Analytica Chimica Acta 1011 (2018) 59–67

Contents lists available at ScienceDirect

Analytica Chimica Acta

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

Time-resolved method to distinguish protein/peptide oxidation during electrospray ionization mass spectrometry

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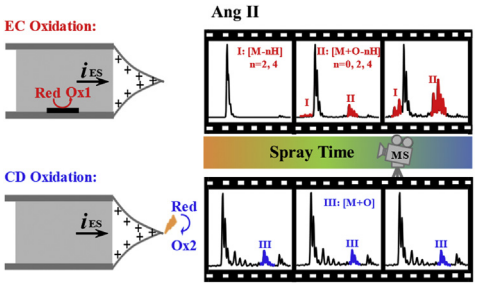
HIGHLIGHTS

- EC- and CD-induced peptide/protein oxidation during ESI-MS was systematically investigated.
- A time-resolved method was proposed to distinguish the two kinds of oxidation.
- EC- and CD-induced peptide/protein oxidations were found to be closely related with the experimental parameters.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 19 October 2017
Received in revised form
5 January 2018
Accepted 8 January 2018
Available online 31 January 2018

Keywords:
Protein/peptide oxidation
Corona discharge
Electrochemistry
Time-resolution
Electrospray ionization
Mass spectrometry

ABSTRACT

Electrospray ionization mass spectrometry (ESI-MS) is one of the most prevalent techniques used to monitor protein/peptide oxidation induced by reactive oxygen species (ROSs). However, both corona discharge (CD) and electrochemistry (EC) can also lead to protein/peptide oxidation during ESI. Because the two types of oxidation occur almost simultaneously, determining the extent to which the two pathways contribute to protein/peptide oxidation is difficult. Herein, a time-resolved method was introduced to identify and differentiate CD- and EC-induced oxidation. Using this approach, we separated the instantaneous CD-induced oxidation from the hysteretic EC-induced oxidation, and the effects of the spray voltage and flow rate of the ESI source on both oxidation types were investigated with a home-made ESI source. For angiotensin II analogue (b-DRVYVHPF-y), the dehydrogenation and oxygenation species were the detected EC-induced oxidation products, while the oxygenation species were the major CD-induced oxidation products. This time-resolved approach was also applicable to a commercial HESI source, in which both CD and EC were responsible for hemoglobin and cytochrome *c* oxidation with upstream grounding while CD dominated the oxidation without upstream grounding.

1. Introduction

Protein/peptide oxidative modification *in vivo* is closely associated with various diseases and aging, for example, Sultana et al. reported that elevated protein nitrotyrosine was found with Alzheimer's disease [1]. Exposure to reactive oxygen species (ROSs) is thought to play a critical role in the modification [2,3]. *In vivo* protein/peptide oxidation modification can be characterized by circular dichroism [4], fluorescence spectroscopy [5], gel electrophoresis [6], UV-vis spectroscopy [4], dynamic light scattering

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https://doi.org/10.1016/j.aca.2018.01.025
0003-2670/

(DLS) [4], nuclear magnetic resonance (NMR) [7] and mass spectrometry (MS) [8]. Among them, MS is one commonly used analytical technique for its capacity of obtaining highly accurate intact masses and the three-dimensional structure of proteins [9]. Electrospray ionization-mass spectrometry (ESI-MS) is preferred for the operation under atmospheric pressure conditions [10–12]. However, a major obstacle for the ESI-MS technique is that two major types of artificial protein/peptide oxidation occur during the ESI process, i.e., electrochemistry (EC)- [13] and corona discharge (CD)-induced [14] oxidation, which can mislead investigations of *in vivo* protein/peptide oxidation. Thus, knowing the properties of these two types of instrument-related oxidation is of great importance to accurately study *in vivo* protein/peptide oxidation.

An ESI source can be viewed as a controlled-current electrolytic cell [13,15]. When an analyte solution flows through the stainless-steel capillary during a typical ESI process, an oxidation reaction occurs at the solution/electrode interface in the positive mode. Moreover, the oxidation ratios of the analytes are proportional to the solution/electrode contact time. In addition to the EC-induced oxidation, the CD in the gas phase can also result in protein/peptide oxidation during the ESI process [14,16,17]. Once the spray voltage in an ESI source exceeds a critical onset value, a plasma containing abundant ROSs is generated in the vicinity of the emitter tip, and the plasma further induces various oxidation reactions (Fig. 1). Unexpected protein oxidations complicate spectrum interpretation, reduce sensitivity by splitting ion current, interfere with peak isolation for MS^2 fragmentation, and alter the chemical state of the proteins. To decrease CD during ESI, organic solvent is generally added, which will change the conformation of proteins/peptides [18], and is unfavorable for native protein analysis [19–21]. On the other hand, protein oxidation during ESI can also be strengthened for protein footprinting investigation. For example, Downard et al. utilized ESI-based oxidation to investigate protein folding/unfolding, protein interactions, impact of oxidation on protein aggregation, and the residue side chain solvent accessibility of proteins based on the oxidation level and rate [14]. Therefore, to effectively avoid or take full advantage of these two types of oxidation, differentiation of them is necessary. However, since the CD- and EC-induced oxidations occur almost simultaneously, their contributions to the ESI-based protein/peptide oxidation are unclear [22–25].

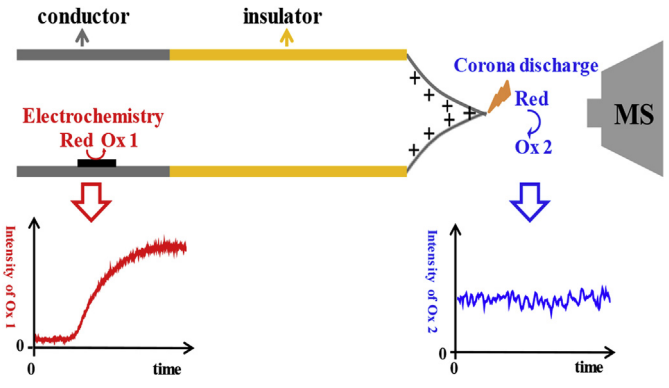

Fig. 1. Schematic diagram of the CD- and EC-induced protein/peptide oxidation during ESI-MS. The EC-induced oxidation occurs at the solution/electrode (conductor) interface, and the analyte is oxidized to Ox1 (labeled in red). Alternatively, the CD-induced oxidation occurs at the tip of the spray emitter, and the analyte is oxidized to Ox2 (labeled in blue). The oxidation extent of the EC-induced oxidation is proportional to the solution/electrode contact time, while that of the CD-induced oxidation does not depend on the spray time. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Kim et al. [26] and Liu et al. [24] observed unexpected protein/peptide oxidation, which was decreased by rearranging LC plumbing, in a conventional liquid chromatography-mass spectrometry (LC-MS) configuration. The oxidations were expected to be related with the enhanced electrochemical reaction of ESI source through construction of an upstream grounding loop between the ion source and the nearest upstream grounded metal element [25,27]. However, Morand et al. favored CD that was responsible for peptide oxidation during ESI, because they observed the appearance of the modified ions with the observation of a faint blue hue near the tip of the electrospray needle [22]. Chen et al. [23] attributed Aβ peptide oxidation to gradual corrosion of stainless steel electrospray emitters that could promote electrical discharge. They also thought that the increased emission current strengthened the electrochemical reactions associated with Aβ peptide oxidation. Use of redox buffers and reduction of electric field were suggested to decrease the oxidation. Lars Konermann et al. systematically determined the effects of EC and CD on protein oxidation [28]. Based on a visible inspection of the light emission to judge whether CD occurred and the record of the voltage-current plots under different nebulizer gas conditions, it was concluded that protein oxidation in ESI is predominantly mediated by CD-generated ROSs. Additionally, an off-line electrolysis experiment was conducted to illustrate that EC cannot account for protein oxidation under typical ESI operating conditions. Although Lars Konermann helped to understand the protein oxidation mechanism in ESI, direct MS proof is still required to investigate the two types of simultaneous oxidation. Previously, we minimized the EC-induced oxidation for MS measurements of proteins using a modified ESI method; however, the CD-induced oxidation was still observed [29]. Considering that the EC-induced oxidation is proportional to the solution/electrode contact time and the CD-induced oxidation is not, we were inspired to design a time-resolved ESI approach to distinguish the CD- and EC-induced protein/peptide oxidation.

The time-resolved method is based on the variation in the analyte oxidation extent with the spray time. To better isolate the two types of oxidation in space and time, a homemade ESI source was used to investigate the CD- and EC-induced oxidation. Using the homemade ESI source, the effects of the spray voltage, flow rate, and solvent on the CD- and EC-induced oxidation were separately investigated. Also, the CD extent was characterized by the luminescence recorded by a photomultiplier. Then, protein oxidation in a commercial HESI source with and without upstream grounding was investigated using the established method.

2. Experimental

2.1. Materials and reagents

HPLC-grade methanol (CH_3OH) was purchased from Honeywell Burdick & Jackson Inc. (U.S.A.). Angiotensin II analogue (Ang II) and KKTCAA were synthetized by Sangon Biotech (Shanghai Sangon Biological Engineering Technology & Services Co. Ltd., Shanghai, China). Cytochrome *c* (Cyt *c*) and hemoglobin (Hb) were purchased from Sangon Biotech. Melittin, reserpine, and formic acid (FA) were obtained from Sigma-Aldrich Chemical Co. Ltd. (U.S.A.). NH_4Ac was obtained from Sinopharm Chemical Reagent Co. Ltd. (Beijing, China). All the reagents were used without any further purification. Distilled water (18.2 MΩ) was produced by a Milli-Q system (Millipore Inc., Bedford, MA, U.S.A.).

Unless noted otherwise, all peptide and protein solutions were prepared in CH_3OH, CH_3OH/H_2O (v/v, 1:1) or H_2O with the addition of NH_4Ac and FA.