Hartmut Jungclas*, Viacheslav V. Komarov, Anna M. Popova and Lothar Schmidt

Non-Statistical Oligopeptide Fragmentation by IR Photons with $\lambda$ = 16–18 $\mu$m

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Abstract: In this article we analyse the vibration excitation and following dissociation of protonated oligopeptide molecules induced by IR photons with $\lambda$ = 16–18 $\mu$m. The analysis is based on our previous works in which we considered a specific non-statistical dissociation process in organic molecules containing substructures consisting of chained identical diatomic dipoles such as (CH$_2$)$_n$. Such dipole chains can serve as IR antennas for external radiation in the IR frequency range. The acquired vibration energy accumulated in IR antennas can be large enough to dissociate molecules within a femtosecond time interval by a non-statistical process, which is driven by a radiationless low-energy transport mechanism inside the peptide molecules. We point out in this article that the suggested IR-induced dissociation mechanism can be applied to obtain sequence information of protonated oligopeptides.

Keywords: IR Radiation; Oligopeptide Fragmentation.

1 Introduction

The general interest in peptide structures has stimulated investigations of dissociation processes and the analysis of fragment ion spectra. There are a lot of publications devoted to the study of protonated peptide dissociation induced by fast atom bombardment [1–3], electron spray ionization [4], and atom collisions [5, 6]. As a result of these works, it was established that in low-energy processes, the main excitation mechanism of the protonated peptides and their following dissociation is statistical [7]. Besides that, ion fragment spectra obtained in these works show that the dissociation sites are located at different places in the backbones and amino acid side chains in the considered protonated peptides. All these dissociation processes are applied for the development of methods to determine or confirm the amino acid sequences, the investigation of dissociation mechanisms, and the analysis of peptide mixtures [7, 8]. Some of these methods combine matrix-assisted laser desorption/ionization with time-of-flight mass spectrometry (MALDI-TOF MS). In high-energy processes with UV photons [9, 10] both statistical and non-statistical peptide dissociations are possible. These processes are not discussed here, as we only consider peptide dissociations induced by very-low-energy IR photons exclusively.

We suggest a different method of protonated peptide dissociation, which is based upon irradiation of the peptides by IR photons with wave length 16–18 $\mu$m. Dissociation can occur as secondary process in this case. We show that the considered dissociation mechanism proceeds in a femtosecond time scale and that it is a non-statistical process driven by a radiationless low-energy transport inside the peptide molecules. We point out that the suggested method of protonated peptide dissociation induced by IR photons can be used for acquisition of peptide sequence information. However, for unknown peptides, this information can be ambiguous.

2 Theory

In the present article we analyse the vibration excitation and the following dissociation of protonated oligopeptide molecules induced by IR photons with $\lambda$ = 16–18 $\mu$m. This treatment is based on our previous works [11–18], in which we considered a specific non-statistical dissociation process in organic molecules containing chain-like substructures of periodically located diatomic identical dipoles (CH$_2$)$_n$, which can serve as antennas for external IR radiation with a proper frequency.

As it is known, protonated oligopeptides are organic molecular ions of the type
where $R_i$ ($i = 1 \ldots m$) are amino acid side chains and $i$ is the number of amino acids in a sequence of $m$ amino acids in the peptide. The hydrocarbon molecular substructures ($CH_2)_n$ of the amino acid side chains are considered as antennas for IR radiation. In these molecular antennas, collective vibration excitations (excimols) are resonantly and coherently produced and accumulated when IR radiation has a frequency matching the excimol frequency.

As was shown in [11], diatomic CH-dipoles in the ($CH_2)_n$ antenna can be considered as a chain of coupled quantum oscillators with a specific band of vibration states, which are produced instead of the first vibration state of the isolated dipoles. This band is a consequence of the dipole–dipole interaction between the diatomic dipoles in the antenna. The lowest vibration state in the band is called excimol, which has energy $E_{ex} = 0.074$ eV, a lifetime $\tau_{ex} = 10^{-11}$ s, and can be excited in each antenna dipole by radiation with a frequency $\omega_{ex} = 1.2 \times 10^{14}$ s$^{-1}$ [12].

A number of $K_i$ excimols in the antenna ($CH_2)_n$ of each peptide amino acid side chain $R_i$ can be excited independently, but they are not accumulated in a single antenna dipole due to its unharmonicity. During the radiation time $\tau_{ex} < \tau_{ex}$ each dipole in the antenna can be excited $N = \tau_{ex}/\tau_{ex}$ times, where $\tau_{ex} = h/E_{ex}$. Here, $\tau_{ex}$ and $E_{ex}$ are the time and the energy of a vibration excitement transition from one dipole to the next [13]. The number of $K_i$ excimols accumulated in an antenna of $R_i$ can be defined from (8) in [14] by the relation

$$K_i = M_iNP_{ex},$$

where $P_{ex} = P_{ex}\tau_{ex}$ and $P_{ex}$ is the probability per unit time to excite one excimol in the antenna. In addition, $M_i$ is a real number of CH dipoles in the antenna of $R_i$. The function $P_{ex}$ can be calculated in the frame of the time depended perturbation theory by the equation

$$P_{ex} = (8\pi^2/3hc)(eD_{0}/r_0)^2\omega_{01}^2(F/E_{ex}),$$

where $\omega_{01}$ is the dipole transition matrix element for the excimol excitation, $eD_{0}$ is the dipole momentum value of the antenna dipole, $r_0$ is the equilibrium CH dipole length, and $F$ is the IR radiation fluency. During the irradiation time $\tau_{ex} < \tau_{ex}$ multi-excimol excitation in the dipole chain of each $R_i$ occurs independently. Consequently, in each antenna of $R_i$ the accumulated excimol energy $E(K_i)$ is defined by the relation

$$E(K_i) = K_iE_{ex} = M_iNP_{ex}E_{ex}\tau_{ex}.$$
in the not excited N-terminal protonated peptide, the H-bond is formed between nitrogen (N) and oxygen in the bond C–O. Thus, the dissociation energy of the bond N–C in peptides protonated at the N-terminal is lower compared to the dissociation energy of this bond in the corresponding neutral peptides. However, the N-terminal protonation does not change the dissociation energy of the bond C2–C3 significantly. For neutral peptides the dissociation energy of the bond N–C is 1.5 times lower than the dissociation energy of the bond C2–C3, and N-terminal protonation increases this ratio of dissociation energies. This difference of the dissociation energies of the bonds N–C and C2–C3 enables the accumulation of excimol energies in R1 up to the energy needed for resonant dissociation of C2–C3.

Then, the immonium fragment ion \([\text{NH}_2–\text{CHR}_1]^+\), where R1 is the side chain of the first amino acid in the oligopeptide sequence, is produced with maximum probability in the considered process by dissociation of C2–C3 bonds in any N-protonated oligopeptide. The intensity of the photon flux must correspond to the accumulated excimol energy in the antenna of R1 equal to 3.35 eV, which is the dissociation energy of the considered bond. Besides the pronounced fragment ion \([\text{NH}_2–\text{CHR}_1]^+\) in the spectrum, there are also fragment ions of R (i = 1 ... m).

As it follows from the presented analysis, a non-statistical mechanism for the transfer of excitation energy combined with the special peptide structure defines the dissociation sites in protonated oligopeptide ions and thus the resulting fragment ion spectra induced by IR photons. These specific spectra of oligopeptide ions do not contain fragments produced by backbone dissociation except the N-terminal immonium fragment ion \([\text{NH}_2–\text{CHR}_1]^+\). The probability of this fragment production has a maximum value for a definite intensity of the IR photon flux. As follows from (6), this flux depends upon the number of the CH dipoles in the IR antenna of R1 for a fixed radiation time less than the excimol lifetime.

Oligopeptides are composed of up to 20 different amino acids. In Table 1 we present the 20 possible m/z values of N-terminal immonium fragment ions \([\text{NH}_2–\text{CHR}_1]^+\) (j = 1 ... 20). In this table we also present the number \(M_{R_j}\) of CH dipoles in R1j. Because the dissociation energy of C2–C3 is known, it is possible to calculate the intensity value \(F_j\) of the IR photon flux, for which the accumulated excimol energy in the IR antenna of any N-terminal amino acid (j) is equal to the dissociation energy \(E_d(B)\). Using (6), we calculated \(F_j\) vs. the possible number of dipoles \(M_{R_j}\) in the IR antenna of R1j for the fixed radiation time \(\tau_{ex}\) and \(E_d = 3.35\ eV\) (Fig. 1). Because the value of \(F_j\) depends upon \(\tau_{ex}\), one can obtain a value of \(F_j\) for any \(\tau_{ex}\) by using Figure 1.

The experimentally obtained ion spectra of oligopeptides contained also possible fragment ions of amino acid

**Table 1:** Calculated grazing velocities \(v_{max}^{gr}\) for a maximum yield of a, fragment cations from oligopeptide cations grazing along a hydrocarbon surface.

<table>
<thead>
<tr>
<th>Amino acid (j)</th>
<th>m/z of immonium fragment ions ([\text{NH}_2–\text{CHR}_1]^+)</th>
<th>Number of CH dipoles in antenna of R1j</th>
<th>Calculated grazing velocities (v_{max}^{gr} \times 10^4\ m/s) for ions ([\text{NH}_2–\text{CHR}_1]^+) maximum yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine (A)</td>
<td>44</td>
<td>4</td>
<td>0.50 ± 0.05</td>
</tr>
<tr>
<td>Arginine (R)</td>
<td>129</td>
<td>7</td>
<td>0.60 ± 0.10</td>
</tr>
<tr>
<td>Asparagine (N)</td>
<td>87</td>
<td>3</td>
<td>1.00 ± 0.10</td>
</tr>
<tr>
<td>Aspartic acid (D)</td>
<td>88</td>
<td>3</td>
<td>1.00 ± 0.10</td>
</tr>
<tr>
<td>Cysteine (C)</td>
<td>76</td>
<td>3</td>
<td>1.00 ± 0.10</td>
</tr>
<tr>
<td>Glutamic acid (E)</td>
<td>102</td>
<td>5</td>
<td>0.65 ± 0.05</td>
</tr>
<tr>
<td>Glutamine (Q)</td>
<td>101</td>
<td>5</td>
<td>0.65 ± 0.05</td>
</tr>
<tr>
<td>Glycine (G)</td>
<td>30</td>
<td>2</td>
<td>1.00 ± 0.10</td>
</tr>
<tr>
<td>Histidine (H)</td>
<td>110</td>
<td>3</td>
<td>1.00 ± 0.10</td>
</tr>
<tr>
<td>Isoleucine (I)</td>
<td>86</td>
<td>8</td>
<td>0.60 ± 0.05</td>
</tr>
<tr>
<td>Leucine (L)</td>
<td>86</td>
<td>8</td>
<td>0.60 ± 0.05</td>
</tr>
<tr>
<td>Lysine (K)</td>
<td>101</td>
<td>9</td>
<td>0.60 ± 0.05</td>
</tr>
<tr>
<td>Methionine (M)</td>
<td>104</td>
<td>5</td>
<td>0.65 ± 0.05</td>
</tr>
<tr>
<td>Phenylalanine (F)</td>
<td>120</td>
<td>3</td>
<td>1.00 ± 0.10</td>
</tr>
<tr>
<td>Proline (P)</td>
<td>70</td>
<td>7</td>
<td>0.60 ± 0.10</td>
</tr>
<tr>
<td>Serine (S)</td>
<td>60</td>
<td>3</td>
<td>1.00 ± 0.10</td>
</tr>
<tr>
<td>Threonine (T)</td>
<td>74</td>
<td>2</td>
<td>1.00 ± 0.10</td>
</tr>
<tr>
<td>Tryptophan (W)</td>
<td>159</td>
<td>3</td>
<td>1.00 ± 0.10</td>
</tr>
<tr>
<td>Tyrosine (Y)</td>
<td>136</td>
<td>3</td>
<td>1.00 ± 0.10</td>
</tr>
<tr>
<td>Valine (V)</td>
<td>72</td>
<td>6</td>
<td>0.60 ± 0.05</td>
</tr>
</tbody>
</table>
side chains. As the probability of backbone dissociation is negligible except for the site C$_2$–C$_3$, the maximum m/z values of fragment ions in these spectra do not exceed m/z 159, which corresponds to the value of m/z for the immo-

3 Experiment

To demonstrate the suggested mechanism of dissociation protonated oligopeptides irradiated by IR photons, we performed a set of experiments in which the periodical Coulomb field with eximol frequency $\omega_{\text{ex}}$ was used. This periodical Coulomb field experienced by grazing molecules, which are sliding at a velocity $v_\text{gr}$ below Bohr velocity along a set of four or more screened charges of surface atoms at a minimum distance of about 2 Å during a time period of about $10^{-12}$ s [12].

Our periodic Coulomb field excitation model for IR antenna dipoles inside amino acid side-chains of grazing protonated oligopeptide molecules is based, firstly, on the well-known quantum theory of velocity-dependent resonant excitation of dipoles passing charged particles [24] and, secondly, on the analytical evaluation of the intensity of Coulomb field harmonic corresponding to the frequency $\omega_{\text{ex}}$ (see appendix in [12]). This permits us to analyse the dissociation fragment spectra of grazing protonated oligopeptide molecules in the frame of the presented theory.

In our experimental device the velocity component of the grazing molecule normal to the surface is close to zero. Under our conditions only the Coulomb interaction between the grazing molecules and the surface atoms is essential. All other processes, e.g. molecular scattering and charge transfer, resonant electron transfer and Auger are negligible [14].

If the accumulated excimol energy in the IR antenna of $R_1$ of the grazing N protonated oligopeptide becomes equal to the dissociation energy of the bond C$_2$–C$_3$, then the fragment spectrum of dissociating peptides contain the peak corresponding to the immonium fragment ion [NH$_2$–CHR$_1$]$^+$, which pronounce with a maximum abundance, comparing with peaks corresponding to accompanying fragments. The dissociation energy for this process is defined by the equation

$$E_d + \hbar \omega_{\text{ex}} = E_{\text{ex}} + F_{\text{coul}}(v_{\text{gr}}) M, N,$$

where $F_{\text{coul}}(v_{\text{gr}})$ is the probability of one excimol excitation in IR antenna during grazing time. The analytical expression for this function was obtained earlier in our work [14]. In this expression the flux intensity of the photons with $\omega_{\text{ex}}$ of the considered periodic Coulomb field depends upon the grazing velocity. The values of the function $F_{\text{coul}}(v_{\text{gr}})$ vs. $v_{\text{gr}}$ are calculated by using the equation

$$F_{\text{coul}} = (8 \pi^2 e^2 / 3 \hbar c E_{\text{ex}})(D_0 / \tau_0)^2 \gamma R_1 F_{\text{coul}} \tau_{\text{eff}},$$

where $F_{\text{coul}}$ is defined by

$$F_{\text{coul}} = Z^2 e^2 c \omega_{\text{ex}} (2 v_{\text{gr}} b^{-1})^{-1} \cdot \exp \left[ -2 R_1 \left( a_{\text{eff}} + (\omega_{\text{ex}} / v_{\text{gr}})^2 \right) \right].$$

Here, $a_{\text{eff}}$ is an effective radius of the screen charges $Z$ of surface atoms, and $b$ is a distance between these charges.

Simultaneous analysis of (7) and (8) by the condition that the grazing time equals $10^{-12}$ s, permits one to obtain for different $R_1$, ($i = 1\ldots20$), values of the grazing velocity $v_{\text{gr}}$ max, for which the accumulated excimol energy is equal to the dissociation energy of the bond C$_2$–C$_3$ in the oligopeptide protonated at the N terminal site. The calculated values of $v_{\text{gr}}$ max are presented in the last column of Table 1.

4 Experimental Results

Using the modified mass spectrometer, we measured the GME-induced dissociation spectra of the oligopeptides YGGFL, YGGFLK, and the YGGFLR for grazing velocities $v_{\text{gr}}$ of 1.05·10$^4$, 0.98·10$^4$, and 0.95·10$^4$ m/s correspondingly. Here, we used the standard type abbreviations for amino acids in the oligopeptides. The aim of this measurement analysis was to prove that the production of N-terminal fragment ions has a maximum probability for $v_{\text{gr}} = v_{\text{gr}}$ max (see Table 1) and that $R_1$ ($i \neq 1$) following $R_1$ do not change the probability of the N-terminal fragment ion production.
The fragment ion spectra are presented in Figure 2. The source of the fragment production is the energy accumulated in IR antennas of the amino acid side chains. For all considered oligopeptides, the N-terminal fragment ion $[\text{NH}_2–\text{CHR}_1(Y)]^+$ with m/z 136 appears with highest intensity for the proper grazing velocity (see Table 1). In addition, the fragment ion $\text{CHR}_1(Y)^+$ with m/z 120 and $R_1(Y)^+$ with m/z 107 are visible in all spectra. Also, in all spectra are fragment ions of the phenylalanine $R(F)$ at m/z 91 and a fragment ion of its dissociation at m/z 77, plus fragment ions of the leucine $R(L)^+$ with m/z 57 and fragment ions of its dissociation with m/z 43 and 30. In the spectrum for the oligopeptide YGGFLK, there are peaks corresponding to the ion of the lysine amino acid $R(K)^+$ with m/z 73 and fragment ions of its dissociation with m/z 56 and 17. The oligopeptide YGGFLKR yields peaks corresponding to the ion of the arginine side chain $R(R)^+$ with m/z 101 and fragment ions of its dissociation with m/z 59 and 44. Its N-terminal fragment ion $[\text{NH}_2–\text{CHR}_1(Y)]^+$ also appears in the spectrum. The spectra in Figure 2 show that the N-terminal fragment ion $[\text{NH}_2–\text{CHR}_1(Y)]^+$ has a maximum abundance for value $\nu_{gr}^{\text{max}}$, which is obtained theoretically in the present work. These spectra also show that a change of amino acid side chains in the sequence following $R(Y)$ does not influence the abundance of the N-terminal fragment ion $[\text{NH}_2–\text{CHR}_1(Y)]^+$. The N-terminal fragment ion $[\text{NH}_2–\text{CHR}_1(Y)]^+$ is not pronounced significantly if the value of the grazing velocity $\nu_{gr}$ is not equal to $\nu_{gr}^{\text{max}}$. We measured the dissociation fragment spectrum of the grazing peptide YR for $\nu_{gr} = 1.54 \times 10^4$ m/s, which does not correspond to $\nu_{gr}^{\text{max}}$. The spectrum shows that the peak corresponding to the N-terminal fragment ion $[\text{NH}_2–\text{CHR}_1(Y)]^+$ is negligible.

**5 Discussion and Conclusions**

In this article the dissociation of protonated oligopeptides induced by IR radiation with $\lambda = 16–18 \mu$m was investigated. This process is possible, because amino acid side chains in peptide molecules contain hydrocarbon substructures, which can serve as antennas for IR photons with the energy 0.07 eV corresponding to a collective vibrational state (excimol) in the mentioned antenna. Earlier in our works we analysed the resonant absorption and accumulation of excimols in IR antennas and suggested a non-statistical picosecond transport mechanism of accumulated excimol energy from antenna dipoles to neighbouring molecular dipoles, which do not belong to the antenna. The results of these works were applied for the explanation and detail analysis of fragment ion spectra of protonated oligopeptides.

The following important properties of the considered process were predicted and supported by experimental evidence. The accumulated excimol energy in one side chain is not transferred to any other side chains. The fragment ion spectra do not contain fragment ions produced by backbone dissociations except the N-terminal immonium fragment ion. The spectra do not contain fragment

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**Figure 2:** Mass spectra of the protonated oligopeptides YGGFL (a), YGGFLK (b), and YGGFLR (c) after GME-induced fragmentation. The peak at m/z 136 in each of the spectra corresponds to the ion $[\text{NH}_2–\text{CHR}_1(Y)]^+$, where $R_1(Y) = \text{CH}_2–\text{C}_6\text{H}_4–\text{OH}$ is the side chain of the N-terminal tyrosine amino acid.

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Ions of the oligopeptide sample are produced by a nitrogen laser pulse, and all cations are accelerated by the potential $U_{acc} = 8$ kV. Molecular ions are selectively deflected by a pair of pulsed deflection plates; thus, these cations reach a converter at an angle $\alpha = 11.5^\circ$. The converter consists of a stretched aluminized foil and a grounded grid mounted parallel to the foil at the distance of 7 mm. The converter surface is covered by a hydrocarbon layer. The screened charges of the hydrogen atoms of the hydrocarbon layer are sources of a periodic Coulomb field. By choosing a positive converter potential, the desired grazing condition for the primary cations is achieved for $U_{acc} = U_{cos} \alpha = 765$ keV. The strength of the homogeneous electric field in the space between the converter surface and the grid is about $10^{-4}$ eV/Å at the mentioned value of $U_{acc}$ and the angle $\alpha$. Due to Coulomb repulsion between the atomic charges of the peptide and atomic charges of converter molecules, the normal component of the projectile velocity comes to zero at a minimum distance about 2 Å to the converter surface. Now the projectiles are grazing along the converter surface molecules for about $10^{-12}$ s. During this grazing period the primary cations are excited in grazing molecule excitation (GME) and dissociated. Only positively charged dissociation products are reaccelerated by the converter potential and finally analysed using the time-of-flight technique. For details, see references [25, 26].

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ions with m/z bigger than the m/z value of the tryptophan immonium ion. The intensity of the IR radiation flux for which the N-terminal immonium fragment ion has a maximum abundance depends upon the number of IR antenna dipoles in R. The established properties of the oligopeptide ion spectra obtained by IR photon irradiation and dependence of these spectra on the irradiation flux intensity by fixed irradiation time \( \tau < 10^2 \) is less than the excimol lifetime, which permits us to determine the amino acid sequence of oligopeptides.

Using the described features of the oligopeptide spectra obtained by IR photons from any source, it is possible to identify the first N-terminal amino acid in a sequence of the considered protonated oligopeptide. This is possible, because the suggested analysis of these spectra permits simultaneously to determine the number \( M_1 \) of IR antenna dipoles in R, the N-terminal immonium fragment ion and the value m/z of this fragment ion. With the knowledge of the first amino acid, the other patterns of the amino acid chain can be analysed in the same way, provided a digestion method is used (e.g. in-source decay [27]), which yields enough molecules.

Repeating this procedure again and again, it is possible to reveal the total amino acid sequence of the oligopeptide. In each step of this procedure a suitable value of the grazing velocity must be applied. By this procedure we can face a problem with definition of \( R_1 \) of isomers Leucine and Isoleucine, as the parameters \( M_1 \) and m/z are equal. These amino acids can be identified, however, if the full fragment ion spectrum is analysed in which the considered N-terminal immonium ion has maximum abundance. The presence of a peak with m/z = 57 in this spectrum indicates that \( R_1 \) in the N-terminal immonium fragment ion belongs to the amino acid Isoleucine [28].

References