Thermolysis

Hypercondensation of an Amino Acid: Synthesis and Characterization of a Black Glycine Polymer

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Abstract: A granular material was obtained by thermal polymerization of glycine at 200 °C. It has been named “thermomelanoid” because of its strikingly deep-black color. The polymerization process is mainly a dehydration condensation leading to conventional amide bonds, and also C=C double bonds that are formed from C=O and CH₂ groups (“hypercondensation”). Spectroscopic data, in particular from ¹³C and ¹⁵N solid-state cross-polarization magic angle spinning (CP-MAS) NMR spectra, suggest that the black color is due to (cross-)conjugated C=C, C=O, and NH groups. Small glycine peptides, especially triglycine, appear to be key intermediates in the formation of the thermomelanoid. This has been concluded by comparing the thermal behavior of glyₙ homopeptides (n = 2–6) and glycine. The glycine polymerization was accompanied by the formation of small amounts of byproducts. Notably, a few percent of alanine and aspartic acid could be detected in the polymer. By using ¹³C-labeled glycine, it was shown that these two amino acids formed through a common pathway, namely C≠C→C bond formation between glycine molecules. The thermomelanoid is hydrolyzed by strong acids and bases at room temperature, forming brown solutions.

Introduction

Peptide formation is arguably the most important chemical reaction of α-amino acids. However, under mild conditions, this reaction does not proceed spontaneously. In water at room temperature, for example, it is both thermodynamically and kinetically unfavorable.[1] In ribosomal and non-ribosomal peptide biosynthesis, this problem has been overcome by chemical activation.[2] Similarly, laboratory (e.g., solid-phase “Merrifield”) syntheses use coupling reagents such as carboimidides to facilitate the formal elimination of water and thus the formation of the amide bond.[2–4]

On the primordial Earth, prebiotic routes to peptides probably existed that were quite different from present-day biological and laboratory syntheses.[5] In simulation experiments, short linear peptides have been obtained from amino acids in the presence of silica.[6] The volcanic gas carbonyl sulfide,[7] and concentrated Cu²⁺-containing sea salt solutions.[8] In flow reactors, which simulate the pressure and temperature conditions of submarine hydrothermal systems, glycine (Hgly) was found to oligomerize into linear peptides glyₙ (Scheme 1) even without condensing agents.[9] Peptides up to n = 10 have been detected in this type of experiment.[10] Even longer oligomers (up to n = 16) have been observed as products of the thermal reaction of glycine molecules adsorbed on catalytically active TiO₂ surfaces.[11] Exposure of glycine–clay mineral mixtures to wet-dry cycles yielded peptides up to gly₈.[12] In a related study, it was found that the cyclic dipeptide of glycine (piperazine-2,5-dione, cyclo-gly₂; Scheme 1) was one of the main products.[13] Cyclic dipeptides are often encountered as products of the thermal condensation of amino acids on mineral surfaces. For example, they were isolated after heating L-alanine, α-aminoisobutyric acid, L-leucine, or L-valine to 230–250 °C in vacuo in the presence of silica.[14] Cyclic dipeptides were also obtained from metal complexes of neutral or anionic amino acids at 320–350 °C.[15,16] Under certain conditions, some amino acids thermally condense to give bicyclic and tricyclic amidines (BCA and TCA in Scheme 1). This reaction probably proceeds through the corresponding cyclic dipeptides.[14,17]

![Scheme 1. Chemical structures of condensation products of amino acids; R¹ = H, R² = alkyl, or R² = R³ = alkyl.](image-url)
In the course of our studies on \(\alpha\)-amino acids in different binding states,\(^{[15, 18-20]}\) we have noticed that information about the solid thermolysis products that form from neat bulk glycine is scarce. This is surprising not only because glycine is the simplest amino acid but also because it is considered highly relevant in prebiotic chemistry studies.\(^{[21]}\) From thermoanalytical measurements it is known that glycine shows a first rapid mass loss between 200 and 300 °C.\(^{[22-26]}\) This process is endothermic. Initially it was solely attributed to the release of \(\text{CO}_2\),\(^{[26]}\) but shortly afterwards \(\text{H}_2\text{O}\) and \(\text{NH}_3\) were described as additional gaseous products.\(^{[27]}\) A further mass loss at temperatures above 300 °C is accompanied by the release of \(\text{CO}\) and \(\text{HCN}\).\(^{[27]}\) These results have been confirmed by use of a combination of thermogravimetry and Fourier transform infrared spectroscopy, which also allowed the identification of the product \(\text{HNCO}\).\(^{[28]}\) Some conflicting data have been reported on the percentage mass loss, for example, 65% at 573 °C,\(^{[26]}\) compared with the probably less realistic value of at least 71% at the much lower temperature of 350 °C.\(^{[24]}\) To our knowledge, the only detailed study of solid thermolysis products obtained from glycine at temperatures \(>150^\circ\)C was performed by Heyns and Pavel.\(^{[29, 30]}\) In 1957, these authors reported the formation of a black substance, named “thermomelanoid”, which shared some properties with humic substances and products of browning reactions, such as those of the Maillard type. Their observations already indicated a certain complexity of the thermal transformation of glycine.

The studies by Heyns and Pavel have not attracted much attention over the past nearly six decades. This may be partly because the authors did not go into detail about the structure of the thermomelanoid. Furthermore, the characterization was based on less efficient classical methods such as fractional dissolution–precipitation and paper chromatography. Heyns and Pavel used reaction temperatures well above the onset of the thermal transformation of glycine. A better defined product can be expected at lower temperatures. We therefore decided to elucidate the chemical nature of the low-temperature thermomelanoid by applying a combination of state-of-the-art analytical techniques. Additional relevant information was obtained from the thermal behavior of short glycine homopeptides. The results of our study are presented in this paper.

Results and Discussion

The Heyns–Pavel experiment revisited

The original experiment was typically conducted with a mixture of glycine (50 g) and quartz (250 g) at 260–280 °C in a stream of nitrogen gas.\(^{[30]}\) Its overall duration was \(\approx 160\) min (90 min at 100–260 °C during the heating and cooling phases and 70 min at \(\geq 260^\circ\)C). It is known that adsorption on high surface area silicas, such as nanoparticulate fumed silica, modifies the reactivity of glycine.\(^{[31, 32-36]}\) Heyns and Pavel, however, used quartz sand, which has a very low surface area, and a comparatively high glycine-to-quartz ratio. Hence, any effects caused by the adsorption of glycine should have been negligible. Nevertheless, the presence of quartz sand is problematic because it may have interfered with the fast removal of volatile products thereby favoring secondary reactions. In fact, it has been suggested that the small amounts of other amino acids that were detected after hydrolysis of the thermomelanoid\(^{[30, 31]}\) had been formed through oligomerization of HCN.\(^{[37]}\) Therefore, we did not add quartz sand in our experiments. Furthermore, we used a thermolysis tube instead of a flask to facilitate the removal of volatiles by the nitrogen stream. Five grams of glycine were thermolyzed at 270 °C for 24 h. We were not primarily interested in the volatile organic products. However, a cursory study using GC-MS revealed the formation of succinimide (pyrrolidine-2,5-dione), \(N\)-methylsuccinimide, acetamide, and cyclo-gly. In one of the original papers, succinic acid, acetamide and several other, partly unidentified organic compounds have been described as components of the “distillate” of the thermolysis reaction.\(^{[30]}\) Despite the differences in the experimental conditions, the mass loss in our experiment (47.0%) was not significantly different from the one given in the literature (46.3% when 2 g of glycine were used).\(^{[38]}\) The infrared spectrum of the black residue obtained by us showed only three very broad bands at 1130, 1540, and 3100 cm\(^{-1}\). The X-ray powder diffractionogram was equally featureless with two extremely broad signals centered about \(d = 2.18\) and 3.45 Å. Taken together, these results demonstrate the diversity of reactions that glycine undergoes at 270 °C and the poorly defined structure of the thermomelanoid obtained at this temperature.

Synthesis and general properties of a low-temperature glycine thermomelanoid 1

Figure 1 compares the thermogravimetric (TG) curves of glycine and \(\alpha,\alpha\)-dimethylglycine (\(\alpha\)-aminoisobutyric acid, Haib). The non-proteinogenic Haib frequently occurs in micro-fungi\(^{[39]}\) and in certain types of meteorites.\(^{[21]}\) Its thermal behavior is characteristic of simple \(\alpha\)-alkyl and \(\alpha,\alpha\)-dialkyl amino acids, which lose virtually all their mass \((>90\%)\) in a single step. This mass loss can be mainly (e.g., alanine) or entirely (e.g., Haib) attributed to sublimation. It is obvious from Figure 1 that glycine behaves very differently. Here, the first TG step already ends at a mass loss of 44% and is not caused by sublimation of the amino acid. A second, less well-defined step, which corresponds to an additional mass loss of approximately 17%, follows. It ends at about 410 °C. At higher temperatures, a further continuous mass loss is observed, which reaches 98% at 900 °C (not shown). In our preparative experiment at 270°C, we measured a mass loss of 47% (see above). This value already falls within the second step of the TG curve. To avoid unnecessary decomposition of the thermomelanoid, the mass loss should therefore be kept below 44%, that is, within the first step. At this point it should be mentioned that, for principal reasons, thermal processes usually appear at higher temperatures in TG curves compared with fixed-temperature experiments. For example, the mass loss of 47%, which was obtained by keeping a glycine sample constantly at 270 °C, occurred at 298 °C in the TG curve of glycine (Figure 1). Higher heating rates further delay mass losses. For example, at 2 Kmin\(^{-1}\) we measured a mass loss of 71% at 573 °C, but at...
10 K min$^{-1}$ only 65% has been reported at the same temperature.$^{[25]}$ To minimize these effects and to improve the resolution, we conducted all of the TG and differential thermal analysis (DTA) measurements at a relatively low heating rate of 2 K min$^{-1}$.

According to the TG analysis, the formation of the thermomelanoid slowly starts at about 207°C and is fast between 234 and 253°C. High-resolution TG and DTA data allowed the identification of three overlapping processes in the first mass-loss step (see the Supporting Information, Figure S1). The first two processes are endothermic and occur at 237 and 239°C. The mass loss associated with them is approximately 16%. The rapid mass loss between 247 and 253°C is caused by a combination of amide bond formation, an unconventional condensation reaction and, to a lesser extent, side reactions. These points will be discussed later in detail.

On a preparative scale, the onset of the thermomelanoid formation was determined by keeping samples of the α-modification of glycine at different temperatures in a slow stream of nitrogen gas for 48 h. The samples turned light gray at 160°C and dark gray at 180°C. The infrared spectrum of the sparingly water-soluble portion revealed that already the light-gray color was caused by the presence of 1. The completely black thermomelanoid 1 was obtained at 200°C, typically from 5 g of α-glycine. The transformation of glycine to 1 at this temperature is accompanied by a mass loss of (38 ± 1)%.

The X-ray diffractogram of 1 resembles those of certain types of charcoal. Charcoals often show a prominent very broad XRD band, which is caused by parallel stacking of graphite-like layers in an otherwise non-ordered material. The $d$ value of $\approx 3.8\,\text{Å}$, around which this band is centered, is interpreted as the mean distance between adjacent layers. Thus, it is related to the $d$ value of the strongest reflection of crystalline graphite ($d_{002} = 3.35\,\text{Å}$).$^{[43]}$ The X-ray diffraction pattern of 1 is similarly dominated by a broad band at $d = (3.42 ± 0.02)\,\text{Å}$. However, the only conclusion that can safely be drawn from the X-ray diffraction is that 1 is definitely non-crystalline. The similarities between 1 and charcoal end when the electrical conductivity and the elemental composition are considered. In contrast to charcoal, compound 1 is electrically non-conducting. Furthermore, its composition (C 47.1, H 4.6, N 24.4, O 23.9%) differs markedly from that of carbonized charcoal, which typically contain C $\geq 90$, $H \approx 1$, $N < 0.6$, and $O < 6\%$ on an ash-free basis.$^{[41]}$

The experimentally determined element ratio in 1 can be expressed as C$_{100}$H$_{110}$N$_{40}$O$_{30}$. It is informative to compare this formula with the composition of some other black or nearly black organic substances. For instance, the insoluble organic matter (IOM) in chondritic meteorites has much higher C/H and C/N ratios.$^{[42]}$ The IOM composition C$_{59}$H$_{25}$N$_{13}$O$_{11}$S$_{2}$ of the Murchison meteorite may serve as an example. Black HCN polymers have been shown to release glycine when hydrolyzed.$^{[44]}$ Glycine is also the main product of the hydrolysis of 1, as will be discussed below. However, no HCN was detected during the formation of 1. Furthermore, the C/N ratio of 1 is about twice as large as that of HCN polymers.$^{[47]}$ Spectroscopic properties also distinguish HCN polymers from 1 (see below). Humic acid and humin, which are dark-colored (brown, gray or black) fractions of humic substances, typically have C/N ratios near 20.$^{[48]}$ In contrast, the relative carbon content of 1 is one order of magnitude lower (C/N $\approx 2$). Similarly, kerogen has a C/N ratio (≥ 20) that is much higher than that of 1.$^{[49]}$ The same is true for the melanoids that are formed as end products of the Maillard reaction between glycine and glucose (C/N $= 7–13$).$^{[50]}$

In summary, it can be said that the elemental composition, especially the carbon-to-nitrogen ratio, clearly distinguishes 1 from carbonized charcoal, meteoritic IOM, HCN polymers, humic acid, humin, kerogen, and Maillard browning products.
An important insight into the chemical structure of 1 was provided by experiments in which samples of 1, Hgly, cyclo-gly, and gly were separately deuterolyzed with DCI (6 mol L⁻¹) in D₂O at 110 °C. After removal of DCI and D₂O, the residues were treated with H₂O to replace the deuterium in the COOD and ND₃⁻ groups with protium. After water removal, the glycine in the samples was converted into the N-trifluoroacetyl glycine methyl ester (TFA-gly-OMe), which was then analyzed by using GC-MS.

The base peak in the EI mass spectrum of TFA-gly-OMe corresponds to the ion [CF₃C(=O)NH-CH₂]⁺. Therefore, we used the single ion traces of the three [CF₃C(=O)NH-CH₂X]⁺ ions (X = H, D) to determine the degree of deuteration at the α-C atom. By comparing the results obtained after different times of deuterolyis, it was found that in the case of Hgly, cyclo-gly, and gly, the α-H atoms were slowly exchanged by deuteron. The rates of deuteration were virtually identical for the three compounds. For example, after 6 h the CH₂/CHD ratio was 91.9% with a negligible proportion of doubly deuterated molecules (<1%). In contrast, after the same reaction time, the glycine that had been released from 1 showed a CH₂/CHD/CD₂ ratio (%) of 64:14:22, that is, a substantial degree of double deuteration. This difference suggests that part of the glycine residues in 1 are interconnected by C=C double bonds. These bonds form from carbonyl and carboxyl groups and are deuterolyzed according to Scheme 2. Because this type of reaction exceeds the conventional condensation of amino acids, we refer to it as hypercondensation. Condensation between C=C(=O)=N and N-CH₂-C=O moieties is rare. It is known to occur, for example, in the solvent-free reaction between imidazolidine-2,4,5-trione and imidazolidine-2,4-dione at 160 °C (220 °C with the 1-methyl derivatives).

During the formation of 1, 2% of the glycine was transformed into the byproduct cyclo-gly, which sublimed. Per mole of the remaining glycine 1.30 mol of H₂O, 0.12 mol of NH₃, and 0.04 mol of CO₂ were released. Only traces of CO and no HCN were detected. The combined masses of H₂O, NH₃, CO₂, and cyclo-gly represent 37.5% of the initial glycine mass, meaning that virtually the entire observed mass loss of (38 ± 1)% can be attributed to these four volatile products. Water alone accounts for 83% of the mass loss (86% if the contribution of the side reaction to cyclo-gly is subtracted out). Thus, the formation of 1 is mainly a dehydration condensation.

The release of NH₃ and CO₂ can, at least in part, be attributed to the formation of alanine (Hala) and aspartic acid (H₂asp). The racemic forms of both amino acids were detected by using chiral GC-MS/FID after the acid hydrolysis of 1. The Hgly/Hala/H₂asp molar ratio in the hydrolysate was 96.9:1.4:1.7. Scheme 3 shows the pathway by which Hala and H₂asp are formed. The crucial step is the C=C bond formation between two glycine groups. This conclusion is strongly supported by GC-MS data from a hydrolysate of isotopically labeled 1. The sample of 1 used had been prepared from (2-¹³C)-substituted glycine (H₂N⁻¹³CH₁⁻COOH). Scheme S1 (see the Supporting Information) shows some diagnostic mass fragments observed for the TFA methyl esters (see the Supporting Information for a detailed discussion).

The conjecture that Hala and H₂asp formed from the thermomelanoid by hydrolysis of an HCN polymer is inconsistent with our MS results. If strictly either the ¹³CH₃ or the COOH group of [2-¹³C]glycine provided the carbon atom of HCN, the resulting amino acids would have shown either 100% ¹³C at all carbon positions or no ¹³C enrichment. If both the ¹³CH₃ and COOH groups contributed carbon atoms to HCN, all carbon positions in the hydrolysate products would have exhibited identical partial isotopic enrichment. Therefore, our experimental observation of simultaneously occurring highly enriched α- and β-positions and non-enriched carbonyl groups excludes a significant contribution of HCN polymers.
The thermal behavior of glycine homopeptides

In a first series of experiments, we exposed the linear peptides gly₂ to gly₆ to the standard conditions under which glycine forms the thermomelanoid (200 °C, nitrogen atmosphere, 48 h). The starting materials were pure white powders. The two longest peptides survived the thermal treatment virtually unchanged. The samples showed negligible mass losses (1%) and had turned only very faintly brown. The next shortest peptide gly₁, lost 4% of its mass and turned dark grey. After washing with water, a small amount of a black residue remained which was identified as 1 by infrared spectroscopy. HPLC analysis showed that the water-soluble portion mainly consisted of the unreacted tetrapeptide. The cyclic dipeptide was also detected. In contrast, the two shortest peptides gly₂ and gly₃ suffered considerable mass losses of 22 and 21%, respectively, which were, however, still lower than the mass loss of glycine under identical conditions (38%). The products obtained from gly₂ and gly₃ were black. The residue of the tripeptide consisted of unreacted starting material, the thermomelanoid 1, and a smaller amount of cyclo-gly₂, whereas the dipeptide had largely transformed into a mixture of 1 and cyclo-gly₂. Thus, only the shortest peptides gly₂ and gly₃ yielded significant amounts of 1.

The mass losses were in part due to the sublimation of the product cyclo-gly₂. For comparison purposes, a sample of cyclo-gly₂ was therefore treated under the same conditions as the linear peptides. In this experiment, a mass loss of 16% was observed, mainly caused by sublimation. The grey residue contained cyclo-gly₂ as the only compound detected by HPLC. In addition, a very small amount of 1 could be isolated, even less than in the case of gly₂. It can be cautiously inferred from these observations that 1 is not built from condensed cyclo-gly₂ rings.

The thermal behavior of linear glyₙ peptides was further studied by using TG and DTA. The TG curves in Figure 2 clearly show that the onset of the thermal transformation continuously shifts to higher temperatures with increasing length of the peptide. This trend in thermal stability can be attributed to a combination of factors; among them is the larger number of hydrogen bonds that longer peptides can form with their environment. The onset temperatures for gly₂, gly₃, and gly₄ (202, 222, and 230 °C, respectively) are still near the temperature at which the transformation of glycine starts (207 °C). The onset temperatures for gly₅ and gly₆, however, are much higher (278 and 282 °C, respectively). This explains why gly₅ and gly₆ remained unchanged in the 200 °C heating experiments. In the TG curves of the glyₙ peptides, three (n = 2, 3) and two mass loss steps (n = 4–6), respectively, are distinguishable up to 400 °C. Beyond this temperature, the mass loss continues without pronounced steps and reaches > 90% at 900 °C. The initial mass loss process is endothermic for all six peptides. For gly₂, the first TG step, which is observed between 202 and 233 °C, is associated with DTA signals at 215 and 225 °C. Thus, the underlying processes proceed at lower temperatures than the formation of 1 from glycine (compare with Figure S1 in the Supporting Information). We presume that these are the incipient cyclization to and the sublimation of cyclo-gly₂, which both start slightly earlier than the thermomelanoid formation. This idea is supported by the observation that gly₂ produced a considerable amount of cyclo-gly₂ in the preparative scale experiment. In contrast, the TG curve of the next larger peptide gly₃ bears some resemblance to that of glycine. Its first step lies between 222 and 250 °C with a sharp DTA signal at 238 °C. Thus, this step overlaps with the temperature range at which glycine forms the thermomelanoid (234–253 °C). For the longer peptides gly₄, gly₅, and gly₆, the first DTA signal is shifted to 255, 290, and 323 °C, respectively. Hence, it is impossible for gly₃, and gly₄, to form the low-temperature thermomelanoid 1. Instead, they will probably yield a poorly defined product, which may resemble the one described by Heyns and Pavel.

The thermoanalytical data support a mechanistic model for the transformation of glycine to 1, which assigns a key role to short linear peptides, especially the tripeptide. The initial mass loss of glycine at around 238 °C fits well with the formation of gly₂ (calcd: 16.0, found: 16%). A second mass loss of 25% immediately follows between 240 and 253 °C (see the Supporting Information, Figure S1). It may be interpreted as the result of hypercondensation and further amide bond formation, both involving gly₁ molecules. Interestingly, the above-discussed first step in the TG curve of gly₂ exhibits a similar mass loss of 22%. After heating glycine at 200 °C, only a small amount of the cyclic dipeptide was observed. This is in contrast to the behavior of gly₃, which yielded cyclo-gly₂ as one of the main products. Thus, we conclude that when gly₂ molecules are formed in situ during the thermolysis of glycine, they are probably efficiently incorporated into the condensation processes that ultimately lead to 1. It is also conceivable that intermediated formed cyclo-gly₂ molecules are rapidly consumed by ring-opening oligomerization. The clear similarities between the infrared spectra of 1 and gly₂, molecules (see below) are consistent with the idea that glycine is transformed into 1 through linear peptides. As a hypothetical alternative to the picture outlined above, glycine may first be more or less com-

Figure 2. Thermogravimetric curves of linear glyₙ peptides. For experimental conditions see the caption to Figure 1.
pletely transformed into relatively long peptides, which subsequently undergo hypercondensation. Given the high stability of gly, and gly, at 200 °C, this idea must, however, be discarded.

Spectroscopic properties and structural details of 1

Acidic and alkaline solutions of 1 are brown and have very similar electronic absorption spectra. The absorption maxima in the 400–500 nm region are reminiscent of conjugated, non-aromatic π-systems (cf., e.g., β-carotene[54]). Raman spectra of 1 did not provide useful information because of strong fluorescence, at least with a laser excitation wavelength of 532 nm. Infrared spectroscopy, however, proved to be particularly informative about the structural details. The IR spectrum of 1 clearly differs from that of the 270 °C product (see above) but resembles the spectra of oligoglycines such as gly, (see the Supporting Information, Figure S2) and gly10. By comparison with oligopeptide data,[55, 56] the absorption maxima at 1627 and 1524 cm⁻¹ can be unambiguously identified as an amide I and amide II band, respectively. Absorptions at 3291 and 1228 cm⁻¹ are assigned to the amide NH stretching vibration and the amide III mode, respectively. Thus, the infrared spectrum confirms that the peptide bond is a major structural element of the thermomelanoid. In addition, a strong shoulder at 1640–1656 cm⁻¹ may be assigned to the stretching vibrations of C=O bonds that are conjugated with amide C=O bonds (cf., Scheme 2). Acrylamide, for example, has a C=O stretching vibration at 1650 cm⁻¹.[57, 58] The shoulder may also contain contributions from the amide I mode of folded segments of peptide chains.[55, 56] There are no indications of insoluble black HCN polymers of the “anhydrous poly-HCN” type. Such polymers give rise to a ν(C≡N) band around 2200 cm⁻¹,[47, 59–61] which is absent in the spectrum of 1.

Based on the analytical and spectroscopic data, we propose that the thermomelanoid mainly consists of oligoglycine chains that form inter- and intramolecular carbon–carbon double bonds (Scheme 4). A minor portion of the amino acid positions is occupied by alanine and aspartic acid residues. This model allows differently (cross-)conjugated systems of C=C, C=O and NH groups, including arrangements that resemble donor–acceptor (“push–pull”) polymers of the mercyanine type R,N-(C(H) ≡C(H)N)=C=O.[62] It is well known that donor–acceptor combinations lead to a strong bathochromic shift (cf., e.g., CH3(CH=CH)≡C=CH (λmax = 312 nm)[63] and (CH3)2N(CH=CH)=C=O (λmax = 422 nm)).[64] In the case of 1, it is probably a combination of different aliphatic π-systems, varying in length, structural detail, and microenvironment, which causes absorption over the whole visible range and thus the deep-black color.

The structural elements shown in Scheme 4 are consistent with the 13C and 15N solid-state CP-MAS NMR spectra of 1. In the 1D 13C NMR spectrum, intense signals at δ ≈169.7 and 42.1 ppm can be unambiguously assigned to amide and methylene carbon atoms, respectively. For comparison, the corresponding resonances of solid gly, have been found at δ ≈167.7, 44.9, and 40.4 ppm.[60] The spectrum of 1 shows a further signal, which is very broad and overlaps with the amide peak; this signal extends down to δ ≈100 ppm and has components at δ = 145.6, 123.5, and 111 ppm and is very probably due to carbon atoms in C=C and certain C=O bonds. This assignment is consistent with the chemical shifts predicted by ACD/lab software for the model in Scheme 4: δ = 159, 151, 150, 142, and 136 ppm (C=C); δ = 165 and 156 ppm (C=O ring carbons).[65] For the C=C bond of the open-chain structure in Scheme 2, δ = 159 and 113 ppm were calculated. The accuracy of the predicted values was between ±4 and ±20 ppm.

The 1D 15N NMR spectrum of 1 exhibits an intense signal at δ = −268.1 ppm with a shoulder at δ = −263 ppm, undoubtedly due to amide groups. The solid-state spectrum of gly, for example, shows the amide signal at δ = −269.4 ppm.[66] Nitrogen atoms in C=C–NH–C=C groups (Scheme 4), for which δ = (−262 ± 31) ppm is calculated,[66] may also contribute to this signal. The same holds true for the enamone nitrogen atoms in Scheme 4 (NH–C=C–C=C–O) with a predicted resonance at δ = (−285 ± 15) ppm. In addition, a weaker and very broad feature around δ = −215 ppm is observed. It is compatible with the presence of the C=C–NH–C=C structural element the theoretical δ value of which varies slightly with the environment: δ = (−221 ± 6) and (−222 ± 8) ppm for ring N atoms (Scheme 4) and δ = (−238 ± 5) ppm for the respective N atom in the open-chain structure shown in Scheme 2. Hydrogen bonds, which are expected to be frequent in compounds with a high density of NH and C=O groups such as 1, can shift 15N resonances by several ppm. For example, it has been shown that hydrogen bonding in oligopeptides can cause considerable downfield shifts (>10 ppm).[67] Therefore, some of the experimental δ(15N) values of 1 may appear slightly too positive compared with the theoretical ones.

An additional candidate structure is the 1,4-dihydropyrazine ring, which in principle could be formed by double condensation of two CH3–NH–C(O) moieties. Most 1,4-dihydropyrazines are, however, notoriously unstable.[68] At the formation temperature of 1 (200 °C), they are expected to react readily, for example, by 1,3-hydrogen shift or aromatization. The resulting imines and pyrazines, respectively, however, have chemical shifts that are not found in the 15N NMR spectrum of 1.[67]
There is also no other evidence for the presence of imines (–N=C<−) or aromatic six-membered N-heterocycles in the thermomelanoid. Likewise, aliphatic amines cannot be among the major functional groups, for the 13N NMR spectrum lacks signals in the relevant ppm range (ca., δ = −300 to −370 ppm). Thus, Dark HCN polymers too have prominent signals in this region. Therefore, it can be concluded, in accordance with other observations (see above), that such polymers do not form major constituents of the thermomelanoid. Nevertheless, one should bear in mind that the solid-state NMR spectra do not provide exact quantitative information. The presence of the main structural elements shown in Scheme 4 is further supported by the 2D 13N–13C correlation spectrum of Scheme 4 (see the Supporting Information). Finally, we note the possibility that additional minor structural elements can be present in that could not be detected by our analytical and spectroscopic methods.

**Conclusion**

We found that on heating the amino acid glycine, a new type of polymer formed. The reaction started at approximately 160 °C under constant temperature conditions and at approximately 207 °C under TG conditions (heating rate of 2 K/min−1). A relatively well-defined black product, the ‘thermomelanoid’, was obtained at 200 °C. It was mainly built from glycine residues. In addition, it contained a few percent of alanine and aspartic acid, which were formed through Cα–Cα bond formation between glycine molecules. The formation of the thermomelanoid was accompanied by the release of an unexpectedly large amount of water. Ammonia, carbon dioxide, and piperazine-2,5-dione were also detected, albeit in much smaller quantities.

From the results of various analytical measurements, we conclude that in two different types of covalent linkages between glycine residues exist, namely, amide bonds and C=C double bonds. The latter form by an unconventional condensation reaction between a carbonyl and a methylene group. This additional ‘hypercondensation’ explains why considerably more water is released than expected from peptide formation alone. The thermal behavior of glycine homopeptides suggests that small peptides, particularly triglycine, may play a central role in the formation mechanism of the thermomelanoid. Spectroscopic evidence indicates that (cross-)conjugation of C=C, C=O, and NH groups is responsible for the black color of the thermomelanoid. In a sense, be regarded as a largely immobile storage form of glycine and glycine homopeptides. On the early Earth, it could therefore have provided some protection against dilution. This may have been important for the prebiotic chemistry on primordial volcanic islands, which are plausible locations for thermomelanoid formation. That glycine is partially transformed into other amino acids during the formation of the thermomelanoid is another aspect of potential prebiotic interest.

The thermomelanoid may find practical applications because it provides a potentially useful combination of physical and chemical properties (formation of stable pellets, non-crystallinity, deep-black color, electrical insulator, insolubility in organic solvents, and hydrolysis in strong acids and bases). In this context, it would be of interest to explore the biological properties of 1, such as biodegradability and toxicity. This is, however, beyond the scope of the present study.

**Experimental Section**

The thermolyses were performed in a tube furnace under pure nitrogen gas. The thermolysis apparatus used has been described elsewhere. Further experimental details are given in the Supporting Information.

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**Keywords:** amino acids · condensation reactions · peptides · polymers · structure elucidation

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