International Journal of Mass Spectrometry 316–318 (2012) 133–139

Contents lists available at SciVerse ScienceDirect

International Journal of Mass Spectrometry

journal homepage: www.elsevier.com/locate/ijms

Gas-phase reactivity of sulfur-based radical ions of cysteine derivatives and small peptides

Sandra Osburn[a], Richard A.J. O'Hair[b,c,d], Victor Ryzhov[a,*]

[a] Department of Chemistry and Biochemistry, and Center for Biochemical and Biophysical Studies, Northern Illinois University, DeKalb, IL 60115, USA
[b] School of Chemistry, The University of Melbourne, Melbourne, Victoria 3010, Australia
[c] Bio21 Institute of Molecular Science and Biotechnology, The University of Melbourne, Melbourne, Victoria 3010, Australia
[d] ARC Centre of Excellence for Free Radical Chemistry and Biotechnology, Melbourne, Victoria 3010, Australia

ARTICLE INFO

Article history:
Received 4 November 2011
Received in revised form 1 December 2011
Accepted 2 December 2011
Available online 10 December 2011

Dedicated to Professor Alex Harrison on the occasion of his 80th birthday and in recognition of his important contributions to gas-phase ion chemistry.

Keywords:
Ion-molecule reactions
Cysteine radical cations
Cysteine radical anions
Radical rearrangement

ABSTRACT

The gas-phase reactivity of sulfur-based radical ions of cysteine derivatives and Cys-containing small peptides was studied via the use of ion-molecule reactions inside a quadrupole ion trap mass spectrometer. Both the radical cations $M^{\bullet+}$ and the anions $(M-2H)^{\bullet-}$ were generated via the gas-phase homolysis of the S—NO bond of the S-nitrosylated precursors of the following: Cys, N-Ac-Cys, CysOMe, Gly-Cys, Cys-Gly, γ-Glu-Cys, γ-Glu-Cys-Gly, and Gly-Cys-Arg. The radical ions were allowed to react with the following volatile neutral reagents: allyl iodide, allyl bromide, nitric oxide, 1-propane thiol, 3-mercaptopropionic acid, dimethyl disulfide and dimethyl sulfide. The charge has little effect on the types of products formed, with typical S-based radical reactivity being observed. In several systems (N-Ac-Cys, Cys-Gly, Gly-Cys, γ-Glu-Cys-Gly, and Gly-Cys-Arg) the radical was found to lose its reactivity very rapidly, which is consistent with S-to-α-carbon radical rearrangement via hydrogen atom transfer. The observed radical reactivity serves as a model for studying the chemistry of cysteine-based thiyl radicals in protein systems.

1. Introduction

Radicals generated in proteins can play a crucial role in their activity [1–12]. In the active site of an enzyme, the radical site is very tightly controlled by the protein architecture allowing it to be used to transform substrates by taking advantage of radical chemistry [13,14]. In the active form the radical is often found on the peptide backbone or the side chain of a reactive amino acid such as the: glycyl radical found in class III ribonucleotide reductase (Scheme 1a) [8], tyrosyl radical involved in class I ribonucleotide reductase (Scheme 1b) [3], tryptophyl radical cation found in cytochrome oxidase (Scheme 1c) [12], and cysteinyl radical involved in pyruvate formate lyase (Scheme 1d) [2]. In some cases the radical rearranges to different positions in the inactive form, specifically to the less reactive sulfur atom on a cysteine residue [5,6,11] or to the captodatively stabilized glycine α-carbon [4,8]. This rearrangement is considered to be a protective mechanism, allowing the radical to be recycled many times. Recycling helps the protein to retain the radical rather than having to regenerate it, thereby saving energy [5].

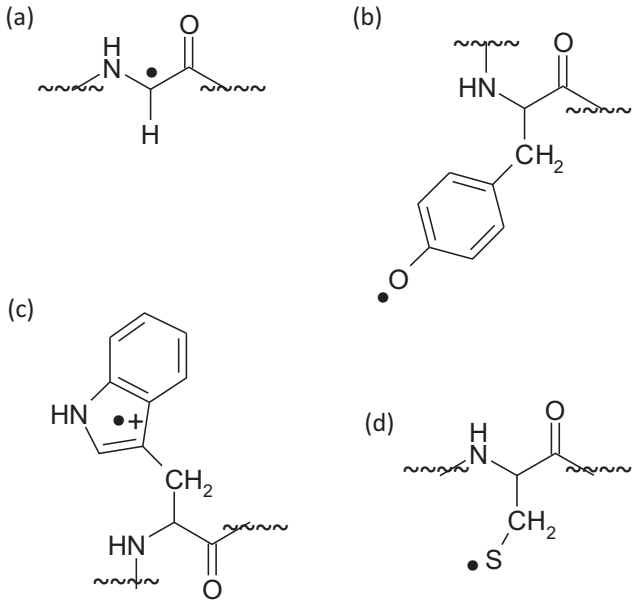
In contrast to these well controlled radical sites in enzymes, uncontrolled radical formation in proteins can lead to oxidative damage, formation of reactive intermediates, protein fragmentation, cross-linking, changes in conformation, and changes in susceptibility to proteolysis [15–21]. All of these processes can lead to the loss of protein function.

Small model systems provide valuable insights into the stability, reactivity and rearrangement of radical sites in proteins [22]. Several different radicals derived from amino acids, peptides and their derivatives have been studied using a variety of experimental [9,10,23] and computational techniques [15–17]. In solution, radicals can be indirectly identified via techniques such as EPR spin-trapping [24] and the use of fluorescent-labeled tags [25–28]. Spin-trapping involves the reaction of short-lived radicals with nitrones or nitroso compounds (spin traps) producing nitroxide-based relatively long-lived radicals (that are paramagnetic). The EPR spectra of these nitroxide-based radicals represent specific structural characteristics of the parent short-lived radical species. These solution-phase methods are often susceptible to unwanted side-reactions which can make their use in identifying radical sites and reactivity challenging [25,29–31].

An alternative approach, which has attracted considerable interest, is the use of mass spectrometry based approaches to gain a

* Corresponding author.
E-mail address: ryzhov@niu.edu (V. Ryzhov).

1387-3806/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijms.2011.12.001



Scheme 1. Key structures of amino acid radicals involve in proteins: (a) α-glycyl radical, (b) tyrosyl radical, (c) tryptophyl radical, and (d) cysteinyl radical.

molecular understanding of the chemistry of small model systems in the gas-phase [22,32]. Radical cations were first generated in the gas-phase over 50 years ago using electron ionization/mass spectrometry (EI/MS) [33]. Since then, electron capture dissociation (ECD) and electron transfer dissociation (ETD) have been used to produce radical cations via reduction of multiply charged cations, $[M+nH]^{n+}$ of peptides and proteins [34,35]. One of the first chemical-based method developed involves carrying out collision-induced dissociation (CID) of copper (II) ternary metal complexes to form radical cations of peptides [36,37] which has been extended to transition metal salen complexes [38]. The limitation of each of these methods is that the initial site of radical formation is generally unknown. To overcome this issue several chemical-based techniques have been developed to regiospecifically generate radical sites in amino acid and peptide ions via CID of derivatives containing bonds susceptible to homolysis. These include the use of: N-terminal azo derivatives [39], N-terminal [40] and lysine side chain [41] carbamate derivatives, serine nitrate ester derivatives [42], and S-nitrosocysteine derivatives [43]. These studies have opened up opportunities to examine the fundamental gas-phase chemistry of side chain and backbone radical sites in peptides.

CID has not only been used to generate radical ions, but also to probe their structure. For instance, CID and deuterium isotopic labeling has been used to examine the structure and reactivity of the radical cation of cysteine [44]. However, the extra energy deposited into the ions during the collisional activation may lead to rearrangement and scrambling of isomeric structures. For example, the two α-carbon-based radicals and the Ala side chain radical of the sodiated dipeptide PhCO-Ala-Gly-OMe, formed via the combined CID losses of $NO_2^{\bullet}$ and CH_2O from the corresponding serine and homoserine nitrate esters, gave virtually identical CID spectra, consistent with their interconversion prior to fragmentation [45]. Similarly, the CID spectra of the S- and α-carbon-based radical cations of GCG [46] and glutathione [47] are identical, highlighting the ability of radical site rearrangement. Such rearrangements do not always occur, as shown in Siu's study on the CID reactions of different isomers of $GGG^{\bullet+}$, which displayed fragmentation patterns characteristic of their structures [48].

Gas-phase ion-molecule reactions offer an alternative, thermal [49] means of probing the structure of radical ions. Several studies by Kenttamaa and co-workers [50,51] have used gas-phase radical reactions with volatile neutrals such as allyl iodide to differentiate between various radical and polyradical structures. In addition, the same group has looked at radical-induced damage to amino acids and dipeptides [52,53]. The sulfur atom in methionine and cysteine was found to be the most susceptible to radical attack [52,53]. Their studies of the reactivity of distonic radical ions provide an important foundation for structure–reactivity relationship studies of small models of protein radicals [50,51,54–57].

We have been examining the gas phase unimolecular [44,46] and bimolecular reactions [58,59] of cysteine-based radical cations and anions. The S-based radical ions can be generated via the gas-phase homolysis of the S—NO bond of S-nitrosylated precursors [44,58,59]. The S-based radical has been found to be very reactive toward neutrals that act as radical probes, such as allyl iodide [58,59] which contrasts with α-carbon-based radicals, which are generally unreactive [45]. This difference in reactivity provides a way of both distinguishing between isomeric S-based and C-based radicals as well as examining their rearrangment. Theoretical calculations and infrared multi-photon infrared dissociation (IRMPD) spectroscopy were used to confirm the position of the radical [58,59].

A key goal in our studies is to model biochemical radical processes that can occur in solution phase in proteins via the careful choice of the neutral used. An example is the use of dimethyl disulfide, which reacts with S-based radical ions in the gas phase, suggesting a potential class of reaction involving thiyl radical attack onto disulfide bridges of proteins [58,59]. The focus of this study is to extend the use of ion-molecule reactions to investigate the reactivity of a wider range of cysteine-based radical cations and anions with an extended range of neutral reagents with different functional groups.

2. Experimental

2.1. Chemicals and reagents

All chemicals and reagents were used as received without any further purification. N-acetyl-cysteine, cysteine, cysteine methyl ester, Cys-Gly, γ-Glu-Cys, reduced glutathione (γ-Glu-Cys-Gly), methanol (HPLC grade), tert-butylnitrite, dimethyl disulfide, dimethyl sulfide, allyl iodide, allyl bromide, 1-propane thiol and 3-mercaptopropionic acid were all purchased from Sigma–Aldrich (Milwaukee, WI). Gly-Cys (90% purity) was obtained from Bachem (Bubendorf, Switzerland). Gly-Cys-Arg (90% purity) was purchased from SynPep (Dublin, CA, USA). Helium containing 1% NO was purchased from Air Liquide (LaPorte, TX).

2.2. Ion-molecule reactions

2.2.1. Northern Illinois University

Ion-molecule reactions were carried out using a Bruker Esquire 3000 quadrupole ion trap mass spectrometer (Bruker Daltonics, Bremen, Germany) modified to conduct ion-molecule reactions as described previously [60]. The nitrosylated precursor was generated by allowing a 1.5:1 mixture of tert-butylnitrite and a 1 mM solution of the cysteine containing molecule (in 50/50 methanol:water with 1% acetic acid) to react for 10 min at room temperature. The reaction mixture was diluted 100-fold using 50/50 methanol:water with 1% acetic acid and introduced into the ESI source of the mass spectrometer at a flow rate of 5 μL min^{-1}. The nebulizer gas, needle voltage, and temperature were adjusted to about 15 arbitrary units, 3.4 kV, and 180 °C, respectively. Radical cations and anions were produced either by either CID or in-source fragmentation. When using CID, the protonated (or deprotonated)