Interaction of thermally evaporated dipeptides with keV-energy $\alpha$-particles

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Abstract. Fragmentation of isolated molecular ions of dipeptides produced by alpha-particles was studied by an experimental technique based on time-of-flight analysis of the charge and mass composition of the fragment ions. The study has shown that the N-Glycylglycine sublimation results in the molecule decomposition into cyclo(GlycylGlycyl) and H$_2$O molecule. We have examined a mass-spectrum of the fragments arising in the cyclo(GlycylGlycyl) interaction with $\alpha$-particles. By analyzing the temperature dependence of the cyclo(GlycylGlycyl) vapor pressure, we have determined sublimation enthalpy $\Delta H_{\text{subl}}$(453K) = (213 ± 9) kJ/mol.

1. Introduction

During the last three decades, investigation was performed of mechanisms for interaction between isolated biologically important molecules (amino-acids, nucleic acid bases) and various types of ionizing radiations, such as UV- and X-rays [1-3], electrons [4,5] and ions [6,7]. It was shown that amino-acid ions formed due to this interaction are highly probable to be fragmented, the fragmentation scheme depending on the type of the amino-acid side chain. First of all, the obtained experimental data were important for check the quantum-chemical calculations (see, e.g., [8]).

Peptides are one of the most important classes of biological molecules and have multiple functions in living organisms. They consist in chains of peptide bonded amino-acid residues. Peptide ions to be studied by mass-spectrometry are typically introduced into the gaseous phase by using Electrospray Ionization or Matrix Assisted Laser Desorption Ionization techniques. In these techniques, ionization of peptide molecules is caused by protonation of the molecule M in question. In studying ions of n-fold protonized peptides (M+nH)$^{n+}$, different mass-spectrometers are used (see, e.g., [9]).

Dipeptides consisting of two amino-acid residues are referred to as elementary peptides. The simplest of dipeptides (N-Glycylglycine C$_4$H$_8$N$_2$O$_3$) (see figure 1b) forms from the residues of two glycine molecules C$_2$H$_5$NO$_2$ (figure 1a). Dipeptides can form cyclic structures that, as distinct from linear dipeptides, have two peptide bonds. As an example of such a cyclic structure, the cyclo(GlycylGlycyl) C$_4$H$_6$N$_2$O$_2$ molecule can be regarded (figure 1c).

In studying sublimation of a number of amino-acids and dipeptides, authors of [10, 11] showed that some of the studied dipeptides are not decomposable in thermal evaporation. Photoemission spectra of
some gaseous dipeptides were analyzed in [12-14]. Mass-spectra of ion fragments of the series of dipeptides arising in molecule ionization by electron impact are presented in the database [15].

The goal of this work was first to acquire experimental data on fragmentation of single-charged ions of elementary peptides being formed in the single electron capture process (1) by keV-energy He$^{2+}$ ions:

$$\text{He}^{2+} + M \rightarrow \text{He}^+ + M^\dagger \rightarrow \text{He}^+ + \text{Fr}^+ + \Sigma_n \text{Fr}_n^0,$$ (1)

where $M^\dagger$ is the intermediate state of the molecular ion arising in the single electron capture by a He$^{2+}$ ion, Fr$^-$ is the fragment ion, Fr$^0_n$ are the neutral fragments accompanying formation of a charged fragment (experimentally non-observable).

Another goal was to obtain the sublimation enthalpy of the substance under study from the experimentally measured temperature dependence of its relative vapor pressure.

**Figure 1.** Schematic structures of (a) Glycine, (b) N-Glycylglycine and (c) cyclo(GlycylGlycyl).

2. Methods of research and materials

The investigation was performed by using the experimental technique described in detail in [7]. The technique is based on the mass-spectrometric analysis of the fragment ions being formed in processes (1) with simultaneously fixing the charge of the projectile after its interaction with the molecule.

As a sample, chemically pure N-Glycylglycine supplied by “Vekton” [16] was used. The effusion molecular beam was obtained by heating the studied substances in an oven. The oven absolute temperature was measured accurately to ±0.12%. Relative density of the molecular beam was measured by a using a quartz microbalance.

Relative cross-sections of elementary processes were deduced from mass-spectra of the fragment ions. After subtracting the background, those spectra were normalized to the integral of the projectile beam current and to the amount of substance that has passed through the interaction region and condensed on the quartz microbalance sensor in the process of measuring.

The sublimation enthalpy was derived from the microbalance reading dependence on the vapor temperature in the oven. The dependence of the oscillation frequency variation on the mass of substance deposited on the microbalance sensor is defined as follows [17]:

$$f(t) = Yf_0^2m(t),$$ (2)

where $f(t)$ is the frequency variation, $Y$ is the parameter depending on the sensor characteristics, $f_0$ is the sensor resonance frequency, $t$ is the time, and $m(t)$ is the condensed substance mass.

The substance flow is related with the sublimation enthalpy at a mean experimental temperature $\Delta H_{\text{sub}}(<T>)$ by the following expression (3) [18]:
\[ R \ln \left( \frac{\dot{m}}{\sqrt{T}} \right) = a - \frac{\Delta H_{\text{sub}}(\langle T \rangle)}{T} , \]  

where \( T(t) \) is the oven temperature, \( R \) is the gas constant.

3. Results and discussion.

Figure 2 (a and b) presents typical mass-spectra of fragment ions formed in process (1) in interaction between He\(^{2+} \) 4 keV/a.m.u. in energy and of the effusion beam of molecules formed in heating N-Glycylglycine (structural form (b) in figure 1). Ion H\(_2\)O\(^+\) dominated in the mass-spectrum of the analyzed substance at relatively low temperatures (spectrum a). Intensities of mass-spectrum peaks of other fragment ions increase rapidly with increasing temperature. Thus the H\(_2\)O\(^+\) relative intensity decreased with increasing temperature (spectrum b).

![Figure 2](image-url)  

**Figure 2.** Mass-spectra of fragment ions observed in thermal evaporation of N-Glycylglycine (a – temperature 449K, b - temperature 463K) and its sublimate (c – temperature 449K, d - temperature 463K).  
Mass-spectra obtained at the same temperatures (a and c, b and d) were normalized to the integral of peak at mass 114 a.m.u.

Such a behavior of the water peak may be explained by decomposition of the substance under study with formation of water molecules and, most probably, cyclo(GlycylGlycyl) molecules (structural form (c) in figure 1). The process of cyclization of fragments formed in decomposition of dipeptides and amino-acids was discussed in papers [14, 20, 7].

To validate this assumption, the initial substance sublimate was analyzed. The sublimate mass-spectra are also shown in figure 2 (c and d). At the temperature of 449 K, both the substances exhibited statistically important peaks at masses m = 30 and 114 a.m.u. (spectrum (a) and (c)). Intensity of the sublimate water peak is below 3% of the N-Glycylglycine water peak. Thus we can
state (accurately to the data processing error) that decomposition was not observed in thermal evaporation of the sublimate.

Analysis of mass-spectra obtained for the case of electron-impact ionization of various \( \text{C}_4\text{H}_6\text{N}_2\text{O}_2 \) isomers (11 substances) [15] showed that the cyclo(GlycylGlycyl) spectrum is most similar to the observed spectrum.

Table 1 presents relative cross-sections of the main channels of the \( \text{C}_4\text{H}_6\text{N}_2\text{O}_2^+ \) ion fragmentation. Cross-sections of the listed fragmentation channels amount up to 78% of the total fragmentation cross-section of a single-charged molecular ion formed in process (1). To identify fragment ions \( m \) in mass, we have chosen compounds whose formation needs the minimal number of ruptured bonds and minimal regrouping of atoms between the arising ion and neutral fragments (figure 1 c).

**Table 1.** Relative cross-sections of the main fragmentation channels of the \( \text{C}_4\text{H}_6\text{N}_2\text{O}_2^+ \) ion.

| Fragment ion | \( m \) (a.m.u.) | Rel. cross sec. % |
|--------------|------------------|-------------------|
| \( \text{CO}^+, \text{CH}_2\text{N}^+ \) | 28 | 7.1±1.0 |
| \( \text{CHO}^+, \text{CH}_2\text{NH}^+ \) | 29 | 6±1 |
| \( \text{CH}_2\text{O}^+, \text{CH}_3\text{NH}^+ \) | 30 | 15.0±1.5 |
| \( \text{CNO}^+, \text{C}_2\text{H}_2\text{O}^+ \) | 42 | 4.4±1.0 |
| \( \text{CHNO}^+, \text{C}_2\text{H}_2\text{O}^+ \) | 43 | 6.3±1.3 |
| \( \text{C}_2\text{H}_3\text{N}_2\text{O}^- \) | 71 | 6±1 |
| \( \text{C}_2\text{H}_4\text{N}_2\text{O}^- \) | 72 | 4.6±1.0 |
| \( \text{C}_3\text{H}_6\text{N}_2\text{O}_2^+, \text{C}_4\text{H}_4\text{NO}_2^+ \) | 86 | 1.6±0.5 |
| \( \text{C}_4\text{H}_6\text{N}_2\text{O}_2^+ \) | 114 | 27.2±2.0 |

To obtain the sublimation enthalpy, data on the substance mass flow from the oven was analyzed in the temperature range of 448 to 458K. The lower limit of the range was chosen because just there statistically important fragments of the studied molecule appear in the measured spectra. The upper limit of the range corresponds to the point of the oven stabilization temperature. The stabilization was necessary to determine the relative fragmentation cross-sections.

**Figure 3.** Value of the equation (3) left part versus the oven inverse temperature. The necessary explanations are given in the text. The points represent the measurements, the straight line is linear approximation corresponding to the equation (3) right part.
In figure 3, the value of the equation (3) left part is shown versus the oven inverse temperature. The oven temperature is a function of time $t$ measured in the course of the experiment. The rate of variation in the substance flow $\frac{dm}{dt}$ to the microbalance sensor depends on the rate of variation in the sensor frequency $\frac{df}{dt}$ as per equation (2). The measurements were fitted by a straight line approximating the right part of equation (3). Thus, the following value of the sublimation enthalpy was found: $\Delta H_{\text{subl}}(453K) = (213 \pm 9) \text{kJ/mol}$.

4. Conclusions
Heating of crystalline N-Glycylglycine results in its decomposition with formation of cyclo(GlycylGlycyl) and an $\text{H}_2\text{O}$ molecule. The formed cyclo(GlycylGlycyl) sublimes and, while interacting with ions $\text{He}^{2+}$, is most probably ionized in the single electron capture process. The single-charged ion of cyclic dipeptide is characterized by a high probability to avoid fragmentation, which is, generally speaking, not characteristic of most biological molecules. Perhaps, this may be explained by that formation of fragmentary ions of cyclic structures always needs rupturing of at least two molecular bonds.

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