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Gas-phase reactions of NO^+ with Glu and γ-Glu–Met

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Dedicated to the memory of Dr. Piet Leclercq

Abstract. The reactivity of the nitrosonium ion, NO^+, with the amino acid Glu and the dipeptide γ-Glu–Met in the gas phase has been investigated using the combination of chemical ionisation mass spectrometry and MS/MS. It is shown that NO^+ reacts efficiently with both Glu and Glu–Met leading to the formation of the nitroso-group containing ions at m/z 159 and 288.

The formation of m/z 159, $(GluNO\text{-}18)^+$, is rationalized by a mechanism involving an electrophilic attack of NO^+ upon the carbonyl oxygen atom of one of the carboxylic groups of Glu and the N-terminal carboxylic group of Glu–Met leading to the neutral losses of H_2O and Met, respectively. The unimolecular decompositions of the metastable and collisionally activated m/z 159 ions lead primarily to the elimination of the neutral species HNO (major) and the (Glu-18) residue (minor). The formation of the m/z 288 ions can be described by a mechanistic scheme which involves the ion-molecule interaction of m/z 159 with Glu and Glu–Met and subsequent losses of H_2O and Met, respectively. Unimolecular and collisionally activated dissociations of m/z 288 suggest the formation of the proton-bridged ion-neutral complex $[(GluNO\text{-}18)\cdots H^+\cdots(Glu\text{-}18)]$.

1. Introduction

Nitric oxide (NO) has provoked considerable interest in various laboratories for many years. Until the discovery of the biological functions of NO, this molecule has been considered as having relevance to environmental and atmospheric chemistry [1]. During the past decade, the biological and physiological significance of NO and its complexes has received much interest in connection with the recognised impact on human health [2–10]. Recent evidence indicates that the oxidised form of NO, the nitrosonium ion (NO^+), is involved in the biosynthesis of nitrosothiols (RS–NO) that are thought to be responsible for harnessing neuroprotective actions and avoiding neurotoxicity in the central nervous system [11, 12] as well as in the cross-linking of DNA [13,14]. NO^+ is a highly reactive species that can modify functional groups of proteins and peptides *in vivo* and can play a key bioregulatory function [6]. In order to understand better the chemistry of this ion with simple biomolecules, we examined its reactivity with the amino acid Glu and the dipeptide γ-Glu–Met, using the combination of chemical ionization (CI) mass spectrometry and MS/MS.

Very little is known about the chemistry of the nitrosonium ion with amino acids and peptides. Recently, Freitas et al. [15] have reported on the gaseous reactions of NO^+ with the amino acid molecules of glycine, alanine and valine and their N-methyl derivatives. Two reaction channels have been observed,

which are (i) hydride abstraction from the α-carbon atom and (ii) attack upon the carboxylic group leading to a concomitant loss of HONO and CO and resulting in iminium ion formation [15].

2. Experimental

All experiments were performed with use of a JEOL SX/SX 102A (Tokyo, Japan) four-sector tandem mass spectrometer ($B_1E_1B_2E_2$ geometry) fitted with a chemical ionization source whose ion exit slit width was reduced to ~0.15 mm. The source was operated with the accelerating voltage set at 10 keV and at a temperature between 110 and 200°C, measured by a thermocouple in contact with the source block. H_2, N_2 and CH_4 were used as reagent gases at the estimated pressures of 1.3–27 Pa. The reactant ion, NO^+, was generated from NO and CH_3NO_2. Formation of NO^+, together with NO_2^+, from CH_3NO_2 in N_2 carrier gas could be represented by the following reaction sequence:

$$N_2^{\cdot+}, (N_4^{\cdot+}) + CH_3NO_2 \rightarrow (CH_3NO_2^{\cdot+})^* + N_2, (2N_2), \quad (1)$$

$$(CH_3NO_2^{\cdot+})^* \rightarrow (CH_3ONO^{\cdot+})^* \rightarrow NO^+ + CH_3O^{\cdot}, \quad (2a)$$

$$\rightarrow NO_2^+ + {}^{\cdot}CH_3. \quad (2b)$$

The samples were introduced into the ion source with a direct insertion probe system with the probe temperature being between 120 and 200°C.

The fragment ions resulting from metastable ion (MI) or collisionally activated (CA) dissociation were mass analysed to record MS/MS product ion spectra for a mass selected ion beam. In the CA experiments, the pressure of argon gas in the collision cell was approximately 9×10^{-3} Pa.

Glu (obtained from Aldrich) and Glu–Met (obtained from Bachem) were used as received.

3. Results and discussion

An examination of the CI mass spectra in Figs 1 and 2 shows that in the case of NO^+-containing systems (Figs 1a and 2a) two peaks are formed of significant abundance at m/z 159 and 288 indicating that these ions result from the reactions of NO^+ with Glu and Glu–Met.

When CH_3NO_2 is used as reagent gas from which the reactants ions NO^+ and NO_2^+ are generated (reactions 1 and 2), also small peaks at m/z 175 and 304 are observed in the CI mass spectra of the Glu/(CH_3NO_2 + N_2) and (Glu–Met)/(CH_3NO_2 + N_2) gas mixtures. These peaks could arise from the reactions of NO_2^+ with Glu and Glu–Met, analogous to those of NO^+ discussed below.

3.1. NO^+/Glu

In the NO^+/Glu case, the m/z 159 ion is presumably generated by the H_2O loss from the excited [Glu + NO]$^{+*}$ adduct ion. The spectrum for the Glu/(N_2 + CH_3NO_2) system in Fig. 1a exhibit a small peak at m/z 177 which may be attributed to this species. In the formation of the excited [Glu + NO]$^{+*}$ adduct ion **1** it is suggested that NO^+ electrophilically attacks a carbonyl oxygen atom of one of the carboxylic groups of Glu as shown in Scheme 1 for the α-carboxylic group of Glu.

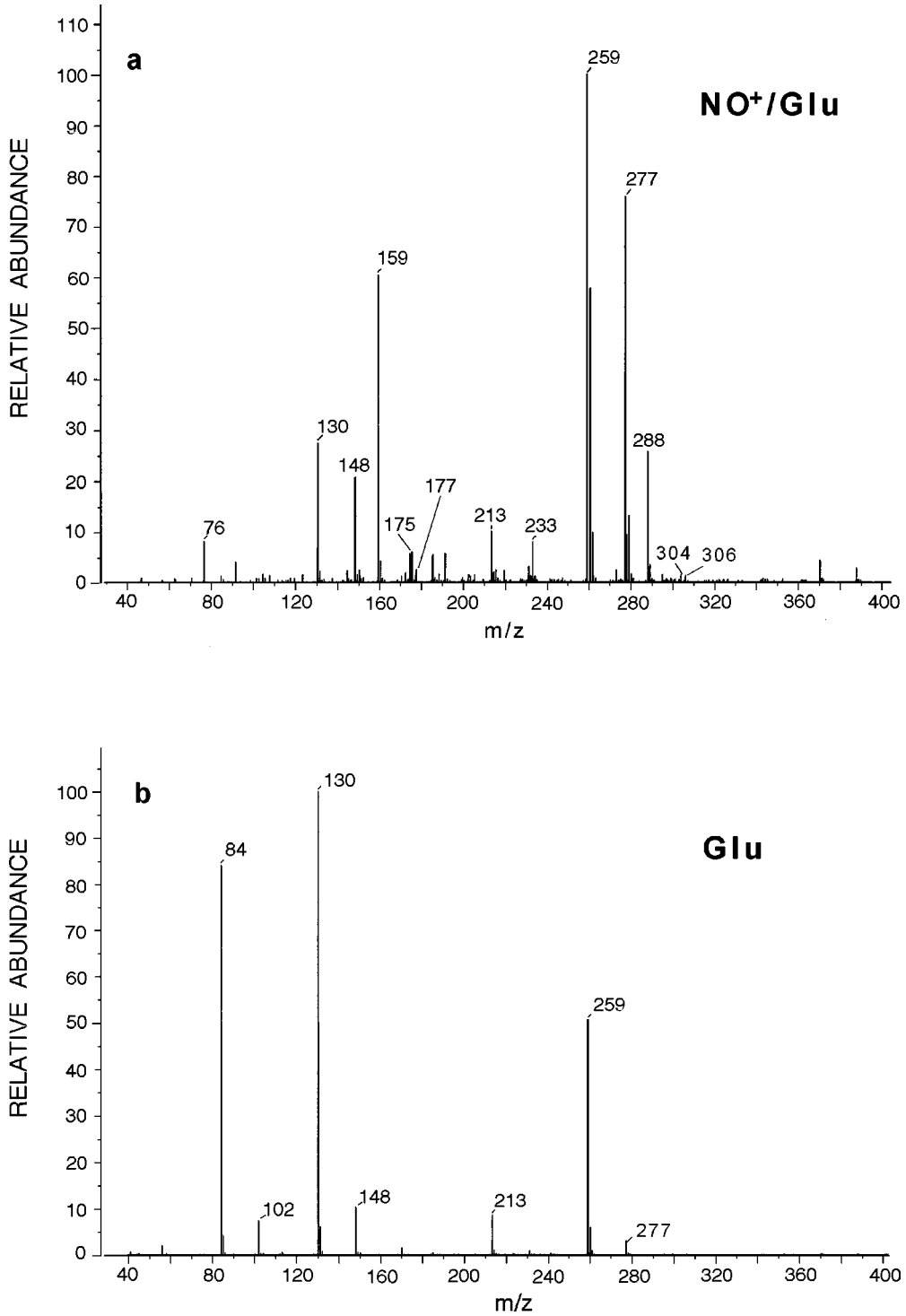


Fig. 1. Mass spectra of Glu obtained in the CI experiments of the gas mixtures: (a) Glu/(N_2 + CH_3NO_2) and (b) Glu/H_2.

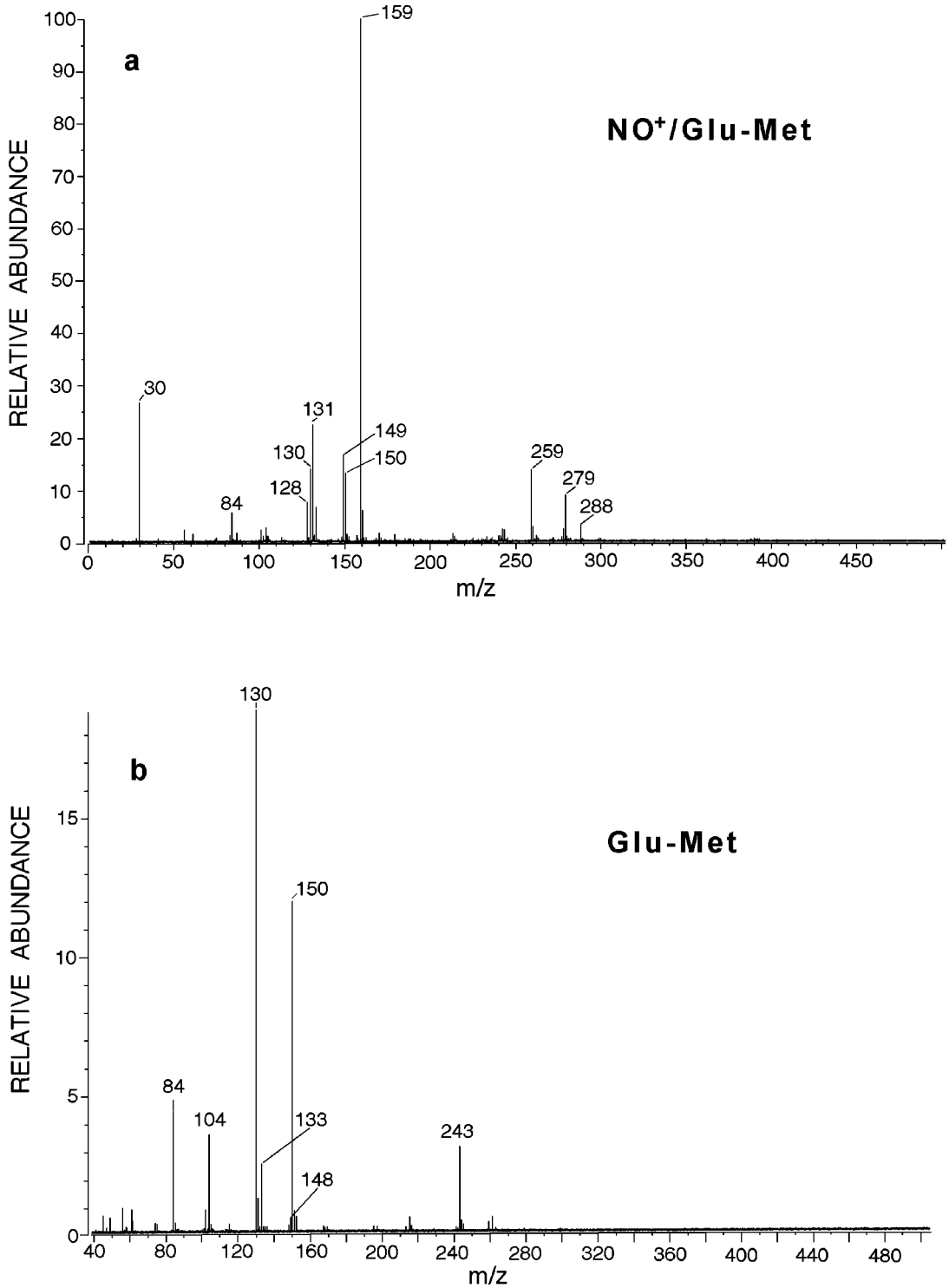
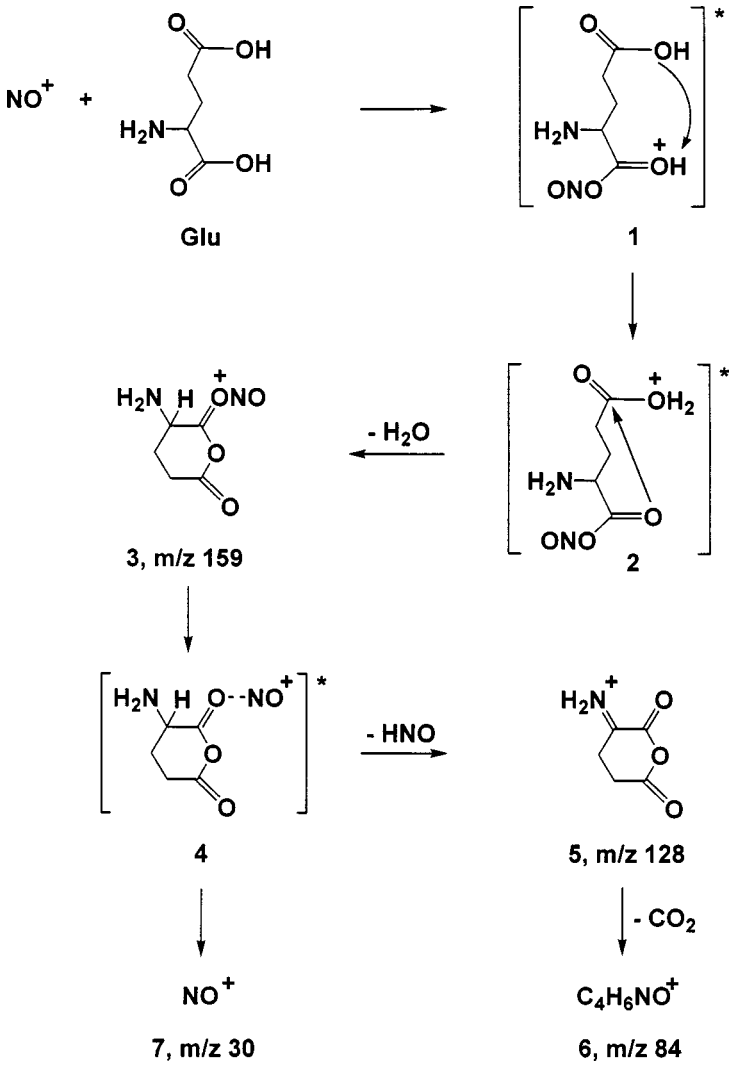


Fig. 2. Mass spectra of γ-Glu–Met obtained in the CI experiments of the gas mixtures: (a) γ-Glu–Met/NO and (b) γ-Glu–Met/H_2.



Scheme 1.

Subsequently an intramolecular proton transfer in **1** can take place to give ion **2** from which H_2O can be eliminated in an intramolecular substitution reaction to give the m/z 159 ion, **3** (an initial attack of NO^+ on the γ-carboxylic group of Glu would lead in this way to the formation of an isomeric ion of **3**, that is the NO group bonded to the other carbonyl group). From the MI and CA spectra in Fig. 3a, together with a comparison of the MI spectra of various mass-selected ions, the sequential fragmentation of **3** has been deduced.

The elimination of HNO is the dominant channel for the MI and CA fragmentations of the m/z 159 ion, **3**. This reaction is proposed to proceed *via* the ion/molecule complex **4** in which NO^+ abstracts a hydride from the α-carbon atom with respect to the NH_2 group to give the m/z 128 ion, **5** (Scheme 1). Such reaction is analogous to the reported hydride abstraction from the α-carbon atom of glycine by NO^+ [15]. The intense peak at m/z 84 is attributed to the loss of CO_2 from the m/z 128 ion, **5**, while the minor peak at m/z 30 originates from the m/z 159 ion *via* the sequence $3 \rightarrow 4 \rightarrow 74$ (Scheme 1).

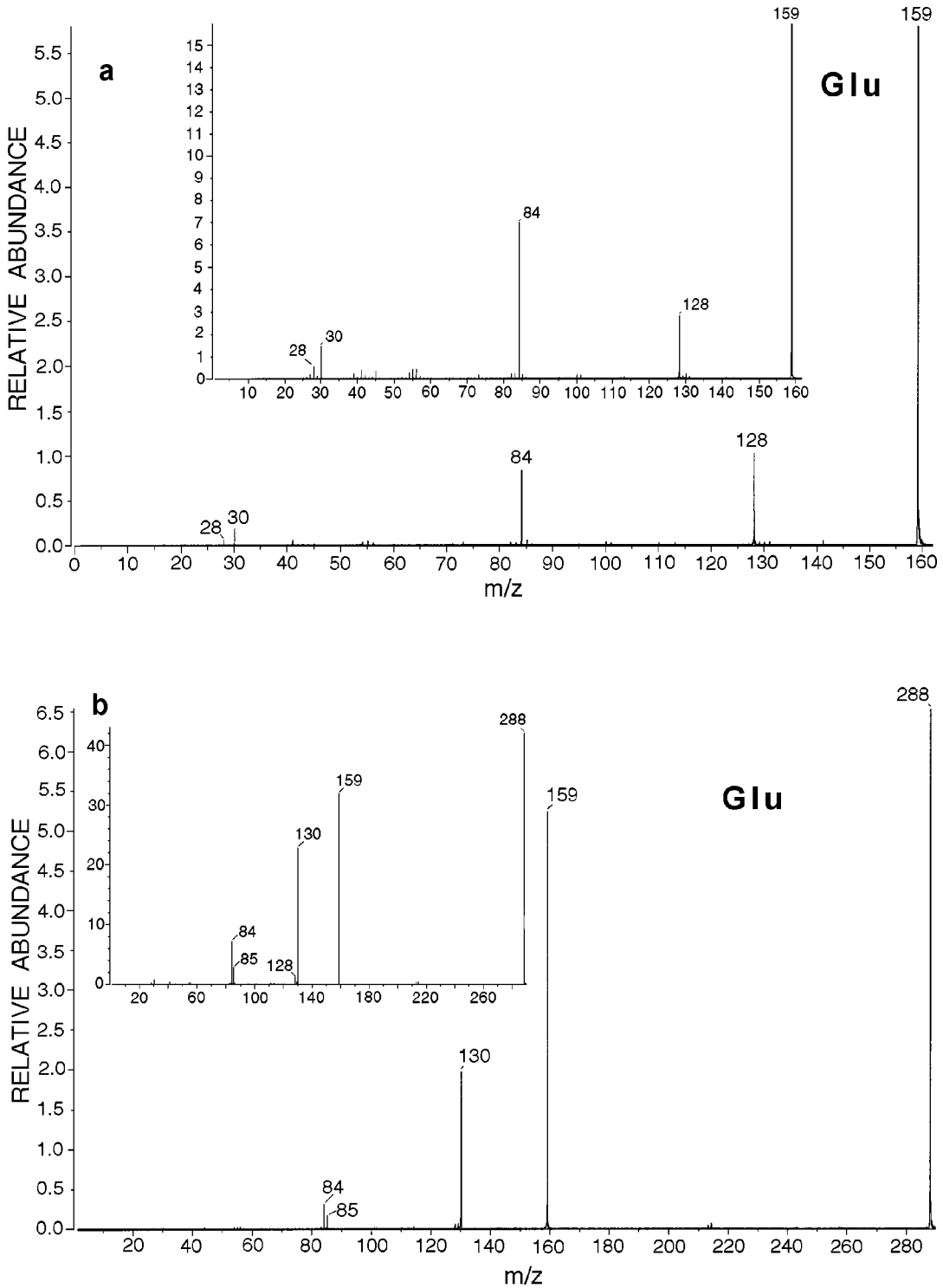


Fig. 3. MI and CA (inset) mass spectra of the ions derived from the gas mixture Glu/(N_2 + CH_3NO_2): (a) m/z 159 and (b) m/z 288.

The CA spectrum of m/z 159 displays practically the same fragmentation pattern as observed in the MI spectrum (see Fig. 3a), but significant differences in the intensity ratio for the main fragment ion peaks at m/z 128/84 are found; the collisionally activated m/z 159 decomposes more extensively to shift the relative abundance of the daughter ions from m/z 128 to m/z 84. This behaviour most likely arises from variation in the internal energy of the precursor ion m/z 159 and is consistent with the mechanistic scenario presented in Scheme 1. Moreover, these data suggest that the presumably six-membered cyclic structure **3** does not change appreciably upon collisional activation apart from stretching the O–NO bond.

It should be mentioned that in contrast to our observations for the NO^+/Glu system, no ions corresponding formally to $[M + NO]^+$, $[(M + NO) - H_2O]^+$ and $[(M - H) - H_2O]^+$ have been reported in a previous CI study [15] of NO^+/M, where M = Gly, Ala and Val. The absence of the $[M + NO]^+$ adduct ion in these systems most probably arises from insufficient adduct ion collisional stabilization. This suggestion is confirmed in our separate NO^+/CI experiments with Gly and Ala showing the formation of the $[Gly + NO]^+$ and $[Ala + NO]^+$ ions, respectively.

The m/z 288 ion most probably is formed through H_2O loss from the excited $[m/z\ 159 + Glu]^{+*}$ adduct ion. In the CI spectrum of NO^+/Glu (Fig. 1a), a very small peak at m/z 306 is observed, which could correspond to such adduct ion. For the H_2O loss from the excited $[m/z\ 159 + Glu]^{+*}$ adduct ion it is very likely that the m/z 159 ion, **3**, transfers within the complex a proton to Glu in view of the decomposition behaviour of the resulting m/z 288 ion given below (see Scheme 2).

Following proton transfer from **3** to Glu to give complex **8**, H_2O may be eliminated in complex **9** yielding the m/z 288 ion, **10**.

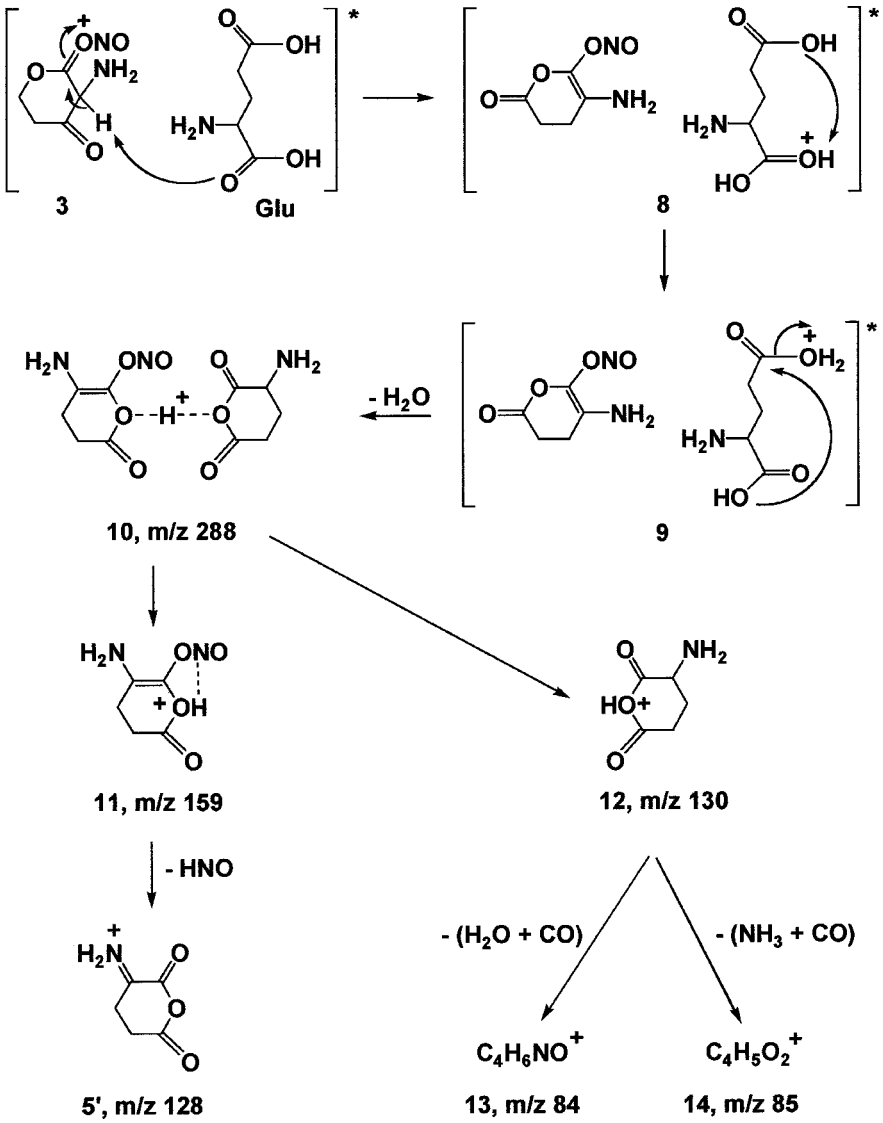
Fig. 3b presents the MI and CA mass spectra of the m/z 288 ion, **10**, generated in the ion source from the Glu/(N_2 + CH_3NO_2) mixture. They show that the m/z 288 ion decomposes *via* two fragmentation pathways leading to the primary product ions m/z 159 and 130. Formation of these ions can be easily rationalized by the proton-bridged ion-neutral complex **10** in Scheme 2, where the ratio of the product ions of **11**/**12** results from the proton affinities, PA, of the corresponding neutral partners 158/129 u, respectively.

As can be seen in Fig. 3b, the MI and CA mass spectra of m/z 288 are qualitatively identical, indicating that a structural rearrangement does not occur during the CA process. Slight differences in the abundances of the m/z 159 ions relative to the m/z 130 ions are probably due to differences in the internal energy content. The internal energy influence on the product ion ratio from dissociation of proton- and metal ion-bound systems has been observed previously in several studies [16–18], and has been attributed to entropy effects. Application of the kinetic method [19], developed by Cooks and co-workers, to the MI mass spectrum of m/z 288 leads to a difference of PA(158 u) − PA(129 u) = 0.8 kcal/mol. Scheme 2 presents the routes for the formation of ions of relatively low abundance at m/z 128 and 84, 85 (Fig. 3b). These routes are proposed on the basis of the MI spectra of the mass selected ions, which yield m/z 159 → 128 and m/z 130 → 84, 85, that is **11** → **5′** and **12** → **13**, **14** in Scheme 2. The fragmentation of the m/z 130 ion, **12**, is similar to that previously observed for m/z 130 derived from $GluH^+$ [20].

3.2. NO^+/(Glu–Met)

In order to obtain further information about the possible formation and destruction of nitroso compounds of potentially biological significance, the reactivity of NO^+ with the dipeptide Glu–Met in the gas-phase has been studied.

As shown in Fig. 2a, the ion at m/z 159 is the most abundant one in the CI mass spectrum of the (Glu–Met)/NO mixture. The MI spectrum of this ion exhibits a peak at m/z 128 which could be due to

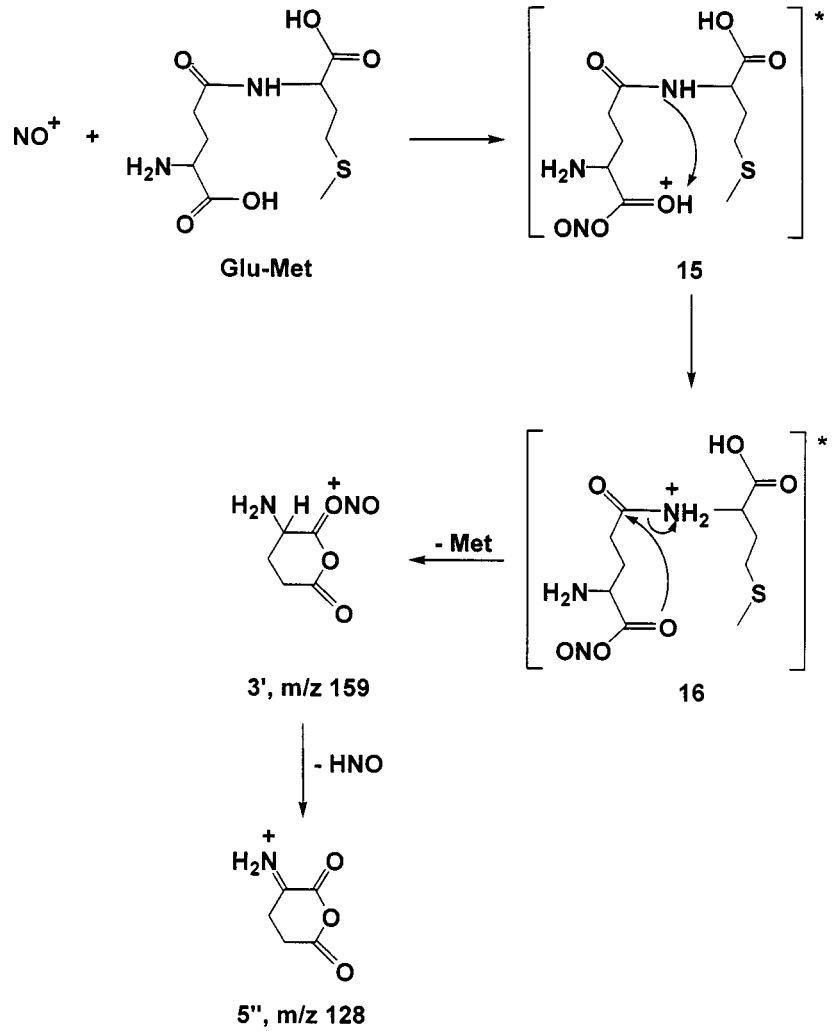


Scheme 2.

the loss of HNO, and thus may be taken as an indication of nitroso ion formation in the interaction of NO^+ with the dipeptide Glu–Met.

Scheme 3 presents a plausible reaction mechanism which commences with NO^+ attack on the carbonyl oxygen atom of the α-carboxylic group of Glu followed by a successive intramolecular proton transfer from the nitrosated carboxylic acid group to the amide nitrogen and amide bond cleavage with the elimination of Met concomitant with the formation of the ring-closed ion m/z 159, **3′**.

In contrast with **3** derived from NO^+/Glu (Fig. 3a), the **3′** ion shows fragmentation on the metastable ion time scale to give only a peak at m/z 128, due to the ion **5″**. This difference may be attributed to the lower internal energy of **3′** generated from the NO^+/(Glu–Met) couple or/and may reflect the different isomeric forms of the m/z 159 ion arising from the different sources (see, for example, ions **3** and **11** in

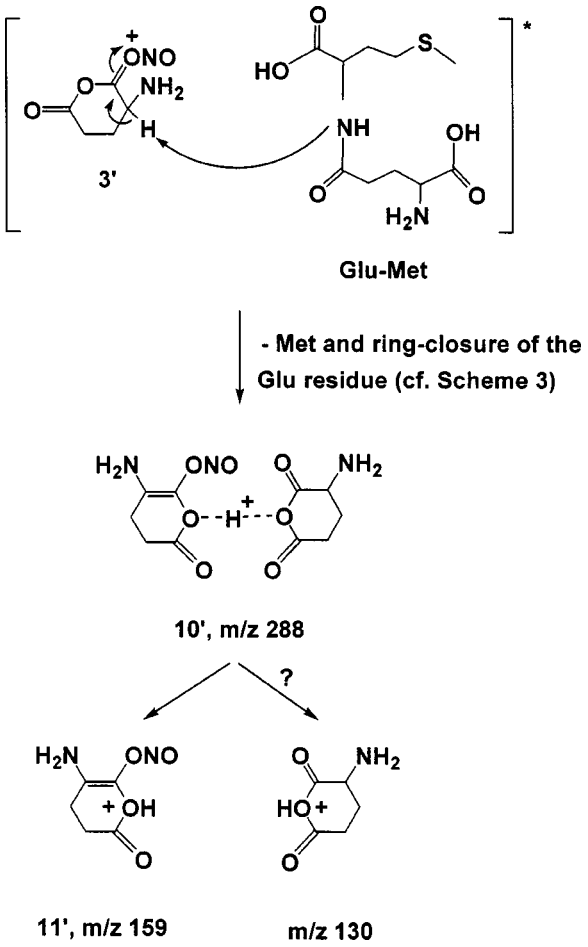


Scheme 3.

Schemes 1 and 2, respectively). Important to note is that our observations indicate that NO is attached to the Glu residue. The relatively high stability of **3** and **3′** is related to the stabilization of these ions by cyclization to a six-membered ring structure (Schemes 1 and 3). Recently, an analogous six-membered ring structure has been proposed [21] for the dehydrated ion (m/z 130) from $GluH^+$.

Similarly as in the NO^+/Glu case (Fig. 1a), the CI mass spectrum of the (Glu–Met)/NO mixture in Fig. 2a shows the formation of the ion at m/z 288, **10′**, although in a very much lower abundance. This again most likely results from a sequential reaction in which **3′** transfers a proton to the amide nitrogen of a second Glu–Met neutral molecule to form **10′** by the amide bond cleavage and the loss of Met, concomitant with ring-closure of the Glu residue as shown in Scheme 4.

The MI mass spectrum of the m/z 288 ion shows a small signal at m/z 159, suggesting that this product ion arises from the interaction of **3′** with Glu–Met. Unfortunately, in the MI spectrum no observable signal corresponding to m/z 130 is found because of the relatively large noise.



Scheme 4.

4. Conclusions

The results presented show the strong effect of nitrosation of the Glu residue by dissociative attachment of gaseous NO^+ to the amino acid Glu and the dipeptide γ-Glu–Met. This effect is based on a nucleophilic attack of NO^+ upon a carboxylic group which activates its acidic hydrogen atom and which triggers the formation of $(GluNO\text{-}18)^+$, **3** and $((Glu–Met)NO–Met)^+$ **3′**. Unimolecular decompositions of these ion structures show a preferential loss of HNO. Furthermore, **3** and **3′** in subsequent ion-molecule reactions detach H_2O from the Glu and Met from the Glu–Met neutral molecules to yield the proton-bridged complexes, **10** and **10′**, respectively. This study provides initial information about the gas-phase chemistry induced by the transfer of the nitrosonium ion to simple biomolecules.

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