J|A|C|S
ARTICLES

Resonant Electron Capture by Some Amino Acids and Their Methyl Esters

Yury V. Vasil'ev,[†,‡] Benjamin J. Figard,[†] Valery G. Voinov,[†,§]
Douglas F. Barofsky,[†] and Max L. Deinzer*,[†]

*Contribution from the Department of Chemistry, Oregon State University,
Corvallis, Oregon 97331, Department of Physics, Bashkir State Agricultural University,
Ufa, Russia, and Pacific Institute of Bioorganic Chemistry, Vladivostok, Russia*

Received December 22, 2005; E-mail: Max.Deinzer@orst.edu

Abstract: Resonant electron capture mass spectra of aliphatic and aromatic amino acids and their methyl esters show intense $[M-H]^-$ negative ions in the low-energy range. Ion formation results from a predissociation mechanism mediated by the low-energy π^*_{OO} resonant state. Methylation in general has little influence on the electronic structure according to quantum chemical calculations, but the corresponding ions from the methyl esters, $[M-Me]^-$, could be ascertained to arise only at higher resonance energies. Aromatic amino acids are characterized by an additional low-energy fragmentation channel associated with the generation of negative ions with loss of the side chain. The complementary negative ions of the side chains are more efficiently produced at higher energies. The results have significant implications in biological systems as they suggest that amino acids can serve as radiation protectors since they have been found to efficiently thermalize electrons.

---

1. Introduction

Structural changes of biomolecules in cellular matrices caused by deposition of ionizing radiation result in alteration of molecular function and eventually cell death. It was well recognized by the middle of the 1980s[1] that the nucleus of the cell is much more sensitive to radiation than the cytoplasmic components. The natural and direct way to rationalize these findings is to examine interactions of biospecies with electrons and free radicals that, being secondary products of ionizing radiation, can very often cause even higher destructive consequences in a cell than direct radiation. Condensed phase reactions of radical and electron additions to nucleobases, the carriers of the genetic information in a cell and the most fragile components of DNA with respect to radiation damage, have been thoroughly studied.[2] Moreover, it was shown recently[3] that even low-energy secondary electrons, i.e., those with energies well below the ionization threshold, can cause significant damage to DNA and lead to single and double strand breaks (SSBs and DSBs). The main mechanistic feature of the processes has been suggested[3] to proceed via formation of an intermediate compound state, known also as resonant state or simply resonance, of the extra electron and DNA followed by dissociation of the DNA bonds.[4] Gas-phase experiments with DNA and RNA bases also pointed to very efficient dissociation of their molecular negative ions (NIs) occurring in the low-energy range.[5] Recent theoretical studies[6] provided rationalizations for possible fragmentation pathways, and Rydberg electron-transfer experiments[7] pointed to a positive electron affinity of the monomeric and dimeric nucleosides as dipole-supported anions.

Most biological systems are composed of about 75−80% water,[1] and as a result, the participation of water molecules in radiation damage to cells has been studied extensively. The main conclusion from these studies is that water is considered to be the source of reactive hydrated electrons (e^-_{aq}), hydroxyl radicals ($^\bullet$OH), and hydrogen atoms ($^\bullet$H)[8] upon radiation of the cell. Therefore, if these reactive species are generated from water, it is somewhat surprising that components in the cytoplasm are more resistant to radiation damage than are those in the nucleus, despite the fact that water predominates in both. The role of

---

[†] Oregon State University.
[‡] Bashkir State Agricultural University.
[§] Pacific Institute of Bioorganic Chemistry.

(1) Holahan, E. V., Jr. In *Military radiobiology*; Conklin, J. J., Walker, R. I., Eds; Academic Press: Orlando, FL, 1987; pp 87−110.

(2) (a) Steenken, S. *Chem. Rev.* **1989**, *89*, 503. (b) Henriksen, T.; Snipes, W. *J. Chem. Phys.* **1970**, *52*, 1997.

(3) Boudaïffa, B.; Cloutier, P.; Hunting, D.; Huels, M. A.; Sanche, L. *Science* **2000**, *287*, 1658−1660.

(4) Because single- and double-strand breaks have been found at energies higher than ca. 3.5 eV, corresponding resonances are hardly shape resonances examined by electron transmission experiments [Aflatooni, K.; Gallup, G. A.; Burrow, P. D. *J. Phys. Chem.* **1998**, *102*, 6205−6207] but rather electronically excited Feshbach resonances (see also papers quoted in ref 6). This means that generation of the resonances competes with the formation of electronically excited states of neutral molecules that can be directly initiated by the initial radiation.

(5) (a) Abouaf, R.; Pommier, J.; Dunet, H. *Int. J. Mass Spectrom.* **2003**, *226*, 397−403. (b) Hanel, G.; Gstir, B.; Denifl, S.; Scheier, P.; Probst, M.; Farizon, B.; Illenberger, E.; Märk, T. D. *Phys. Rev. Lett.* **2003**, *90*, 188104-1−188104-4. (c) Denifl, S.; Ptasinska, S.; Cingel, M.; Matejcik, S.; Scheier, P.; Märk, T. D. *Chem. Phys. Lett.* **2003**, *377*, 74−80. (d) Abdoul-Carime, H.; Huels, M. A.; Illenberger, E.; Sanche L. *J. Am. Chem. Soc.* **2001**, *123*, 5354−5355. (e) Abdoul-Carime, H.; Huels, M. A.; Brüning, F.; Illenberger, E.; Sanche L. *J. Chem. Phys.* **2000**, *113*, 2517−2521. (f) Huels, M. A.; Handorf, I.; Illenberger, E.; Sanche L. *J. Chem. Phys.* **1998**, *108*, 1309−1312.

(6) (a) Berdys, J.; Anusiewicz, I.; Skurski, P.; Simons, J. *J. Am. Chem. Soc*. **2004**, *126*, 6441−6447. (b) Berdys, J.; Skurski, P.; Simons, J. *J. Phys. Chem. B* **2004**, *108*, 5800−5805.

(7) (a) Desfrançois, C.; Abdoul-Carime, H.; Schulz, C. P.; Schermann, J. P. *Science* **1995**, *269*, 1707−1709. (b) Desfrançois, C.; Abdoul-Carime, H.; Schermann, J. P. *J. Chem. Phys.* **1996**, *104*, 7792−7794.

10.1021/ja058464q CCC: $33.50

proteins in the cytoplasm when interacting with slow electrons is poorly understood, but a protective effect toward double-strand breaks in DNA has been attributed to histones as well as to nonhistone proteins.[9]

Very little is known about low-energy electron interactions with uncharged proteins or amino acids. However, such gas-phase reactions of amino acids with electrons are supposed to be models for charge-transfer and charge-induced processes that may occur in cellular proteins. Resonant electron capture (REC) by neutral molecules with electrons of well-defined energies results in formation of NIs. An important advantage of the method of NI production is that statistical and nonstatistical fragmentation of molecular negative ions (resonances) can be distinguished because of the competition between the decay channels, namely dissociation of the NIs into smaller charged species and their neutral counterpart(s) on one hand and electron autodetachment on the other. Indeed, negative ion resonance states with shorter lifetimes normally exhibit nonstatistical fragmenatation[10] via rapid direct bond cleavages since complete and uniform sampling of the phase space and complex rearrangement processes are prevented by very efficient electron autodetachment.

Information on experimentally observed NIs of amino acids is indeed very scanty; just a few papers relating to the subject have been found. The first investigation of NIs of amino acids was carried out with fixed electron energies in the range 2−4 eV,[11] but ions were generated in a plasma at such pressure that even normally unstable molecular NIs were observed. With the exception of cysteine, methionine, asparatic acid, and asparagine, the most intense NI peaks in the mass spectra of all other amino acids were those of fragment ions formed by hydrogen atom loss. Other fragmentation channels were found to be weak and associated with losses of NH_2 and COOH radicals, H_2O, and glycine moieties and the formation of an "acid fragment" (m/z 74).

NI chemical ionization (CI) mass spectra[12] with NH_2^- or, as in the case of deuterated amino acids, OD^- ions were found to be qualitatively similar to their fast atom bombardment (FAB) spectra[13] but reportedly several orders of magnitude more intense. The last finding proved that amino acids are very good scavengers of negatively charged OH^-/OD^- ions. Recent combined theoretical and experimental work[14] provided detailed insight into the structural composition of deprotonated NIs of glycine.

At the time this paper was submitted glycine was the only amino acid that had been studied by REC mass spectrometry.[15−17] After submission, several papers appeared in the literature dealing with the investigation of REC by cysteine,[18a] proline,[18b] *N*-acetyl tryptohan,[18c] and alanine.[18d] In the first study of glycine by Muftakhov et al.[15] only cross-section formation of $[M-H]^-$ NIs as a function of electron energy was reported, whereas two others[16,17] also dealt with the formation cross-sections of different NIs. Direct comparison of glycine NI mass spectra obtained by CI,[12] FAB,[13] and broad band low energy electron capture[11] with that of the REC spectrum showed similarity in that $[M-H]^-$ was the most abundant ion generated at energies 1.31,[15] 1.4,[16] or 1.25 eV.[17] However, in contrast to the low-pressure plasma experiments,[11] careful electron energy ramping from near zero to 4[15] or 15 eV[16,17] showed no evidence for long-lived (>1 μs) molecular NIs.[19] In addition there were no fragment NIs resulting from loss of hydrogen molecules. Other fragmentation channels in the REC spectra[16,17] associated with the abstraction of NH_3, H_2O, and CO_2H_2 were established only with simultaneous or sequential loss of a hydrogen atom. Peaks due to low mass NIs, like O^-/NH_2^-, OH^-, CN^-, that were of greater intensity than those mentioned above except for $[M-H]^-$ were another peculiarity of the REC spectra.[16,17]

The REC spectra of glycine (Gly), alanine (Ala), phenylalanine (Phe), tyrosine (Tyr), and tryptophan (Trp) have been studied with the aim of examining the unique characteristics of individual amino acids in their elementary resonant reactions with electrons. With the exception of Gly and Ala, all other data are reported for the first time as far as the authors know. The methylated esters of the amino acids were also studied to determine the possible effects of esterification on the electronic structures of amino acid NIs.

2. Experimental Section

2.1. Materials. Amino acid samples were purchased from Sigma/Aldrich Chemical Co. (St. Louis, MO) with a stated purity of 99% or greater (Gly) and 98% (Gly-2,2-d_2). Methyl esters were purchased from Sigma/Aldrich with a stated purity of 98% (Me-Phe, Me-Trp) and 99% (Me-Gly, Me-Ala) or greater (Me-Tyr). The compounds were used without further purification.

2.2. Instrumentation. All REC spectra were obtained using a custom-made trochoidal-electron-monochromator/reflectron-time-of-flight mass spectrometer.[24] Briefly, the instrument was built on the platform of a JEOL JMS-DX300 mass spectrometer retrofitted with a trochoidal electron monochromator (TEM)[25] so that a quasi monoen-

---

[8] See, for example, papers in: *Physical mechanisms in radiation biology*; Cooper, R. D., Wood, R. W., Eds; Conference Proceedings, Airlie, Virginia, USA, 1972, Technical Information center, United State Atomic Energy Commission, 1974.

[9] Sak, A.; Stuschke, M.; Wurm. R.; Budach, V. *Int. J. Radiat. Biol.* **2000**, *76*, 749−756.

[10] Kalamarides, A.; Marawar, R. W.; Durham, M. A.; Lindsay, B. G.; Smith. K. A.; Dunning, F. B. *J. Chem. Phys.* **1990**, *93*, 4043−4046.

[11] Voigt, D.; Schmidt, J. *Biomed. Mass Spectrom.* **1978**, *5*, 44−46.

[12] Kulik, W.; Heerma, W. *Biomed. Environ. Mass Spectrom.* **1988**, *15*, 419−427.

[13] Eckersley, M.; Bowie, J. H.; Hayes, R. N. *Int. J. Mass Spectrom. Ion Processes* **1989**, *93*, 199−213.

[14] O'Hair, R. A. J.; Blanksby, S.; Styles, M.; Bowie, J. H. *Int. J. Mass Spectrom.* **1999**, *182/183*, 203−211.

[15] Muftakhov, M. V.; Vasil'ev, Y. V.; Mazunov, V. A. *Rapid Commun. Mass Spectrom.* **1999**, *13*, 1104−1108.

[16] Gohlke, S.; Rosa, A.; Illenberger, E.; Brüning, F.; Huels, M. A. *J. Chem. Phys.* **2002**, *116*, 10164−10169.

[17] Ptasinska, S.; Denifl, S.; Abedi, A.; Scheier, P.; Märk, T. D. *Anal. Bioanal. Chem.* **2003**, *377*, 1115−1119.

[18] (a) Abdoul-Carime, H.; Gohlke, S.; Illenberger, E. *Phys. Chem. Chem. Phys.* **2004**, *6*, 161−164. (b) Abdoul-Carime, H.; Illenberger, E. *Chem. Phys. Lett.* **2004**, *397*, 309−313. (c) Abdoul-Carime, H.; Gohlke, S.; Illenberger, E. *J. Am. Chem. Soc.* **2004**, *126*, 12158−12161. (d) Ptasinska, S.; Denifl, S.; Candori, P.; Matejcik, S.; Scheier, P.; Märk, T. D. *Chem. Phys. Lett.* **2005**, *403*, 107−112.

[19] One of the possible explanations for the discrepancy is low-pressure plasma conditions may have provided stabilization of so-called dipole-bound NIs via third body collisions. Although the zwitterionic form of Gly is known to possess a very large dipole moment, 9.3 D (see ref 20), it hardly serves as precursor for the dipole-bound NIs since the zwitterions would not survive in the gas phase without the stabilizing environment of water molecules. Simons and co-workers (ref 20) reported the dipole moment (5.5 D) of a polar isomer ($\mathbf{B}_n$, Scheme 1 and Chart 1 in Supporting Information), which is only 394 cm^{-1} (ca. 0.049 eV) less stable than the most stable Gly isomer (**C**, Scheme 1 and Chart 1 in Supporting Information). Experimental evidence of the gas phase equilibrium between these Gly isomers is well-known (ref 21). Such a large dipole moment of isomer $\mathbf{B}_n$ exceeds the critical magnitude (ref 22) necessary for a dipole-bound anionic state of Gly. In light of the recent success of Rydberg electron-transfer spectroscopy for investigating such species (ref 23), it is quite possible that stable molecular radical anions of amino acids can exist under very specific conditions even though earlier and the present gas-phase experiments suggest otherwise.

[20] Gutowski, M.; Skurski, P.; Simons, J. *J. Am. Chem. Soc.* **2000**, *122*, 10159−10162.

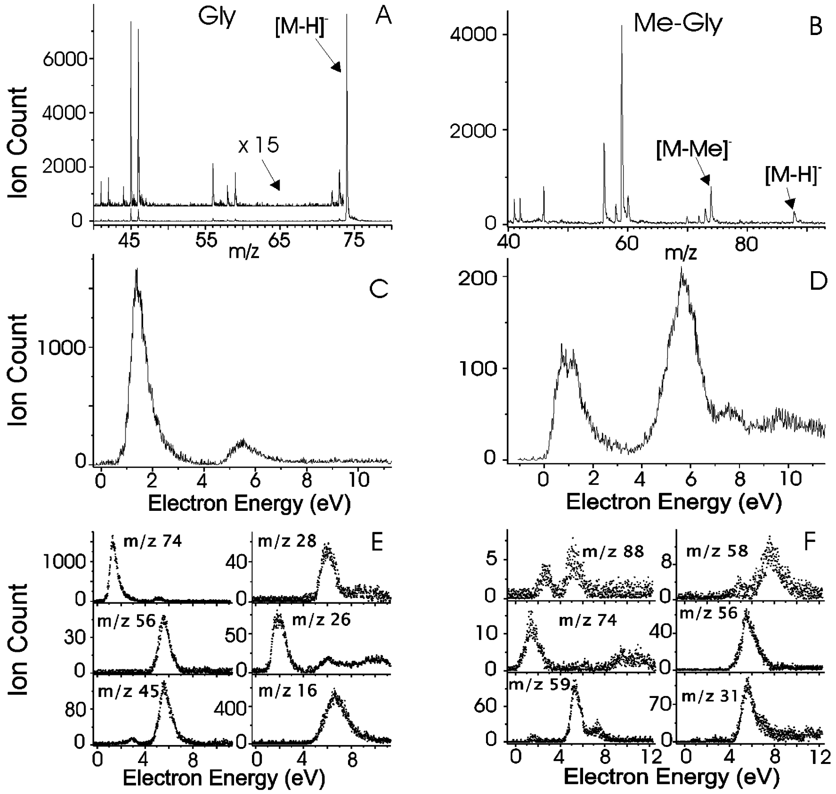


Figure 1. Resonant electron capture mass spectrum of glycine (A) and glycine methyl ester (B) summarized over the 0−12 eV electron energy range for the ion peaks from *m*/*z* 40 to 95. Total dissociative electron capture cross-section of glycine (C) and glycine methyl ester (D) for the negative ions displayed in (A) and (B). Peaks with *m*/*z* 42 and 46, generated with near zero energy, are strong functions of temperature and are not included in the integrated curves. Effective yield curves of selected negative ions (*m*/*z* are shown) from glycine (E) and glycine methyl ester (F) as functions of electron energies

ergetic electron beam could be generated. Samples of amino acids were initially loaded into a glass capillary, which in turn was placed into a homemade direct insertion probe that was inserted into the ionization chamber through a vapor lock. The ionization chamber and direct insertion probe have autonomous heating systems. The TEM is able to generate an electron beam with an energy spread of less than 50 meV, but for these experiments, the energy resolution was adjusted to ca. 200 meV with an electron current of ca. 50 nA in order to provide better sensitivity. The electron beam and sample vapor diffused from the direct insertion probe interact in the ionization chamber, and NIs formed after electron attachment are drawn in a direction orthogonal to the electron beam into the time-of-flight analyzer by an electric field at ca. 200 V applied to an extraction electrode. The ionization chamber was kept at 100−250 °C depending on the compound under examination; heating the chamber prevented condensation of sample on the walls. Depending on the compound studied, the temperature of the inlet system was set as follows: 150 °C (Gly), 90 °C (Me-Gly), 170 °C (Ala), 80−90 °C (Me-Ala), 180 °C (Phe), 115 °C (Me-Phe), 220 °C (Tyr), 140 °C (Me-Tyr), 215 °C (Trp), 140 °C (Me-Trp). The mass and energy scales were calibrated using SF_6 and CCl_4 whereby positions of the maxima in the yield curves of SF_6^-/SF_6 and Cl^-/CCl_4 were chosen as zero energy. In the case of measurements of the NI appearance energies, the onsets of SF_6^-/SF_6 or Cl^-/CCl_4 were taken as zero energy. For analysis of esters, CCl_4 was used only at the initial stage of the experiments; after achieving the inlet system temperature for effective sample vaporization, the CCl_4 effusion was shut off to prevent Cl^- signal from this compound from interfering with the signal from HCl that was present with the methylated amino acids as a salt. The pressure measured with an external ion gauge near the ionization chamber did not exceed 5×10^{-6} Torr thereby providing single-collision conditions throughout the experiment.

3. Results and Discussion

3.1. Aliphatic Amino Acids. The glycine REC mass spectrum integrated over the energy range 0−12 eV (Figure 1A) was identical to the earlier published spectra[16,17] except for some additional ion mass peaks (see Supporting Information[26]) that were not observed earlier. A comparison with the corresponding mass spectrum of Gly methyl ester (Figure 1B; Tables 1, 1S) demonstrates the uniqueness and similarity of the NI formation processes involved in the gas-phase resonant reactions of electrons with these compounds. In accordance with the reported findings for Gly,[15−17] long-lived molecular NIs were not observed for either Gly or Ala or their methyl esters.

3.1.1. Even-Electron NIs and Radicals. $[M-H]^-$ Negative Ions and Atomic $H^•$. The similarity between the total dissociative electron capture cross-section (Figure 1C) and the $[M-H]^-$ effective yield curve (*m*/*z* 74, Figure 1E) of Gly shows that formation of these NIs that peaked at 1.28 eV is the main decay channel among all fragmentation products of Gly resonance states formed by electron capture within the energy range ∼0

---

(21) (a) Schäfer, L.; Sellers, H. L.; Lovas, F. J.; Suenram, R. D. *J. Am. Chem. Soc.* **1980**, *102*, 6568−6569. (b) Suenram, R. D.; Lovas, F. J. *J. Am. Chem. Soc.* **1980**, *102*, 7180−7184.

(22) Crawford, O. H.; Garret, W. R. *J. Chem. Phys.* **1977**, *66*, 4968−4970.

(23) Desfrançois, C.; Carles, S.; Schermann, J. P. *Chem. Rev.* **2000**, *100*, 3943−3962.

(24) (a) Voinov, V. G.; Vasil'ev, Y. V.; Morré, J.; Barofsky, D. F.; Deinzer, M. L.; Gonin, M.; Egan, T. F.; Führer, K. *Anal. Chem.* **2003**, *75*, 3001−3009. (b) Voinov, V. G.; Vasil'ev, Y. V.; Ji, H.; Figard, B.; Morré, J.; Egan, T. F.; Barofsky, D. F.; Deinzer, M. L. *Anal. Chem.* **2004**, *76*, 2951−2957.

(25) Stamatovic A.; Schulz, G. J. *Rev. Sci. Instrum.* **1970**, *41*, 423−427.

(26) All tables and figures mentioned in the text that are in the Supporting Information have letter S with the running numbers.

Table 1. Relative Intensity (RI) of the Most Characteristic Negative Ions from Amino Acids (X = H) and Their Methyl Esters (X = Me),[a] $H_2N{-}CHR{-}C(O){-}OX$, Formed via Resonance Electron Capture at Electron Energy (E_m) Where the Ions Have Their Maximum Yield from a Resonance

	Gly		Ala		Phe		Tyr		Trp	
	X = R = H	X = Me; R = H	X = H; R = Me	X = R = Me	X = H; R = $C_6H_5CH_2$	X = Me; R = $C_6H_5CH_2$	X = H; R = $HOC_6H_4CH_2$	X = Me; R = $HOC_6H_4CH_2$	X = H; R = $C_8H_6NCH_2$	X = Me; R = $C_8H_6NCH_2$
ions	RI, % (E_m, eV)	RI, % (E_m, eV)	RI, % (E_m, eV)	RI, % (E_m, eV)	RI, % (E_m, eV)	RI, % (E_m, eV)	RI, % (E_m, eV)	RI, % (E_m, eV)	RI, % (E_m, eV)	RI, % (E_m, eV)
$[M-H]^-$	100 (1.28) 5.6 (5.15)	3 (2.6) 4.8 (5)	100 (1.2)	4.4 (3.3) 9.5 (6)	100 (1.16)	1.6 (3.4) 5 (6)	100 (1.12)	100 (1.35)	100 (1.2)	100 (1.55)
$[M-X]^-$	100 (1.28)	15 (1.3) 3 (8−12)^b	100 (1.12)	51 (1.2) 7 (8−12)^b	100 (1.16)	13 (1.2) 2.7 (9−11)^b	100 (1.12)	1.2 (1.4) <1 (6−11)^b	100 (1.2)	6 (1.25) 3 (∼2) *sh.*
$[M-H-XOH]^-$	2.7 (5.5)	53 (5.45)	2.3 (6) 2.5 (8.5)	21 (1.2) 40 (5.5)	0.7 (6)	11 (96) <3 (7−10)^b		8 (5.5) 5 (7−10)^b	<1 (5−10)^b	4 (6) 1.8 (9) *sh.*
R^-	d	d			1.8 (1.4) 2.9 (6.4)	4 (1.55) 28 (6.3)	2.5 (1.35) 1.4 (5)	10.1 (1.45) 6.7 (5.5)	0.74 (1.2) 0.43 (9)	2.5 (5−12)^b
$[M-R]^-$	5.6 (5.15)	4.8 (5)		7 (8−12)^b	12 (1.45)	20 (1.7) 6 (6.8)	11.3 (1.25)	11(1.55) 3.2 (5−8)^b	5.8 (1.2) 2 (6.2)	9 (1.85) 2.6 (6.3) *sh*
$[M-CO_2X]^-$							4.5 (1.4) <1 (∼8)		1.3 (1.2) 0.6 (5)	
$[M-HCO_2X]^-$								6 (5.6) 2 (7−9)^b	1.5 (5−12)^b	2 (2.5) 2 (5.5)
CO_2X^-	1 (2.8) 7.7 (5.6)	87 (5.3) 19 (7.4)	1.6 (2.3) 3.5 (5.7)	79 (5.8) 54 (8)	2 (6.5)	9 (5.45) 32 (8.2)	<0.6 (5−10)^b	6.5 (7.7) 6.4 (8.8)	<1 (5−12)^b	3.8 (8.4)
CO_2^-	1.1 (4−7)^b		<1 (5−11)^b		1 (6.5−7)^b		<0.3 (5−10)^b			
$OX^{-\,c}$	4.4 (6) 1.9 (10)	100 (5.45) 47 (7) *sh.*	2 (8.5) 2.5 (6.7)	23 (3.1) 100 (6)	9.5 (6)	100 (6.65) 40 (9.5) *sh.*	4.2 (6)	33 (6) 11 (9)	<1 (5−11)^b	18 (6.3
$NH_2^-/O^{-\,c}$	10−80 (6.7)	18 (6) 15 (9.2)	10 (6.6)	50 (6.6) 80 (7−11)^b	49 (6.6)	12 (6.4)	4.7 (4.22) 6.4 (5.7)	74 (6.65)	<1 (5−11)^b	5 (6.5) 2 (9)

[a] Me stands for CH_3. [b] Precise determination of resonance maxima was impossible. [c] Intensity of OH^- and O^- negative ions depends on the presence of water vapor in the samples. [d] Formation of H^- from Gly and its methyl ester was impossible to study in these experiments.

to 12 eV. Ala showed very similar behavior as the $[M-H]^-$ NI was also produced in the low energy range. Electron transmission spectroscopy of selected amino acids, yielding NI total formation cross-sections as functions of electron energy,[27] showed that these compounds are characterized by a low energy resonant state at 1−2 eV. In the language of molecular orbital theory, this is a shape resonance associated with electron capture into a π^* molecular orbital of the carboxylate group, which by analogy with the corresponding occupied π orbital localized on the same group[28] can be named as a π^*_{OO} "acidic orbital". This resonance was observed in the Gly electron transmission spectrum with the cross-section maximum at 1.93 eV. The displacement between maximum yield for fragment NIs and the center of the parent resonance is quite common[29] and normally is associated with a competition between fragmentation and electron autodetachment, where the latter becomes especially dominant at higher energies. Comparison with the $[M-H]^-$ and $[M-CH_3]^-$ NIs from the Gly methyl ester (Figure 1F) unambiguously indicates the $[M-H]^-$ NIs for Gly at 1.28 eV result in the carboxylate anions, $[H_2NCH_2CO_2]^-$. Earlier[12,14,30] and present quantum chemical calculations using a semiempirical method (PM3 level), density functional theory, B3LYP with the 6-31G and 6-311G basis sets, and ab initio SCF calculations at the 6-31G level support this interpretation. In addition these computations provided proof that the carboxylate anion is a more stable structure than either the enolate, $[H_2NCHCO_2H]^-$, or amide, $[HNCH_2CO_2H]^-$, anions. Indeed, comparison of the thermochemical balance for the formation of carboxylate $[M-H]^-$ NIs derived on the basis of the gas-phase acidity of Gly (1431.0 kJ/mol[31]) with experimentally determined appearance energy of $[M-H]^-$ NIs (1.10 ± 0.05 eV) indicates that only carboxylate anions can be formed at this energy from the π^*_{OO} resonance and, moreover, these NIs are formed practically at the thermochemical threshold.

The formation of $[M-H]^-$ NIs from OH group-containing molecules has been considered in detail,[32] and it has been established that the effective yield curves of the NIs indicate vibrational fine structure associated with the excitation of the ν(OH) stretching modes, thereby serving as an indicator of a predissociation mechanism. This means that the π^*_{OO} shape resonance only mediates the formation of $[M-H]^-$ ions, whereas the real driving force for dissociation is predissociation of the bound π^*_{OO} resonant state that evolves into a repulsive state along the OH-bond surface potential. The present and earlier[15,16] energy resolving powers of instrumentation used in these experiments were not sufficient to distinguish vibrational fine structure on the $[M-H]^-$ yield curve, whereas much better energy resolution applied by the Märk group[17] unambiguously showed the structure, though this fact was not emphasized in their report.

Based on thermochemical grounds and a comparison of relative intensities of the $[M-H]^-$ from Gly and its ester (Figure 1), $[M-H]^-$ NIs from Gly that peak at ca. 5.5 eV are enolate anions. In contrast to Gly, Ala, which has a methyl group on the α carbon, has no apparent peak at higher energies, and this fact also supports the enolate structure for $[M-H]^-$ from Gly at 5.5 eV. Finally, taking into account a comparison of the

---

(27) Aflatooni, K.; Hitt, B.; Gallup, G. A.; Burrow, P. D. *J. Chem. Phys.* **2001**, *115*, 6489−6494.
(28) Cannington, P. H.; Ham, N. S. *J. Electron Spectrosc. Relat. Phenom.* **1983**, *32*, 139−151.
(29) Aflatooni, K.; Burrow, P. D. *J. Chem. Phys.* **2000**, *113*, 1455−1464.
(30) Yu, D.; Rauk, A.; Armstrong, D. A. *J. Am. Chem. Soc.* **1995**, *117*, 1789−1796.
(31) O'Hair, R. A. J.; Bowie, J. H.; Gronert, S. *Int. J. Mass Spectrom.* **1992**, *117*, 23−36.
(32) Vasil'ev, Y. V.; Muftakhov, M. V.; Tuimedov, G. M.; Khatymov, R. V.; Abzalimov, R. R.; Mazunov, V. A.; Drewello, T. *Int. J. Mass Spectrom.* **2001**, *205*, 119−135.

effective yield curves for $[M-H]^-$ (*m/z* 76) and $[M-D]^-$ (*m/z* 75) anions from C_α-di-deuterated glycine, H_2NCD_2COOH (C_α-D_2-Gly), the higher energy hydrogen loss can only come from the α-carbon. The origin of these NIs is most probably an electronically excited Feshbach resonance since amino acids are known to have weak $n-\pi^*$ transitions near 2100−2200 Å (5.64−5.9 eV) and a very strong $\pi-\pi^*$ transition at 1900 Å (6.5 eV).[33] It is known[34,35] that triplet and, singlet excited states of a molecule can create an attractive field for an extra electron and generated in such a manner, electronically excited Feshbach resonances (intershell[36] or nonvalence[37] resonances) lie within ca. 0.5 eV of the corresponding excited states.

Taking into account the earlier reported[17] dissociative electron capture cross-section, $\sigma_{dec}(H)$, for the formation of $[M-H]^-$ at electron energy 1.25−1.3 eV where $\sigma_{dec}(H) = 5 \times 10^{-16}$ cm^2, one can estimate the formation cross-section of $[M-D]^-$ from C_α-D_2-Gly at 5 eV as $\sigma_{dec}(D) \approx 1.25 \times 10^{-17}$ cm^2. This value is more than 2 times lower than $\sigma_{dec}(H)$ of Gly with formation of $[M-H]^-$ at 5 eV (ca. 2.8×10^{-17} cm^2). The dissociative electron attachment cross-section, σ_{dec}, can be defined via the total electron attachment (reaction) cross-section, σ_{tot}, and the resonance width of the molecular NI state, Γ:[38]

$$\sigma_{dec} = \sigma_{tot} \exp(-\Gamma\tau_c/\hbar), \tag{1}$$

where the expression under the exponent is the survival probability[39] and τ_c is the time required to reach a point on the parent anion potential surface for autodetachment to become inaccessible. Taking into account that τ_c is proportional to $\sqrt{M_R}$ (M_R is the reduced mass of the fragmentation products)[39] and magnitudes of σ_{dec} for $[M-H]^-$ from Gly and $[M-D]^-$ from C_α-D_2-Gly at 5 eV, the total electron attachment cross-section at this energy will be of the order of 2×10^{-16} cm^2. This value is very large and practically identical with the unitarity limit ($\pi/k^2 \approx 2.4 \times 10^{-16}$ cm^2; where k is the electron wavenumber) for the reaction cross-section of the s-wave electron scattering value at 5 eV and correspondingly only 3 times lower than the unitarity limit of the p-wave electron scattering. Estimation of the mean autoneutralization lifetime of the resonance at 5 eV in Gly made on the basis of eq 1 is of the order of 10^{-14} s. This delay time is much longer than the time required for the 5 eV electron to pass a region equal to the size of the Gly molecule and enough to excite some vibrational modes in Gly. The very high total electron attachment cross-section and a relatively low dissociative electron attachment cross-section make Gly a very efficient electron scavenger in the low-energy range.

$[M-CH_3]^-$ Negative Ions. The relative intensity of the low energy resonance of what appears to be $[M-Me]^-$ from the Gly methyl ester is much weaker than that of the corresponding $[M-H]^-$ ions from the acid (Figure 1E and 1F). This is especially obvious when comparing the total dissociative electron capture cross-sections (Figure 1C and 1D). Quantum chemical calculations indicate there is little difference in the energies of frontier orbitals between Gly and its methyl ester and the low-energy resonance in the vicinity of 1.3 eV in the ester could decay with the formation of $[M\text{-}Me]^-$. However, considerable doubt remains since the underivatized acid, if present at only 1% as an impurity, could produce an $[M-H]^-$ of the intensity shown. A similar situation prevails for the ion of Ala methyl ester. Previously[40,41] reported $[M-Me]^-$ ions from simpler organic esters have been observed only at higher energies. Whether $[M-Me]^-$ ions from those compounds were formed at low energies cannot be determined from the reported experimental conditions.

$[M\text{-}16]^-$. Loss of Oxygen Atom and/or Amino Group. NIs of esters from which the equivalent of a methane molecule may be lost cannot be distinguished on the basis of mass from ions associated with the loss of the amino group or one of the oxygen atoms of the carboxyl group. For amino acids, however, only the last two processes can occur. NIs with *m/z* 59 and *m/z* 73 arise from Gly and its methyl ester, respectively (Figure 1A and 1B), both at high (∼5 eV) and low (1.5−2.0 eV) energies (Table 1S). Surprisingly, the analogous NIs from Ala with *m/z* 73 and its ester with *m/z* 87 have only been detected at low (1.5−2.0 eV) energies (Table 2S). According to quantum chemical calculations, the neutral residue resulting from the loss of the carbonyl oxygen should have a positive electron affinity. But loss of this oxygen atom demands too much energy and can only take place with electrons of energies higher than 6.5 eV. Hence, it may be concluded that NIs formed by loss of a neutral species of 16 amu from aliphatic amino acids are primarily $[M-NH_2]^-$ anions, although the loss of methane from the ester cannot be ruled out at this point.

$[M\text{-}17]^-$. Loss of NH_3 or OH Radical. Illenberger and co-workers[16] have proposed two possible structures for the low intensity NIs with *m/z* 58 in Gly namely $H_2NCH_2CO^-$ and $CHCOOH^-$ from loss of neutral $^{\bullet}OH$ and NH_3 groups, respectively. The observation that both $[M\text{-}17]^-$ and $[M\text{-}18]^-$ are produced from di-deuterated C_α-D_2-Gly, and that NIs with *m/z* 72 and *m/z* 58 arise from nondeuterated Gly methyl ester and the *m/z* 72 ions arise from Ala and its methyl ester, confirms that both structures are present. Moreover, comparison of the $[M\text{-}17]^-$ NIs from Gly and $[M\text{-}OCH_3]^-$ NIs from its methyl ester (Tables 1S) immediately shows that loss of NH_3 occurs at low energy (1−2 eV), whereas loss of OH (or OCH_3 in the ester) occurs predominantly from higher resonances (>5 eV). This finding is in accord with earlier analyses[16] suggesting loss of NH_3 from the Gly anion as a less energy-demanding process than formation of the $^{\bullet}OH$ radical.

$[M\text{-}19]^-$. Processes Leading to Losses of Hydrogen and Water from Acids and Methanol from Esters. A characteristic decay of molecular NIs of fatty acids[42] and simple organic acids[41,43] generated by capturing high energy electrons (7 eV and higher) is loss of a 19 amu fragment. Gentisic acid, a

---

[33] (a) Wetlaufer, D. B. In *Advances in protein Chemistry*; Anfinsen, C. B., Jr., Anson, M. L., Bailey, K., Edsal, J. T., Eds.; Academic Press: New York, London, 1962; Vol. 17, pp 303−390. (b) Saidel, L. J.; Goldfarb, A. R.; Waldman, S. *J. Biol. Chem.* **1952**, *197*, 285−291.

[34] Dressler, R.; Allan, M.; Tronc, M. *J. Phys. B: At. Mol. Opt. Phys.* **1987**, *20*, 393−402.

[35] Khvostenko, V. I.; Vorob'ev, A. S.; Khvostenko, O. G. *J. Phys. B: At. Mol. Opt. Phys.* **1990**, *23*, 1975−1977.

[36] Brunt, J. N. H.; King, G. C.; Reed, F. H. *J. Phys. B: At. Mol. Opt. Phys.* **1977**, *10*, 433−448.

[37] Nesbet, R. K. *J. Phys. B: At. Mol. Opt. Phys.* **1978**, *11*, L21−L25.

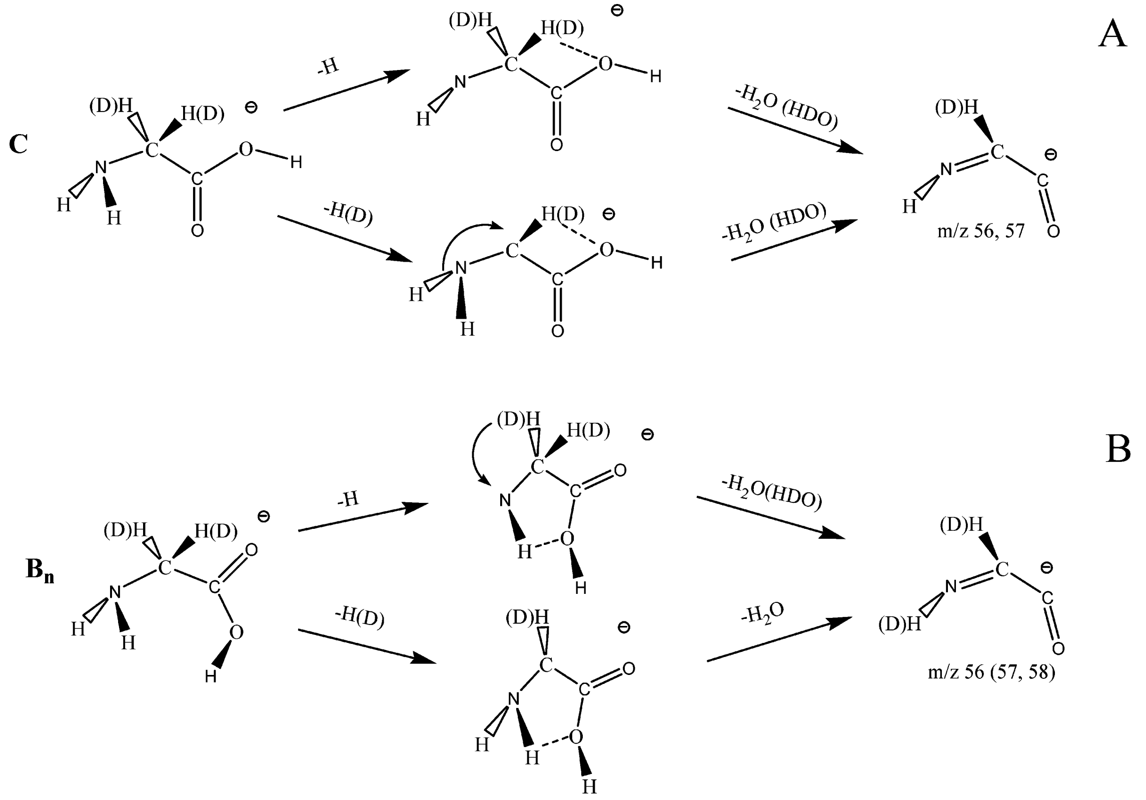
[38] (a) Bardsley, J. N.; Herzenberg, A.; Mandl, F. In *Atomic Collision Processes*; McDowell, M. R. C., Ed.; North-Holland Publishing Company: Amsterdam, 1964; pp 415−427. (b) O'Malley, T. F. *Phys. Rev.* **1966**, *150*, 14−29.

[39] Demkov, Yu. N. *Phys. Lett.* **1965**, *15*, 235−236. (b) Chen, J. C. Y.; Peacher, J. L. *Phys. Rev.* **1967**, *163*, 103−111.

[40] Takhistov, V. V.; Muftakhov, M. V.; Krivoruchko, A. A.; Mazunov, V. A. *Russ. Chem. Bull.* **1991**, 1812−1817.

[41] Muftakhov, M. V.; Vasil'ev Y. V.; Khatymov, R. V.; Mazunov, V. A.; Takhistov, V. V.; Travkin, O. V.; Yakovleva, E. V. *Rapid Commun. Mass Spectrom.* **1999**, *13*, 912−923.

Scheme 1. Loss of a Water Molecule from Parent Gly (or C_α-D_2-Gly) Negative Ions Occurs after Hydrogen Abstraction from the Amino Group or Deuterium Abstraction from the C_α-Position, Which Results in Generation of NIs with *m*/*z* 56 (or *m*/*z* 57, 58)[a]



[a] Top (A) and bottom (B) panels indicate the fragmentation pathways for the **C** and $\mathbf{B}_n$ isomers[20] of Gly, respectively.

MALDI matrix, also showed loss of 19 amu, in REC mass spectrometry studies.[44] Both Gly and Ala molecular NIs decay via this fragmentation channel yielding ions with *m*/*z* 56 and *m*/*z* 70, respectively, after capturing electrons of energies greater than 5 eV (Table 1, $[M\text{-}H\text{-}XOH]^-$). Observation of peaks of metastable NIs in the REC mass spectra of fatty acids[42a] unambiguously proved that the first step of the reaction is H-atom elimination followed by loss of a water molecule. The spectra[42c] indicated that H-atom loss is not from the carboxylic acid group but rather from a position on the aliphatic chain, after which loss of water to yield either a cyclic ketone or a double bond along the chain takes place. A similar mechanism may prevail for the molecular NI fragmentation in aliphatic amino acids. Inspection of C_α-D_2-Gly indicated an involvement in the dissociation of hydrogen/deuterium atoms at the C_α position and from the amino group (Scheme 1). The participation of these hydrogens was confirmed by observation of NIs with *m*/*z* 57 in C_α-D_2-Gly which are produced in greater abundance in comparison with the NIs with *m*/*z* 56 (Figure 3S). A small amount of *m*/*z* 58 is also produced. There is also a parallel between esters of fatty acids and amino acids, where similar fragmentation channels are again associated with loss of a hydrogen atom and subsequent loss of methanol with *m*/*z* 56 and 70 for Gly and Ala methyl esters (Table 1 $[M\text{-}H\text{-}XOH]^-$). In addition, Ala molecular NIs dissociate via loss of a water molecule or perhaps via abstraction of a H-atom and ammonia yielding an $(M\text{-}18)^{-\bullet}$ ion even more efficiently at high energies again suggesting that the hydrogen on the alpha carbon is likely lost.

3.1.2. Odd-Electron NIs and Even-Electron Neutrals. $[M-H_2]^{-\bullet}$ Negative Ions and H_2. In contrast to that previously reported,[16,17] very weak $[M-H_2]^{-\bullet}$ NIs were observed from Gly with a maximum yield at an energy of ~1 eV (Table 1S). Production of the hydrogen molecule must be a concerted process at these low electron energies, since energy balance of the reaction is close to zero, whereas loss of two separate hydrogen atoms would require about 4.5 eV. On the basis of simple chemical intuition, there are three possible structures for these ions (Scheme 2 **A**, **B** and **C)**. Although structures **C** or **A** are energetically favorable according to PM3 or B3LYP/6-31++G** calculations, the difference in calculated magnitudes of the total energies as well as the heats of formation for all three structures is small assuming they all can arise from the low-lying π^*_{OO} shape resonance. Studies with C_α deuterated Gly resulted in the generation of NIs with *m*/*z* 74. These ions can have either structure **A** or **C**. However, more intense NIs with *m*/*z* 75 at low energies (~1 eV) can only be associated with H_2 loss. This provides proof that structure **B** is definitely formed at low energies and the Gly isomer $\mathbf{B}_n$ (Scheme 1)[20] is the most probable precursor for these ions. As discussed above, NIs of the same nominal mass generated at energies > 5 eV

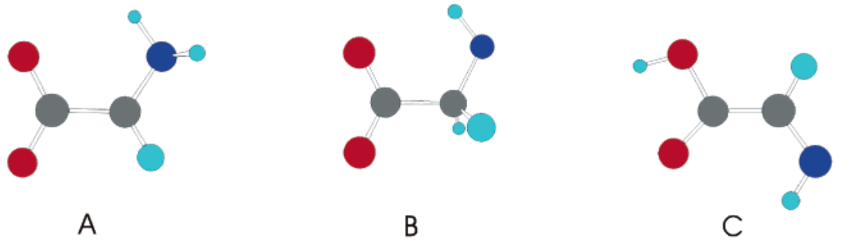
---

(42) (a) Voinov, V. G.; Boguslavskiy, V. M.; Elkin, Y. N. *Org. Mass Spectrom.* **1994**, *29*, 641−646. (b) Voinov, V. G.; Clayes, M. *Int. J. Mass Spectrom.* **2000**, *198*, 23−32. (c) Voinov, V. G.; Heuvel Van Den, H.; Clayes, M. *J. Mass Spectrom.* **2002**, *37*, 313−321.

(43) (a) Muftakhov, M. V.; Tuktarov, R. F.; Mazunov, V. A. *Chem. Phys. Reports* **1999**, *18*, 413−433. (b) Pelc, A.; Sailer, W.; Scheier, P.; Mason, N. J.; Illenberger, E.; Märk, T. D. *Vacuum* **2003**, *70*, 429−433. (c) Sailer, W.; Pelc, A.; Probst, M.; Limtrakul, J.; Scheier, P.; Illenberger, E.; Märk, T. D. *Chem. Phys. Lett.* **2003**, *378*, 250−256.

(44) (a) Asfandiarov, N. L.; Pshenichnyuk, S. A.; Fokin, A. I.; Lukin, V. G.; Fal'ko V. S. *Rapid Commun. Mass Spectrom.* **2002**, *16*, 1760−1765. (b) Pshenichnyuk, S. A.; Asfandiarov, N. L.; Fal'ko V. S.; Lukin, V. G. *Int. J. Mass Spectrom.* **2003**, *227*, 281−288. (c) Pshenichnyuk, S. A.; Asfandiarov, N. L.; Fal'ko, V. S.; Lukin, V. G. *Int. J. Mass Spectrom.* **2003**, *227*, 259−272. (d) Pshenichnyuk, S. A.; Asfandiarov, N. L. *Eur. J. Mass Spectrom.* **2004**, *10*, 477−486.

Scheme 2. Three Isomers for $[M-H_2]^-$ NIs of Gly with Heats of Formation Calculated According to PM3 Theory and Total Energies (in Parentheses) Using B3LYP/6-31++G** Level as −95.4 kcal/mol (−7707.361 eV), −91.2 kcal/mol (−7707.073 eV), and −95.8 kcal/mol (−7707.289 eV) for A, B, and C, Respectively[a]



[a] Carbon, hydrogen, oxygen, and nitrogen atoms are labeled gray, light blue, red, and blue, respectively

are $[M-D]^-$ ions. Registration of NIs generated by loss of a neutral species of 16 amu from Gly and Ala methyl esters yielding ions with *m*/*z* 73 and 87, respectively, have been discussed above for the production of an NH_2 species or an oxygen atom. But the loss of a methane molecule of the same nominal mass from the esters, which could not be distinguished by mass, can be assumed to occur in a fashion analogous to the formation of $[M-H_2]^-$ NIs via structure **A** or **B** (Scheme 1).

Loss of Ammonia. As already mentioned, analysis of both amino acids and esters showed that loss of ammonia occurs predominantly at low energies. Another fragmentation channel involving the amino group is associated with the registration of NIs of *m*/*z* 71 and 85 from Ala and its ester at very high energies (~9 eV; Table 2S). This process must involve either loss of an additional hydrogen atom together with NH_3 or abstraction of H_2 and NH_2. These NIs in Ala, however, could also be due to the loss of a water molecule.

3.1.3. Generation of Low Mass Negative Ions. The generation of NIs of low mass is characteristic of REC processes in all amino acids studied. The main difficulty in the investigation of these ions is being able to recognize their neutral counterpart(s) and minimizing potentially false processes resulting in generation of the ions that are thermal decomposition products. The following fragment ions are produced from both amino acids and their esters: $COOX^-$, OX^- (X = H or Me), NH_2^-/O^- (Table 1) and other NIs with *m*/*z* 46, 44, 42, 41, 39, 26 (Tables 1S,2S). With the exception of the ion with *m*/*z* 44, the remainder of these ions arise because of the high electron affinities of the neutral species. Negatively charged fragments $COOX^-$ and OX^- (Table 1) are typical products of REC reactions of acids and esters, and these have been studied extensively.[45] In Gly and Ala methyl esters, OCH_3^- ions result in major peaks in REC mass spectra (Table 1). The very low abundance NIs with *m*/*z* 44 are probably CO_2^- ions on the basis of charge reversal experiments,[13] even though the adiabatic electron affinity of CO_2 is known to be negative.[46] Cooper and Compton[47] were among the first investigators to observe these species experimentally and also the first to determine the lifetimes of the metastable NIs from different precursors.

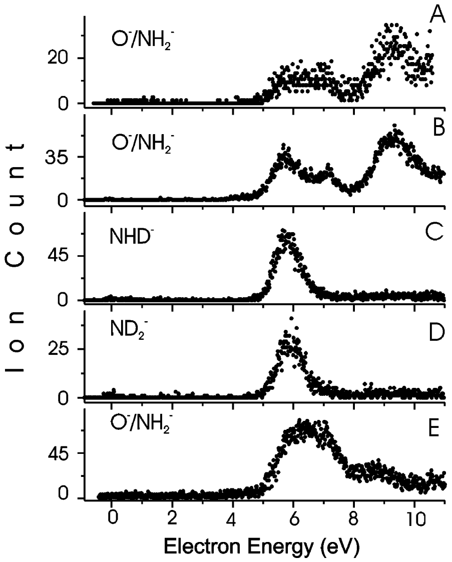


Figure 2. Effective yield curves of negative ions with *m*/*z* 16 (A and B), 17 (C), and 18 (D) generated from partially deuterated (deuteration of the amino group) triglycine methyl ester as function of electron energy. Water content in sample A was higher than in sample B. Shown is the relative intensity of the resonance at 6 eV relative to the resonance at 10 eV when more water was present, suggesting that the resonance at 6 eV is due to the NH_2^-. This is confirmed by the deuterium experiments in (C) and (D) (see text). (E) Effective yield curve of negative ions with *m*/*z* 16 from tryptophan methyl ester.

The ions with nominal mass 16 amu may be due to NH_2^-, O^-, or a combination of both. Amino acids easily absorb water from ambient air, and therefore, one of the sources for the ion with *m*/*z* 16 could be O^- from H_2O. Indeed, the yield of the ions has been found to be strongly dependent on how water-free the particular amino acid was before introducing it into the mass spectrometer. The analysis of partially deuterated methyl ester of glycine trimer, which we had available, helped answer this question. The NIs with *m*/*z* 17 and 18 from the compound were unambiguously assigned to NHD^- and ND_2^-, respectively (Figure 2). Effective yield curves of ions with *m*/*z* 16 from the compound have been found to be different depending on the water content (Figure 2); but it is still unclear whether O^- can be formed from the sample or whether the ion with *m*/*z* 16 is primarily the NH_2^- ion, since the shapes of the effective yield curves for O^- and NH_2^- can be identical.

The yields of other NIs from amino acids with small masses are much more sensitive to the inlet system temperature, especially for anions with *m*/*z* 46 ($NHCH_2OH^-$ or $CHOOH^-$) and 42 ($C_2H_4N^-$, $C_2H_2O^-$, CNO). Generation of these anions necessarily involves rather complex rearrangement processes. Comparison of NIs with *m*/*z* 46 from Gly and *m*/*z* 60 from its methyl ester indeed supports this finding. Formation of these anions at very low energy (<0.1 eV), however, is probably

---

(45) Burgers, P. C.; Holmes, J. L.; Szulejko, J. E. *Int. J. Mass Spectrom. Ion Processes* **1984**, *57*, 159−166.

(46) (a) Krauss, M.; Neumann, D. *Chem. Phys. Lett.* **1972**, *14*, 26−28. (b) Gutsev, G. L.; Bartlett, R. J.; Compton, R. N. *J. Chem. Phys.* **1998**, *108*, 6756−6762.

(47) (a) Cooper, C. D.; Compton, R. N. *Chem. Phys. Lett.* **1972**, *14*, 29−32. Cooper, C. D.; Compton, R. N. *Bull. Am. Phys. Soc.* **1972**, *17*, 109−110. Cooper, C. D.; Compton, R. N. *J. Chem. Phys.* **1973**, *59*, 3550−3565.

associated with thermal activation or even thermal degradation of the samples on hot surfaces of the ion source or the inlet system.

In conclusion, there is a qualitative similarity of the REC mass spectra of aliphatic amino acids and their methyl esters. Apparently esterification of these amino acids does not dramatically change the electronic structure of molecular NIs and, therefore, has little effect on the order of the low energy unoccupied molecular orbitals. Otherwise one would expect a different arrangement of all the available NI state energy levels and completely different fragmentation mechanisms or decay channels.

3.2. Aromatic Amino Acids. REC mass spectra of aromatic amino acids and their methyl esters (Table 1) have certain similarities with those of the aliphatic amino acids discussed in the previous section. Despite the presence of aromatic rings, no long-lived molecular NIs are formed from the compounds via REC processes over the energy range 0−12 eV. By analogy with aliphatic amino acids, all ion-formation processes in aromatic amino acids are considered on the basis of the electronic nature of NIs and their neutral counterparts.

3.2.1. Even-Electron NIs. Production of $[M-H]^-$ through Hydrogen Atom Loss and $[M-CH_3]^-$ Negative Ions through Methyl Radical Loss. The most intense NI peaks in the REC mass spectra of aromatic amino acids are $[M-H]^-$ generated with electrons of 1−1.2 eV energies that result in carboxylate anions. Again, it is not clear whether $[M-Me]^-$ is produced at low energies, but in accord with the aliphatic counterparts, the relative yield of the NI from Phe methyl ester is lower with respect to the acid counterparts $[M-H]^-$. This result is again related to the predissociation mechanism for their formation.[32] The scenario is totally different in the case of Tyr or Trp methyl esters, since these compounds have hydrogen atoms attached to oxygen and nitrogen that are located in the side chains. Tyr methyl ester efficiently generates $[M-H]^-$ NIs via resonant low-energy electron capture. The origin of these NIs, however, is associated with the phenol ring.[32] The Trp methyl ester also exhibits efficient formation of $[M-H]^-$ NIs at low energies. Quantum chemical calculations predict that these ions can only be produced through loss of a hydrogen atom from the indole ring. Comparison of $[M-H]^-$ NIs from Tyr and Trp with that from the esters shows that formation cross-sections of the NIs from esters have their maxima at slightly higher energies and have marginally lower relative values with respect to other fragment NIs. The last result is not surprising since acids have two sources for $[M-H]^-$ production: from the carboxyl groups and from side chains, whereas the esters of Tyr and Trp have only the one. The $[M-H]^-$ NIs for these methyl esters are interesting and need additional examination because hydrogen atom loss from the radical anions of such compounds is supposed to be associated with vibrational Feshbach resonances,[48] i.e., excitation of ν(NH) or ν(OH) stretching modes, the nature of which and role at low-energy electron−molecule interactions have not yet been fully recognized.[49]

Generation of H atoms at higher energies has been found to be even less efficient than for Gly, and there was no apparent resonance-like peak but rather a very flat and broad hump in the $[M-H]^-$ effective yield curve. Since these processes in Gly have been proved to be associated with the formation of enolate anions, the presence of the side chains in aromatic amino acids changes the fragmentation into different pathways, thereby, preventing the loss of H atoms at energies > 3 eV.

There is, however, another possibility for production of hydrogen atoms of low intensity occurring with simultaneous or consecutive loss of water (acids) or methanol (esters) in full analogy with aliphatic compounds. These NIs are similar to the *m*/*z* 56 ions from Gly and its ester and appear with *m*/*z* 146 and *m*/*z* 185, respectively, in the REC spectra of Phe and Trp and their esters. This reaction in Tyr is different from Phe but similar to that in Ala where only the ion with *m*/*z* 163 resulting from loss of a water molecule without hydrogen atom abstraction is observed.

Loss of the Side Chains. According to electron transmission spectroscopy[27] the side chains in aromatic amino acids change the electronic structure of the molecular NIs, although the anionic state associated with π^*_{OO} "acidic orbital" is itself only slightly stabilized with respect to that of Gly. The limited stabilization is a result of a stronger positive inductive effect of the aromatic substituent in comparison to the destabilization from resonance interaction. The main anomaly is the appearance of lower-energy states of molecular NIs that can be described as electron attachment into molecular orbitals having benzene/toluene or indole-like character. The NIs that are definitely associated with the aromatic side groups and adjacent backbone residues are R^- and $[M-R]^-$, respectively, where R corresponds to the aromatic substituents: $C_6H_5CH_2^\bullet$, $OHC_6H_4CH_2^\bullet$ and $C_8H_5NHCH_2^\bullet$ for Phe, Tyr, and Trp, respectively. The production of these complementary $[M-R]^-$ and R^- NIs from Phe and its methyl ester has been observed at low (1.4−1.7 eV) and high (6.3−6.8 eV) energies (Table 1); the first one is more effectively produced at lower energies, whereas the latter is predominantly formed from higher energy resonances. To give a more mechanistic insight into the formation of these anions, one may compare their appearance energies. The $[M-C_6H_5CH_2]^-$ NIs from Phe have an experimental appearance energy of 0.7 ± 0.2 eV that is practically identical to the onset energy of the quasi-degenerate $\pi_{1,2}{}^*(-\phi)$ shape resonances located at ca. 0.6−0.7 eV with a maximum cross-section at 0.87 eV.[27] The experimental appearance energies of the anions fall between those estimated by quantum chemical calculations using PM3 (0.18 eV) and B3LYP/6-31++G** (1.04 eV). These results indicate that the experimental appearance energy of $[M-C_6H_5CH_2]^-$ is determined by the corresponding characteristics of the parent states, which undoubtedly involve $\pi_{1,2}{}^*(-\phi)$ shape resonances, since the next π^*_{OO} shape resonance lies at the higher energy, 1.85 eV.[27] The complementary $C_6H_5CH_2^-$ has an experimental appearance energy of 0.95 ± 0.25 eV, which is higher than the onset of the $\pi_{1,2}{}^*(-\phi)$ shape resonances, but it is close to its calculated thermochemical thresholds of 1.09 eV (PM3) or 1.58 eV (B3LYP/6-31++G**). This simple comparison indicates that R^- anions again are generated from the $\pi_{1,2}{}^*(-\phi)$ shape resonances and their appearance is determined only by the energetics of the process. These findings are interesting in two respects. First, an extra electron captured via a resonance process involves molecular orbitals located mainly on the phenyl ring followed by orbital mixing with an orbital

---

(48) Wilde, R. S.; Gallup, G. A.; Fabrikant, I. I. *J. Phys. B: At. Mol. Opt. Phys.* **2000**, *33*, 5479−5492.

(49) (a) Fabrikant, I. I.; Hotop, H. *Phys. Rev. A* **2001**, *63*, 022706-1−022706-9. (b) Hotop, H.; Ruf, M.-W.; Allan, M.; Fabrikant, I. I. *Adv. At., Mol., Opt. Phys.* **2003**, *49*, 85−216.

located on the backbone of the molecule, and the charge is accommodated predominantly in these parts after dissociation. A process of this kind was invoked earlier[50] in the case of phthales, whereby loss of the alkyl group resulted from $\pi^*-\sigma^*$ mixing. Second, these observations are the first experimental demonstration of low-energy decay occurring from an intermediate resonant state associated with side chains of isolated amino acids.

It is of interest that the behavior of fragment anions, $[M-R]^-$ and R^-, from Phe at higher energies is reversed when R^- anions have larger formation cross-sections. The reasons for this phenomenon are two-fold. First, since formation of R^- is a more energy demanding process, less energy is left to be partitioned between fragments of the reaction and consequently excitation of the fragments will be less. Second, since the side group, R, has a larger number of atoms in its structure than in the amino acid backbone, the excess energy in the R^- anion will be disposed of through larger numbers of vibrational degrees of freedom than in $[M-R]^-$. Both these factors should lead to greater survival of R^- NIs with respect to electron autodetachment; hence, R^- will possess a higher dissociative electron capture cross-section. Another important finding is that the appearance energy of the R^- NI generated from the resonance state at 5.4 ± 0.4 eV is higher than the center of the π_4^* $(-\phi)$ shape resonance associated with the benzene ring (4.51 eV[27]). For this reason, it is not possible to draw a connection to the origin of these two anions, and it is more likely that the R^- fragment ions are formed from Feshbach resonances associated with electron transfer that occurs predominantly on the aromatic ring. Indeed, transmission of slow (<10 eV) electrons through thin Trp films carried out by the Sanche group[51] at 300 K pointed to the presence of three optically allowed $\pi-\pi^*$ transitions at 4.4, 6.1, and 7.5 eV. The last two neutral-excited states could be parent states for resonances that decay into R^- NIs.

The same applies to R^- and $[M-R]^-$ ions in Tyr and Trp as well as in Trp methyl ester. Intensities of these NIs in Tyr methyl ester (Table 1), however, were practically identical. In going from Phe to Tyr and their esters, it is observed that relative intensities of the R^- NIs are greater in the low energy resonant state than in the higher energy states. This is particularly pronounced in the case of another type of ion associated with the side chains that differ from R^- NIs by abstraction of a hydrogen atom. These NIs have *m*/*z* 106 in the case of Tyr and 129 in Trp. Instead of the H atom generated from Gly anion at 5 eV, aromatic amino acids expel even-electron R^- NIs.

New Fragmentation Channels Involving the Side Chains. Spectra of Tyr and Trp and their methyl esters indicate there is another fragmentation channel at high energies that is associated, respectively, with the negatively charged phenolic (*m*/*z* 93) and indolic (*m*/*z* 116) residues. An interesting counterpart to these types of NIs with characteristic involvement of only part of the side chain is that in which the α-carbon atom from the amino acid backbone is attached to the side chain. These ions were observed at low energies for all three aromatic amino acids and their esters, which are *m*/*z* 103 (Phe), 119 (Tyr), and 142 (Trp). These ions may be represented as $[M-NH_3-COOH]^-$ or $[M-NH_2-HCOOH]^-$ in the case of amino acids and $[M-NH_3-COOCH_3]^-$ or $[M\text{-}NH_2-HCOOCH_3]^-$ for the esters.

Loss of COOH Radical. The fragmentation channel associated with the loss of the $^{\bullet}COOH$ radical has been observed only in Tyr and Trp (*m*/*z* 136 and 159, respectively; Table 1). The abstraction of the $^{\bullet}COOH$ group through dissociation of molecular NIs from aliphatic amino acids and similar loss of the $^{\bullet}COOCH_3$ counterpart from esters was not detected most likely because the electron affinities of the corresponding neutral radicals $NH_2CH_2^{\bullet}$ for Gly and $NH_2CH(CH_3)^{\bullet}$ for Ala are negative. The same is probably true of Phe and its ester; for these the loss of the $^{\bullet}COOX$ groups (X = H or Me) have been found to be accompanied by abstraction of the hydrogen molecule (*m*/*z* 118) at electron energies 5−7 eV (Table 3S). Similar processes occur for Tyr and Trp methyl esters in addition to abstraction of a hydrogen atom,[$M-H-COOX]^-$ with *m*/*z* 134 and $[M-H_2-COOX]^-$ with *m*/*z* 135 for Tyr methyl ester and *m*/*z* 157 and *m*/*z* 158 for Trp methyl ester with electrons of 5−9 eV (Table 4S). Loss of the $^{\bullet}COOX$ group is important in the sense that this type of dissociation has some relation to the well-known cleavage of $C_\alpha-C$ bonds in the electron capture dissociation (ECD) of protonated peptides.[52] Localization of the negative charge at a different site of a dissociative NI leading to formation of $HCOO^-$ (acids) or $COOCH_3^-$ (esters) was observed in all aromatic amino acids and esters all at electron energies 5−9 eV (Tables 3S−5S).

Other Fragmentation Channels. Fragmentation channels of molecular NIs of aromatic amino acids associated with the loss of atomic oxygen and NH_2 radicals have been found to be similar with those for the aliphatic counterparts, which proves they are characteristics of the backbone rather than the side chains. Loss of a neutral species with a nominal mass of 17 amu, $^{\bullet}OH$ radical or ammonia, has been observed only in the case of Phe (Tables 3S−5S).

3.2.2. Odd-Electron NIs. Aromatic amino acids did not show any significant signs of $[M-H_2]^{-\bullet}$ in contrast to Gly. Instead, relatively abundant formation of $[M-RH]^-$ NIs with *m*/*z* 73 in the low energy range was observed in all aromatic amino acids, suggesting that the processes are comparable to hydrogen molecule production in Gly. REC by Tyr and Trp methyl esters did not lead to the detection of these NIs, thus providing evidence this ion-formation mechanism involves the carboxyl hydrogen in the amino acids. Observation of NIs with *m*/*z* 87 in Phe methyl ester shows, however, that there must be an alternative pathway for the generation of $[M-RH]^-$ NIs, at least in Phe methyl ester; for example, a hydrogen atom from the α carbon could be involved.

Loss of a neutral species with a nominal mass of 18 amu has been observed at high energies in the case of Phe, Tyr, Tyr methyl ester, and Trp methyl ester. This decay process can be associated with the production of H_2O or $H^{\bullet}$ and NH_3 or H_2 and NH_2.

3.2.3. Generation of Low Mass Negative Ions. NIs of low masses produced under REC by aromatic amino acids and their esters have been found to be identical to those from aliphatic amino acids and their esters and will not be discussed further. It is worth mentioning, however, that analogous to the CH_2N^- NIs from Gly, peaks with *m*/*z* 118, 134, and 157 from Phe,

---

(50) Mazurkiewicz, P. H.; Deinzer, M. L. *J. Am. Chem. Soc.* **1999**, *121*, 3421−3427.

(51) Leclers, G.; Goulet, T,; Cloutier, P.; Jay-Gerin, J.-P.; Sanche, L. *J. Phys. Chem.* **1987**, *91*, 4999−5001.

(52) Zubarev, R. A.; Kelleher, N. L.; McLafferty, F. W. *J. Am. Chem. Soc.* **1998**, *120*, 3256−3266.

Tyr, and Trp and their esters, respectively, appear. Production of the OCH_3^- NI in Phe methyl ester is likely similar to the pathways observed for the aliphatic counterparts and again is represented by the most intense peak in the integrated mass spectrum. Generation of these ions in Tyr and Trp methyl esters is not as abundant as the above-discussed $[M-H]^-$ NIs, but it is still rather intense.

The question of the interpretation of NIs with m/z 16 has already been discussed at length for aliphatic amino acids. Since Trp and its methyl ester have a NH-group and assuming a possible H-shift, it is reasonable to suppose there is an interference of the NH_2^- NIs generated directly from the N-terminus and the indole ring of Trp. The shape of the effective yield curve of the NIs with m/z 16 from Trp methyl ester (Figure 2E) indeed indicates a difference when compared to that from Gly (Figure 1) and that deuterated via the amino-group tri-Gly methyl ester (Figure 2). The oxygen in the phenolic ring of Tyr can also be involved in generating NIs with m/z 16. The shape of the effective yield curves of these ions from Tyr are rather complex, but the high temperature used in these experiments could cause partial decay through loss of CO_2. Similar processes have been observed previously in electron transmission spectra of some amino acids.[27] Indeed, effective yield curves of NIs with m/z 16 from Tyr have a shape that can be constrained by addition of the well-known curve of O^- from CO_2 [53] to that of m/z 16 from Gly (Figure 1).

4. Conclusions

The simplest aliphatic amino acid NIs generated in the energy range from zero to ca. 12 eV yield a variety of different fragmentations, and the aromatic amino acids undergo additional dissociations associated with the side chains. The relative intensities of these dissociation processes have not been found to be high in comparison with hydrogen atom losses from the carboxylic acid groups that occur in the energy range 1.2−1.3 eV. On the other hand, a study of isotopically labeled compounds for comparison of $[M-H]^-$ and $[M-D]^-$ formation at 5 eV in Gly and deuterated C_α-D_2-Gly, respectively, made it possible to conclude that the total electron capture cross-section in Gly at 5 eV is close to the unitarity limit. This fact together with an estimation of the mean lifetime of the Gly resonant state using the same approach suggested that Gly, and probably other amino acids, is a very effective scavenger of free electrons. Hence, amino acids and proteins can conceivably act as radiation protectors in living cells. In addition, resonant electron capture by all amino acids investigated here leads to generation of H-atoms that can be involved in recombination processes with free radicals, again with the prospect of reducing radiation damage to the cell. Recent experiments[9] with variation of DNA-associated proteins indeed pointed out increased DNA damage when the protein content in the nucleus decreased. The present experiments suggest that such behavior may be associated with low-energy electron capture processes in the cellular nucleus.

Peaks of fragment NIs from Tyr and Trp and their methyl esters originating from a precursor resonant state associated with the phenolic and indolic rings, respectively, are the next most intense in the mass spectrum of these compounds. This observation is in line with recent reports[54] on the correlation between localization of a positive or negative charge and the distance from the indole ring as observed by variation of the fluorescence quantum yield in different derivatives of Trp.

It was found that esters and the underivatized amino acid analogues experienced similar processes in the formation of NIs. The major difference is that the relative intensities of the $[M-H]^-$ NIs from acids are much greater than the corresponding $[M-Me]^-$ NIs from esters. Such a difference, however, is not related to a difference in the anion electronic structures but may be related to a predissociation mechanism[32] involved in their formation, if the amino acid itself present as an impurity was not responsible for the equivalent ion.

Acknowledgment. This project was supported by Grants NIH ES09536 and ES10338 and by the NIEHS Environmental Health Sciences Center Grant ES00210. This publication was made possible in part by Grant Number P30 ES00210 from the National Institute of Environmental Health Sciences, NIH. The authors wish to acknowledge the Mass Spectrometry Facility of the Environmental Health Sciences Center at Oregon State University.

Supporting Information Available: Total resonant electron capture mass spectra of all compounds studied (Tables 1S−5S) and effective yield curves of some negative ions from the amino acids and their esters under study (Figures 1S−11S). This material is available free of charge via the Internet at http://pubs.acs.org.

JA058464Q

---

(53) (a) Chantry, P. J. *J. Chem. Phys.* **1972**, *57*, 3180−3186. (b) Stamatovic, A.; Schulz, G. J. *Phys. Rev. A* **1973**, *7*, 589−592.

(54) Callis, P. R.; Liu, T. *J. Phys. Chem. B* **2004**, *108*, 4248−4259.