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SERS study of transformation of phenylalanine to tyrosine under particle irradiation

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HIGHLIGHTS

- SERS has been for the first time applied in the radiobiological research.
- SERS quantitatively measured Tyr isomers produced from Phe solution irradiated by particle irradiation.
- The study confirms the role of ·OH attack and so explains the higher efficiency by electron beam induced Tyr production.

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ABSTRACT

Surface enhanced Raman scattering or spectroscopy (SERS) is a very powerful analytical tool which has been widely applied in many scientific research and application fields. It is therefore also very intriguing for us to introduce SERS technique in the radiobiological research, where in many cases only a very few of biomolecules are subjected to changes which however can lead to significant biological effects. The radiation induced biochemical reactions are normally very sophisticated with different substances produced in the system, so currently it is still a big challenge for SERS to analyze such a mixture system which contains multiple analytes. In this context, this work aimed to establish and consolidate the feasibility of SERS as an effective tool in radiation chemistry, and this purpose, we employed SERS as a sensitive probe to a known process, namely, the oxidation of phenylalanine (Phe) under particle irradiation, where the energetic particles were obtained from either plasma discharge or electron-beam. During the irradiation, three types of tyrosine (Tyr), namely, *p*-Tyr, *m*-Tyr and *o*-Tyr were produced, and all these tyrosine isomers together with Phe could be identified and measured based on the SERS spectral analysis of the corresponding enhanced characteristic signals, namely, 1002 cm^{-1} for Phe, 1161 cm^{-1} for *p*-Tyr, 990 cm^{-1} for *m*-Tyr, and 970 cm^{-1} for *o*-Tyr, respectively. The estimation of the quantities of different tyrosine isomers were also given and verified by conventional method such as high performance liquid chromatography (HPLC). As for comparison of different ways of particle irradiation, our results also indicated that electron-beam irradiation was more efficient for converting Phe into Tyr than plasma discharge treatment, confirming the role of hydroxyl radicals in the Phe–Tyr conformation. Therefore, our work has not only demonstrated that SERS can be successfully applied in the radiobiological study, but also given insights into the mechanism for the interaction between particle radiation and biological systems.

Introduction

Energetic particle radiation is ubiquitous and implicates various biological effects, such as somatic or genetic effect [1–3], and so it may affect our environment and human health profoundly [4]. Generally, the influence imposed by particle radiation on organisms is either through direct or indirect ways. The direct way refers to the direct energy deposition and ionizing reactions in the biomolecules, whereas indirect way is due to radiation induced reactions through reactive chemical species, mainly reactive oxygen species (ROS), in the medium (mainly water) surrounding the biomolecules [5]. It has been observed that indirect effect may play a dominant role in living organisms. For example, it was reported that one-third of the damage in the genome of a living cell was

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directly caused by ionizing radiation and two-thirds of the damage were caused indirectly [6]. Therefore, the indirect effects mediated by ROS related biochemical reactions of biomolecules have gained special interest in the research. In particular, because DNA, lipids and proteins are the most important biological macromolecules in biological systems, and also because the physico-chemical changes of these biomolecules in the early primary processes are responsible for the observable biological effects, the indirect effects on DNA, lipids and proteins have been investigated intensively and extensively in the past, and they have been definitely rendered as the major radiation targets in living organisms [7–11].

In order to investigate the radiation caused indirect effects on the important biomolecules such as DNA, lipids and proteins, a variety of methods and tools have been utilized. For example, radiation induced DNA strand breakage and cracking were investigated via fluorometric measurement [12], microelectrophoresis or the single cell gel electrophoresis/comet assay [13,14] and etc. Lipid, especially unsaturated lipids are liable to undergo peroxidation initiated by reactive free radicals such as hydroxyl radicals [15], and peroxidation of membrane lipids (LPO) can be detected based on high performance liquid chromatography–electrochemical detection, gas chromatography–mass spectrometry (GC-MS), liquid chromatography–tandem mass spectrometry [16] and etc. For example, Tyurina et al. employed oxidative lipidomics to qualitatively and quantitatively characterized phospholipid peroxidation in a radiosensitive tissue, the small intestine, of mice exposed to total body irradiation via electrospray ionization mass spectrometry [17]. As for proteins, they are also highly reactive with chemical reactive species upon ionizing irradiation [18]. Because they are of high content within living organisms and perform a vast array of functions including catalyzing metabolic reactions, replicating and repairing DNA, responding to stimuli and etc, the role of proteins in the radiobiology is now well recognized and of special concern. For example, the study by Eon et al. [19] reported that the damage of protein, but not DNA, was the reason to the disruption of *Escherichia coli* lactose repressor-operator complex (DNA–protein complex) induced by γ rays. Besides, relative to the radiation chemistry of proteins, the indirect effects on amino acids, the basic building blocks of protein polymer chains, have also been extensively investigated [20–22]. In these studies, the ROS-mediated oxidation reactions are generally investigated via conventional analytic methods including high performance liquid chromatography and mass spectrometry. For instance, the ROS induced hydroxylation of aromatic groups, sulfoxidation of methionine residues and formation of disulfide bond have been determined through MS/MS, GC/MS and HPLC methods [23,24]. Although these methods show advantages in reproducibility and accurate quantitative analysis, they have disadvantages of being very time-consuming, costing large amount of materials, labor-intensive and demanding stringent specification of experimental conditions. Neither could these methods achieve measurements in vivo. Nowadays a new trend emerges to study the radiation induced biological effects at the single-cell level, and for such circumstance, normally only a very few of biomolecules are involved in the biochemical reactions, so it becomes indispensable to apply alternative ultra-sensitive analytical tools that can probe the changes of the involved biomolecules at trace amount of level within a very tiny volume of bio-samples. This, as one can imagine, presents a new challenge for researchers to develop new analytical tools for radiobiological studies.

Recently, surface enhanced Raman spectroscopy or scattering (SERS) technique has been widely used as a powerful tool for ultra-sensitive chemical analysis down to the single-molecule level [25,26]. The rapid development of SERS-based biosensors has led to its realm of applications from chemical–biochemical analysis to biomedical applications [27–30]. The SERS technique is a very effective analytical tool owing to its high sensitivity and selectivity, so that it only requires a very small amount of samples but can reach extremely low detection limit. Also, more and more studies have also proved the great potential of SERS technique in protein identification and detection of protein-interactions [31]. For example, Xu et al. [32] reported single-molecule SERS of hemoglobin, and Etchegoin et al. [33] observed dynamic oxygen release in hemoglobin via SERS. Moger et al. [34] claimed that the short scanning and processing time associated with SERS makes it an attractive and a highly sensitive alternative for near-real-time measurement at sub-micro-molar concentrations for detecting peptide phosphorylation at serine and tyrosine residues at micromolar concentrations.

Based on the advantages, advance and development of SERS technique, this work aimed to introduce SERS spectroscopy to the study of radiation reactions of biomolecules. And for this purpose, we chose one aromatic amino acid, phenylalanine (Phe), and employed SERS technique to analyze the oxidation of Phe under energetic particle irradiation. Phe was selected as the representative because the main radiation reaction products including tyrosine (Tyr) isomers (ortho-, meta-, and para-Tyr) have been investigated and clearly determined [35,36], so it may helps us to consolidate the feasibility of our method. The particle irradiation by either plasma discharge or electron beams caused oxidation of Phe mainly by hydroxyl radicals, leading to the production of three tyrosine isomers, which were identified and quantitatively analyzed based on their characteristic bands from SERS measurements. Our results demonstrated that SERS may not only be utilized as a new and effective way for the study of radiation chemistry in radiobiology, but also shed light on the mechanism for the indirect interactions between particle radiation and biological systems.

Materials and methods

Materials

L-Phenylalanine (L-Phe), L-*p*-Tyrosine (L-*p*-Tyr), DL-*m*-Tyrosine (DL-*m*-Tyr), DL-*o*-Tyrosine (DL-*o*-Tyr), Dityrosine (DT), Silver nitrate ($AgNO_3$), Sodium borohydride ($NaBH_4$), Catalase (CAT), acetonitrile, Coumarin-3-carboxylic acid (3-CCA) were purchased from Sigma–Aldrich Co, Ltd. Nitroblue tetrazolium chloride (NBT) was purchased from Sangon Biotech (Shanghai) Co., Ltd. Titanium sulfate was purchased from Sinopharm Chemical Reagent Co., Ltd. All the chemicals were used without further purification.

Irradiation treatment of samples

For the treatment of Phe solution by discharge plasma, the same instrument was employed as described previously [35]. Briefly, 25 mL Phe solution (10^{-2} M) in water was placed into the circular reactor. The anode and the cathode, both made of stainless steel, were placed ≈3–5 mm above the solution surface and submerged in the solution, respectively. When the discharge was steady, the current was about 40 mA, and the total voltage was about 1300 V (±10%). During the plasma treatment, the solution was magnetically stirred and certain volume of samples was drawn periodically from the reactor for analysis.

Electron beam irradiation of Phe solution was carried out using a Ju-Neng high energy electron beam accelerator provided by Shidai Ju-Neng Co., Ltd. (Hefei, China) (10 MeV, 10 kW, pulse duration of 13 ms, pulse frequency of 211 Hz, dose rate of 3.5 kGy min^{-1}) with doses of 0.5, 1, 1.5, 2, 4, 6, 8, 10 kGy at room temperature.

After irradiation, each sample was added with certain amount of catalase solution (1 mg/mL) to remove H_2O_2 in the sample. Then, the catalase was removed with a centrifugal filter device