# Resonant dissociative electron capture by the simplest amino acids and dipeptides

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The formation of ions from amino acids (glycine and alanine) and dipeptides (glycylglycine, alanylalanine, and glycylalanine) under the resonant electron capture conditions was studied by negative ion resonant electron capture mass spectrometry. The isobaric ions were found, their effective yield curves were experimentally separated, and the elemental composition was determined. The thermochemical aspect of ion formation was considered, and probable dissociative channels of fragmentation ion formation and their structures were established on the basis of this aspect. Bond cleavage reactions only and H-shift processes were revealed. The rearrangements occur presumably through the stage of formation of intramolecular hydrogen bonds. The cross-sections of formation of ions $[M - H]^-$ were measured in the energy range 1.1—1.3 eV. The metastable decay channels of ions $[M - H]^-$ and $[M - COOH]^-$ were found in the energy range 4.5—7.5 eV for dipeptides, which enabled establishing the genetic relationship between the parental and daughter ions and revealing hidden fragmentation pathways.

**Key words:** mass spectrometry, resonant electron capture, negative ions, glycine, alanine, glycylglycine, alanylalanine, glycylalanine.

Recent studies in the field of radiation biology showed[1] that biomolecules can decompose in electron-molecular reactions upon the interactions with electrons having an energy considerably lower than the ionization potentials of the molecules. In this case, the molecules dissociate due to processes of resonant electron capture,[2] which usually occurs in the region of electron energies ≤15 eV. This is related to the formation of the short-lived negatively charged molecular ions that further decay in the accessible dissociative channels or due to the ejection of the captured electron (autoneutralization). This discovery has earlier initiated studies of resonant electron capture by various chemical compounds of biological significance (amino acids, nucleic bases, *etc.*) and related compounds (organic acids, five-membered heterocycles, *etc.*). The mechanisms of electron capture by molecules of some amino acids (glycine,[3—6] alanine,[5,7] valine,[8] proline,[9] tryptophane,[10] and phenylalanine[5]) have been established and their fragmentation processes were studied to date. The next stage is the study of electron-molecular reactions involving peptides.

In the present work, we studied processes of resonant capture of energy-controlled electrons by dipeptide molecules: glycylglycine (Gly-Gly), glycylalanine (Gly-Ala), and alanylalanine (Ala-Ala). The study of larger peptides by the resonant electron capture method is prevented by their decomposition upon thermal sublimation *in vacuo*. The process of electron capture by glycine (Gly) and alanine (Ala) are also considered for comparison.

## Experimental

Mass spectra were recorded on an MI-1201 magnetic mass spectrometer (Sumy, Ukraine) modified for the detection of negative ions (NI)[11]; the possible detection of positive ions conserved as well. The scheme of the apparatus is presented in Ref. 12. The fast switch from one mode to another was carried out by changing the polarity of the electrode potentials of the ion-optical system and the current polarity of the analyzing electromagnet. The mass spectrometer is equipped with two ion sources, where one of them contains a trochoidal electron monochromator. The electron beam emitted from the Th/W cathode passed through the trochoidal electron monochromator decreasing the electron scatter with respect to kinetic energies. The electron energy scale was calibrated by maxima in the effective yield curves (EYC) of the ions $SF_6^-/SF_6$ (~0 eV) and $[M - H]^-/CH_3COOH$ (~1.55 eV, see Ref. 13). The appearance energy (AE) of the negative ions was determined relative to the beginning of the yield maxima of the ions $SF_6^-/SF_6$ (~0 eV). The mass resolution of the instrument was ~2000 with the narrow open slits of the ion receiver, which was used for the separation of the EYC of isobaric ions. Since the intensity of the ion signals is decreased in the case, the main work was carried out with the widely open receiver slit, which made it possible to detect reliably

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low-intensity ions. The procedure of determination of the cross-section of the fragment NI formation has been described previously.[14]

Commercial samples of the studied compounds (Sigma/Aldrich Chemical Co.) were used. The sample under study was placed on the bottom of the ionization chamber from which it was thermally evaporated. Unlike the use of the tube of direct inlet of solid samples, this procedure made it possible to obtain the necessary vapor pressure in the chamber at comparatively low heating temperatures, which is very important for the work with peptides. The effective yield curves of the NI from Gly (86 °C) and Ala (85 °C) as functions of the electron energy were obtained using a monochromator, $\Delta E_{1/2}$ = 0.30—0.32 eV; the yield curves of the NI from Gly-Gly (175 °C), Ala-Ala (139 °C), and Gly-Ala (141 °C) were obtained without a monochromator,

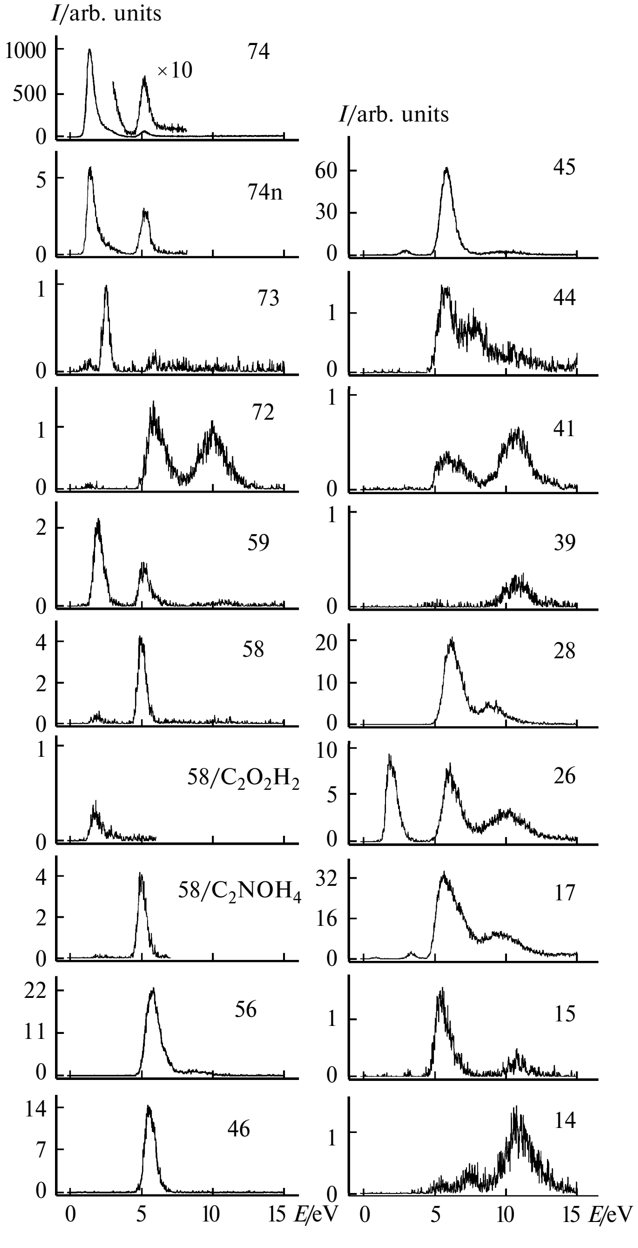

**Fig. 1.** Effective yield curves (EYC) for the NI from Gly (an ion source with an electron monochromator, $\Delta E_{1/2}$ = 0.32 eV). The total EYC and individual curves recorded with the broad and narrow slit of the ion receiver, respectively, are presented for the isobaric ions $C_2O_2H_2^-/C_2NOH_4^-$ (*m/z* 58). Here and in Figs 2—5, the mass number (*m/z*) of ions is given at upper right; *E* is the energy of electrons, and *I* is the intensity.

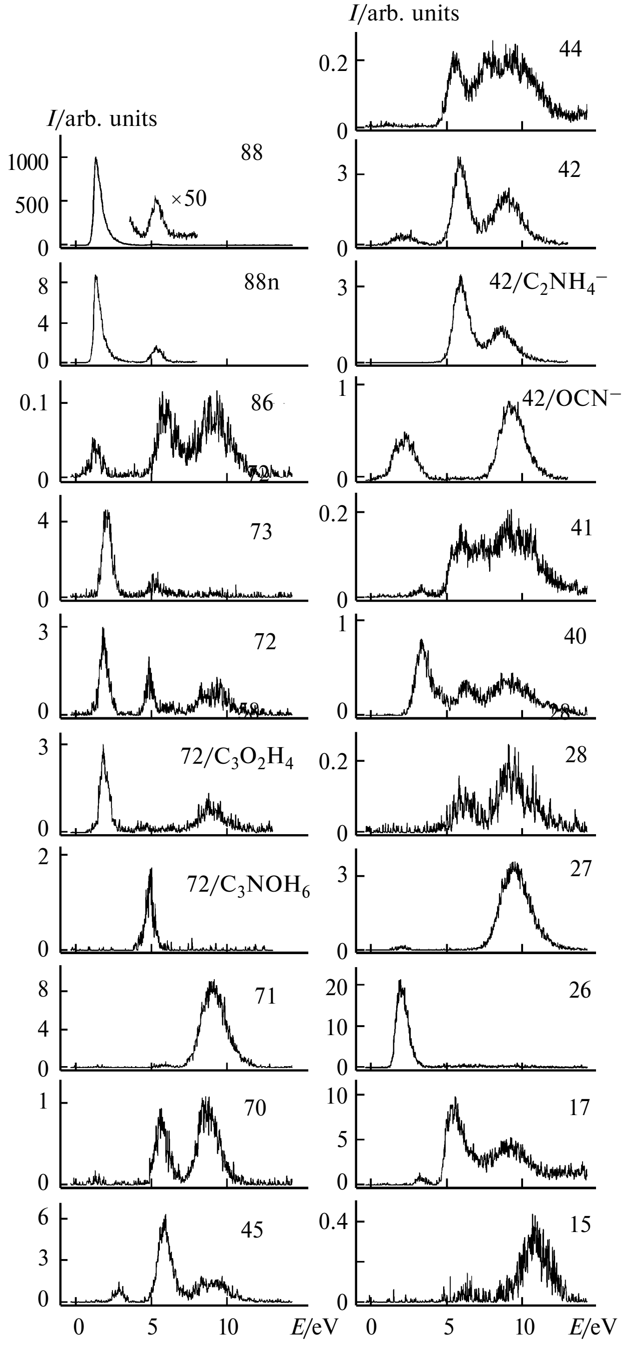

**Fig. 2.** Effective yield curves for the NI from Ala (an ion source with an electron monochromator, $\Delta E_{1/2}$ = 0.3 eV). The total EYC and individual curves recorded with the broad and narrow slit of the ion receiver, respectively, are presented for the isobaric ions $C_3O_2H_4^-/C_3NOH_6^-$ (*m/z* 72) and $C_2NH_4^-/OCN^-$ (*m/z* 42).

$\Delta E_{1/2}$ = 0.52—0.58 eV. The yield curves of the $[M - H]^-$ ions with $\Delta E_{1/2}$ = 0.11—0.12 eV from amino acids (85—86 °C) and with $\Delta E_{1/2}$ = 0.12—0.13 eV from peptides (110—128 °C) were obtained in additional experiments.

The pressure in the region of the ion source was monitored by a magnetic ionization sensor; in experiments it did not exceed $8 \cdot 10^{-5}$ Pa, the residual pressure being $5 \cdot 10^{-5}$ Pa.

## Results and Discussion

Resonant electron capture by molecules of the studied amino acids and peptides occurs at the energies ≤12 eV and produces various fragmentation NI (Figs 1—5). The whole energy range in which the resonance NI peaks were detected was arbitrarily divided into three regions with low energy (0—4.5 eV), medium energy (4.5—7.5 eV), and high energy (>7.5 eV). The main criterion for the elucidation of the structures of molecular ion dissociation products is the energy of the processes (the thermochemical parameters of ions and neutral species necessary for calculations are given below).

The principle of least structural changes during reactions is the next in significance: the processes of just the bond cleavage rather than rearrangements were preferred. The mass numbers of ions that can be formed due to the simple cleavage of this or another bond in dipeptides are shown in Fig. 6. The classification of ion formation processes from peptides is shown in Fig. 7.

**Ions $[M - H]^-$.** At the energy of 1.14 eV, peptides form the ions $[M - H]^-$ with high efficiency due to H atom elimination from the carboxy group, which is characteristic of organic acids[15—18] and amino acids studied earlier.[3—10] We determined the cross-section of dissociative electron capture for these ions as $2.8 \cdot 10^{-18}$ (*m/z* 131, Gly-Gly and *m/z* 159, Ala-Ala) and $3.1 \cdot 10^{-18}$ cm$^2$ (*m/z* 145, Gly-Ala). These values are approximately twice as large as analogous values for the $[M - H]^-$ ions from amino acids in the energy region 1.3 eV: $1.3 \cdot 10^{-18}$

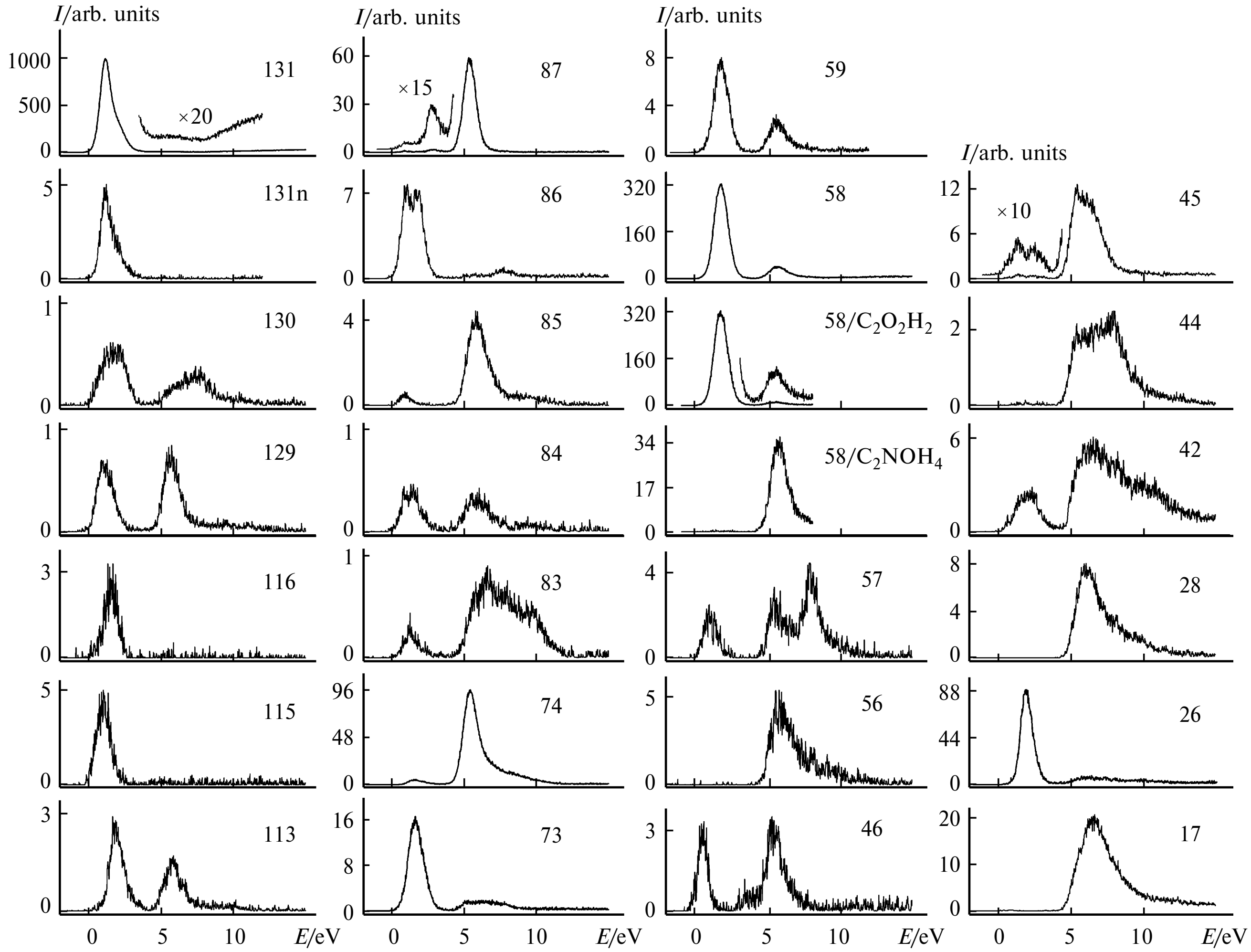

**Fig. 3.** Effective yield curves for the NI from Gly-Gly (an ion source with an electron monochromator, $\Delta E_{1/2}$ = 0.58 eV). The total EYC and individual curves recorded with the broad and narrow slit of the ion receiver, respectively, are presented for the isobaric ions $C_2O_2H_2^-/C_2NOH_4^-$ (*m/z* 58).

(*m/z* 74, Gly) and $1.6 \cdot 10^{-18}$ cm$^2$ (*m/z* 88, Ala). The latter values agree well with the values $3.9 \cdot 10^{-19}$ and $4.4 \cdot 10^{-19}$ cm$^2$ (Ref. 19). However, they considerably differ with the values $5 \cdot 10^{-16}$ (see Ref. 4) and $10^{-16}$ cm$^2$ (see Ref. 3). It is mentioned[19] that the discrepancy with the published[3,4] values of measured cross-sections of the $[M - H]^-$ ions are probably due to the incorrect determination of the vapor pressure of the sample. According to the literature data,[20] the $[M - H]^-$ ions in the energy region 1.3 eV are formed from amino acids due to the decay of the dipole-bound state of molecular ions. It is most likely that the discussed ions formed from peptides in the energy region 1.14 eV have the same origin.

The effective yield curve of ions from Gly exhibits a shoulder at 3 eV and a peak at $E = 5.2$ eV. As mentioned earlier,[5,20] the origin of the both varieties is caused by H atom elimination from the $C_\alpha$ atoms. Similar processes occur in Ala but with a lower efficiency, perhaps, due to the deficient of the hydrogen atoms at the $C_\alpha$ atom. At 2 eV, the EYC of ions from peptides exhibit explicit inflections caused by the abstraction of the carboxylic H atom with a small contribution of the loss of the H atom

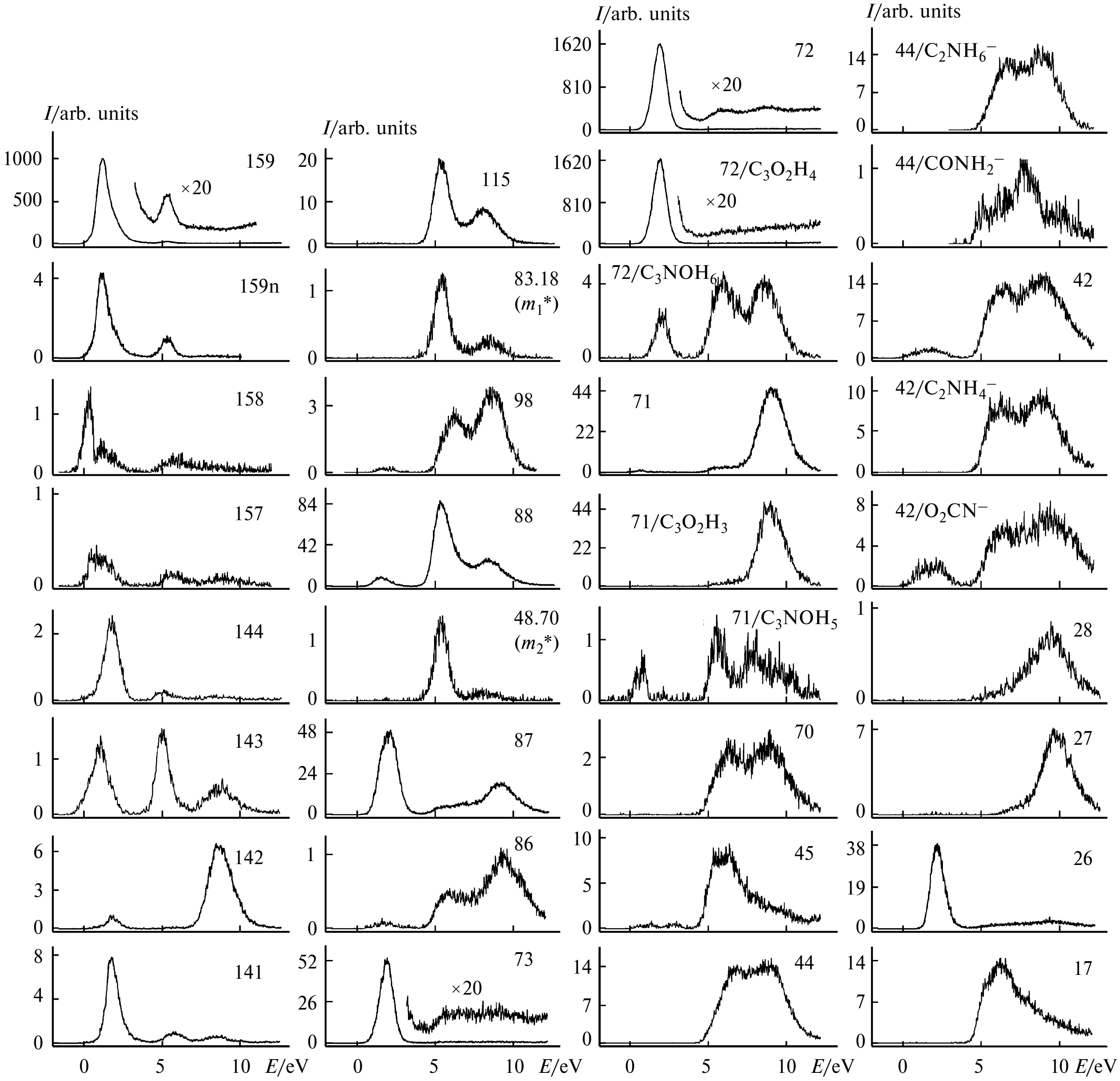

**Fig. 4.** Effective yield curves for the NI from Ala-Ala (an ion source with an electron monochromator, $\Delta E_{1/2} = 0.52$ eV). The total EYC and individual curves recorded with the broad and narrow slit of the ion receiver, respectively, are presented for the isobaric ions with *m/z* 72, 71, 44, and 42.

at the peptide nitrogen atom.[13] The low-intensity peak for Gly-Ala and Ala-Ala was detected at 5 eV. Perhaps, it has the same origin as that in amino acids. For Gly-Gly no similar peak was observed in the medium-energy region against the background of intense secondary processes, and it remains unclear whether long-lived ions are formed or not (the term "long-lived" means that the ions can be detected mass spectrometrically). This question is clarified to a greater extent by the EYC of the neutrals due to the loss of electrons by ions in the field-free region (marked as 74n, 88n, 131n, 145n, and 159n, see Figs 1—5). In the low-energy region, they match completely the yield curves of ions but with the intensity two orders of magnitude lower. In this case, ions are formed near the threshold

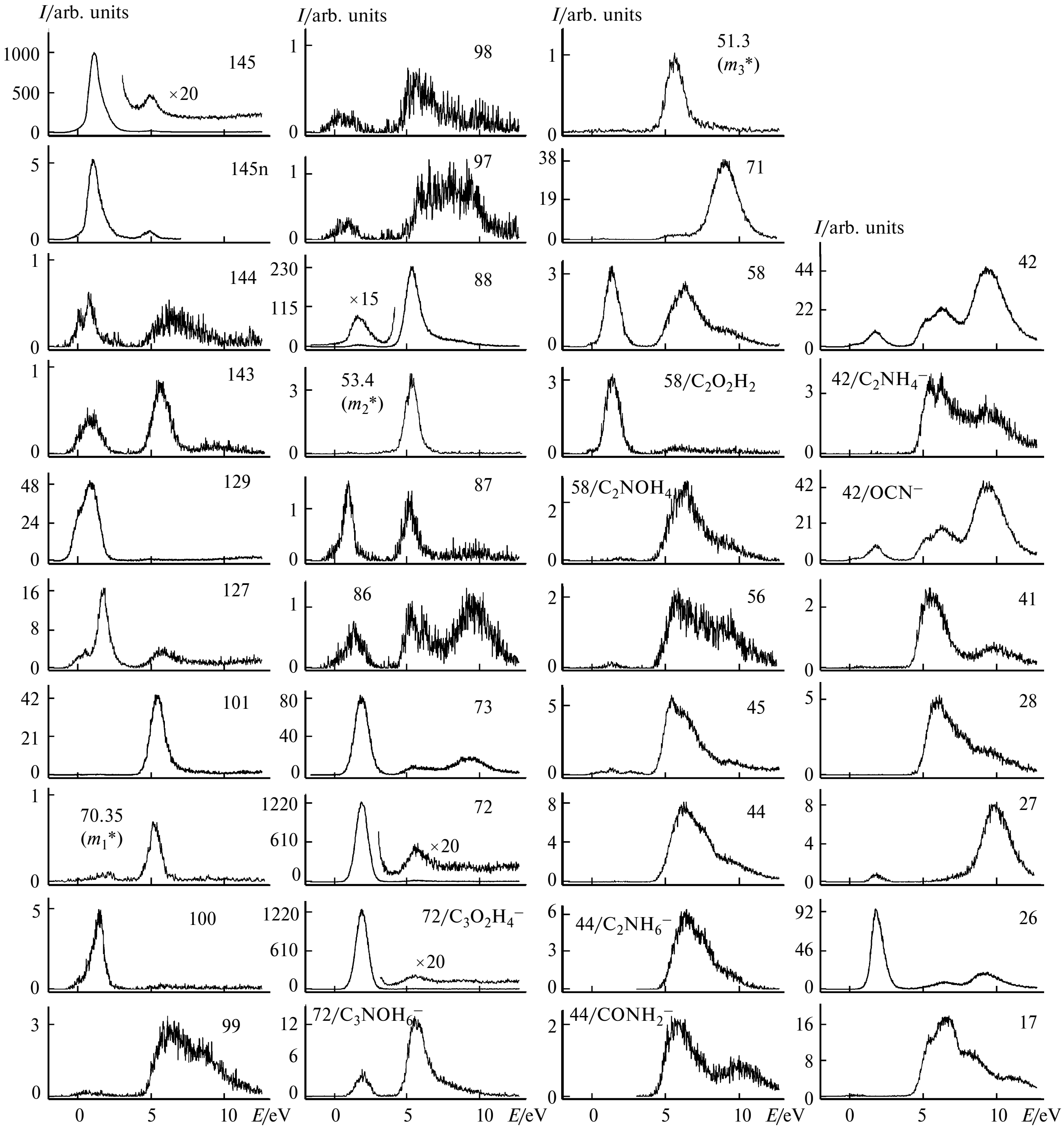

**Fig. 5.** Effective yield curves for the NI from Gly-Ala (an ion source with an electron monochromator, $\Delta E_{1/2}$ = 0.54 eV). The total EYC and individual curves recorded with the broad and narrow slit of the ion receiver, respectively, are presented for the isobaric ions with *m/z* 72, 58, 44, and 42.

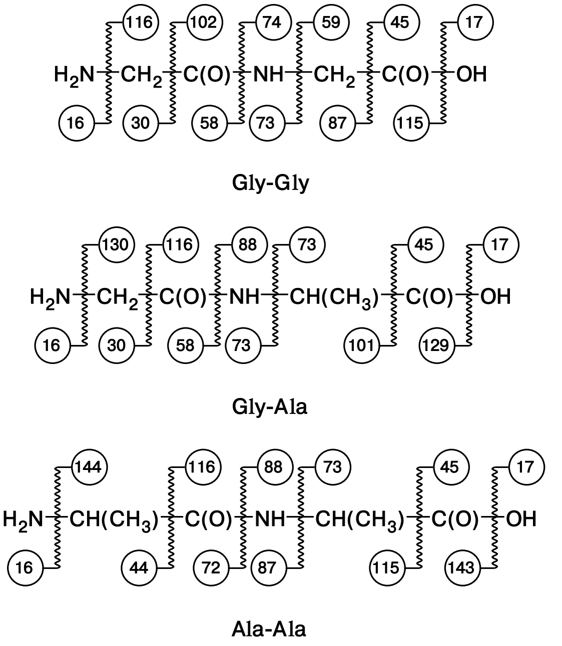

**Fig. 6.** Mass numbers of ions that can be formed due to the cleavage of a particular bond only in the dipeptides under study.

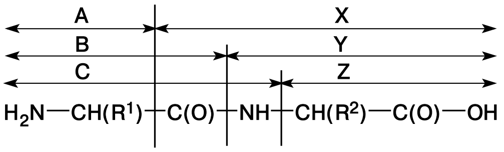

**Fig. 7.** Classification of process of ion formation from the peptides under study.

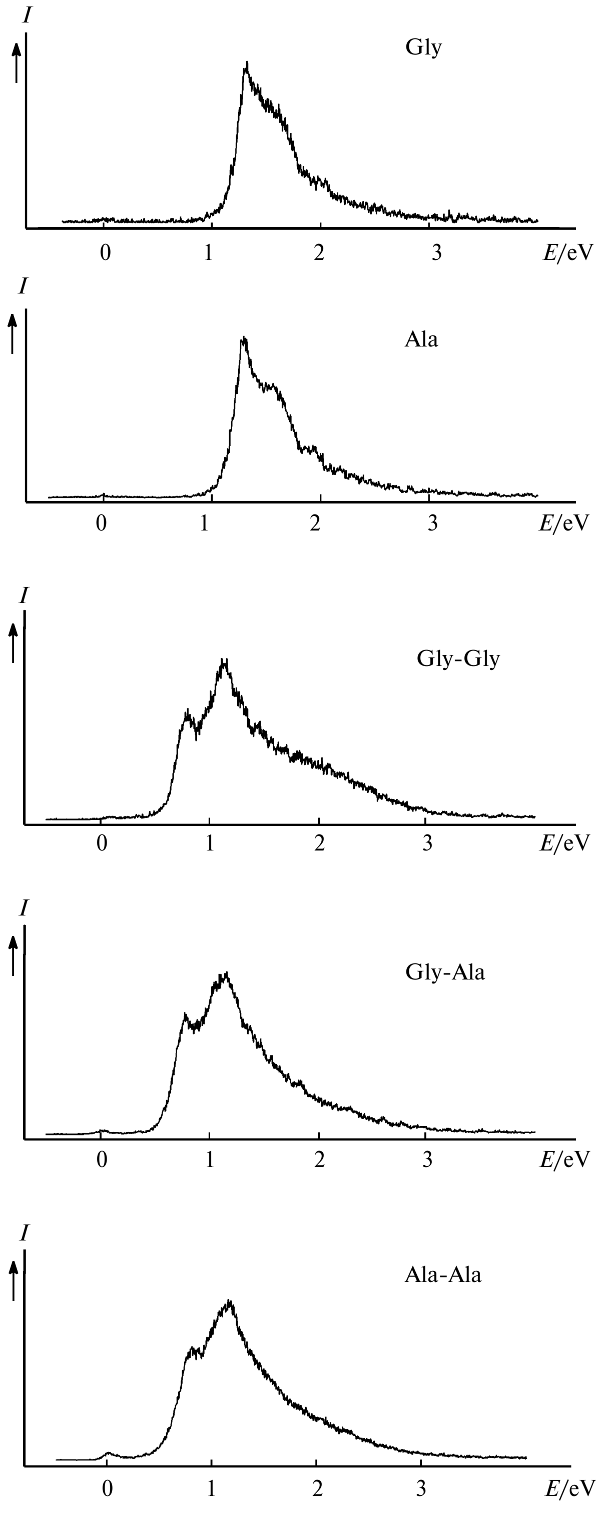

**Fig. 8.** Effective yield curves for the $[M - H]^-$ ions from amino acids ($\Delta E_{1/2}$ = 0.11—0.12 eV) and dipeptides ($\Delta E_{1/2}$ = 0.12—0.14 eV) obtained using an ion source with an electron monochromator.

and, hence, the electron loss is possible only as a result of the collision of ions with molecules of the own or residual atmospheric gas. In the medium-energy region, the ratio of intensities of peaks of ions and peaks of their neutrals differs from that in the low-energy region and indicates that the spontaneous electron ejection by ions of all discussed compounds, except for Gly-Gly, occurs along with the collision process. Thus, neutrals act as indicators of the formation of long-lived NI in the high-energy region. For instance, the neutrals in Ala-Ala show that the long-lived ions are also formed in the region 8 eV; however, this is not evident from the yield curve of ions. The absence of the resonance peak in the yield curve of the $[M - H]^-$ ions and their neutrals from Gly-Gly in the medium-energy region does not mean that these ions are not formed in this case. It is most likely that these decompose rapidly to smaller fragments, whose short lifetime prevents their detection.

Experiments with the electron beam monochromator reveal additional specific features in the EYC of the discussed ions in the low-energy region (Fig. 8). A shoulder at 1.65 eV along with a sharp maximum is observed for Gly. A similar result has been obtained earlier.[5,20] These specific features were identified[20] as a fine structure corresponding to the excitation of vibrations of the O—H bond in the molecular ion. Perhaps, the specific feature (a peak in the implicit form) at 2 eV is related to this fine structure and continues the vibrational progression. However, this specific feature can be related to another resonance state of molecular ions, which are identified[20] as the $\pi_{00}$*-resonance of the form.

It was expected that a similar vibrational progression will be observed in the case of peptides. However, the broad smooth peak extending to ~3 eV does not allow one to reveal the fine structure. The origin of this peak is related, most likely, to the $\pi_{00}$*-resonance of the form as in amino acids, but the efficiency of fragmentation ion formation in peptides is higher. Another distinction of the EYC of ions from peptides from the EYC of ions from amino acids is the sharp peak at 0.8 eV. We believe that this peak is temperature-dependent and continues the vibrational progression of the O—H bond extension. The appearance of such peaks below the threshold dissociation energy at elevated temperatures has been discussed in detail earlier[21] for the OH-containing compounds. Glycine and alanine were studied at a higher temperature than in the present case,[4,7] but no similar temperature-dependent peak was observed. It is most likely that the reason for the anomalously high intensity of the peak of ions at 0.8 eV in peptides is the pre-dissociation mechanism of the process. According to Ref. 21, no stretching vibrations of the O—H bond appear directly upon the formation of molecular ions. Excitation occurs due to the interaction with active vibrations, which appear at the moment of electron capture. The efficiency of this interaction depends on several factors, one of which is the value of the overlapping integral of the corresponding vibrational wave functions. Another important factor is time. A substantial difference in the processes that occur above and below the energy threshold is that the latter require additional time to concentrate an excess of the internal ion energy on the vibrational mode responsible for dissociation. Since the cross section of the fragment NI formation depends exponentially on the lifetime of molecular ions relative to the autodetachment of an electron, an insignificant change in the latter can result in a substantial change in the cross section. Probably, the lifetime of molecular ions in peptides is longer than that in amino acids, because its value increases with an increase in the number of degrees of freedom of the NI. In addition, the additional acceptor group in dipeptides compared to amino acids also favors the more prolonged containment of the captured electron.

**Medium-mass ions from dipeptides.** The effective yield curves of the $[M - 45]^-$ ions (*m/z* 87, Gly-Gly; *m/z* 101, Gly-Ala; *m/z* 115, Ala-Ala), which are formed due to the C—C$_\alpha$ bond cleavage (see Fig. 6), are characterized by a sharp peak in the medium-energy region*

$$\mathrm{M + e \rightarrow NH_2CH(R^1)C(O)NHCH(R^2)^- + {}^{\bullet}COOH} \qquad (1)$$
$(\mathrm{AE_{Gly\text{-}Ala}} = 3.17\ \mathrm{eV})$.

An analog of this process in amino acids should be the formation of the $NH_2CH_2^-$ and $NH_2CH(CH_3)^-$ ions. However, no peak of ions with *m/z* 30 was observed in the mass spectrum of the NI of Gly, and the ions with *m/z* 44 in the mass spectrum of Ala have another elemental composition, although the peaks of the ions with *m/z* 30 ($NH_2CH_2^+$) or 44 ($NH_2CH(CH_3)^+$) predominate in the mass spectra of positive ions of these amino acids. Evidently, the absence of these NI is related to the negative electron affinity of the radicals $NH_2CH_2^{\bullet}$ and $NH_2CH^{\bullet}(CH_3)$.

The metastable peaks with apparent *m/z* 83.18 and 70.35 in the mass spectra of Ala-Ala and Gly-Ala, respectively, indicate that the ions $[M - 45]^-$ in these peptides are formed also by the consecutive decay through the intermediate ion $[M - H]^-$

$$\mathrm{M + e \rightarrow NH_2CH(R^1)C(O)NHCH(CH_3)COO^- + H \rightarrow}$$
$$\mathrm{\rightarrow NH_2CH(R^1)C(O)NHCH(CH_3)^- + CO_2} \qquad (2)$$
$(\mathrm{AE_{Gly\text{-}Ala}} = 3.40\ \mathrm{eV})$.

The metastable peaks found are very important because they directly suggest the formation of the ions $[M - H]^-$ with the structure of the carboxyl anion in the medium-energy region, which, according to the published data,[5] is not characteristic of amino acids. An analogous metastable decay of carboxylate anions with the elimination of the $CO_2$ molecule has been observed previously in benzylacridone acetate[22] and in derivatives of di- and tetrafluorocinnamic acids.[23]

In Gly-Gly, another structure of the ions $[M - 45]^-$ corresponds to the low-intensity peak in the energy region 2.8 eV. These ions are formed due to the rearrangement in the molecular ion

$$\mathrm{M + e \rightarrow NH_2CH_2C(O){=}NCH_3^- + {}^{\bullet}COOH} \qquad (3)$$
$(\mathrm{AE_{Gly\text{-}Gly}} = 1.61\ \mathrm{eV})$.

The both varieties of the ions $[M - 45]^-$ are ions of the series A (see Fig. 7). The group of ions with low-intensity peaks with *m/z* 86—83 in the mass spectrum of Gly-Gly includes ions of the series A with the elemental composition different from the composition of the ions $[M - 45]^-$ by 1—4 hydrogen atoms. This is inferred from the similarity of the EYC of these ions with the EYC of the ions with *m/z* 100—97 from Gly-Ala.

The ions with *m/z* 74 in Gly-Gly and the ions with *m/z* 88 in Gly-Ala and Ala-Ala are most intense in the medium-energy region. They are formed due to the cleavage of the peptide bond only and are similar to the ions $[M - H]^-$ in the corresponding amino acids

$$\mathrm{M + e \rightarrow NH_2CH(R^1)C^{\bullet}(O) + NHCH(R^2)COOH^-} \qquad (4)$$
$(\mathrm{AE_{Gly\text{-}Ala}} = 2.84\ \mathrm{eV})$.

The metastable peaks with apparent *m/z* 48.7 and 53.4 in the mass spectra of Ala-Ala and Gly-Ala indicate, correspondingly, that in these peptides the ions with *m/z* 88

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* Hereinafter $R^1 = R^2 = H$ (Gly-Gly); $R^1 = R^2 = Me$ (Ala-Ala); $R^1 = H$, $R^2 = Me$ (Gly-Ala).

can be formed by the consecutive decay through the intermediate ions $[M - H]^-$ due to the cleavage of the peptide bond only

$$M + e \rightarrow NH_2C(R^1){=}C(O)NHCH(CH_3)COOH^- + H^{\bullet} \rightarrow \rightarrow NH_2C(R^1){=}C{=}O + NHCH(CH_3)COOH^- \quad (5)$$
$(AE_{Gly\text{-}Ala} = 4.43\ eV)$.

However, one cannot exclude a possibility of the H-shift in the intermediate ion, due to which ions with *m/z* 88 with a more stable structure are formed

$$M + e \rightarrow NH_2C(R^1){=}C(O)NHCH(CH_3)COOH^- + H^{\bullet} \rightarrow \rightarrow NH_2C(R^1){=}C{=}O + NH_2CH(CH_3)COO^- \quad (6)$$
$(AE_{Gly\text{-}Ala} = 2.49\ eV)$.

This assumption is based on the fact that in the low-energy region only the structure of the carboxylate anion corresponds to the discussed ions with *m/z* 74 and 88. These ions are formed due to the H-shift in the molecular ion

$$M + e \rightarrow NH_2CH(R^1)C^{\bullet}(O) + NH_2CH(R^2)COO^- \quad (7)$$
$(AE_{Gly\text{-}Ala} = 0.90\ eV)$.

The both varieties of the ions discussed belong to the series Y (see Fig. 7).

The effective yield curve of the ions with *m/z* 73 from Gly-Gly in the low- and medium-energy regions resembles the EYC of the ions with *m/z* 73 from Gly-Ala and the EYC of the ions with *m/z* 87 from Ala-Ala, but the latter has no common features with the EYC of the ions with *m/z* 87 from Gly-Ala. This suggests that the ions discussed are formed due to the cleavage of the $N—C_\alpha$ bond only and represent ions of the series C (see Fig. 7)

$$M + e \rightarrow NH_2CH(R^1)C(O)NH^- + {}^{\bullet}CH(R^2)COOH \quad (8)$$
$(AE_{Gly\text{-}Gly} = 1.04\ eV)$.

It should be mentioned that the EYC of the discussed ions from Gly-Ala contains a large contribution from the isotope peak of ions with *m/z* 72 in the low-energy region.

**Ions $[M - 16]^-$, $[M - 17]^-$, $[M - 18]^-$, and $[M - 19]^-$ in amino acids and their analogs in dipeptides.** The ions $[M - 16]^-$, $[M - 17]^-$, $[M - 18]^-$, and $[M - 19]^-$ in amino acids are formed due to the ejection of the terminal $NH_2$ and OH groups and the H atoms in various combinations.[3–7] Therefore, the listed ions in peptides have the same origin. The ions with *m/z* 59, 58, 57, 56 (from Gly-Gly), 73, 72, 71, 70 (from Ala-Ala), and 73, 72, 71, 58, 56 (from Gly-Ala) are of greater interest, because they are formed due to the cleavages of the central bonds in peptides.

*Analogs of ions $[M - 17]^-$*. In Gly-Gly the ions with *m/z* 58 are the most intense in this group. Their EYC demonstrate resonance peaks in the low- and medium-energy regions as in the case of the ions $[M - 17]^-$ with *m/z* 58 in Gly. Two possible structures were proposed[3] for the latter: $H_2NCH_2C^{\bullet}O^-$ (*i.e.*, $[M - OH]^-$) and $^{\bullet}CHCOOH^-$ (*i.e.*, $[M - NH_3]^-$). From experiments with glycine with deuterium labels and amino acid esters, it was suggested[5] that elimination of ammonia occurs in the low-energy resonance (1—2 eV), whereas the loss of the OH fragments takes place for resonances in the region of high energies (>5 eV). To prove this hypothesis, we carried out an experiment with a narrow open slit of the ion receiver and with better mass resolution than that in Refs 3—6 and detected separately the yield curves of the isobaric NI (see Fig. 1). The instrument resolution higher than 2400 is required for the complete separation of the mass peaks of ions with the nominal mass *m/z* 58. In the present work, the resolution was lower (the use of better resolution considerably decreases the instrument sensitivity). However, when separating the effective yield curves of the NI, we used the following advantage of mass spectrometry of resonant electron capture: the ions $C_2H_4NO^-$ and $C_2H_2O_2^-$ are formed at different energies of electrons.

Assuming that, as in Gly, the peak with *m/z* 58 in Gly-Gly represents two isobaric ions, we carried out a similar experiment: its results are shown in Fig. 3. It follows that in the low-energy region the resonance peak is caused only by the ions $C_2H_2O_2^{\bar{}}$. By analogy to the process in Gly, they are formed upon the rearrangement in the molecular ion

$$M + e \rightarrow NH_2CH_2C(O)NH_2 + CH_2COO^{\bullet -} \quad (9)$$
$(AE = 1.15\ eV)$.

In the medium-energy region, the ions $C_2H_2O_2^-$ and $C_2H_4NO^-$ contribute to the resonance peak, and the latter ion derives upon the bond cleavage only

$$M + e \rightarrow NH_2CH_2C(O)^- + {}^{\bullet}NHCH_2COOH \quad (10)$$
$(AE = 3.36\ eV)$.

The ions with *m/z* 72 from Ala-Ala are analogs of the ions $[M - 17]^-$ with *m/z* 72 in Ala and are similar to the ions with *m/z* 58 from Gly-Gly. As for the ions with *m/z* 58 from Gly-Gly, we separated the yield curves of the isobaric ions with *m/z* 72 for Ala and Ala-Ala (see Figs 2 and 4). However, the experimental result turned out to be somewhat worse than that in the previous case, because the complete separation of the yield curves of these ions requires a higher mass resolution (>3000) than that for the ions with *m/z* 58 (>2400). In the obtained yield curve of the ions $C_3H_6NO^-$ for Ala-Ala, only two broad peaks in the energy region >4 eV are directly related to these ions, whereas the peak in the low-energy region is caused by the ions $C_3H_4O_2^-$. The yield curve of the latter represents an intense peak with a maximum at 1.9 eV, while in the energy region >4 eV the curve is structureless and caused by secondary processes.

Based on the data obtained for peptides Ala-Ala and Gly-Gly, one can assume that each ion with *m/z* 72 and 58 in the mass spectrum of Gly-Ala has the only possible elemental composition. However, in the experiment with the narrow receiver slit we found that these peaks corresponded to pairs of the isobaric ions: $C_3H_4O_2^-$/$C_3H_6NO^-$ (*m/z* 72) and $C_2H_2O_2^-$/$C_2H_4NO^-$ (*m/z* 58) (see Fig. 5). The origin of the ions $C_2H_2O_2^-$ at the energy of 1.3 eV is related to either a possible impurity of Ala-Gly in the sample under study or (which is most likely) the rearrangement of the molecular ion with methyl group migration to the peptide nitrogen atom

$$\mathrm{M + e \rightarrow NH_2CH_2C(O)NH(CH_3) + C_2H_2O_2^{-}}. \qquad (11)$$

The relatively high effective yield of the ions $C_3H_6NO^-$ in the energy region 5.5 eV cannot be explained by an impurity of Ala-Gly or the complex rearrangement of the molecular ion. Based on the metastable peak with apparent *m/z* 51.33 found in the mass spectrum, we assumed three possible variants of formation of isomeric ions $C_3H_6NO^-$ from the ions $[M - 45]^-$.

$$\mathrm{M + e \rightarrow NH_2CH_2C(O)NHCHCH_3^{-} +}$$

$$\mathrm{+ {}^{\bullet}COOH} \begin{cases} \xrightarrow{\mathrm{AE\,=\,4.16\;eV}} \mathrm{NH{=}CH_2 + C(O)NHCH_2CH_3^{-}} & (12)\\ \xrightarrow{\mathrm{AE\,=\,4.46\;eV}} \mathrm{NH{=}CH_2 + HC(O)NHCHCH_3^{-}} & (13)\\ \xrightarrow{\mathrm{AE\,=\,2.76\;eV}} \mathrm{NH{=}CH_2 + HC(O){=}NCH_2CH_3^{-}} & (14)\end{cases}$$

These ions are assigned to the so-called internal ions and can be formed only due to the hydrogen atom shift in the intermediate ion. From the data obtained it seems impossible to reveal which of three listed processes occurs really. From the viewpoint of energy of the process, the latter reaction is more preferable. However, the double H-shift is necessary for this reaction to occur, unlike two former reactions.

Up to now we considered the ions with *m/z* 72 and the elemental compositions $C_3H_4O_2^-$ and $C_3H_6NO^-$ in Ala-Ala and Gly-Ala ignoring possible ions with the elemental composition $C_2H_4N_2O^-$. This was a consequence of the analogy with the processes in Ala, whose molecule contains only one nitrogen atom. Dipeptides contain two nitrogen atoms, and the ions with the elemental composition $C_2H_4N_2O^-$ can be possible candidates for this ion in Gly-Ala. Hypothetically, this ion can be formed directly from the molecular ion due to the H-shift and the N—C$_\alpha$ bond cleavage

$$\mathrm{M + e \rightarrow NH_2CH_2C(O)N^{\bullet -} + CH_3CH_2COOH} \qquad (15)$$

or upon the successive decay from the intermediate ion $[M - 45]^-$

$$\mathrm{M + e \rightarrow NH_2CH_2C(O)NHCH(CH_3)^{-} + {}^{\bullet}COOH \rightarrow}$$
$$\mathrm{\rightarrow NH_2CH_2C(O)N^{\bullet -} + {}^{\bullet}CH_2CH_3}. \qquad (16)$$

However, two facts reject the hypothesis about the formation of ions $NH_2CH_2C(O)N^{\bullet -}$. The first fact is that we found no ions with *m/z* 72 in Gly-Gly and the assumed candidate with *m/z* 86 in Ala-Ala has more common features with the ion *m/z* 86 rather than with the ions *m/z* 72 in Gly-Ala. The second fact is that in the experiment with the narrow receiver slit we should detect in the yield curve of the ions $C_2H_4N_2O^-$ a considerably greater contribution from the ions $C_3H_4O_2^-$ in the low-energy region, because the mass resolution >6400 is necessary for their complete resolution.

*Analogs of ions $[M - 16]^-$ and $[M - 19]^-$.* We did not detect the EYC of the ions with *m/z* 59 and 56 from Gly-Gly with the narrow receiver slit because of their low intensity. Based on the available data on Gly, it can be assumed that in Gly-Gly the ions with *m/z* 59 in the medium-energy region are formed due to the cleavage of the N—C$_\alpha$ bond only

$$\mathrm{M + e \rightarrow NH_2CH_2C(O^{\bullet}){=}NH + CH_2COOH^{-}} \qquad (17)$$
$$\mathrm{(AE = 1.85\;eV)}.$$

The ions with *m/z* 56, most likely, have the elemental composition $C_2H_2NO^-$ and are formed due to the H-shift in the intermediate ion $[M - H]^-$

$$\mathrm{M + e \rightarrow H^{\bullet} + NHCHC(O)^{-} + NH_2CH_2COOH} \qquad (18)$$
$$\mathrm{(AE = 4.23\;eV)}.$$

Modeling this reaction, we based on the results of detailed analysis of a similar process in deuterium-substituted glycine ($NH_2CD_2COOH$)[5]

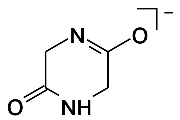
$$\mathrm{NH_2CH_2COOH + e \rightarrow NHCHC(O)^{-} + H^{\bullet} + H_2O}$$
$$\mathrm{(AE = 3.94\;eV)}.$$

The yield curve of the ions with *m/z* 73 from Ala-Ala exactly coincides with the yield curve of the ions with *m/z* 72 in a certain scale (see Fig. 4). It follows from this that the peak with *m/z* 73 in the whole energy range is the isotope peak from the peak with *m/z* 72, possibly, with an insignificant contribution of the ions $C_3H_5O_2^-$. Based on the available data on Ala, we assume that in Ala-Ala the ions with *m/z* 70 have the elemental composition $C_3H_4NO^-$. Probably, they are formed due to the H-shift in the intermediate ion $[M - H]^-$, as the ions $C_2H_2NO^-$ in Gly-Gly and Gly-Ala.

The yield curves of the ions $[M - 19]^-$ in peptides differ from those in amino acids by the resonance peak with a maximum about 1.8 eV, whose origin is unclear. In this case, the formation of ions $[M - H - H_2O]^-$ with the structure $NH{=}C(R^1)C(O)NHCH(R^2)CO^-$ according to Eq. (18) is impossible because of the high threshold. The threshold energy of other assumed reactions with the formation of linear ions is also high. For instance, for the reaction of isolation of ions with the minimum enthalpy of formation

$$\mathrm{M + e \rightarrow NH{=}C(R^1)C(O){=}NCH(R^2)C(O)H^{-} + H^{\bullet} + H_2O} \qquad (19)$$
$$\mathrm{(AE_{Gly\text{-}Ala} = 3.06\;eV)}$$

the estimated AE value exceeds 3 eV. Another hypothesis concerning the peak discussed is based on the formation of the cyclic ion. In Gly-Gly this ion can have the structure of the ion $[M - H]^-$ from piperazine-2,5-dione

M + e →


$+ H^{\bullet} + H_2O$ (20)

(AE = 1.39 eV).

According to the third assumption, the peak under discussion can correspond to the ion $[M - NH_3 - H_2]^-$ formed in the reaction

$$M + e \rightarrow CH(R^1){=}C(O)N{=}C(R^2)COO^{\bullet -} + NH_3 + H_2 \quad (21)$$

($AE_{Gly\text{-}Gly}$ = 1.74 eV).

It is impossible to experimentally check this assumption, because for the complete separation of the isobaric ions $[M - H - H_2O]^-$ an $[M - NH_3 - H_2]^-$, for example, in Gly-Gly, the mass resolution should be ~5500.

*Analogs of ions [M – 18]⁻*. The effective yield curves of the ions with *m/z* 71 in Ala-Ala, Gly-Ala, and Ala are very similar; in amino acid the single elemental composition $C_3H_3O_2^-$ corresponds to the ions $[M - 18]^-$. By analogy to Ala, let us assume that in peptides in the high-energy the $C_3H_3O_2^-$ ions are formed due to the bond cleavage only

$$M + e \rightarrow NH_2CH(R^1)C(O^{\bullet}){=}NH + 2\,H^{\bullet} + \\ + CH_2{=}CHCOO^- \quad (22)$$

($AE_{Gly\text{-}Ala}$ = 6.13 eV).

This process is caused by the presence of the methyl group in the molecules and, hence, it is impossible in Gly and Gly-Gly. This conclusion is confirmed by the characteristic shape of the yield curve of the ions $[M - 18]^-$ from Ala-Ala and the absence of peaks of the ions $[M - 18]^-$ in the mass spectra of two other dipeptides. For Ala-Ala, the broad peak in the high-energy region reflects the formation of the ions $[M - NH_2 - 2\,H]^-$, as in Ala

$$M + e \rightarrow CH_2{=}CHC(O)NHCH(CH_3)COO^- + \\ + {}^{\bullet}NH_2 + 2\,H^{\bullet} \quad (23)$$

(AE = 6.21 eV).

In the experiment with the narrow receiver slit, along with the contribution of the ions $C_3H_3O_2^-$, an insignificant contribution of the ions $C_3H_5NO^-$ was observed (see Fig. 4). The effective yield of the latter in Gly-Ala (it is omitted in Fig. 5) is almost an order of magnitude lower than that in Ala-Ala. In Gly no ions $[M - 18]^-$ with *m/z* 57 were observed. In order to identify the elemental composition of the ions with *m/z* 57 from Gly-Gly, we recorded the region of the mass spectrum in the range of 55—60 amu at the fixed energy equal to 5.5 eV with the narrow receiver slit. In this case, the peaks of the ions with *m/z* 58 and 56 were used as the internal standard. It turned out that the ion considered has the composition $C_2H_3NO^-$. We assume that the discussed ions from peptides are analogs of hypothetical ions $[M - H_2O]^-$ from amino acids. In Gly-Gly and Ala-Ala they are possibly formed due to the H-shift reaction and the peptide bond cleavage in the molecular ion

$$M + e \rightarrow NHCH(R^1)C^{\bullet}(O)^- + NH_2CH(R^2)COOH. \quad (24)$$

In Gly-Ala, we found no ions with *m/z* 57, and the low-intensity ions $C_3ONH_5^-$ with *m/z* 71 can appear due to the skeletal rearrangement in the molecular ion

$$M + e \rightarrow NH_2CH_2COOH + NHCH(CH_3)C^{\bullet}(O)^-. \quad (25)$$

The above discussed ions with the elemental composition $C_2H_nO_2^-/C_3H_{n+2}O_2^-$ are formed upon the cleavage of the N—$C_\alpha$ bond. They are ascribed to ions of the series Z (see Fig. 7), whereas the ions with the elemental composition $C_2H_mNO^-/C_3H_{m+2}NO^-$ are formed due to peptide bond dissociation and represent ion of the series B.

**Ions with small masses.** Processes of formation of ions with small masses from amino acids have earlier been discussed to certain extent. In particular, it was assumed[5] that in Gly and Ala the ions with *m/z* 46 can have the elemental composition $CH_2O_2^-/CH_4NO^-$, the ions with *m/z* 44 are $CO_2^-$, and three composition varieties were ascribed to the ions with *m/z* 42, namely, $C_2H_4N^-$, $C_2H_2O^-$, and $CNO^-$. Experiments with the narrow receiver slit made it possible to answer these questions. The mass spectral regions recorded at two fixed energies in the range 40—46 amu for all studied compounds are shown in Fig. 9, and the ions $CHO_2^-$ (*m/z* 45) and $C_2HO^-$ (*m/z* 41) were used as the internal standards. The ions with *m/z* 45 in the medium-energy region are formed due to the cleavage of the C—$C_\alpha$ bond only. In the low-energy regionm they are probably the reaction products of the H-shift in the molecular ion. The both structural isomers, *viz*., $COOH^-$ and $HCOO^-$, are ions of the series X (see Fig. 7). In Gly and Gly-Gly the ions with *m/z* 46 have the elemental composition $CH_4NO^-$ with an insignificant contribution of the isotope peak from ions with *m/z* 45. No ions with *m/z* 46 were recorded in other objects. The $CO_2^-$ ions are characterized by the low intensity, and the high-quality EYC was obtained for them only in the case of Ala due to the absence of other isobaric ions. In Glym the ions $CH_2NO^-$ were found along with the ions $CO_2^-$, whereas in Gly-Gly the both isobaric ions equally contribute to the peak with *m/z* 44. In two other peptides, the peak with *m/z* 44 is mainly presented by the ion $C_2H_6N^-$ with a small contribution of the ions $CH_2NO^-$ (see Figs 4 and 5) and, possibly, the ions $CO_2^-$. No ions with *m/z* 42 were observed in Gly, while in Gly-Gly they

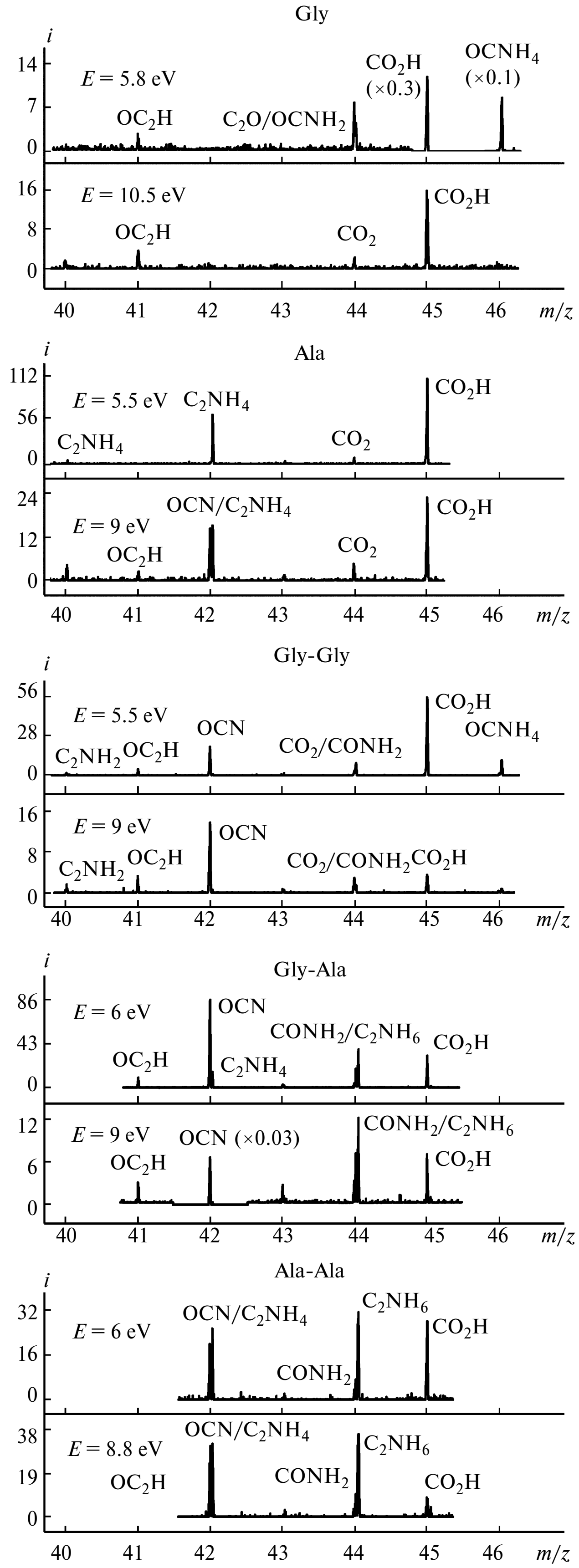


**Fig. 9.** Regions of the mass spectra of the NI from amino acids and dipeptides in the range $m/z$ 40—46 at the fixed electron energies with the closed slit of the ion receiver ($i$ is the ionic current).

were identified as $CNO^-$. In other objects, the ions $C_2NH_4^-$ were observed along with the ions $CNO^-$ (see Figs 2, 4, and 5).

The ions with $m/z$ 28 from Gly were identified[6] as $CH_2N^-$; we found them for all compounds studied. The ions with $m/z$ 27 were detected only in Ala, Gly-Ala, and Ala-Ala, *i.e.*, in the objects containing the lateral Me group. The assumption that they have the elemental composition $C_2H_3^-$ was confirmed in the experiment with the narrow receiver slit. The ions $CN^-$ ($m/z$ 26) and $CH_2N^-$ ($m/z$ 28), as well as the isotope peak from the ions $CN^-$, were used as the internal standards when recording the mass spectra in the range 25—29 amu.

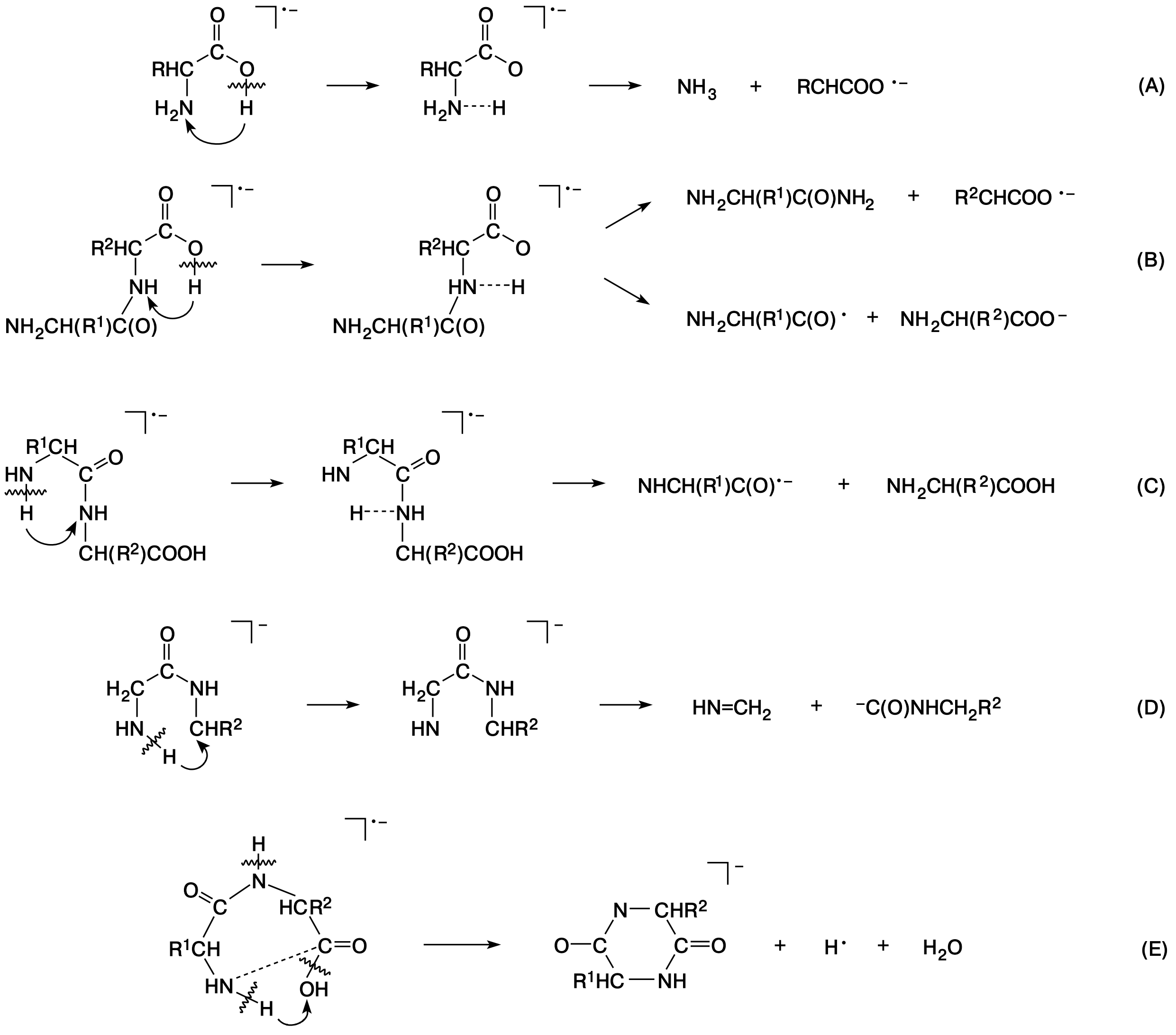
The ions with $m/z$ 41, 40 ($C_2H_2N^-$), 39 ($C_2HN^-$), 15 ($CH_3^-$), and 14 ($CH_2^-$) are characterized by the low effective yield.

**Scheme of rearrangement processes.** According to the rules of NI formation under the conditions of resonant electron capture,[24] the autodetachment of an electron prevents rearrangement processes in molecular ions and, therefore, predominantly fast reactions of simple bond cleavage can occur in the latter. These rules were formulated about thirty years ago and are based in the results of studies of simple molecules with a small number of atoms. Numerous examples of rearrangement processes in molecular NI with the relatively short lifetime are presently known. It can be inferred whether one or another process occurs from the formation of dissociation products with the corresponding structures. An analysis of the experimental data and the calculation of the thermodynamic parameters suggested that the rearrangement reactions in the low-energy region occur in the molecular ions of dipeptides (see Eqs (3), (7), (9), and (20)). A possibility of other isomerization processes in the fragmentation or molecular ions (see Eqs (6), (11)—(14), (18), (24), and (25)) can only be assumed because of the absence of reliable proofs. Reactions (7) and (9) occur in the same energy region (~1—2.5 eV) and are due to the migration of the H atom of the carboxyl group to the peptide nitrogen atom to form ions $NH_2CH(R^2)COO^{\bullet-}$ and $CH(R^2)COO^{\bullet-}$, respectively. Reaction (9) is an analog of the formation of ions $[M - NH_3]^-$ in amino acids in the energy range ~1.5—2.5 eV, but in dipeptides this reaction occurs with much higher efficiency: the relative intensities of ions $CH_2COO^-/CH(CH_3)COO^-$ in the mass spectra of amino acids and peptides differ by almost three orders of magnitude. This fact needs to be explained.

The rearrangement with the migration of the H atom of the carboxyl group to the nitrogen atom can occur more efficiently if these atoms are maximally brought together. In amino acids this situation takes place in conformer IIn (see Refs 25 and 26). Owing to the closeness of these atoms, an intramolecular hydrogen bond appears between them (due to a lone electron pair of the N atom). This conclusion was drawn as a result of the study of Ala by gas electron diffraction.[27] Upon capture of an electron with the energy ~1.5—2.5 eV, the resonance state $^2[\pi_{00}{}^*]$

is formed[28] in amino acids and decays following several channels (see Figs 1 and 2). The most intense channel is related to the formation of the ions $[M - H]^-$ of the carboxyl group. According to the published data,[20] they are formed due to the over-barrier predissociation from all conformers, including IIn, thus creating the competition for the isomerization process. The assumed mechanism of rearrangement is shown in Scheme 1 (reaction (A)). The concentration of the internal energy of the molecular ion Gly on the O—H bond elongates this bond, and the H atom approaches the N atom. If the O—H bond cleavage does not involve the hydrogen bond, the cluster ion with the elemental composition of the molecular ion is formed, *i.e.*, the H-shift occurs. The low intensity of the ions $[M - NH_3]^-$ in amino acids indicates that the hydrogen bond is very weak. Unlike amino acids, in peptides the carboxylic H atom interacts with the whole peptide fragment rather than with the particular N atom, which is due to the conjugation effect of the lone electron pair of the nitrogen atom with the neighboring double bond. Thus, the hydrogen bond in peptides involving the carboxylic H atom should be stronger than that in amino acids, and the probability of rearrangement should be high (see Scheme 1, reaction (B)). The isomerized molecular ion decomposes in the channels described by Eqs (7) and (9), whose intensity ratio is determined by the stability of the corresponding fragments. The intensity of the ions $CH(R^2)COO^-$ in Gly-Ala and Ala-Ala is four- to fivefold higher than the intensity in Gly-Gly. We explain this by the lateral methyl group: it cannot be excluded

**Scheme 1**



$R^1 = R^2 = H$ (Gly-Gly); $R^1 = R^2 = Me$ (Ala-Ala); $R^1 = H$, $R^2 = Me$ (Gly-Ala) that its presence insignificantly distorts the molecular geometry, thus favoring the greater approach of the H atom of the carboxyl group and the peptide N atom. Perhaps, due to this, the molecules have more stable conformers with an appropriate geometry or their molar fraction is large.

The efficiency of other rearrangement processes in peptides is low. The assumed reaction described by Eq. (24) in the molecular ion can be initiated by the migration of the H atom of the amino group to the peptide fragment (see Scheme 1, reaction (C)). The H-shift follows the same scheme in the fragment ion $[M - H]^-$, which was formed due to the elimination of the hydrogen atom bound to the $C_\alpha$ atom at the terminal amino group (see Eq. (18)). As mentioned above, this process in peptides is analogous to the formation of the ions $[M - H - H_2O]^-$ in amino acids with the only difference that in the latter the H atom of the amino group migrates to the OH group.[5] In the ion $[M - H]^-$ the H-shift can follow Scheme 1, reaction (A) (see Eq. (6)). In Gly-Ala the ions $C_3H_6NO^-$ are formed not only from the molecular ions but also from the fragment ions $[M - COOH]^-$ due to the H-shift. The simplest variant of rearrangement is shown in Scheme 1, reaction (D) (see Eq. (12)): here the H atom of the amino group migrates to the $C_\alpha$ atom. In the above considered reactions, first the rearrangement and then the fragmentation occur. In the reactions described by Eqs (3), (13), (14), and (20) it is difficult to represent the intermediate ion due to the stepped character of the process. Therefore, the concerted mechanism seems more probable, *i.e.*, the H-shift occurs simultaneously with fragmentation. For example, during reaction (3) in the molecular ion the $C_\alpha$—C and N—H bonds are cleaved and the new bond $C_\alpha$—H is formed. Thus, the elimination of the COOH group is accompanied by the migration of the H atom bound to the peptide nitrogen atom to the $C_\alpha$ atom. The fragmentation of the molecular ion in the reaction described by Eq. (20) proceeds in a more complicated manner and is initiated, most likely, by the migration of the H atom of the amino group to the OH group with the intermediate formation of an intramolecular hydrogen bond (see Scheme 1, reaction (E)). The products of this

**Table 1.** The $\Delta H_f°$, $\Delta H_{acid}$, and EA values for various species according to Ref. 33 used for the calculation of the energy balance of NI fragmentation from amino acids and peptides

| Species A | $\Delta H_{acid}$(AH) | $\Delta H_f°$(AH) | $\Delta H_f°$(A) | EA(A) /eV | $\Delta H_f°(A^-)$ /kJ mol$^{-1}$ |
|---|---|---|---|---|---|
| | kJ mol$^{-1}$ | | | | |
| $NH_2CH_2COO^\cdot$ | 1433 | −391 | −165 | 3.35 | −488 |
| $NH_2CH(CH_3)COO^\cdot$ | 1425 | −414 | −188 | 3.42 | −519 |
| $CH_2{=}CHCOO^\cdot$ | — | −331[a] | −107 | 3.57[a] | — |
| $^\cdot CH_2COOH$ | — | −432 | −243[b] | — | — |
| $CH_3COO^\cdot$ | 1459 | −432 | −504 | 3.07 | −504 |
| $^\cdot CH_2COO^\cdot$ | — | — | — | — | −256[a] |
| $HCOO^\cdot$ | 1444 | −379 | −153 | 3.23 | −464 |
| $^\cdot COOH$ | — | −379 | −198[b] | — | — |
| $NH{=}CH_2$ | — | — | 109[a] | — | — |
| $CH_3C^\cdot O$ | — | — | −23[b] | — | — |
| $CH_2CO$ | — | — | −88[b] | — | — |
| $CO_2$ | — | — | −394[b] | — | — |
| $HO^\cdot$ | — | −242[b] | 39[b] | — | — |
| $^\cdot NH_2$ | — | −46[b] | 193[b] | — | — |
| $H^\cdot$ | — | — | 218 | — | — |
| $HC(O^\cdot){=}NH$ | 1506 | −186 | — | — | −210 |
| $NH{=}CO^\cdot OCH_3$ | 1514 | −425[a] | — | — | — |
| $NH{=}CO^\cdot OC_2H_5$ | 1514 | −447[a] | — | — | — |
| $CH_2{=}CO^\cdot OCH_3$ | 1556 | −410 | −210 | 1.80[c] | −384 |
| $CH_2{=}C(O^\cdot)CH_3$ | 1544 | −217 | −24 | 1.758[c] | −203 |
| $CH_2{=}C(O^\cdot)N(CH_3)_2$ | 1569 | −255[a] | — | — | — |
| $HC(O)N(CH_3)CH_2^\cdot$ | 1670 | −192 | — | — | −52 |

*Note*. Here and in Table 2, A is the neutral species (molecule or radical), $A^-$ is the negative ion of species A, AH is species A + atoms H, and $\Delta H_{acid}$(AH) is the proton affinity (acidity) of species A; $\Delta H_f°$(A), $\Delta H_f°$(AH), and $\Delta H_f°(A^-)$ are the standard enthalpies of formation of species A, AH, and $A^-$, respectively; EA(A) is the electron affinity of species A.

[a] According to Ref. 34.

[b] According to Ref. 32.

[c] According to Ref. 35.

concerted reaction is the ion with the geometry of a six-membered ring, the water molecule, and the hydrogen atom. It is not excluded that the molecular ion with the structure of piperazine-2,5-dione in Gly-Gly or its C-methyl derivatives in Gly-Ala and Ala-Ala is formed in the intermediate step of this reaction.

Although in the most part of the rearrangement reactions considered the enthalpies of formation of the decay products correspond to the experimental energy of the appearance of ions, the isomerization barrier can be higher than the internal energy of the decaying ion. In these cases, the H-shift can occur due to the tunneling mechanism by analogy to the formation of the ions $[M - H]^-$ from amino acids in the region of 1.3 eV. However, the efficiency of skeletal rearrangements that follow the mechanism of skeletal rearrangements should be very low because of the large mass of the tunneling particle.

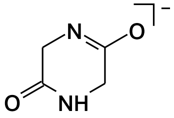
**Thermochemical aspect.** For the calculation of the energy balance of the processes of NI fragmentation, we used the standard enthalpies of formation ($\Delta H_f^\circ$) and the electron affinities (EA) of neutral species, as well as the $\Delta H_f^\circ$ values of ions taken from Refs 29—32 (Table 1). The thermochemical parameters calculated in the present work are given in Table 2. The enthalpies of formation of neutral species were calculated by the additive schemes using the known increments of groups and bonds[29] and the $\Delta H_f^\circ$ values of the starting molecules and radicals.[29,30] For instance, the enthalpy of formation of the Gly-Gly molecule was calculated by the Benson scheme[33] using the enthalpy of formation of the starting Gly molecule

$$\begin{aligned}\Delta H_f^\circ(NH_2CH_2C(O)NHCH_2COOH) &\approx \Delta H_f^\circ(NH_2CH_2COOH) - \\ &- \Delta H_f^\circ(N(C)(H)_2) + \Delta H_f^\circ(N(C)(CO)(H)) + \\ &+ \Delta H_f^\circ(CO(N)(C)) + \Delta H_f^\circ(C(CO)(N)(H)_2) + \\ &+ \Delta H_f^\circ(N(C)(H)_2) = (-391) - (20.1) + (-18.4) + \\ &+ (-137.3) + (-21.3) + (20.1) = -568 \text{ kJ mol}^{-1}.\end{aligned}$$

The enthalpy of formation of the Gly-Ala molecule was calculated by the Rosenstock scheme[34] from the enthalpies of formation of the starting molecules Gly-Gly, Gly, and Ala

$$\begin{aligned}\Delta H_f^\circ(NH_2CH_2C(O)NHCH(CH_3)COOH) &\approx \\ &\approx \Delta H_f^\circ(NH_2CH_2C(O)NHCH_2COOH) - \\ &- \Delta H_f^\circ(NH_2CH_2COOH) + \Delta H_f^\circ(NH_2CH(CH_3)COOH) = \\ &= (-568) - (-391) + (-414) = -591 \text{ kJ mol}^{-1}.\end{aligned}$$

**Table 2.** Calculated thermochemical parameters ($\Delta H_f^\circ$ and EA) of the studied species

| Species A | $\Delta H_f^\circ(AH)$ | $\Delta H_f^\circ(A)$ | EA(A) /eV | $\Delta H_f^\circ(A^-)$ /kJ mol$^{-1}$ |
|---|---|---|---|---|
| | kJ mol$^{-1}$ | | | |
| $NH_2CH_2C(O)NHCH_2COO^{\cdot}$ | −568 | — | — | −682 |
| $NH_2CH_2C(O)NHCH(CH_3)COO^{\cdot}$ | −591 | −365 | — | −709 |
| $^{\cdot}NHCH_2C(O)NHCH(CH_3)COOH$ | −591 | −392 | — | −510 |
| $NH_2CH{=}C(O^{\cdot})NHCH(CH_3)COOH$ | −591 | — | — | −552 |
| $NH_2CH_2C(O^{\cdot}){=}NCH(CH_3)COOH$ | −591 | — | — | −607 |
| $NH{=}CHC(O^{\cdot}){=}NCH(CH_3)C(O)H$ | −256 | — | — | −272 |
| $CH_2{=}CHC(O)NHCH(CH_3)COO^{\cdot}$ | −526 | −307 | — | −644 |
| $NH_2CH(CH_3)C(O)NHCH(CH_3)COO^{\cdot}$ | −614 | — | — | −732 |
| $CH_2C(O^{\cdot})N{=}CHCOO^{\cdot}$ | — | — | — | −354 |
| $NH_2CH_2C(O)NHCH^{\cdot}CH_3$ | −227 | −33 | 0.40 | −87 |
| $^{\cdot}NHCH_2C(O)NHCH_2CH_3$ | −227 | −28 | — | −146 |
| $NH_2CH_2C(O^{\cdot}){=}NCH_2CH_3$ | −227 | −9 | 2.57 | −243 |
| $HC(O)NHCH^{\cdot}CH_3$ | −212 | −18 | 0.49 | −72 |
| $HC(O^{\cdot}){=}NCH_2CH_3$ | −212 | 6 | 2.60 | −236 |
| $C^{\cdot}(O)NHCH_2CH_3$ | −212 | −31 | 0.72 | — |
| $^{\cdot}NHCH_2COOH$ | — | −192 | 1.22 | — |
| $^{\cdot}NHCH(CH_3)COOH$ | — | −214 | — | −333 |
| $NH_2CH_2C(O^{\cdot}){=}NCH_3$ | −199 | 15 | 2.71 | −215 |
| $NH_2CH_2C(O)NHCH_2^{\cdot}$ | −199 | −7 | 0.52 | −59 |
| $NH_2CH_2C(O^{\cdot}){=}NH$ | −201 | 16 | — | −225 |
| $NH_2CH_2C^{\cdot}O$ | −123 | 15 | 0.69 | — |
| $NH{=}CHC^{\cdot}O$ | — | 94 | 0.84 | — |
| $NH_2CH{=}C{=}O$ | — | −50 | — | — |
| $^{\cdot}CH_2COOH$ | — | — | — | −406 |
|  | −342 | −128 | 2.92 | — |

The $\Delta H_f^\circ$ value for gaseous piperazine-2,5-dione was calculated from the enthalpy of formation for the crystalline form (−446 kJ mol$^{-1}$) and the heat of sublimation (103.8 kJ mol$^{-1}$).[34]

The electron affinity of molecules and radicals were estimated from the calculated enthalpies of formation of ions and the corresponding neutral species by the density functional theory in the B3LYP/6-311++G(3df,3p) basis set.

The enthalpies of formation of some NI were determined by the proton affinity values ($\Delta H_{acid}$) similar in structures of molecules and ions. For the calculation of $\Delta H_f^\circ$ of the $HC(O)NHCH(CH_3)^-$ ion, we used the value $\Delta H_{acid}$ = 1670 kJ mol$^{-1}$ obtained[30] in the reaction

$$HC(O)N(CH_3)CH_2^- + H^+ \rightarrow HC(O)N(CH_3)_2.$$

* * *

The resonant electron capture by molecules of the compounds studied generates short-lived molecular NI that undergo fragmentation predominantly by the bond cleavage only because of the competition with the process of autodetachment of an electron. The dissociation of "central" bonds in dipeptides gives rise to ions similar in structure and elemental composition to the ions from amino acids that are formed by the elimination of terminal groups and hydrogen atoms. The mass spectra of the NI of two groups contain peaks of the same-type ions $[M - H]^-$, $[M - NH_2]^-$, $[M - OH]^-$, $CHO_2^-$, $OH^-$, *etc.* No long-lived molecular NI were detected; however, experiments on capturing low-energy electrons indicate that their lifetime is sufficient for relatively slow predissociation and rearrangement reactions to occur. In the high-energy region, the fragment ions that are stable with respect to electron autodetachment undergo isomerization. Almost all revealed rearrangements are reactions of H-shift and can be due to intramolecular hydrogen bonds, which are initially present in some conformers of molecules or appear at the stage of NI formation.

The intense ions $[M - H]^-$ of the carboxyl structure characteristic of carboxylic acids, for which relative cross-sections were measured, were detected in the low-energy region. Experiments with the high energy resolution of the electron beam revealed the fine vibrational structure on the yield curves of these ions, indicating the predissociation mechanism of their formation. The highly efficient channel for the decay of molecular NI, which is due to the migration of the hydrogen atom of the carboxyl group to the peptide group followed by the $N—C_\alpha$ bond cleavage and the formation of radical anions $CH(R^1)COO^{\cdot -}$ ($R^1$ = H, Me) observed also for amino acids but with considerably lower intensity, was found in dipeptides.

In the region of high energies of captured electrons, the ions $[M - H]^-$ in dipeptides represent a mixture of various structures and the efficiency of their yield is lower than that in the low-energy region. They undergo fragmentation due to the bond cleavage only or rearrangements, as in the case of amino acids. Metastable decays of these ions were observed for Gly-Ala and Ala-Ala, which are not characteristic of amino acids (only the fragmentation in the mass spectrometric time interval of the ions $[M - H]^-$ from valine is known[35]). The efficiency of these processes depends on several factors[36]; in the presented examples, it is determined, most likely, by the number of active vibrational degrees of freedom of the decaying ion capable of accumulating its internal energy, if other conditions are approximately equivalent.

Two specific fragmentation channels caused by the presence of the methyl group were found for alanine and its derivatives. In addition, its presence affected the efficiency of some reactions.

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