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Formation of radical anions of radiosensitizers and related model compounds via electrospray ionization

Linda Feketeová[a,b,*], Abigail L. Albright[a], Brita S. Sørensen[c], Michael R. Horsman[c], Jonathan White[a], Richard A.J. O'Hair[a,b], Niels Bassler[c]

[a] School of Chemistry and Bio21 Institute of Molecular Science and Biotechnology, The University of Melbourne, 30 Flemington Road, Victoria 3010, Australia
[b] ARC Centre of Excellence for Free Radical Chemistry and Biotechnology, Australia
[c] Department of Experimental Clinical Oncology, Aarhus University Hospital, DK-8000 Aarhus C, Denmark

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Dedicated to Professor Tilmann D. Märk, on the occasion of his 70th birthday and in recognition of his important scientific contributions in the fields of ion processes and mass spectrometry, as well as his commitment to the mass spectrometry community.

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ABSTRACT

Radiosensitizers are used in radiotherapy to enhance tumour control of radioresistant hypoxic tumours. While the detailed mechanism of radiosensitization is still unknown, the formation of radical anions is believed to be a key step. Thus understanding the ionization reactions of radiosensitizers is crucial in evaluating the radiosensitization potential and in developing new and more effective drugs. The present work investigates the negative and positive electrospray ionization and subsequent collision-induced dissociation and electron-induced dissociation reactions of ions derived from nimorazole, misonidazole and related compounds using a hybrid linear ion trap – Fourier Transform Ion Cyclotron Resonance mass spectrometer (Finnigan-LTQ-FT). A key finding is that negative electrospray ionization of these radiosensitizers leads to the formation of radical anions, allowing their fragmentation reactions to be probed for the first time.

1. Introduction

Around 50% of patients diagnosed with cancer are treated with radiation. Nevertheless, a difference in response of tumour cells to radiation quality (radiation dose, type of radiation, energy of radiation) is often an issue, likely arising from different distributions of the initial ionization events, leading to differences in the nature, yields, and spatial distribution of damage from the free radicals produced. Absorption of a high energy particle or a photon can lead to serious biological consequences, for example, damage to DNA can take place either through the direct effect of ionization or indirectly through formation of H•, •OH and carbon radicals [1,2]. These radicals can attack the nucleobases or the sugar backbone leading to DNA strand breaks. Oxygen can react with free radicals and modify the response of irradiated cells. However, if oxygen is not present, as in the case of hypoxic tumour cells, the same dose of radiation has been shown to be considerably less effective [3].

Radiosensitizers are a class of compounds that are capable of differentially sensitizing hypoxic cells to enhance the lethal effects of ionizing radiation [4]. Nimorazole has been tested in the clinic and is in clinical use in radiotherapy in Denmark (following the DAHANCA 5 clinical trial) [5] to enhance tumour control of radioresistant hypoxic tumours in head and neck cancer. A recent review by Overgaard summarizes the clinical use of radiosensitizers for over 10,000 patients treated for head and neck carcinoma [6]. Meta-analysis of sufficient data provided convincing evidence in favour of an improved overall tumour control and patient survival. Radiosensitizers used in the treatment of hypoxic cells are called 'electron-affinic' since studies in bacteria, bacterial spores, and cell cultures have shown a relationship between the

* Corresponding author at: School of Chemistry and Bio21 Institute of Molecular Science and Biotechnology, The University of Melbourne, 30 Flemington Road, Victoria 3010, Australia. Tel.: +61 3 9035 7651; fax: +61 3 9347 5180.
E-mail address: linda.feketeova@gmail.com (L. Feketeová).

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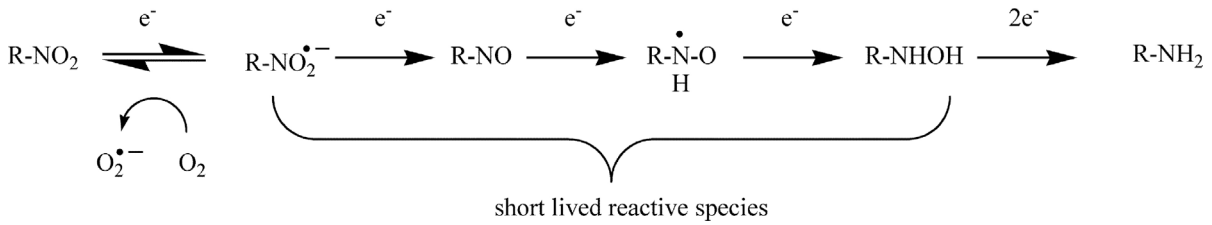

Fig. 1. General scheme for the reduction of nitroimidazole compounds. The ability of oxygen to reverse the formation of the radical anion R—$NO_2^{\bullet -}$ to produce superoxide $O_2^{\bullet -}$ and R—NO_2 is shown.

efficiency of sensitization and the electron affinity. This has aided the search for new compounds considerably [7]. A number of nitroimidazoles and related compounds were studied in vitro as well as in vivo, and partially with high-Linear Energy Transfer (high-LET) radiation, with encouraging results [8]. However, the detailed mechanism of the actual radiosensitization is still unknown. A working hypothesis is that these compounds undergo redox reactions inside the cells that are deficient in oxygen, and that the nitroimidazole ring facilitates reduction via the formation of radical anions. New potential radiosensitizers were suggested by Ramalho et al. based on the calculated adiabatic and vertical electron affinities of nitrofurans and nitroimidazoles in solution [9]. Nitroaromatic compounds bind selectively to hypoxic cells, and anaerobic bacteria, via complex redox chemistry, which is postulated to involve several steps via reduction of the nitro-group (—NO_2) to an amine (—NH_2) through a series of reactive intermediates. The reduction involves sequential addition of an electron and protonation [10,11]. The nitro group may accept up to six electrons in a complete reduction to the corresponding amine and some of the key intermediates shown in Fig. 1 include: the radical anion, $RNO_2^{\bullet -}$; the nitroso compound, RNO; the nitroxyl radical, RNHO•; and the hydroxylamine, RNHOH. Despite considerable effort, no stable intermediates have yet been characterized.

In addition to their use as radiosensitizers and antibacterial drugs [12], nitroimidazolic compounds have attracted recent interest as potential agents for imaging of hypoxia in tumours by positron emission tomography (PET) [13–16], as well as energetic materials (explosives, propellants and pyrotechnics) [17–19]. Despite their use in biological applications, little is known about the fundamental properties and reactions of nitroimidazolic compounds. Gas-phase studies of these compounds appear to be limited to the pioneering photoelectron spectroscopy work of Kajfež et al. [20] on methyl-nitroimidazoles and a very recent nanosecond energy resolved spectroscopic study of nitroimidazoles and 1-methyl-5-nitroimidazole [18,19].

The present work investigates the formation of positive and negative ions of nimorazole and misonidazole (Fig. 2) in electrospray ionization (ESI), and their fragmentation using collision-induced dissociation (CID) and electron-induced dissociation (EID). Importantly, ESI in negative mode has lead to the formation of radical anions of both radiosensitizers. It is well known that ESI transfers analyte ions from solution to gas phase, and the types of ions that are commonly observed are pseudomolecular even electron ions, such as protonated/deprotonated ($[M+nH]^{n+}$, $[M-nH]^{n-}$) or cationized analytes such as $[M+Na]^+$. In contrast, molecular radical ions are rarely observed. ESI formation of radical cations, $M^{\bullet +}$, occurs for easily oxidizable compounds [21–25], and is promoted by high ESI voltage, high analyte concentration and selection of solvent(s) that stabilize the radical ion. Formation of radical anions, $M^{\bullet -}$, in the negative ion mode of ESI was observed for molecules with high electron affinity. The first reports involved anions and dianions of fullerenes [26–29], followed by a variety of compounds [30–36] with the growing understanding of the electrochemical nature of the ESI [37]. Thus, the formation of $M^{\bullet -}$ and $[M-H]^-$ of simple related compounds shown in Fig. 2, i.e. *x*-nitroimidazoles and 1-methyl-*x*-nitroimidazoles (*x* = 2, 4, 5), was investigated in electrospray ionization and complemented by computational chemistry calculations.

2. Materials and methods

2.1. Materials

Misonidazole (**1**) and nimorazole (**2**) were a gift of Prof. Michael Horsman of Aarhus University Hospital and were used as received. 2-Nitroimidazole (**3**) and 4-nitroimidazole (**4**), which is a regioisomer of 5-nitroimidazole (**5**), were purchased from Aldrich (98%).

2.2. Synthesis of methylated nitroimidazoles

1-Methyl-2-nitroimidazole (**6**), 1-methyl-4-nitroimidazole (**7**), were synthesized via deprotonation of the parent nitroimidazole followed by methylation with methyl iodide using a modified procedure from Benjes and Grimmett [38], while 1-methyl-5-nitroimidazole (**8**) was prepared by the method of Groziak and Ding [39].

2.3. Mass spectrometry

All experiments were carried out on a Finnigan-LTQ-FT (Thermo, Bremen, Germany) mass spectrometer equipped with electrospray ionization (ESI) source [40,41] described in detail elsewhere [42,43]. Samples were dissolved in methanol right before infusing the reaction mixture into the mass spectrometer. In the case of nimorazole and misonidazole different solvents were used in order to determine the best solvent for promotion of the formation of radical anion $M^{\bullet -}$. The samples were immediately introduced to the mass spectrometer via the ESI source, using a flow rate of 5.0 μL/min. Typical ESI conditions used were: spray voltage, 3.0–5.0 kV, capillary temperature, 250–330 °C, nitrogen sheath pressure, 5–30 (arbitrary units). The capillary voltage and the tube lens offset were tuned to maximize the desired peak. The injection time was set using the automatic gain control function. The LTQ-FT mass spectrometer consists of: (i) linear ion trap (LTQ); (ii) ion transfer optics; and (iii) FT-ICR mass analyser. For the tandem mass spectrometry experiments, the desired ions produced via ESI were mass selected, trapped in the LTQ and subjected to CID at a He bath gas pressure of ca. 5×10^{-3} Torr. CID was carried out by mass selecting the desired ions with a 1.5–6 *m*/*z* units window and subjecting them to the following typical conditions: normalized collision energy between 16% and 40%, activation (*Q*) 0.25–0.35, and activation time of 30 ms. For high-resolution mass analysis, the ions were transferred via the ion optics transfer region ($\sim 2 \times 10^{-7}$ Torr) into an FT-ICR cell at a pressure below 1.5×10^{-9} Torr. The FT-ICR cell is supplied with low energy electrons produced by an indirectly heated emitter cathode located downstream of the FT-ICR cell. The energy of electrons is given by the potential difference between the emitter cathode with *ECD offset* of −4.2 V and the grid positioned