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High-energy collision-induced dissociation of radiosensitizer anions: Nimorazole and metronidazole

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ABSTRACT

The formation of radical anions of nitroimidazolic radiosensitizers, nimorazole and metronidazole, and their unimolecular dissociation reactions upon high-energy collisions with He gas were investigated with a double focusing mass spectrometer equipped with an electrospray ionization (ESI) source. Radical anions of both, nimorazole and metronidazole, are readily formed in the ESI process, while for the metronidazole also the deprotonated anion is formed. A variety of dissociation channels is observed and the associated kinetic energy released in the dissociation for most of these channels is reported. The marker anion NO_2^- is observed in the dissociation of all studied anions. The release of NO• radical from the radical anions is associated with surprisingly small kinetic energy release in comparison to simple nitroimidazoles. On the other hand, the highest kinetic energy release is noted for the release of the neutral side group from N1 position of the imidazole ring, which suggests that the substitution at the N1 plays a crucial role in the decomposition of anions of radiosensitizers.

1. Introduction

Half of the patients diagnosed with cancer are treated with radiation. The treatment is often based on the combination of surgery, chemotherapy and radiotherapy. The response of tumor cells to the radiation quality, such as radiation dose, type of radiation and energy of radiation, shows a wide variability from patient to patient [1]. In radiation therapy, the absorption of a high energy particle can lead to serious biological consequences, *e.g.*, damage to DNA through direct effect of ionization or indirectly through the formation of H•, •OH and carbon radicals [2,3] and secondary electrons [4]. These radicals can attack the nucleobases or the sugar backbone leading to the DNA strand breaks. Moreover, the oxygen present in cells can react with radicals and modify the response of the irradiated cells. When oxygen is not present, as in the case of hypoxic tumor cells, the same dose of radiation has been shown to be significantly less effective [5]. Thus, compounds that are capable of differentially sensitizing hypoxic cells and enhance the lethal effects of ionizing radiation, commonly called radiosensitizers, are in the development and under investigation [6].

One class of compounds that showed the ability to differentially accumulate in hypoxic cancer cells (or any cells deprived of oxygen) are nitroimidazolic compounds. They are under investigation not only as potential radiosensitizers [7] but also as potential imaging agents for hypoxic tumors [8]. Their ability to selectively accumulate in hypoxic cells is due to the radical induced chemistry taking place in an oxygen deficient environment [9] and the formation of radical anion is believed to be the first key step. These compounds are termed 'electron-affinic' as they have shown a relationship between the efficiency of sensitization and their electron affinity. One of the nitroimidazolic compounds, nimorazole, has been tested in the clinic and is in clinical use in radiotherapy in Denmark [10]. Other nitroimidazoles and related compounds were studied *in vitro* as well as *in vivo* with encouraging results [11]. However, the detailed mechanism of the actual radiosensitization is still unknown, which hinders the progress in the design of more efficient and less toxic treatment compounds.

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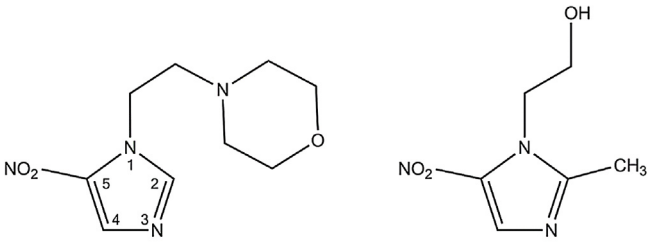

Scheme 1. Molecular structures of investigated radiosensitizers: Nimorazole (**1**) and metronidazole (**2**).

Recently, it has been shown that negative electrospray ionization (ESI) of a solution of nitroimidazolic radiosensitizers can lead to the formation of radical anions [12,13]. It is well known that ESI transfers analyte ions from solution to gas phase, and the type of ions that are commonly observed are pseudomolecular even electron ions. However, there are few examples of radical formation in the ESI process. While the formation of radical cations has been observed in positive ESI for easily oxidizable compounds [14,15], the formation of radical anions has been observed in negative ESI for molecules with high electron affinity [16–18] including nimorazole and other model nitroimidazoles [12,13]. The formation of radical anions of nitroimidazolic radiosensitizers in the gas phase offers the opportunity to study their production and fragmentation reactions, which are crucial in understanding the complexity of processes involved in the radiosensitization ability.

Nimorazole (Nimo = $[C_9H_{14}N_4O_3]$, **1**) and metronidazole (Metro = $[C_6H_9N_3O_3]$, **2**) are 5-nitroimidazolic compounds, *i.e.*, they have $-NO_2$ group attached at the C5 position of the imidazole ring (Scheme 1). They differ in the group attached to the N1 position. In addition, Metro has also an extra $-CH_3$ group attached at the C2 position. The formation of radical anions of both compounds in the negative ESI has been observed recently [12,13]. However, some instrumental differences have been also noted previously, namely, with an ESI source from Finnigan both anions of Nimo, $M^{\bullet -}$ and $[M-H]^-$ (M = molecule), were observed [12], while in the case of the Z-spray ESI source built by Waters only the radical anion of Nimo has been observed [13]. The fragmentation reactions of anions derived from Nimo, $M^{\bullet -}$ and $[M-H]^-$, have been investigated so far only in the low-energy collision-induced dissociation (CID) highlighting the significant differences in the decomposition of a radical anion $M^{\bullet -}$ in comparison with the even electron anion $[M-H]^-$ [12].

Here we investigate the fragmentation reactions of anions of Nimo and Metro after high-energy collisions with He atoms using a recently home build ESI source adapted to a double focusing mass spectrometer [19]. We have also evaluated the kinetic energy release (KER) for the most important dissociation channels.

2. Materials and methods

2.1. Materials

Nimorazole (purity ≥ 99%) was used as received from Toronto Research Chemicals (Canada). Metronidazole (purity ≥ 99%) was used as received from SIGMA-ALDRICH (Austria). The spray solution contained 10 mM of sample dissolved in methanol (HPLC grade). For the deprotonated metronidazole the solution was changed to a mixture of 1 H_2O (HPLC grade): 1 methanol (HPLC grade), while the sample concentration was the same.

2.2. Mass spectrometry

All experiments were carried out using a VG-ZAB-2SE (V.G. Analytical Ltd., Manchester, UK) double focusing mass spectrometer equipped with a home build ESI source, recently described in detail [19]. Briefly, the samples were introduced to the spray needle *via* an Elite Pump 11 syringe motor, using flow rates between 3 μL/min and 5 μL/min. A spray needle voltage in the range of −5.15 kV and −5.66 kV allowed continuous spray conditions towards the inlet capillary with an inner diameter of 750 μm. Furthermore the inlet capillary was heated up to 155–158 °C to prevent the capillary from clogging.

The mass spectrometer is built in reversed Nier-Johnson geometry, *i.e.*, ions are analyzed first by their momentum in a magnetic sector, then analyzed regarding their energy in an electric sector and finally detected with a secondary electron multiplier. The acceleration voltage for the ions to be analyzed in this instrument is −6 kV, therefore, the complete ESI-source is isolated from the analyzer to receive the required acceleration of the ion beam. For the investigation of CID a collision cell was mounted before the electric sector and was filled with helium as collision gas. The exact pressure in the cell is unknown. All pressure values mentioned in the Section Results and Discussion were determined with a pressure gauge mounted in the vacuum chamber of the collision cell. The stated values may be up to three orders of magnitudes lower than the real pressure in the cell [20].

The charged products formed in CID were probed using the mass analyzed ion kinetic energy (MIKE) scan method. The ratio of the precursor ion mass m_p and the resulting product ion mass m_f are related to the ratio of the corresponding electric sector field voltages E_0 and E, where those ions are transmitted through the electric sector:

$$E/E_0 = m_f/m_p \quad (1)$$

We note that for each precursor ion complete electric sector field voltage scans were done. Subsequently, only scan regions indicating a product ion were selectively measured with better statistics. The latter scans are shown below.

For the most abundant product ions we calculated the KER of the reaction by the following formula:

$$KER = \frac{y^2 m_1^2 eV}{16 x m_2 m_3} \left(\frac{\Delta E}{E}\right)^2 \quad (2)$$

where m_1 denotes the mass of the precursor ion and m_2 and m_3 respectively the masses of the neutral and charged products. The charge state of the precursor ion and the product ion (in our case both −1) are denoted by x and y and V is the acceleration voltage (−6 kV). E is the corresponding electric sector voltage and in the case of a Gaussian peak shape ΔE is the width of the peak in the MIKE scan (of which the width of the precursor beam has to be subtracted). See Ref. [21] for further details.

3. Results and discussion

3.1. Formation of anions $M^{\bullet -}$ and $[M-H]^-$

Fig. 1 shows negative ion ESI mass spectra of 10 mM solution of Nimo (Fig. 1a) and Metro (Fig. 1b) dissolved in methanol. In the case of Nimo (Fig. 1a) the radical anion is formed $Nimo^{\bullet -}$ in the ESI. No deprotonated anion is observed. This observation is in agreement with the recent study [13]. In the case of Metro (Fig. 1b), both anions are observed, *i.e.*, the radical anion $Metro^{\bullet -}$ and the deprotonated anion $[Metro-H]^-$. Both anions of Metro have been observed in the recent study, however, with different intensities, where the deprotonated anion $[Metro-H]^-$ showed to be the most abundant anion. Both of the compounds, Nimo and