Angewandte Chemie

DOI: 10.1002/ange.201311089

Amino Acid Chemistry | Hot Paper

The Formation and Self-Assembly of Long Prebiotic Oligomers Produced by the Condensation of Unactivated Amino Acids on Oxide Surfaces**

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Dedicated to Luca Daveggio

Abstract: *In situ IR and mass spectrometry evidence for the catalytic formation on SiO_2 and TiO_2 surfaces of glycine oligomers (poly-Gly) up to 16 units long by successive feeding with monomers from the vapor phase is presented. Parallel experiments carried out on hydroxyapatite resulted in the unreactive adsorption of Gly, thus indicating that the oligomerization was specifically catalyzed by the surfaces of SiO_2 and TiO_2. Furthermore, the poly-Gly moved on the surface when contacted with H_2O vapor and formed self-assembled aggregates containing both helical and β-sheet-like structural motifs. These results indicate that polypeptides formed by the condensation of amino acids adsorbed on a mineral surface can evolve into structured supramolecular assemblies.*

The polymerization of amino acids under abiotic conditions is among the topics that need to be addressed when investigating the origin of life.[1] Since Bernal's seminal 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: 1) the limited extent of the catalytic oligomerization (up to 6 monomers) when unactivated amino acids are considered[5] and 2) 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 reagents with poor atom economy, as well as for well-established solid-phase peptide synthesis methods.[6]

In a previous study, we revealed the role of the (101) and (001) facets of anatase TiO_2 nanoparticles in activating adsorbed carboxylate moieties toward nucleophilic attack by amines to give amides.[7] Herein, we extend the investigation to the adsorption of glycine (Gly) vapor (with molecules in the nonionic HOOC-CH_2-NH_2 form) by adopting the experimental procedure established for adsorbing Gly onto hydroxyapatite (HA) nanoparticles.[8] 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 through drying (e.g., by exposure to the sun under prebiotic conditions).[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 onto mineral matter.[3,10,11] TiO_2 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, TiO_2 may have actually been present on prebiotic Earth.[12] However, we also studied amorphous SiO_2 (see Materials in the Supporting Information) because of its importance in the abiotic polymerization of amino acids.[3,13] The primary results for the adsorption of Gly vapor onto HA (for comparison), SiO_2, and TiO_2 are summarized in Figure 1.

For HA, the spectral profile resulting from the adsorbed Gly arose from monomeric amino acid molecules in the zwitterionic form.[8] By contrast, the spectra collected for SiO_2 and TiO_2 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/SiO_2 and Gly/TiO_2, these signals are accompanied by a partner at 3310/3307 cm^{-1} (Figure 1, insets), 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/SiO_2 spectrum. This component was initially assigned to the $\nu C{=}O$ mode of grafted glycine $\{Si_{surf}{-}O{-}C({=}O){-}CH_2NH_2\}$ species (the so-

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[**] The University of Torino and the Compagnia di San Paolo are acknowledged for funding (Project No. ORTO11RRT5). The authors are grateful to Prof. P. Ugliengo for fruitful discussion.

Supporting information for this article, including experimental details, is available on the WWW under http://dx.doi.org/10.1002/anie.201311089.

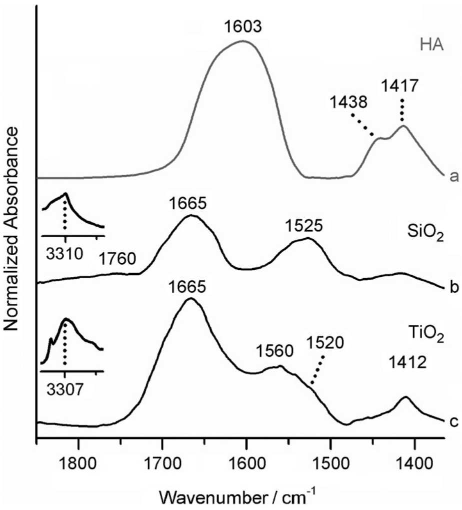

Figure 1. IR spectra resulting from the adsorption of Gly from the vapor phase on HA (a), SiO_2 (b) and TiO_2 (c). Inset: 3500–3000 cm^{-1} range of the Gly/TiO_2 spectrum. The intensity of the spectra has been normalized with respect the specific surface area of the materials and the optical thickness (mg cm^{-2}) of the self-supporting pellets used for the measurements. The spectra of the materials obtained before Gly adsorption were subtracted as background. In the case of SiO_2, 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 TiO_2, see Figure S1.

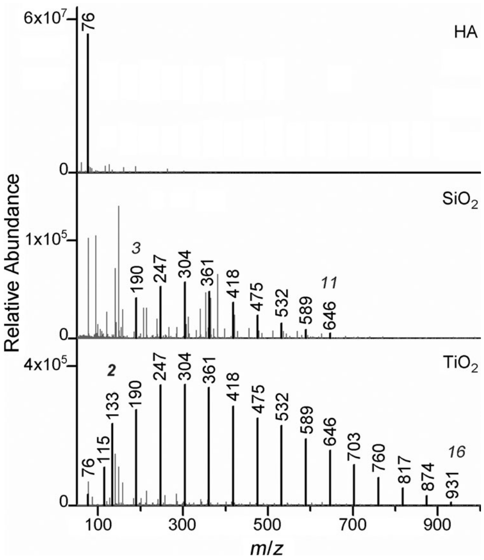

Figure 2. HR-MS spectra of the solutions resulting from washing (with pure water) of the samples produced by adsorbing Gly from the vapor phase onto HA (top), SiO_2 (middle), TiO_2 (bottom). Numbers on the black bars are the *m*/*z* values (without decimal digits; complete data in Figure S3) of (-Gly-)$_n$ peptides. Consecutive signals are separated by an *m*/*z* value of 57, which corresponds to a {-(C=O)−CH$_2$−NH-} unit. The number of monomers present in the shortest and longest peptide detected for Gly/SiO_2 and Gly/TiO_2 is indicated in italics above the corresponding signal. In all cases, the grey bars are the *m*/*z* values resulting from water contaminants.

called "surface mixed anhydride"),[18,19] whereas more recently, it has been related to specific H bonding between the C=O of the carboxylic groups and surface silanols.[20,21]

In order to observe only the results of the Gly–surface interaction, the exposure of the HA and TiO_2 to the Gly vapor had to be limited to ensure that less than a monolayer was adsorbed (Figure S1 in the Supporting Information). By contrast, SiO_2 was significantly less active in retaining the Gly molecules and the spectrum recorded corresponds 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%; Figure S2). The resulting solution was analyzed by high-resolution mass spectrometry (HR-MS; see Methods in the Supporting Information). 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 Supporting Information) were present in the extract from the Gly/HA system (Figure 2, top), whereas peptides containing 3 ($m/z = 190$) to 11 ($m/z = 646$) monomer units and 2 ($m/z = 133$) to 16 ($m/z = 931$) monomer units were detected in the aqueous phases resulting from washing the Gly/SiO_2 and Gly/TiO_2 systems, respectively (Figure 2, center and bottom). The signal at $m/z = 115$ present in the Gly/TiO_2 system is due to cyclic Gly–Gly dimers (diketopiperazines).

Because the same experimental conditions were adopted for adsorbing Gly onto the three materials, the formation of peptides on SiO_2 and TiO_2 clearly indicates that these oxides exhibit catalytic activity for the formation of poly-Gly. The catalytic activity of TiO_2 in the formation of long polypeptides was observed by Leyton et al.,[22] whose work operated in a higher temperature range (448–463 K versus 403 K in the present case) with the amino acids in the molten state, that is, in conditions under which uncatalyzed thermal polymerization also occurs at a significant rate.

As for the catalytic mechanism, TiO_2 has already been demonstrated to activate adsorbed carboxylates toward amidation,[7] and determining the mechanistic steps that occur during amidation on silica surfaces will be an extension of this work. Because of its ability to adsorb a larger quantity of Gly, with the consequent advantages for analyzing the products, TiO_2 was also sequentially exposed to Gly and Glycine-1-^{13}C (^{13}Gly) vapor. The detection of (Gly)$_n$-(^{13}Gly)$_m$ oligomers (see Figure S4 and Table S1 in the Supporting Information) clearly indicates the effectiveness of successive monomer "feedings". A similar process was also reported by Ferris et al.,[1] but in that case, the polymerization of activated amino acids and nucleotides on minerals was achieved by adding an excess of carbonyl diimidazole as an activator.

Based on the idea of "fluctuating environments",[23] Gly/SiO_2 and Gly/TiO_2 systems with as-formed poly-Gly 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/TiO_2 on exposure to water, while those obtained for Gly/SiO_2 are reported in Figure S5.

In the presence of water vapor, most of the spectral pattern of surface Gly and poly-Gly was overshadowed by the signals arising from the stretching and deformation modes of H_2O molecules adsorbed in multilayers (data not shown).

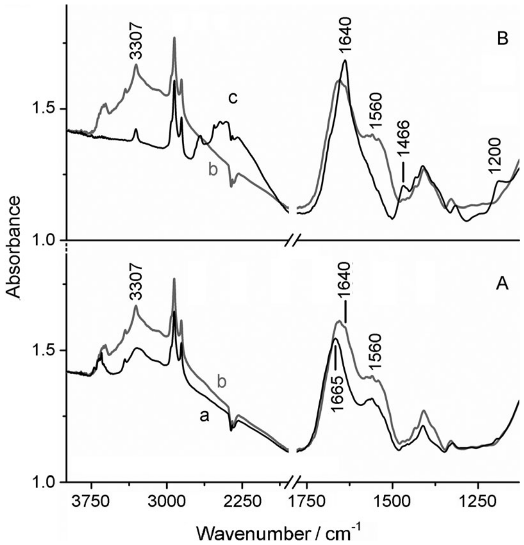

Figure 3. IR spectra measured directly after glycine sublimation on TiO_2 (a), after subsequent contact with water vapor and outgassing for 30 min at beam temperature (ca. 323 K; b), and following H/D exchange and then outgassing of the D_2O for 30 min at beam temperature (c).

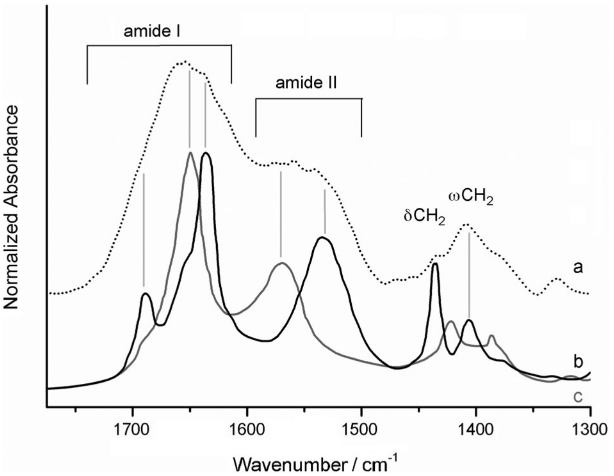

Figure 4. IR spectra of Gly adsorbed on TiO_2 after subsequent contact with water vapor pressure and outgassing (a; same as curve b in Figure 3); poly-Gly I hexaglycine (b), and poly-Gly II hexaglycine (c). Spectra (b) and (c) were adapted from Ref. [15]. Amide I: C=O stretch of the amide group; amide II: coupling between the C–N stretching mode and C–N–H in-plane deformation mode of the amide group; δCH_2: bending mode of CH_2 group; ωCH_2: wagging mode of CH_2 group.

However, by outgassing the excess water, it was possible to observe that the hydration of the sample resulted in a narrowing of the band at 3307 cm^{-1} that arises from the N–H stretching in the peptide units, accompanied by the appearance of a sub-band at 1640 cm^{-1} in the $\nu C=O$ range at the expense of the high frequency side of the component at 1665 cm^{-1} (Figure 3, panel A). Moreover, with subsequent D_2O adsorption/desorption cycles (Figure 3, panel B), these signals shifted to a lower wavenumber as expected (the νD_2O band appeared in the 2800–2000 cm^{-1} range and the weak δD_2O band appeared at ca. 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} exhibited a significant decrease in intensity but did not disappear completely, and the amide II′ signal appeared at 1466 cm^{-1} as a result of deuterated peptide linkages.[24] Furthermore, the amide I band with a maximum at 1640 cm^{-1} underwent a further modification in shape and intensity, thus suggesting the occurrence of some decoupling with vibrations involving N–H moieties, now transformed into 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 packed tightly enough to prevent diffusion of the D_2O molecules. Polyglycines are characterized by high hydrophobicity[25] and the previously described behavior could be explained by the self-assembly of the poly-Gly species formed on the surface through contact with the water molecules.

Moreover, the spectrum obtained after outgassing the excess of adsorbed H_2O molecules was compared with the IR spectra of hexaglycine in poly-Gly I (β-sheet) and poly-Gly II (helical) conformations[15] (Figure 4). Hexaglycine was examined because it is the longest Gly oligomer commercially available in pure form. The Gly/TiO_2 spectrum (Figure 4, curve a) exhibits components similar in position to the signals present in the spectral profiles of both the poly-Gly I (curve b) and ploy-Gly II (curve c) forms. The comparison focused on the amide I and amide II band regions because these features differed, thus allowing the differentiation of the two poly-Gly forms.[15] Differences with respect to the selected references should also arise from the presence of Gly oligomers in which the number of monomer units has a value other than six. Interestingly, peptide chains with β-sheet structures have been reported as being more resistant to hydrolysis than those with random-coil or helical conformations, a difference that likely results in an extended lifetime and may have been utilized by primordial life.[26] Moreover, it has been reported that β-sheet structures could operate as stereoselective templates in the formation of homochiral peptides.[27,28]

In conclusion, to our knowledge, this study is the first to report the sequential occurrence of 1) the catalytic formation of polypeptides (poly-Gly) up to 16 units long on a surface through successive monomer feedings from the vapor phase and 2) the self-assembly of the formed polypeptides into closely packed aggregates containing both helical and β-sheet-like patches. Both occurrences can provide useful insights into the general problem of the surface reactivity of amino acids, and in particular, into the formation of biopolymers in prebiotic times and their subsequent self-organization as a consequence of changes in environmental conditions.

Received: December 20, 2013

Keywords: amino acids · catalytic polymerization · self-assembly · silica · titanium dioxide

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[28] Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201xxxxxx. It contains description of the materials, the IR and HRMS methods, and additional results, that is: IR spectra of increasing amounts of Gly on TiO_2 and evaluation of coverage; IR spectra of Gly adsorbed on HA, SiO_2 and TiO_2 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 ^{12}Gly and ^{13}Gly sublimation on TiO_2; IR spectra of H_2O and D_2O adsorbed on Gly/SiO_2 system.