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The ion source of nitrogen direct analysis in real time mass spectrometry as a highly efficient reactor: generation of reactive oxygen species

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Abstract

An innovative strategy for sustainably active oxygen capture using nitrogen (N_2) instead of helium (He) as direct analysis in real time (DART) gas is demonstrated in this work. DART MS was carried out to analyze different polarity compounds including organophosphorus pesticides, amino acids, hormones, and poly brominated diphenyl ethers by using He and N_2 as DART gas, respectively. The unexpectedly characteristic ionization reactions including replacement reaction where the sulfur atom of P=S group was replaced by oxygen atom, oxidation ($[M+nO+H]^+$ or $[M+nO-H]^-$ ($n = 1, 2, 3, 4, 5$)), and hydrogen loss (loss of two hydrogens) rapidly occurred in situ in the presence of N_2 under ambient conditions without any additives. The reaction mechanisms were proposed and further confirmed by high resolution tandem mass spectrometry. Our study under high temperature and high voltage provides a powerful tool for generating unique ionic species that may be difficult to form by other means, which also creates favorable conditions for the future study of the mechanism of DART MS.

Keywords: Nitrogen direct analysis in real time mass spectrometry; Oxygen species; Replacement; Oxidation; Hydrogen loss.

Introduction

Ambient mass spectrometry, which allows for direct and rapid analysis of raw samples in their native environment with high specificity and minimal or no sample preparation, is an invaluable tool for analysis of a large variety of compounds [1-6]. In particular, direct analysis in real time (DART) ionization source coupled with high resolution mass spectrometer is popular due to its unique advantage in facile identification [7-11]. Usually, working gas plasma is created inside a ceramic flow chamber by an atmospheric pressure glow discharge initiated by applying a potential of several kilovolts. The excited beam is heated by passing through a heater chamber and flows through a grid electrode. When the DART working gas comes into contact with a sample placed between the ion source outlet and the mass spectrometer interface inlet, the sample will be ionized [12-15].

The commonly used DART gas is helium (He), which has been successfully applied to analysis of various compounds. However, prominent fragmentation can occur in the ion source, which makes it a challenge to figure out the structure exactly [16-20]. Some researchers have reported that clean spectra [21,22] and selective ionization ability [23,24] can be obtained using argon as operation gas due to its relatively low ionization energy (IE) (11.55 eV $^3\text{P}_2$ state and 11.72 eV for the $^3\text{P}_0$ state) compared with that of He (19.8 eV) [12,23]. In an effort to understand these phenomena, gaining an insight into the mechanism ionization information is important and desirable. At this time, the DART mechanism has not yet been fully understood, but the widely acceptable mechanism is Penning ionization and proton transfer, based on the interactions of metastable (excited state) atoms or molecules with

atmospheric gases and the analytes [25-27].

Nitrogen (N₂) is usually used as DART gas in the standby mode to replace working gas (He or Ar) in order to decrease the cost of research. The excited nitrogen has an IE of 8.5 to 11.5 eV, even up to 15 eV in some states [28]. N₂ as working gas was seldom investigated in DART MS. While N₂-DART ion source coupled with ion mobility MS has been successfully used to detect toxic chemicals [29]. Recently, ionization mechanism of positive-ion N₂-DART has been described by Song et al. [30], where [M+H]⁺ ions and [M+NH₄]⁺ ions were generated by the direct Penning ionization followed by self-protonation or proton transfer. However, a lack of knowledge concerning the ionization characteristics in N₂-DART leads to difficulty in the interpretation of N₂-DART mass spectra, which limits its analytical utility.

In order to overcome these issues for future applications, it is necessary to investigate the ionization characteristics of N₂-DART in detail, i.e., to identify the unknown regular species and characteristic ions formed in N₂-DART sources and to fully understand the influence of N₂ in the role of characteristic ion formation. Herein, DART MS was carried out to analyze different polarity compounds including organophosphorus pesticides, amino acids, hormones, and poly brominated diphenylethers by using He and N₂ as DART gas, respectively. Unexpectedly characteristic ionization reactions, occurring in N₂-DART MS rather than in He-DART MS, inspire more interest in investigation of N₂-DART MS.

Experimental

Chemicals and reagents

Organophosphorus pesticides (purity, 96.8-99.5%), including phoxim, pirimiphos methyl, dimethoate, quinalphos, sulprofos, tolclofos-methyl, methidathion, diazinon, phostebupirim, triazophos, methamidophos, acephate, and dichlorvos, were purchased from Accu Standard (New Haven, USA). Amino acids, including glycine (Gly), alanine (Ala), phenylalanine (Phe), serine (Ser), tryptophan (Trp), tyrosine (Tyr), valine (Val), asparagine (Asn), glutamine (Gln), glutamic acid (Glu), aspartic acid (Asp), lysine (Lys), cysteine (Cys), histidine (His), isoleucine (Iso), leucine (Leu), methionine (Met), proline (Pro), and threonine (Thr), were purchased from Sigma (St. Louis, MO, USA). 17 α -hydroxyprogesterone, estriol, chlormadinone acetate, estrone, 17-beta-estradiol, medroxyprogesterone-17-acetate, megestrol-17-acetate, and 17-beta-estradiol 3-benzoate were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Tetrabromobisphenol A (TPPBA) and three poly brominated diphenyl ethers (PBDEs) including 3,3',4-tribromodiphenyl ether, 2,2',4,4'-tetrabromodiphenyl ether, and 2,2',4,4',6-pentabromodiphenyl ether were purchased from Accu Standard (New Haven, CT, USA). Acetonitrile, methanol, and acetone (LC/MS grade) were obtained from Fisher Scientific (New Jersey, USA). High-purity helium, argon and nitrogen (99.999%) were supplied by Changchun Juyang Gas Co., Ltd. (Changchun, China). Ultrapure water (specific conductivity, 18.2 M Ω /cm) was produced by a MilliQ device (Millipore, Milford, MA, USA).

DART MS Analysis

All mass spectra were acquired using a Q-Exactive Orbitrap mass spectrometer (Thermo

Fisher Scientific, San Jose, CA) equipped with a fourth generation DART source with standard voltage, ambient pressure and angling capability (SVPA) (IonSense, Inc., Saugus, MA, USA). Instrumental control and data acquisition were performed using Xcalibur 2.2 system software (Thermo Fisher Scientific, San Jose, CA). The orifice of the DART source was positioned in line with the ceramic ion transfer tube that was equipped on the Orbitrap MS. The distance between the DART cap and the ceramic transfer tube was approximately 7 mm. Analyte desorption and ionization were accomplished by the insertion of a closed end of a melting point capillary tube (sealed end) containing the analyte into the heated gas stream between the DART cap exit and the ceramic ion transfer tube. The resulting gas-phase analyte ions were introduced into MS through the ceramic ion transfer tube. For each sample analysis, the melting point capillary tube was held close to the DART cap for about 30 s.

The instrument parameter settings were optimized to obtain sensitive and accurate determination of the analytes. The DART source was operated with a needle voltage of 3.8 kV and exit grid electrode voltages of 350 V (positive mode) and -300 V (negative mode). Gas temperatures were optimized to 300 °C for pesticides, 350 °C for amino acids and hormones, and 450 °C for TPPBA and PBDEs. Gas flow rates were set to 2 L/min. The optimized Orbitrap MS operating conditions were as follows: capillary voltage 30 V (positive ion mode) or -35 V (negative ion mode), capillary temperatures at 250-320 °C depending on the analyte, S-lens voltage at 55 V, and resolution 70000. Tandem mass spectra were obtained by high-energy collision dissociation (HCD) with nitrogen as the carrier gas after isolation of the desired precursor ion (isolation window, 0.4 m/z), and the collision energy was set at 10-30 eV for the suitable dissociation of all the compounds.

Results and discussion

Replacement

As for the replacement reaction, the representative N₂-DART mass spectra are shown in Figure 1. In the mass spectrum of quinalphos (Figure 1a), the abundant ions at m/z 299.0613 and 283.0789 were assigned to $[M+H]^+$ and an unknown ion $[M-15.9774+H]^+$, respectively. In the mass spectrum of methidathion (Figure 1b), the primary peaks at m/z 286.9919, 302.9692, and 319.9956, corresponded to a unknown ion $[M-15.9773+H]^+$ (base peak), a protonated methidathion $[M+H]^+$, and an ammonium adduct ion $[M+NH_4]^+$, respectively. The N₂-DART mass spectra of the other eight organophosphorus pesticides (Supplementary Figure S-1) also exhibited a series of dominant unknown ions of $[M-15.977+H]^+$, which were summarized in Table S1 in supplementary information. The average mass difference value between protonated analytes $[M+H]^+$ and corresponding unknown ions is 15.9772 Da, with a deviation of 0.0176 Da from the exact mass of O¹⁶ (15.9948 Da), but exactly corresponding to the value of S³²-O¹⁶ (31.9720-15.9948). Therefore, the unknown ions are $[M-S+O+H]^+$ other than $[M-O+H]^+$. Without question, for the organophosphorus pesticides which contain only one sulfur atom in their structures, the sulfur atom of P=S group was replaced by the oxygen atom (Figure 1a, Supplementary Figure S-1a-f). Nevertheless, it is unclear that the replacement reaction involved the sulfur atom of P=S group or any of the other sulfur atoms with regard to the compounds containing more than one sulfur atom (Figure 1b, Supplementary Figure S-1g and h). To further investigate this, methamidophos bearing one sulfur atom not belong to P=S group was determined by N₂-DART MS (Supplementary

Figure S-2), and $[M-S+O+H]^+$ was not observed, indicating that this replacement reaction only occurring in N_2 -DART MS of the compounds which contain the P=S group.

Based on our experimental data, we proposed a replacement reaction mechanism of the formation of $[M-S+O+H]^+$ ions in N_2 -DART (Scheme 1). In N_2 -DART, carrier gas N_2 is excited by the high electrode voltage, and excited N_2 is formed. The IE of O_2 (3P_2) is 12.6 eV, so O_2 can be activated by N_2^* ($4S_{3/2}$, IE=15.58 eV) (Scheme 1a). O^* is originated from O_2^* (Scheme 1b), then O^* attacks the sulfur-phosphor double bond, resulting in a three body transition state (Scheme 1c). The bond length of P-S bond is longer than the P-O bond in the three-membered ring transition state, because higher electronegativity of O can enhance the interaction between P and O. Vibrational degree of freedom of diatomic nitrogen molecules can quench the energy of the transition state, generating the $[M-S+O+H]^+$ species (Scheme 1c).

Oxidation

Wang et al. has investigated and reported eight typical deprotonated amino acids which could react with ground state nitrogen and oxygen atoms in mildest condition [31]. Stemmler and Buchanan also investigated the oxidation reaction in the chemical ionization source when N_2 is used as a buffer gas in the absence of oxygen [32,33]. During the N_2 -DART ionization process, some analytes can react with O^* resulting in the formation of the oxygen attachment products, appearing as $[M+nO+H]^+$ or $[M+nO-H]^-$ ($n = 1, 2, 3, 4, 5$) in the mass spectra. The 20 common amino acids, which contain sulphur atom, heterocyclic amines, aromatic and aromatic heterocyclic structure, were analyzed by N_2/He -DART MS. The oxidation reactivity of individual amino acid (M) in N_2 -DART MS was estimated by relative abundance of

$[M+nO+H]^+$ or $[M+nO-H]^-$. The reactivity ratios ($100 \times SI_{[M+O+H]^+} / (SI_{[M+O+H]^+} + SI_{[M+H]^+})$; Signal Intensity, SI) obtained here are tryptophan (Trp, 52.2%) > methionine (Met, 48.2%) > tyrosine (Tyr, 38.7%) > phenylalanine (Phe, 30.9%) > histidine (His, 22.3%) > other amino acids. Additionally, in the N₂-DART mass spectra of 8 hormones and 4 brominated flame retardants, oxygen attachment products were also detected obviously (Supplementary Figure S-3 and Supplementary Figure S-4), confirming that the oxygen addition reactions occurred for a variety of compounds during the ionization process of N₂-DART. Met and Trp were selected as models to illustrate the specific oxidation reaction which only occurred when N₂ was used as DART gas. Both in positive and negative ion mode N₂-DART mass spectra of Met, oxygen addition products of Met at m/z 166.0520 ($[M+O+H]^+$) in Figure 2a, m/z 164.0397 ($[M+O-H]^-$) and m/z 162.0227 ($[M-2H+O-H]^-$) in Figure 2b were observed. Figure 2c depicts positive ion mode N₂-DART mass spectrum of Trp, where the base peak at m/z 221.0918 corresponds to $[M+O+H]^+$. The notable oxygen attachment $[M+O-H]^-$ of Trp in negative ion mode N₂-DART mass spectrum was also observed at m/z 219.0795 (Figure 2d).

High resolution tandem mass spectrometric (MS²) experiments were further employed to clarify the accurate oxidation position and the exploration of the oxidation mechanism in N₂-DART MS. In the tandem mass spectrum of oxidized product of Met, the ion at m/z 102.0539 was observed (Figure 3a). The difference in molecular weight between the precursor ion and the ion at m/z 102.0539 is 63.9971, which is consistent with the formula weight of CH₃SO (MW=63.9977), confirming that oxygen atom is attached to the sulphur atom (formation of sulfoxide). In case of Trp, the tandem mass spectrum and the proposed fragmentation pathway of its oxidation product were displayed in Figure 3b. Due to C-C

cleavage, the ion at m/z 130.0650 which was assigned as $[\text{C}_9\text{H}_8\text{N}]^+$ and the ion at m/z 146.0598 corresponding to $[\text{C}_9\text{H}_8\text{NO}]^+$ were generated, as indicated in Figure 3b. We can infer that the latter is the oxidized product of the former on the basis of the 15.9948 Da mass difference. In addition, the observation of the ions at m/z 132.0429 and 116.0500 (Figure 3b,) more directly specified that the O atom was attached to the heterocyclic aromatic structure. Taking these results into consideration, oxidation reaction mechanism was proposed as displayed in Scheme 2. As mentioned above, in the N_2 -DART process, high density N_2^* could rapidly react with O_2 , producing O_2^* . Then active oxygen (O^*) can react with analytes. Active oxygen is apt to attach to the heteroatom (N, S) and hydrogen, leading to the formation of an excited four-membered-ring transition state. Amount of diatomic molecule N_2 , whose $\text{N}\equiv\text{N}$ stretching vibration constantly keeps active, can quench the energy of excited transition state to produce a stable product. Thus in N_2 -DART, Met containing S prefers to generate Met sulfoxide. Trp, whose unsaturated five-membered ring structure contains one nitrogen atom, can be oxidized to oxindole-3-alanine.

Hydrogen loss

Interestingly, in negative-ion mode N_2 -DART MS, deprotonated analytes with loss of two hydrogens, $[\text{M}-2\text{H}-\text{H}]^-$, as dominant peaks, were observed when analyzing hormones (Supplementary Figure S-3) and amino acids (Figure 4). After careful comparison of the structures of hormones and phenomena, we found that $[\text{M}-2\text{H}-\text{H}]^-$ were detected in the mass spectra of hormones containing aromatic residues, as displayed in Supplementary Figure S-3b, d, e, h. In the high resolution mass spectra of glycine (Gly), alanine (Ala), Phe, valine (Val),

isoleucine (Iso), and leucine (Leu), the peak abundances of $[M-2H-H]^-$ were much higher compared with those of $[M-H]^-$ (Figure 4). As for the other amino acids, the $[M-2H-H]^-$ peaks were also observed with relatively low abundances (Figure 4). It is noteworthy that arginine cannot be detected using DART MS, for which the corresponding studies are ongoing in our laboratory. From the results, it is evident that amino acids with aliphatic side chain prefer to produce base peak of $[M-2H-H]^-$.

Considering these results, previous reports about decarboxylation of amino acids in aqueous solution [30], and those phenomena observed in tandem electrospray ionization-selected ion flow tube [34]. Electron abstraction from the amine nitrogen followed by rearrangement with decarboxylation to form a carbon-centered radical amine is a reported proposed mechanism [34,35]. This radical amine could react with oxygen or, in the absence of oxygen, abstract a hydrogen from another amino acid molecule to form an amine and the apparent hydrogen abstraction products observed by others [35]. In N_2 -DART, the 2H extraction reaction could be rationalized by the mechanism described in Scheme 3. In N_2 -DART, a carbon-centered radical amine is formed by electron abstraction from the amine nitrogen atom followed by rearrangement with decarboxylation. Then, this radical amine can abstract a hydrogen atom from another amino acid molecule to form an amine. Thus, the apparent hydrogen abstraction product $[M-H-H]^-$ is produced. In the presence of oxygen, the $[M-H-H]^\bullet$ can react with oxygen, the H from NH_2 is extracted, generating the $[M-2H-H]^-$ ion. To better understand this reaction mechanism, threonine (Thr) was taken as an example of performing MS^2 experiment. In the MS^2 spectrum of Thr (Figure 5), the base peak at m/z 74.0230 was interpreted as the product ion due to loss of R group (CH_3OHCH^-) from $[M-H]^-$.

ion. The presence of the abundant ion at m/z 72.0078 ($C_2H_2NO_2$) corresponding to the loss of R group from the precursor ion at m/z 116.0313 substantiates our proposed mechanism.

Conclusions

In summary, we have demonstrated a highly efficient strategy for the sustainable capture of active oxygen using N_2 -DART MS. We have revealed the characteristic ion reactions including replacement, oxidation, and hydrogen loss in the gas phase of N_2 -DART ion source and elucidated the specific reaction mechanisms in N_2 -DART. The mechanism proposed in the present study not only can be used to explain some puzzling reactions in N_2 -DART ion source but also gives a new insight into the field of gas-phase ion chemistry, which is very beneficial for the researchers to elucidate structures of unknown compounds in N_2 -DART MS. Our study opens the exciting door to obtain some compounds which are usually difficult to be synthesized or cannot be synthesized.

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Figure and Scheme Legends

Figure 1. N₂-DART mass spectra of (a) quinalphos and (b) methidathion.

Scheme 1. Proposed mechanism of replacement reaction for the compound containing P=S double bond.

Figure 2. N₂-DART Orbitrap MS spectra of Met in (a) positive and (b) negative ion modes as well as Trp in (c) positive and (d) negative ion modes.

Scheme 2. Proposed mechanism of oxidation reaction generated in N₂-DART MS.

Figure 3. Tandem MS spectra and proposed fragmentation mechanisms of oxidized products of (a) Met and (b) Trp using N₂-DART Orbitrap mass spectrometer.

Figure 4. Schematic illustration of the relative abundance of hydrogen extraction products and [M-H]⁻ of the 19 amino acids studied.

Scheme 3. Mechanism of the hydrogen abstraction reaction in N₂-DART MS.

Figure 5. MS² spectrum of Thr in N₂-DART MS.

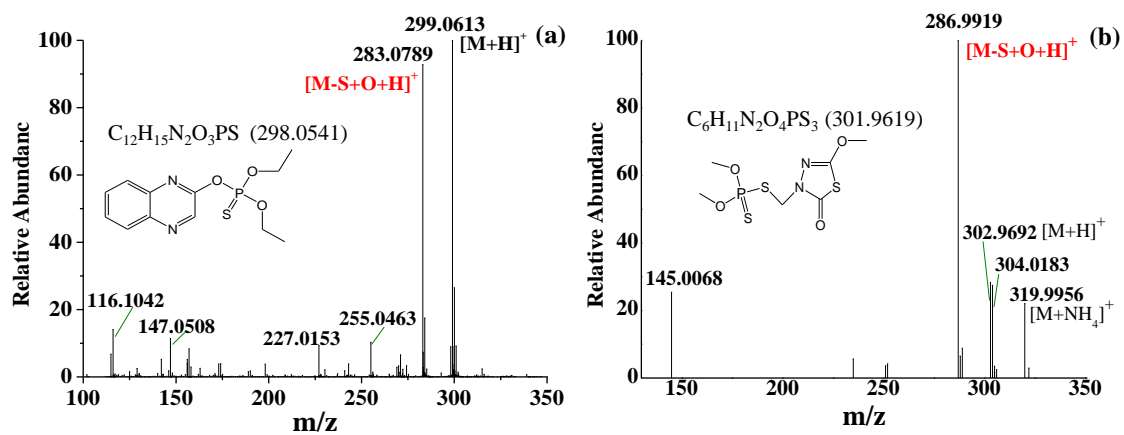
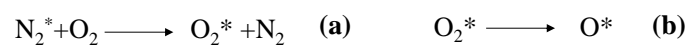
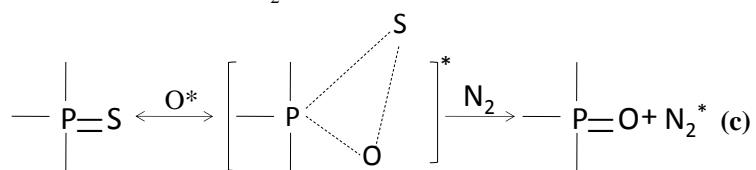


Figure 1



N_2 : 15.58 eV

O_2 : 12.2 eV



Scheme 1

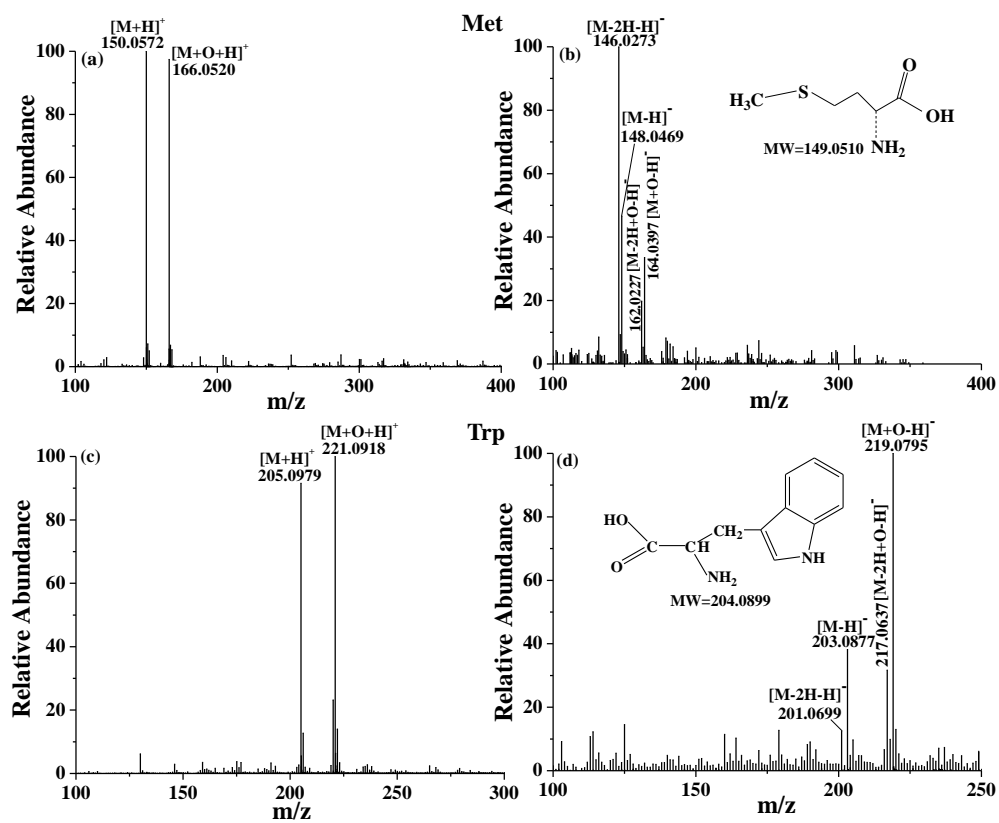
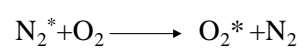


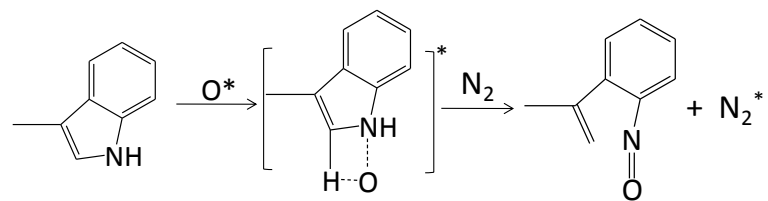
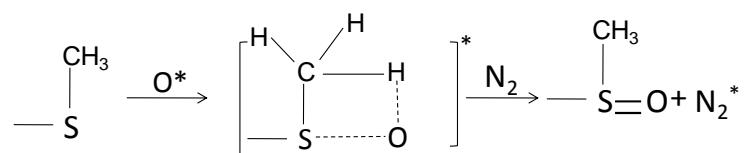
Figure 2

N₂



N₂: 15.58eV

O₂: 12.2eV



Scheme 2

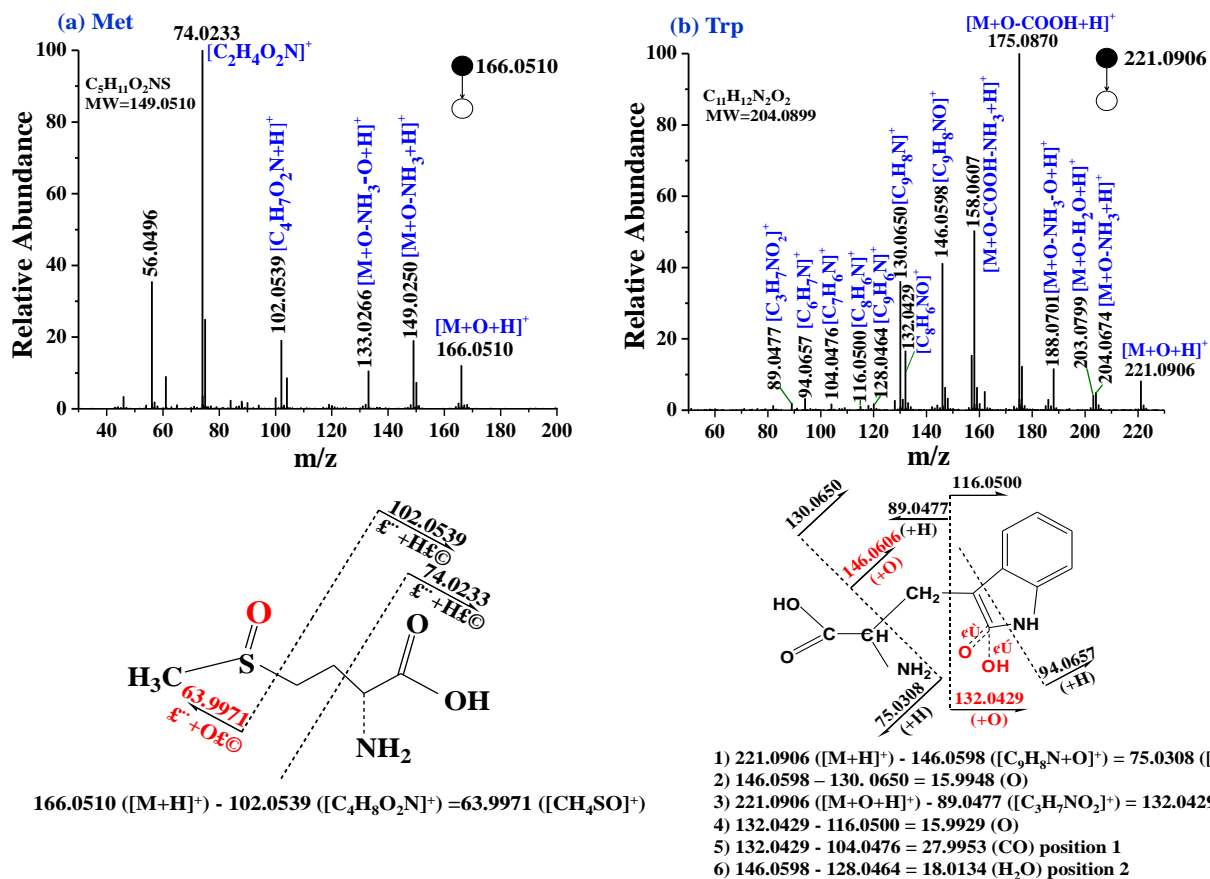


Figure 3

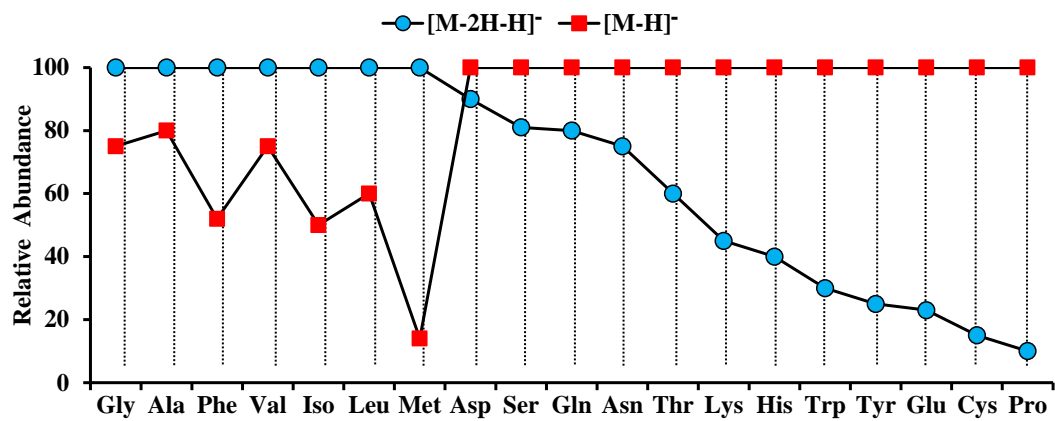
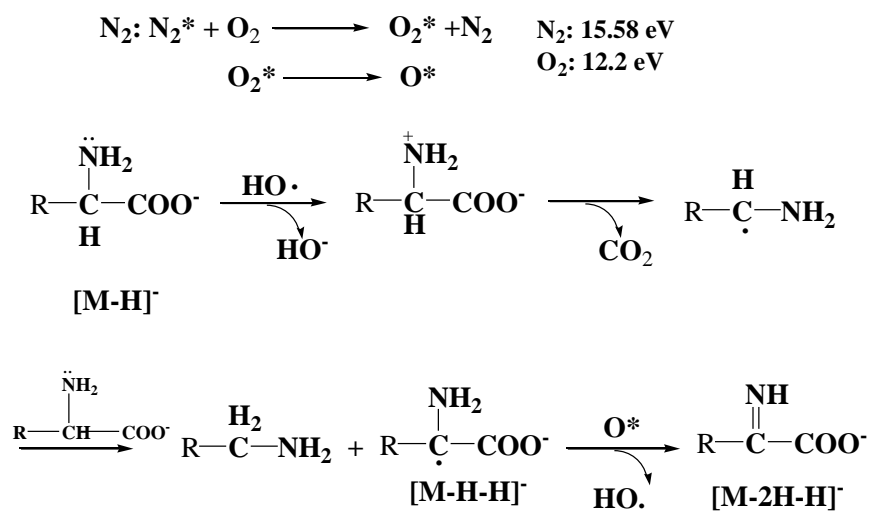


Figure 4



Scheme 3

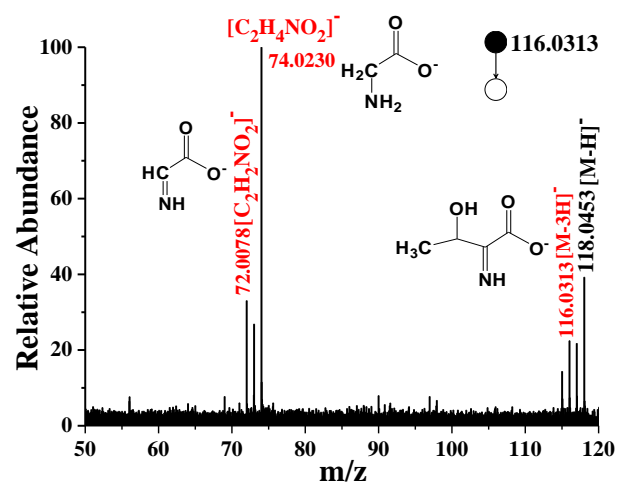


Figure 5

