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To cite this version:
Stéphane Le Gac, Btissam Najjari, Nicolas Motreff, Patricia Remaud-Le Saec, Alain Faiivre-Chauvet, et al.. Unprecedented incorporation of $\alpha$-emitter radioisotope 213Bi into porphyrin chelates with reference to a daughter isotope mediated assistance mechanism.. Chemical Communications, Royal Society of Chemistry, 2011, 47 (30), pp.8554-6. <10.1039/c1cc12455b>. <hal-00682434>
Unprecedented incorporation of α-emitter radioisotope $^{213}$Bi into porphyrin chelates with reference to a daughter isotope mediated assistance mechanism†

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Received 27th April 2011, Accepted 16th June 2011
DOI: 10.1039/c1cc12455b

For the first time, α-emitter radioisotope $^{213}$Bi has been incorporated into porphyrin chelates, with rates matching with the short period of the radionuclide. An in situ transmetalation mechanism involving the daughter isotope $^{209}$Pb is expected to boost the $^{213}$Bi radiolabeling process.

Compared with β-emitters, which have shown promising results in the radio-treatment of cancers, α-emitters have certain theoretical advantages as α particles (high energy helium nuclei) present higher linear energy transfer and shorter path lengths. These properties make targeted α-therapy appropriate for elimination of minimal residual or micrometastatic diseases. Bismuth α-emitter radioisotope $^{213}$Bi is one of the few α-particle emitting radionuclides that meet the parameters for a use in therapy, and clinical studies have demonstrated therapeutic activity of $^{213}$Bi labeled immunoconjugates. However, among various complex obstacles, further advances will require improved chelation techniques. Indeed, all studies have been performed with carriers derived from two chelating agents known as DTPA and C-DOTA. These poly-carboxylic acid ligands were developed in the early 80’s, and no innovating mechanisms, kinetics and stability lie in the coordination of the intramolecular carboxylate group on the bismuth cation (X-ray structure, Fig. 1) is easily purified on silica gel and is remarkably resistant to the addition of a large excess of trifluoroacetic acid (ESI†). Both kinetics and stability lie in the coordination of the intramolecular carboxylate group on the bismuth cation (X-ray structure, Fig. 1). Its bifunctional analogue 2 was recently synthesized (Fig. 1), and similar binding properties towards bismuth(III) were observed. Ligand 1 was also shown to complex lead(II) instantaneously at room temperature. Such coordination properties are of great interest in the context of α-radiotherapy since a $^{212}$Pb isotope has been evaluated as an in-vivo generator for the production of a $^{212}$Bi α-emitter isotope, which results in an artificially extended half-life of ~11 hours. In addition, we found that no demetalation of complex 1Bi occurred upon

Fig. 1 Left: chemical structures of over-hanged carboxylic acid porphyrins 1 and 2. Right: X-ray structure of 1BiDMSO (hydrogen atoms removed for clarity).
standing 8 hours in cell culture media (ESI†). This allowed us to determine the cytotoxicity of both the free-base and its bismuth complex (prepared with the cold isotope $^{209}$Pb) using HT29 colon carcinoma and HeLa cervical carcinoma cell lines (ESI†). I and IB have a low cytotoxic effect on both cancer cells with an IC$_{50}$ value for compound I between 25–50 μM depending on the cell line, and an IC$_{50}$ value close to 100 μM for compound IB in both cell lines.

For all these reasons, ligands I and 2 were thought to be good candidates for $^{213}$Bi complexion assays. It is worth noting that such experiments are scarcely reported due to limited supply of $^{213}$Bi isotope. The α-emitter $^{213}$Bi isotope was eluted from an $^{225}$Ac/$^{213}$Bi generator with a solution of HCl/NaI (1 : 1, 0.1 M), following a standard protocol. From radioactivity counting, the initial concentration of $^{213}$Bi in the eluate was determined as ca. 0.3 nM. Nanomolar affinity is obviously difficult to achieve and, in this first study, we used a rather high concentration of ligands (1.2 μM). $^{213}$Bi incorporation was monitored by radio-TLC (Fig. 2a). In a CH$_2$Cl$_2$/MeOH (9 : 1, v/v) mobile phase, complexes $^{213}$Bi and $^{213}$Bi are identified as a $R_f$ ≈ 0.5 spot, whereas the remaining $^{213}$Bi salts are observed as a $R_f$ ≈ 0 spot.

The initial low pH value (≈ 1) of the $^{213}$Bi eluate prevents metal incorporation into our porphyrin ligands and pH first has to be adjusted. Four pH values ranging from 5 to 8 were studied at 75 °C while the reactions were monitored over 30 min (Fig. 2b). The first observation concerns the pH 5 value. Whereas this value is optimal for DTPA, with chelate I, no incorporation occurred even after 30 min. This observation is consistent with a first protonation $pK_a$ of the macrocycle around 4.5. Conversely, at pH 6, the percentage of incorporation increased at an almost constant value of 25%. To our delight, a significant improvement of the incorporation was measured when pH was raised up to 7 or 8 since, after only 5 min, incorporation was found as high as 80%. These fast kinetics are obviously of great interest considering the short half-life of $^{213}$Bi (45.6 min). Over a 30 min period, incorporation has decreased down to 60% and 50% at pH values 7 and 8, respectively. This indicates that pH 7 is an optimal value for this type of porphyrin chelate.

Secondly, the effect of temperature at a fixed, optimal pH value was investigated. For targeted radiotherapy, tumor-specific conjugates are coupled to the chelate of interest prior to radioisotope incorporation. Whereas radiolabeling of hapten-functionalized chelates can be performed at high temperatures (≈ 80 °C), chelate-appended antibodies do not tolerate temperatures above 40 °C. As shown in Fig. 2c, the percentage of $^{213}$Bi incorporation into chelate I was half as important at 40 °C as at 75 °C. However, approximately 35% of incorporation was observed after only 10 min, which remains quite remarkable. Lowering the temperature down to 20 °C led to a slower rate of incorporation but yet a ca. 25% value was found.

These investigations were extended to the related bifunctional chelate 2 (ESI†). No significant differences were observed: at pH 7, up to 75% and 30% $^{213}$Bi incorporation values were obtained at 75 °C and 40 °C, respectively, in less than 10 minutes. At this early stage, these results indicate that with such chelates, both targeting strategies mentioned above should be investigated to deliver the radionuclide.

Fig. 2 (a) Radio-TLC at t = 10 min corresponding to the monitoring of $^{213}$Bi insertion in ligand I, at T = 75 °C, for different pH values. Influence of pH (b) and temperature (c) on the rate of $^{213}$Bi incorporation into ligand I (b: experiments performed at 75 °C; c: experiments performed at pH 7).

These fast complexation rates were unexpected working with porphyrin ligands at nanomolar concentration of metal ions. Additional kinetic studies were performed with cold isotopes of bismuth and lead and revealed an interesting metalation mechanism. It should first be reminded that $^{209}$Pb is a daughter nuclide of $^{213}$Bi, with a half-life of 3.3 h, and therefore accumulates during the radiolabeling process (see decay scheme in ESI†). The rates of Bi(III) and Pb(II) insertion into porphyrin I were monitored by UV-vis spectroscopy at μM concentration in dimethylsulfoxide solution. A half-time of reaction of approximately 10 min was found for bismuth (Fig. 3a), while lead insertion proceeded in only a couple of seconds (Fig. 3b, 1st step). Very interestingly, the addition of the bismuth salt to the lead complex of I led instantaneously to the formation of the bismuth complex (Fig. 3b, 2nd step): now, the half-time of reaction is lower than 2 seconds! The same behavior was observed with the bifunctional chelate 2 (ESI†). The half-time of reaction for bismuth insertion dropped from ∼7 min for the direct complexation down to ∼12 seconds when lead was previously inserted. It thus appears that the transmetalation reaction proceeds spectacularly faster, that is with ∼300 and ∼35 fold increases in the rate of bismuth insertion for ligands I and 2, respectively. In such a lead-mediated bismuth insertion process, two successive mechanisms of activation actually occur: (i) the instantaneous lead complexation likely proceeds via a deconvolution mechanism involving a hanged carboxylate group and facilitating a sitting atop complex. Indeed, heating is required in the case of related...
compounds with hanged ester groups,\(^{10}\) (ii) we postulate that, in addition to this deconvolution mechanism, the deformation of the porphyrin core induced by the out-of-plane coordination of lead\(^{66}\) promotes bismuth insertion. Such a distortion-mediated insertion mechanism is involved in chelatase-catalyzed insertions of metal ions into porphyrins.\(^{11}\) It has also been evaluated for instance in the case of the mercury-mediated zinc insertion,\(^{12}\) as well as in the case of the lead-mediated zinc and manganese insertion.\(^{13}\) Here, in contrast to the quasi in-plane complexation displayed by e.g. zinc, bismuth and lead cations are both complexed significantly out-of-plane. However, the bismuth complexes of 1 and 2 are far much stable than their lead counterparts which makes such an assistance mechanism possible.

Based on these results, a similar process might be anticipated for the labeling of ligand 1 with \(^{213}\)Bi, which is a daughter-mediated parent isotope insertion mechanism (Fig. 4). Indeed, as mentioned above, the daughter \(^{209}\)Pb isotope accumulates in the eluate from the generator while \(^{213}\)Bi decays. \(^{209}\)Pb is therefore expected to enhance the rate of \(^{213}\)Bi incorporation through an in-situ transmetalation process. To our knowledge, such a possibility of having a daughter isotope mediated assistance mechanism has never been described with the classical chelates used for \(^{213}\)Bi complexation (DOTA and DTPA derivatives). For the latter, complexation of lead and bismuth cations has been only studied in the context of an in-vivo generator for the production of \(^{212}\)Bi from the parent \(^{212}\)Pb.

In conclusion, we have evidenced for the first time that a suitably functionalized porphyrin macrocycle can be efficiently labeled with an \(\alpha\)-emitter radionuclide of particular interest for cancer radiotherapy. The fast rate of \(^{213}\)Bi incorporation is remarkable and matches with the short half-life of the radionuclide. An unprecedented transmetalation mechanism involving a daughter radionuclide is expected to increase the rate of \(^{213}\)Bi complexation. These first results pave the way for further investigations with related porphyrin chelates, and work towards improved chelation properties and biodistribution assessment is underway. All in all, this contribution shows that innovative chelates for a radioelement might come from a completely divergent approach. We hope that this work also may serve as a source of new inspirations for the design of radiolabeling agents.

We thank Gwénaëlle Le Moigne-Muller for technical assistance. CNRS, Région Bretagne and La Ligue are acknowledged for financial support.

**Fig. 4** Schematized assistance mechanism transposed from the cold isotopes to the case of the hot isotopes: \(^{213}\)Bi insertion into chelate 1 mediated by the daughter isotope \(^{209}\)Pb.

**Notes and references**