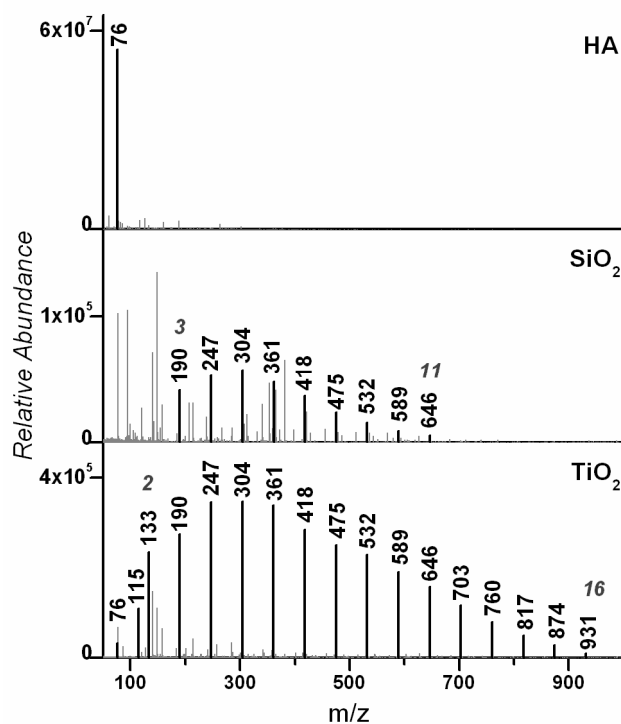


Figure 2. HR-MS spectra of the solutions resulting from the washing with pure water of the samples produced by adsorbing Gly from the vapor phase on HA (top), SiO₂ (middle), TiO₂ (bottom). Numbers on the black bars are the m/z values (without decimal digits; complete data in Figure S3 in the SI) of (-Gly)_n peptides. Consecutive signals are separated by an m/z value of 57, which corresponds to a -(C=O)-CH₂-NH- unit. The number of monomers present in the shortest and longest peptide detected for Gly/SiO₂ and Gly/TiO₂ is indicated above the correspondent signal. In all cases the grey bars are the m/z values due to water contaminants.

As for the catalytic mechanism, TiO₂ has already demonstrated an ability to activate adsorbed carboxylates toward amidation,^[17] and determining the mechanistic steps that occur during amidation on silica surfaces will be an extension of this work. Due to its ability to adsorb a larger quantity of Gly, with the consequent advantages for analyzing the products, TiO₂ was also sequentially exposed to Gly and Glycine-1-¹³C (¹³Gly) vapors. The detection of (Gly)_n-(¹³Gly)_m oligomers (see Figure S4 and Table S1 in the SI) clearly indicated the effectiveness of successive monomer “feedings,” similar to the polymerization of activated amino acids and nucleotides on minerals performed by Ferris et al.^[11]

Based on the idea of “fluctuating environments”,^[23] Gly/SiO₂ and Gly/TiO₂ systems with as-formed polyglycines were exposed to water vapor to investigate the molecular surface events that occur upon hydration. Figure 3 presents the results of the in situ IR study of the evolution of Gly/TiO₂, while those obtained for the Gly/SiO₂ are reported in Figure S5 in the SI.

In the presence of water vapor most part of the spectral pattern of surface Gly and poly-Gly was overshadowed by the signals due to the stretching and deformation modes of H₂O molecules adsorbed in multilayers (not shown).

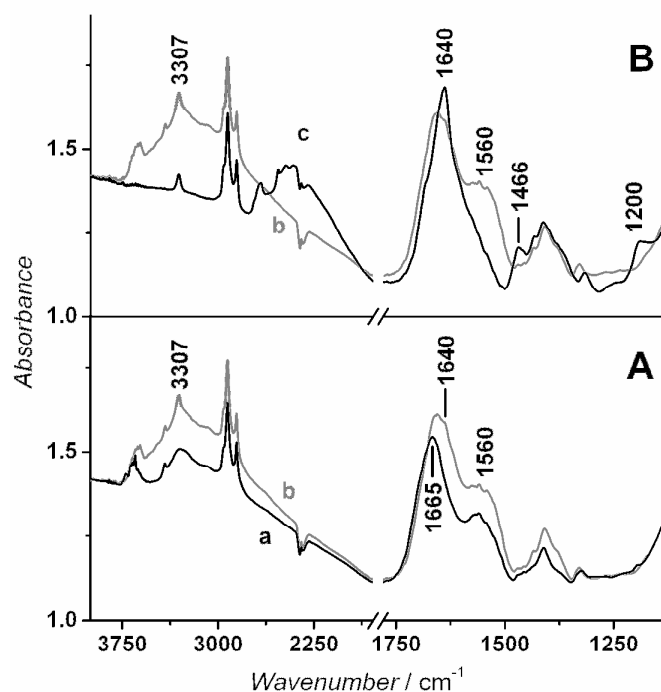


Figure 3. IR spectra of (a) the last step of glycine sublimation on TiO₂, (b) after contact with water vapor pressure and subsequent outgassing for 30 minutes at beam temperature (ca. 323 K, b.t.), (c) after H/D exchange and then outgassing of D₂O for 30 minutes at b.t..

However, by outgassing the excess of water it was possible to observe that the hydration of the sample resulted in a narrowing of the band at 3307 cm⁻¹ due to the N-H stretching in the peptide units, accompanied by the appearance of a sub-band at 1640 cm⁻¹ in the νC=O range at the expense of the high frequency side of the component at 1665 cm⁻¹ (panel A, curves a,b). Moreover, with subsequent D₂O adsorption/desorption cycles (panel B), these signals downshifted as expected (the νD₂O band appearing in the 2800–2000 cm⁻¹ range and the weak δD₂O band appearing at ~1200 cm⁻¹), with an even narrower peak remaining at 3307 cm⁻¹ that appeared almost unaffected. The complex amide II signal at 1560 cm⁻¹ simultaneously exhibited a significant decrease in intensity,^[24] but did not disappear completely, and the amide II' appeared at 1466 cm⁻¹ due to deuterated peptide linkages.^[24] Furthermore, the amide I band with a maximum at 1640 cm⁻¹ underwent a further modification in shape and intensity, suggesting the occurrence of some decoupling with vibrations involving N-H moieties, now transformed in N-D. The appearance of narrower components and the resistance of part of the νN-H and amide II bands to the H/D exchange reflect the movement of poly-Gly on the surface to form more ordered structures (with a series of structurally similar C=O...H-N interactions) that are intra- and/or inter-molecularly tightly packed enough to prevent diffusion of the D₂O molecules. Polyglycines are characterized by a high hydrophobicity,^[25] and the previously described behavior can be explained through the self-assembly of the poly-Gly species formed on the surface by contact with the water molecules.

Moreover, the spectrum obtained after outgassing the excess of adsorbed H₂O molecules was compared with the IR spectra of hexaglycine in the poly-Gly I (β-sheet conformation) and poly-Gly II (helical conformation)^[15] (Figure 4). Hexaglycine was examined because it is the longest Gly oligomer commercially available in pure form. The Gly/TiO₂ spectrum (curve a) exhibits components similar in position to the signals present in the spectral profiles of both the poly-Gly I and II forms (curves b and c, respectively). The comparison focused on the amide I and amide II band regions because these features differed allowing distinction between the two poly-Gly forms.^[15] Differences with respect the selected references should also arise from the presence of Gly oligomers where the number of monomers varies from 6. Interestingly, peptide chains with β-sheet structures have been reported as more resistant to hydrolysis than those with random coil or helical conformations, likely resulting in an extended lifetime and may have been utilized in the first life.^[20]

In conclusion, to the best of our knowledge, this study is the first to observe the sequential occurrence of (i) the catalytic formation of polypeptides (poly-Gly) up to 16 units long on a surface via successive monomer feedings from a vapor phase and (ii) the self-assembling of the formed polypeptides into closely packed aggregates containing both helical and β-sheet-like patches. Both aspects can provide useful insights on the general problem of the surface reactivity of amino acids, and, in particular, on the formation in pre-biotic times of biopolymers and subsequent self-organization as a consequence of changes in environmental conditions.

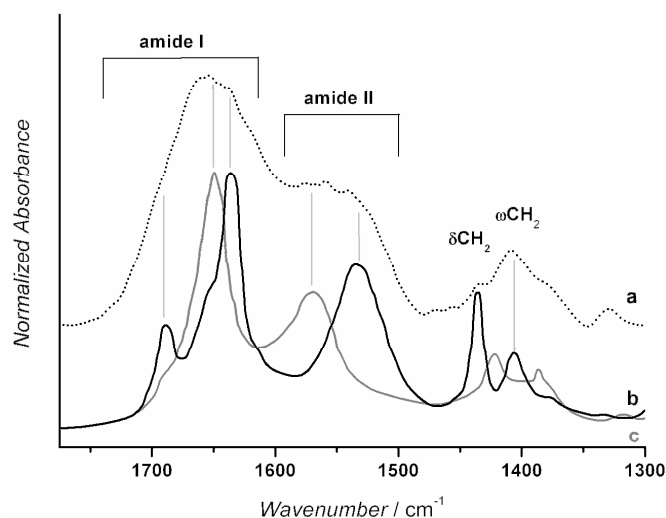


Figure 4. IR spectra of (a) Gly adsorbed on TiO₂, subsequent contact with water vapor pressure and outgassing (same as curve *b* in Figure 3); (b) poly-Gly I and (c) poly-Gly II hexaglycine. Spectra (b) and (c) were adapted from ref.^[15] Amide I: stretching C=O of amide group; amide II: coupling between the C-N stretching mode and C-N-H in-plane deformation mode of amide group; δCH_2 : bending mode of CH₂ group; ωCH_2 : wagging mode of CH₂ group.

Experimental Section

Experimental Details are available in the Supporting Information.

Keywords: amino acids · catalytic polymerization · silica · titania · self-assembly

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Supporting information for this article is available on the WWW under <http://dx.doi.org/10.1002/anie.201xxxxx>. It contains description of the materials, the IR and HRMS methods, and additional results, that is: IR spectra of increasing amounts of Gly on TiO₂ and evaluation of coverage; IR spectra of Gly adsorbed on HA, SiO₂ and TiO₂ and evaluation of extraction yield; HR-MS spectra of the solutions resulting from the washing with complete decimal digits; HR-MS spectra of surface products obtained after ¹²Gly and ¹³Gly sublimation on TiO₂; IR spectra of H₂O and D₂O adsorbed on Gly/SiO₂ system.