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Radiolysis of phenylalanine in solution with Bragg-Peak energy protons

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ABSTRACT

Phenylalanine aqueous solutions were irradiated with Bragg-Peak energy protons (0.6–5.6 MeV). An innovative circulation setup has been developed for the irradiation of these solutions with low-energy protons. Every detectable radiolysis product has been identified and quantified after irradiation. While tyrosines are well-known products of phenylalanine radiolysis, 2,5-dihydroxyphenylalanine and dimers were detected in high quantities, for the first time. The track-yields (G'-values) of all products formed under proton irradiation were determined and compared to those obtained with gamma-rays, and 2,5-dopa and dimers production seems to be specific to accelerated ions. A bi-radical mechanism could explain their higher production with a higher density of energy deposition in the track structure.

1. Introduction

Since the development of radiation-therapy, much interest has been dedicated to the radiolysis of biomolecules and the understanding of radio-induced degradation mechanisms. Proteins are by far the most abundant biomolecules in the cell, and its second constituent by mass, after water. Therefore, they are likely to endure many radio-induced damages when the cell is subjected to any ionizing radiation. Amino acids being the building blocks of proteins, studies of their radiolysis offer an insight in the behaviour of proteins under irradiation. In diluted solutions, the damage observed are caused by *indirect effects*, that is reaction of the molecules with the species formed by water radiolysis: $HO^{\bullet}$, e_{aq}^{-}, H_2O_2 etc. Most of the radiolysis products described arise from reactions with hydroxyl radical (Garrison, 1987). In aerated medium, e_{aq}^{-} and $H^{\bullet}$ are very efficiently trapped by oxygen (Elliot, 1989) to yield superoxide $O_2^{\bullet-}$ radical, which does not react directly with amino acids (Bielski and Shiue, 1979).

While amino acids, and in particular aromatic amino acids, have been extensively studied under gamma rays and electron irradiation (Garrison, 1987; Bergès et al., 2011; Chrysochoos, 1968; Maskos et al., 1992a, 1992b; Solar, 1985; Solar et al., 1984; Wang et al., 1993), work about accelerated ions are very scarce (Taguchi et al., 2001; Nomura et al., 2016, 2017).

Ions have been used since more than 25 years for the treatment of tumours by particle therapy, or hadron therapy, especially carbon ions and protons. Indeed, compared to X-Rays or gamma, they show better ballistic, allowing better preservation of healthy tissues (Fokas et al., 2009), and better relative biological effectiveness (RBE), especially in the case of carbon ions (Paganetti, 2003; Weyrather and Kraft, 2004). While these phenomena have been well studied at the tissue and the cell levels, the ions specificity is far from being totally understood at the molecular scale. Yet, in aqueous solution, if water radiolysis products are the same, whatever the nature of the ionizing radiation, these quantities and densities vary (Muroya et al., 2006). The ion track structure leads to modifications in the radiolysis mechanisms, due to local radical densities (LaVerne, 2000). Therefore, the yields of radiolysis products are different (Baldacchino et al., 2009; Taguchi et al., 2009), which could lead to the formation of specific products.

This is why we chose to study the radiolysis of an amino-acid under low energies proton irradiation, corresponding to energies in the Bragg peak region, with average Linear Energy Transfers (LET) about one order of magnitude higher than that of most gamma rays. Phenylalanine is an aromatic amino-acid, and was chosen for this study as it is known to react very efficiently with hydroxyl radical (Solar, 1985; Buxton et al., 1988). Its radiolysis has been well described under high-energy photons and electron irradiations (Chrysochoos, 1968;

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Maskos et al., 1992a; Solar, 1985; Wang et al., 1993). Phenylalanine diluted solutions were irradiated in aerated medium, the radiolysis products were identified, as exhaustively as allowed by analytical methods, and quantified, as a function of the ion energies.

2. Experimental

2.1. Chemicals

L-Phenylalanine (Sigma Aldrich, > 98%) was used without further purification. Phenylalanine solutions were prepared at 2 mM . These solutions were prepared in 66 mM phosphate buffer, well known to be inert under radiolysis, at pH 6.8. Buffer solutions allow to keep a constant pH, despite acido-basic species formed by water radiolysis. Fricke solution was prepared as described by Fricke and Hart (1966) using reagent grade $Fe(NH_4)_2(SO_4)_2.6H_2O$, NaCl, and H_2SO_4 (Sigma Aldrich) and ultra pure water. Reagent grade tryptophan, p- and m-tyrosine 3,4-dihydroxyphenylalanine (TCI) and o-tyrosine (Sigma Aldrich) were used for calibration.

2.2. Proton irradiations

Proton irradiations were performed on two accelerators: a 4 MV Van de Graaff accelerator (Icube, Strasbourg, France) and the HIMAC (NIRS-QST, Chiba, Japan). Later they will be referred to respectively as setup 1 and setup 2.

An irradiation setup was developed by our team on one line of the Van de Graaff accelerator, to allow irradiation of aqueous solutions with low energy protons (0.5–3 MeV, Fig. 1). The beam is extracted from vacuum through a 12 µm aluminized mylar window, and penetrates the solution through an identical window closing a lab-made flow-through cell. Proton beam was produced as a continuous spot of 5 mm diameter, sweeping at a vertical frequency of 517 Hz and a horizontal of 64 Hz. A ring-Faraday cup placed right before the exit of the line, together with an electron repeller, allowed continuous measurement of the ion currents. The energy of the protons can be easily adjusted by the Van de Graaff accelerator terminal voltage, and the small energy loss resulting from the passing through the windows was computed with SRIM code (Ziegler et al., 2010).

To avoid track overlapping, the solutions were quickly circulated at 40 mL/min, using a peristaltic pump (Watson-Marlow 100 series). The setup included a flow-through quartz-cell, allowing UV–Vis absorption measurements during the irradiations (Agilent DAD Cary 8454).

The very same irradiation setup was used on the HIMAC. Experiments were performed in the Medium Energy Room of the installation, with protons of 5.63 MeV after extraction through a Havar(R) foil window (Konishi et al., 2005). Protons were produced as 700 µs pulses every 1.6 s, with a fluence up to $8.3{\cdot}10^9$ion/pulse/cm^2 (calibrated using solid-state dosimeter CR39 (HARZLAS TD-1/Fukuvi, Japan) (Yamauchi, 2003). Energies of the protons were selected by adjusting the distance between the flow-through cell and the beam extraction window, using a XYZ stage (Yasuda et al., 2005).

In both kinds of experiments, the dose deposited was determined by on-line monitoring of the ion charges.

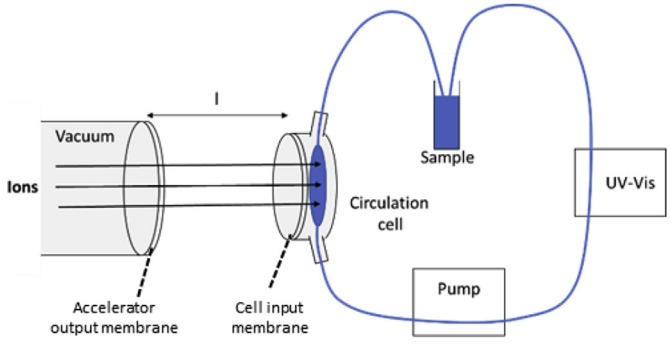

Fig. 1. Irradiation set-up with an air extracted ion beam and a lab-made circulation circuit.

2.3. Gamma ray irradiations

Gamma ray irradiations were performed with a ^{137}Cs source (NIMBE, CEA Saclay), with a dose rate of 4.9 Gy/min, at a typical dose of 200 Gy. Calibration was performed with Fricke dosimeter (Fricke and Hart, 1966) using a yield of 15.3 species/100 eV (ICRU report 14). Irradiations were performed in polypropylene vials, and the typical volume irradiated was 5 mL.

2.4. Post irradiation analyzes

2.4.1. HPLC-PAD-Fluo-CAD/mass spectrometry analyses (setup 1)

After irradiation, solutions were analysed using a Thermo Fisher Dionex Ultima 3000 RS system, equipped with a WPS autosampler, coupled to a photodiode array detector (PAD), a fluorescent detector JASCO FP4020 (Fluo), a charged aerosol detector Corona Veo RS (CAD) and a LTQ Orbitrap XL Thermo Electron mass spectrometer with an ESI source (ESI-FTMS). The eluent was split after the fluorescent detector, a fraction going to the CAD, and the rest to the ESI-FTMS. Separations were performed on a Hypersil Gold C18 column (250 × 4.6 mm, i.d. 5 µm), at a flow rate of 0.8 mL min^{-1} at 10 °C. The gradient used mixed solution A (water with 0.2% acetic acid) and solution B (acetonitrile with 0.1% acetic acid) as follows: 0–26 min 99% A, 27–33 min 50% A, 34–48 min 99% A. 100 µL of each irradiated solution were analysed. Identification of radiolysis products was possible thanks to ESI-FTMS measurements (full scan over the mass range of 120–500 *m/z* and MS measurements) and UV–Visible spectra. Quantification of all species was possible with CAD after calibration with commercial amino acids standards (phenylalanine, tryptophan, o-, m-, p-tyrosines, 3,4-dihydroxyphenylalanine). Under isocratic conditions, the CAD provides an uniform response, which only depends on the mass of the analytes, regardless of the nature/structure of the species allowing quantification of all species using the same calibration curve (Ligor et al., 2013). This allowed also calibration of the PAD and fluorescence detectors for the radiolysis products, which were used for low-dose quantifications, being far more sensible than CAD detector.

2.4.2. HPLC-DAD/spectrofluorimeter (setup 2, Chiba)

Solutions were analysed with a Hitachi L-2450 HPLC-DAD. For fluorescence detection, a Hitachi F-2700 spectrofluorimeter equipped with a 20 µL flow-through cell (Firefly) was used as a fluorescence detector. The same columns and separation conditions were used as in Setup 1. Calibrations made using Setup 1 allowed quantification by fluorescence measurements. In the case of 2,5-dopa, the quantities measured were re-evaluated, to take into account the difference in ions fluences between the two irradiation setups (Cf. Supp info.).

3. Results and discussion

Irradiations of aerated solutions were performed with protons of various energies, ranging from 0.6 MeV to 5 MeV, typical doses of 200 Gy, and led to complex mixtures of radiolysis products (Fig. 2). Phenylalanine concentration was 2 mM, which corresponds to an average capture time of the hydroxyl radical by phenylalanine of 72 ns (k = $6.9.10^9$ M^{-1} s^{-1}) (Solar, 1985). Such time corresponds to the physicochemical stage of water radiolysis, when the species formed are not yet homogenized in the solution, and therefore, when the LET effects are expected to be especially pronounced. p-, m- and o-Tyrosines, as well as 3,4-Dihydroxyphenylalanine (3,4-dopa) were identified with commercial standards. Two other dopa were also identified, 2,5-dopa and 2,3-dopa, using MS analysis together with UV-spectra, by

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