Comparative study of the thermal decomposition behaviour of different amino acids and peptides

Alexander Schaberg\textsuperscript{1}, Robert Wroblowski\textsuperscript{1} and Roland Goertz\textsuperscript{1}

\textsuperscript{1}Chemical safety and fire defence, University of Wuppertal, Germany

schaberg@uni-wuppertal.de

ABSTRACT

Thirteen nitrogen-containing compounds, including 2 peptides and 11 amino acids, were analysed for their thermal decomposition behaviour using TGA/DSC coupled with MS and FTIR. Various decomposition courses have been found. Glycine, cysteine, cystine, proline, D- and L-threonine decomposed in two stages, whereas arginine, asparagine, glutamine, histidine and tryptophan decomposed in three stages. The peptides of glycine both decomposed in four decomposition steps, except for the glycylglycine under oxidative conditions, which decomposed in three stages. Proline proved to be exceptional, as it needed only one decomposition step under nitrogen to decompose (99.5\% mass loss).

The decomposition products of amino acids and peptides described in literature could be confirmed in this work. These were mainly carbon dioxide, water, ammonia, hydrocyanic acid and nitrogen oxides. In traces, also isocyanates and nitriles could be detected, but no clear identification of these substances could be made due to superposition of the signals. For example, in the absorption spectrum at 3000 cm\textsuperscript{-1}, a superposition of the hydrocarbons and amines occurred and in the two sulphur-containing amino acids cysteine and cystine at 2100 cm\textsuperscript{-1}, a superposition of the resulting sulfur compounds could be observed.

It has also been shown that with increasing chain length of the peptides, in this case of glycine, the molecule becomes thermally more stable. Concerning cystine, something similar was observed for cystine, this is due to the strong sulphur bonding of the monomers. In addition, glycine and its peptides have been shown to have doubled or tripled the volume of the resulting carbon foam formed as length increased, a feature interesting for future research involving intumescent materials.

KEYWORDS:

fire chemistry; evolved gas analysis; thermogravimetric analysis; material characteristics; combustion
INTRODUCTION

In thermal decomposition by combustion, a vast variety of compounds is formed influenced by both combustion parameters and source material. In order to estimate how proteins or peptides behave thermally depending on their structure, one has to understand how amino acids, which are the building blocks of proteins and peptides, behave thermally. Amino acids are organic compounds of simple design with at least one amino group (-NH$_2$) and one carboxyl group (-COOH) (fig. 1). This gives them a remarkable physicochemical reaction behaviour, utilised by every life form known to humankind. [1]

All compounds, except glycine, have at least one chiral centre, while naturally occurring α-amino acids are predominantly L-configured. Therefore, unless specified otherwise, the amino acids referred to in the following text are of the L-configured form.

Their behaviour under heat has been studied over a wide temperature range, though mostly under pyrolytic conditions. A study of glycine, alanine and serine in the temperature range of 200-300 °C in vacuum found mostly water, carbon dioxide, ammonium and methylamine for glycine and even less for the remaining amino acids. The condensed phase for glycine yielded formamide, acetamide, N-methylacetamide, n-butylamine and propanamide. [2] Another study of valine, leucine and isoleucine showed a wider emission spectrum, as displayed in table 1, under similar conditions, utilizing a gas chromatographic system.

| valine          | leucine                              | isoleucine                                      |
|-----------------|--------------------------------------|-------------------------------------------------|
| ammonia         | carbon dioxide                       | ammonia                                         |
| carbon dioxide  | carbon monoxide                       | carbon dioxide                                  |
| carbon monoxide | isobutane                            | carbon monoxide                                 |
| propane         | isobutylene                          | isobutene                                       |
| propene         | acetone                              | isobutanol                                      |
| isobutane       | isobutyraldehyde                     | 3-methyl-1-butene                               |
| isobutylene     | isovaleraldehyde                     | acetone                                         |
| acetone         | isobutylamine                        | isobutylamine                                   |
| isobutyraldehyde| isoamylamine                         | N-isobutylidene-isoamylamine                    |
| isoamylamine    | N-isobutylidene-isoamylamine         | N-isobutylidene-isoamylamine                    |
| N-isobutylidene-isoamylamine | | N-isobutylidene-isoamylamine |
| diisobutyramine |                                      | diisobutyramine                                 |

Another study [4] of poly-L-leucine and poly-L-proline performed under pyrolytic conditions showed a conversion of the nitrogen inventory to HCN (58%) and NH$_3$ (31%) at 700 °C, with an even higher ratio of over 2 parts HCN per ammonia at further elevated temperatures in the poly-L-leucine. Poly-L-proline even yielded 10 parts HCN per ammonia, proving the high diversity and dependence on the chemical structure of the amino acid.
In addition, HCNO was identified as an effluent, whereas NO or N₂O did not form or were below the detection limits of the used IR-spectrometer.

Although many interesting findings have been reported as mentioned before, none of those studies were conducted under combustion-like conditions, e.g. under typical atmospheric conditions or (synthetic) air. Therefore, our aim was to study differences under pyrolytic and combustion conditions to further the understanding of the fire effluents and behaviour of natural products. As amino acids (or peptides) make up often-used textile household and clothing products like feathers, leather, wool and silk, their general behaviour and especially the occurrence of toxic gases are of high interest and play a major role in understanding how effluents might be prevented or why high concentrations of ammonia and cyanides may occur.

To measure the combustion behaviour, a thermogravimetric analyser, coupled with differential scanning calorimetry (TGA/DSC) and evolved gas analysis (EGA) was utilised. Using this setup, the change in mass of the compound under influence of an applied heat rate under pre-set atmospheric conditions and flow rates can be studied. In addition, monitoring heat fluxes between the compound containing crucible and a reference crucible, thus giving information of the energetic changes, is possible. The effluents of the analytes are driven towards the EGA and can be identified by their charge-to-mass-ratio using mass spectrometry (MS) and their response to infrared irradiation by a Fourier-transform infrared spectrometer (FTIR). [5-7]

**EXPERIMENTAL**

The experiments were performed in a TGA/DSC 1 (Mettler Toledo). The starting temperature was set to 30 °C. With a heating rate of 10 K/min, the temperature was ramped to 800 °C under a flow of 40 mL/min synthetic air or nitrogen. The compounds (analytical grade, 99% purity, Sigma Aldrich/Alfa Aesar/VWR) were placed in 70 µL aluminium oxide crucibles. To analyse the effluents, a ThermoStar GSD 320 (Pfeiffer Vacuum) in SEM-mode with 65 eV ionisation energy and heated capillary (150 °C) and a Nicolet™ iS™50 (Thermo Scientific) with TGA-IR-module (heated transfer line, 200 °C and heated gas cuvette, 220 °C) were used.

In order to prove the usability in the TGA/DSC, preliminary experiments over a Bunsen burner were carried out, which also showed interesting clues of the general combustion behaviour of the amino acids and peptides. Therefore, 10 mg of each compound was transferred in similar crucibles as used in the TGA/DSC experiments.

**RESULTS AND DISCUSSION**

In the following text, a short overview of the different combustion and pyrolysis characteristics of the studied compounds is given, as well as an in-depth review of the data obtained for glycine and the resulting peptides.

**Glycine, glycyglycine and glycyglyglycine**

The most basic amino acid (glycine, see fig. 1 with R = H) and its peptide derivatives showed interesting behaviour under oxidative conditions in the preliminary experiments. Once exposed to direct heating, a black carbon foam formed rapidly (fig. 2). The volume of this foam seemed to have a direct relation to the amount of linked glycine monomers – although the total mass used for each test stayed the same. In addition, the foam remained stable when further exposed to heat for another 20-30 seconds. These intumescing features were also present in similar extend with histidine; most other amino acids did form a much less stable foam during combustion which disappeared within seconds.

![Fig. 2. Decomposition of glycine (left), glycyglyglycine (mid) and histidine (right).](image)
When those compounds are subjected to TGA/DSC, the following data can be obtained (see table 2). A typical TGA-curve is also displayed for glycine (fig.3).

![TGA/DSC curve of glycine.](image)

**Table 2. Decomposition of glycine and its derivatives.**

| Compounds     | Synthetic air | Decomposition step 1 | Decomposition step 2 | Decomposition step 3 |
|---------------|---------------|----------------------|----------------------|----------------------|
| **Glycine**   |               |                      |                      |                      |
| Mass loss (in % of remaining mass) | 50            | 100                  |                      |                      |
| Effluents     | NH₃, H₂O, HCN, NO, NO₂, CO₂, isocyanates | NH₃, H₂O, HCN, CO, CO₂, isocyanates, nitriles |                      |                      |
| Temperature range (in °C) | 220-280, ΔH > 0 | 280-650, ΔH < 0    |                      |                      |
| **Nitrogen**  |               |                      |                      |                      |
| Mass loss (in % of remaining mass) | 50            | 30, 20% remaining |                      |                      |
| Effluents     | NH₃, H₂O, NO₂, CO₂, isocyanates, nitriles | CO₂, NO₂, isocyanates, buten |                      |                      |
| Temperature range (in °C) | 220-280, ΔH > 0 | 280-800, ΔH > 0    |                      |                      |
| **Glycylglycine** |             |                      |                      |                      |
| Mass loss (in % of remaining mass) | 19            | 20                   | 100                  |                      |
| Effluents     | NH₃, H₂O, HCN, NO, CO₂ | NH₃, H₂O, CO₂ | NH₃, H₂O, HCN, CO, CO₂, isocyanates, nitriles |                      |
| Temperature range (in °C) | 220-250, ΔH > 0 | 250-310, ΔH < 0    | 310-650, ΔH > 0 |                      |
| **Glycylglycine** |             |                      |                      |                      |
| Mass loss (in % of remaining mass) | 19            | 25                   | 13                   | 22, 21% remaining   |
| Effluents     | NH₃, H₂O, NO₂, CO₂ | NH₃, H₂O, NO₂, CO₂ | NH₃, H₂O, HCN, NO₂, CO₂, isocyanates, nitriles | NH₃, H₂O, HCN, NO₂, CO, CO₂, isocyanates, nitriles |
| Temperature range (in °C) | 220-250, ΔH > 0 | 250-320, ΔH > 0 | 320-380, ΔH > 0 | 380-800, ΔH < 0    |
Glycylglycylglycine

| Synthetic air | Decomposition step 1 | Decomposition step 2 | Decomposition step 3 | Decomposition step 4 |
|---------------|----------------------|----------------------|----------------------|----------------------|
| Mass loss (in % of remaining mass) | 20 | 13 | 23 | 100 |
| Effluents | NH₃, H₂O, HCN, NO, NO₂, CO₂, isocyanates | NH₃, H₂O, HCN, NO, NO₂, CO₂, isocyanates | NH₃, H₂O, CO₂, isocyanates, nitriles | NH₃, H₂O, HCN, NO₂, CO, CO₂, isocyanates |
| Temperature range (in °C) | 240-260, ΔH > 0 | 260-310, ΔH < 0 | 310-440, ΔH < 0 | 440-670, ΔH < 0 |

Glycin and the different peptides show an interesting behaviour in many aspects studied by TGA/DSC. For example, a longer chain results in higher thermal stability, whereas the small, easily formed inorganic products remained relatively similar, with the main difference resulting from the surrounding atmosphere. The amount of complex structures in the effluents seems to be a function of the chain length, with a more frequent occurrence and higher concentration of isocyanates, nitriles and HCN in the biggest peptide and at higher temperatures. Under inert atmosphere, neither glycine nor glycyglycine showed the formation of HCN.

Remaining amino acids

The remaining amino acids (arginine, asparagine, cysteine, cystine, glutamine, histidine, proline, D and L-threonine, tryptophane) showed, as could be expected by more complex compounds, a more diverse spectrum of effluents. Those involved complex alkanes and alkenes (methane, butane, butylene, and pentane), aromatic compounds (benzene, toluene), amines and other nitrogen containing compounds (e.g. isobutylamine, urea, HONO, aromatic nitriles). Furthermore, sulphur-containing amino acids yielded SO₂, H₂SO₃, thiocyanates and thioureas. Most amino acids required 2-4 decomposition steps in total. Proline decomposed in an explosive manner, losing 99.5% mass between 220-290 °C. All amino acids were fully oxidised under synthetic air without any residue, under nitrogen a variable amount of carbon remained. For the threonines, the remainds had a mass of less than 1%, while histidine had a residue of 40% under inert conditions. Many amino acids began decomposing as their respective melting point was reached, but cystine and tryptophan began pyrolysing 20-40 °C before melting.

Cysteine proved to be less stable than its dimer (cystin), having double its mass loss rate at a lower initial temperature. This can be traced back to the strong sulphur bonding between the monomers.

Looking at the D- and L-configured isomers of threonine, no differences in effluents were found, but the decomposition mechanism seemed to differ slightly – both began their mass loss at roughly 225 °C, but the L-threonine showed a second, small step at 240 °C, which may be caused by better intermolecular bonding or interactions.

CONCLUSIONS

The experiments showed the high diversity even within the small group of relatively similar compounds, those are also shown in figure 4. The first and main products of decomposition were carbon dioxide, water and ammonia. Carbon monoxide occurred as a trace in most analyses; nitrogen oxides were even less frequently
detected. In addition, isocyanates and nitriles were found at elevated temperatures, just like the hydrocyanic acids.

Although EGA is able to monitor analytes of high volatility during a TGA/DSC-analysis, further equipment is needed to show the even more diverse field of lower volatile compounds. Therefore, gas chromatography and a so-called VCI-oven will be employed to analyse the effluents of a simulated smouldering fire including amino acids. Preliminary experiments with tryptophane already showed high amounts of heterocycles, nitriles and mixtures of both, which may be specific for the used amino acid. Furthermore, this type of analysis is capable of separating the different isocyanates and nitriles obtained by TGA/DSC, which cannot be separated and thus quantified by EGA due to superpositions. In general, the EGA of most researched compounds within this paper showed a much smaller amount of effluents under inert conditions. However, those decompositions products were often much more complex than those under synthetic air, which corresponds with findings under smoldering fire conditions, as simulated by the VCI-apparatus.

To also monitor the complete nitrogen inventory of the compounds, further experiments will be carried out under argon atmosphere to measure the generation of pure nitrogen by decomposition.

Fig. 4. Decomposition of all featured compounds, showing their melting point (blue), begin (grey) and end (orange) of combustion temperatures and decomposition steps (dotted green) under synthetic air

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