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Quantification of urinary *o,o′*-dityrosine, a biomarker for oxidative damage to proteins, by high performance liquid chromatography with triple quadrupole tandem mass spectrometry
A comparison with ion-trap tandem mass spectrometry

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Abstract

We recently described an isotope dilution reversed-phase liquid chromatography–atmospheric pressure chemical ionization–ion-trap-tandem mass spectrometry (HPLC–APCI–MS/MS) method for the quantitative determination of oxidized amino acids in human urine, including *o,o′*-dityrosine, a specific marker of protein oxidation. In the present study, we investigated the possibility to use a triple quadrupole instrument for the analysis of this biomarker in urine. The two instruments were compared in terms of sensitivity, specificity and reproducibility. Results showed that the triple quadrupole instrument reaches 2.5-fold higher sensitivity (LOD = 0.01 μM) compared to the previously used ion-trap instrument. Precision of the present assay is as follows: in-day variation is 4.6% and inter-day variation is 17%. The currently developed method was applied to a group of smoker urine samples. The mean urinary *o,o′*-dityrosine concentration was 0.08 ± 0.01 μM. Expressed per urinary creatinine concentration, this corresponds to 10.1 ± 0.4 μmol/mol creatinine. This is comparable to the previously reported values of 5.8 ± 0.3 μmol/mol creatinine in non-smokers night-time urines, and 12.3 ± 5 μmol/mol creatinine in day-time urines measured by the ion-trap instrument.

Keywords: *o,o′*-Dityrosine; Atmospheric pressure chemical ionisation; Triple quadrupole tandem mass spectrometry; Isotope dilution; Biomarker; Smoker

1. Introduction

o,o′-Dityrosine (dityrosine) cross-links naturally occur in proteins in many macro- and micro-organisms [1–7]. Vertebrate animal proteins known to contain dityrosine include elastin [8], collagen [9], and a storage form of thyroglobulin [10]. Interest in dityrosine is based on its potential as a specific marker for oxidatively damaged proteins and their selective proteolysis [11,12]. Thus, dityrosine may mark oxidatively damaged proteins in red blood cells exposed to hydrogen peroxide (H_2O_2) for proteolytic destruction [13]. Dityrosine has also been proposed as a biomarker of organismal oxidative stress, since its concentrations were found 100-fold higher in low-density lipoproteins isolated from atherosclerotic lesions than in normal ones [14]. Also, humans suffering from systemic bacterial infections had twice the concentration of dityrosine in urine than those of healthy individuals [14]. Beside these pathologies, endurance exercise, which is associated with increased oxygen consumption and subsequent increased reactive oxygen species (ROS) formation, caused elevation in urinary dityrosine concentrations for several days

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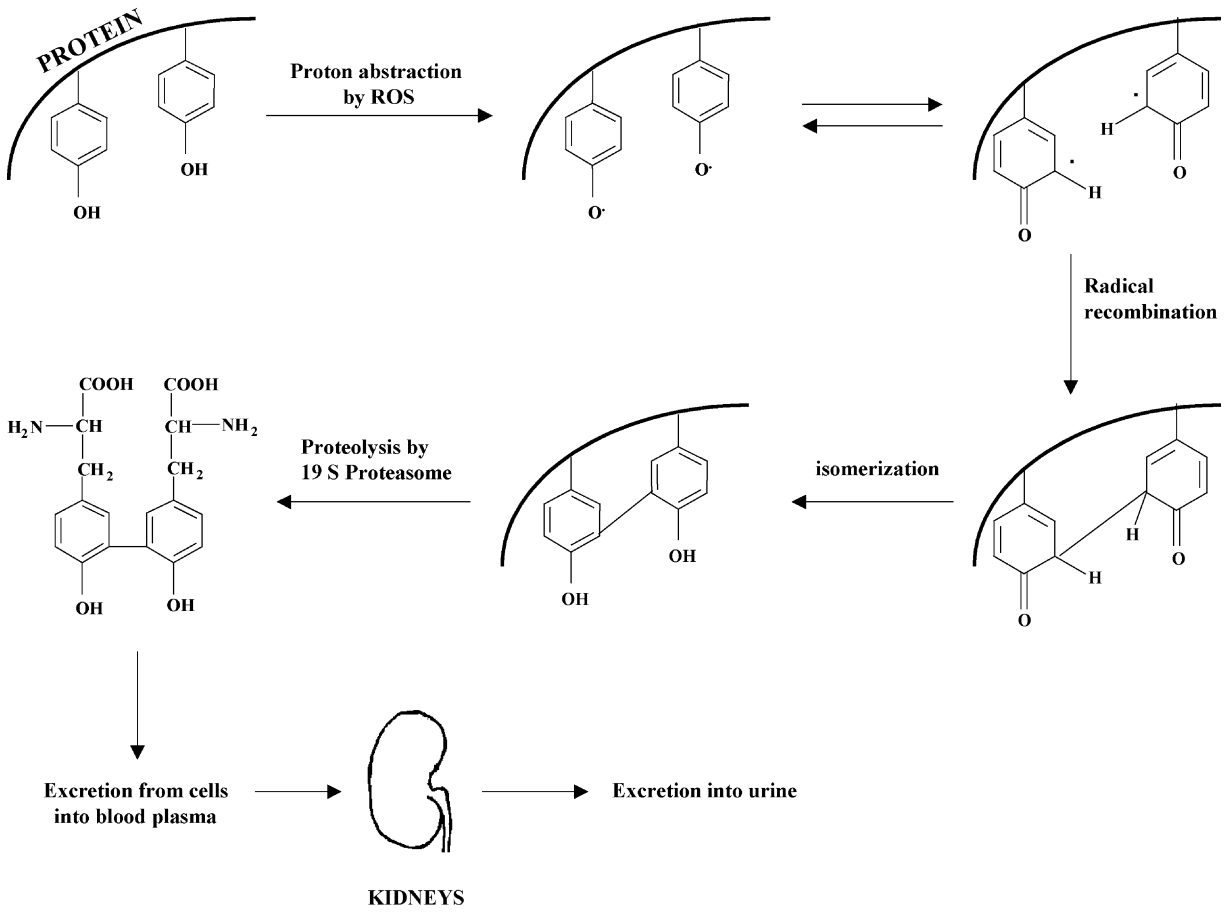

Fig. 1. Mechanism of dityrosine formation from tyrosine residues of proteins and its possible metabolic fate in the body. ROS, reactive oxygen species.

following the exercise [15]. Furthermore, tissue levels of this oxidized amino acid are elevated in prevalent diseases such as atherosclerosis, inflammatory lung disease, neurodegenerative disorders, and aging [16–20].

Dityrosine formation begins with the generation of tyrosyl radicals, followed by radical isomerization and diradical reaction, and finally enolization [21] (Fig. 1). Alternative pathways such as covalent binding of tyrosine to the heme group in myoglobin or addition of oxygen have also been proposed [22]. The reduction potential of the tyrosineO$^{\bullet}$/tyrosineOH couple is 0.88 V [23] indicating that the coupled reaction needs to be highly oxidative to be thermodynamically favourable. In this regard, hydroxyl radical, oxoferryl moiety, porphyrin π cation radical, peroxynitrite, nitrogen dioxide, and nitryl cation, are some of the species that fulfil this requirement [11]. Oxidants generated by activated phagocytes generate dityrosine cross-links in model proteins and lipoproteins in vitro [16,24–26]. NADPH oxidase of phagocytes, which plays a key role in host defences against microbial pathogens, converts oxygen into superoxide anion radical ($O_2^{\bullet -}$) [27,28]. The $O_2^{\bullet -}$ may then dismutase into H_2O_2, which is an oxidizing substrate for myeloperoxidase, a heme protein secreted by activated phagocytes [27,29]. Myeloperoxidase uses H_2O_2 to produce dityrosine cross-links from the tyrosine residues of target proteins [16,24,25]. This pathway was proved to exist also in vivo [30].

Despite the knowledge about their formation, little is known about the metabolic fate of oxidized amino acids released from tissue proteins. One possibility is that oxidized amino acids are excreted from cells and filtered from blood into urine [30]. Alternatively, they may be reabsorbed by the kidney, metabolized to other compounds, or reused for protein synthesis. This has recently been shown for *m*-tyrosine [31]. However, recent rat studies demonstrated that dityrosine is secreted into urine rather than being recycled into proteins [17,32]. As a biomarker, dityrosine has the advantage over other protein oxidation products of being a stable compound and once the 3′-3′ carbon–carbon bond is formed in dityrosine it is resistant to hydrolysis by lytic enzymes [12,13].

Because its universal formation and use as biomarker for oxidative (protein) damage, there is a need for sensitive and reliable analytical method(s) to determine dityrosine in biological media. Several analytical methods have been developed to quantify this product in vivo and in vitro. These include HPLC either with UV or fluorescence detections