^{211}At-Rh(16-S4-diol) COMPLEX AS A PRECURSOR FOR ASTATINE RADIOPHARMACEUTICALS

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^{211}At is one of the most promising radionuclides in α-radioimmunotherapy (α-RIT). Its 7.2 h half-life is sufficient for the radionuclide production, transportation, synthetic chemistry, quality control and biological application in the treatment of certain cancer diseases. The α particles with a mean energy of 6.4 MeV have a mean range in human tissue of 65 μm. Therefore, this nuclide may be useful for the treatment of small clusters of cells or single cells, micrometastatic diseases, leukemias, and lymphomas. Dosimetry calculations and preclinical therapeutic research with ^{211}At have demonstrated its highly toxic effects on tumor cells [1-5]. The short path length of the α particles also limits their toxicity to neighboring normal tissue. The additional electron capture (EC) decay gives rise to high intensity X-rays from the daughter ^{211}Po, making ^{211}At easy to follow with gamma-cameras [6].

^{211}At labeled immunoconjugates have been synthesized and evaluated for their therapeutic potential. Unfortunately, biomolecules labeled by direct electrophilic astatination are unstable due to the rapid loss of ^{211}At under both *in vitro* and *in vivo* conditions [7]. Better stabilization of the weak astatine-carbon bond is observed for proteins astatinated by acylation with a variety of astatobenzoic acid derivatives prepared from trialkylstannyl precursors [8,9].

The purpose of this work was to bind astatide anion At^-, the most stable oxidation state of astatine, with a biomolecule by attaching At^- to a metal cation in a chelate. It can be expected that At^-, similarly to iodide anion I^-, should demonstrate soft ligand properties and form strong complexes with soft metal cations, like Hg^{2+}, Pt^{2+}, Rh^{3+}, Ir^{3+}. In a previous work, we have shown that Hg^{2+} cations form strong complexes with At^-, much stronger than those with I^- [10]. The present paper describes the results of our studies on attaching At^- to the rhodium(III) complex with thioether ligand: 1,5,9,13-tetrathiacyclohexadecane-3,11-diol (16-S4-diol). Rh^{3+} was chosen as a moderately soft metal cation which should form very strong bonds with soft At^- anions, but first of all because of the kinetic inertness of low spin rhodium(III) d^6 complexes. The 16-S4-diol ligand was selected due to formation of stable complexes with Rh^{3+}, as reported in [11]. Additionally, this macrocyclic tetrathioether with the diol functionality offers a site for chemical modification in the synthesis of bifunctional chelating ligand.

Because the availability of ^{211}At is limited, the experiments related to optimization of the reaction conditions were performed with the ^{131}I, basing on a chemical similarity of I^- to At^-. The experiments with ^{211}At were then carried out under the conditions found optimal for I^-.

^{131}I-Rh(16-S4-diol) and ^{211}At-Rh(16-S4-diol) complexes were prepared by addition of $^{131}I^-$ or $^{211}At^-$ activity to the mixture of rhodium(III) nitrate and 16-S4-diol in water-ethanol solution. After adjusting pH to 4.0 by dropwise addition of 0.01-0.1 M nitric acid, the solution was heated for 1-2 h at 80°C. The syntheses were optimized to increase the yield of the obtained complexes with respect to time, temperature, pH, and the concentrations of rhodium(III) and the ligand. The complexes obtained were analyzed by thin-layer chromatography (TLC), paper electrophoresis and ion exchange chromatography (IC). The stability of the ^{131}I-Rh(16-S4-diol) complex was studied at different temperatures in phosphate-buffered saline (PBS) at pH 7.4.

In the complexes obtained, the sulfur donor atoms of the 16-S4-diol ligand occupy equatorial positions, whereas ^{211}At (^{131}I) and OH^- anions are in the axial positions (Fig.1). The formation of ^{131}I and ^{211}At rhodium complexes with thioether ligand

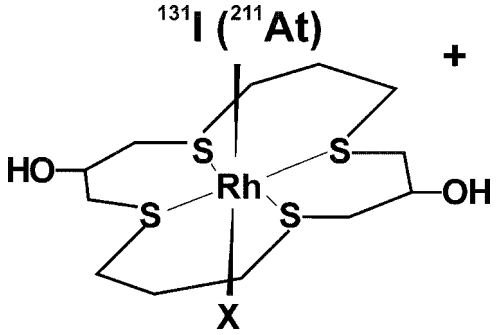

Fig.1. The proposed structure of the complexes, where X=NO_3^-, OH^- or H_2O.

was studied using mainly the electromigration method. As shown in Fig.2A, the ^{131}I-Rh(16-S4-diol) complex was a cation and migrated to the cathode, whereas uncomplexed $^{131}I^-$ migrated to the anode. The obtained results are consistent with the data on rhodium(III) chloride complexation with 16-S4-diol ligand, previously published [11].

The interaction of $^{131}I^-$ with Rh^{3+} cations in the absence of the 16-S4-diol ligand has been studied in control experiments. The products of the reaction between rhodium and iodide (Fig.2B) were neutral and remained at the starting point. These results indicate that $Rh(OH)_2I$ is probably formed under these conditions.

The results obtained by the TLC method confirm the formation of a complex between $^{131}I^-$ and Rh^{3+} with the macrocyclic thioether ligand. The experiments on cellulose plates gave the value R_f=0.55-0.6 for ^{131}I-Rh(16-S4-diol) complex, while for uncomplexed $^{131}I^-$ R_f=1.0. The rhodium-iodide species remained at the origin, when eluted with PBS or methanol-PBS (80-20%).

The kinetics of ^{131}I-Rh(16-S4-diol) formation was studied as a function of time (15-120 min) and temperature (30-90°C). The equilibrium was attained after 50 min heating the solution at 80°C. The kinetics of formation sharply increases at a temperature higher than 40°C. The high yields of

the complex (>90%) can be obtained even when the complexation reaction was performed at a 1:1 stoichiometry ratio of rhodium(III) nitrate to 16-S4-diol at pH 4.0, but the rhodium concentration should not be less than 10^{-4} M; otherwise the yield decreases rapidly. The experiments under physiological conditions show that the complex obtained is stable for a long time. The complex was stable almost for 5 days of incubation at room temperature in 0.02 M PBS at pH 7.4. The experiment was performed by paper electrophoresis.

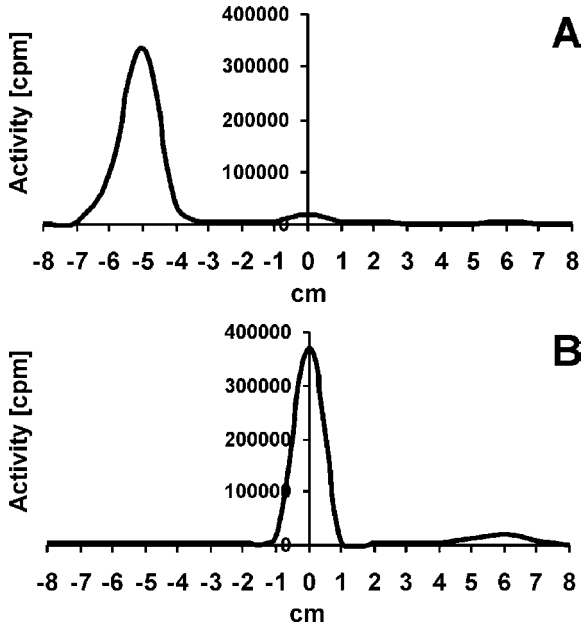

Fig.2. Electrophoretic analysis: (A) cationic complex ^{131}I-Rh(16-S4-diol), (B) mixture of rhodium(III) with ^{131}I, heated for 2 h at 80°C without the thioether ligand. Zero point on each chromatogram is the origin, while the directions of the cathode and anode are toward the -8 and +8 points, respectively.

Preliminary experiments on the synthesis of the ^{211}At-Rh(16-S4-diol) complex were performed using the same procedure as that elaborated with ^{131}I. Astatine was reduced to At^- by sodium sulfite or sodium borohydride in a methanol or water solution. The At^- solution was added to a mixture of rhodium(III) nitrate with 16-S4-diol in ethanol and acidified to pH=4.0, and heated for 1-2 h at 80°C. The synthesis products were analyzed by electrophoresis (Fig.3A). Control experiments were also performed to check whether any interactions occur between ^{211}At and rhodium(III) (Fig.3B).

The obtained results confirmed a similar behavior under the same conditions of the astatine compounds with that of the ^{131}I complexes. The ^{211}At-Rh(16-S4-diol) complex was also cationic (Fig.3A). Reaction of rhodium(III) with ^{211}At in the absence of 16-S4-diol gave the same results as with ^{131}I. The formed compound did not migrate to none of the electrodes (Fig.3B).

The preliminary results with ^{211}At are promising, and indicate a possibility for astatination of

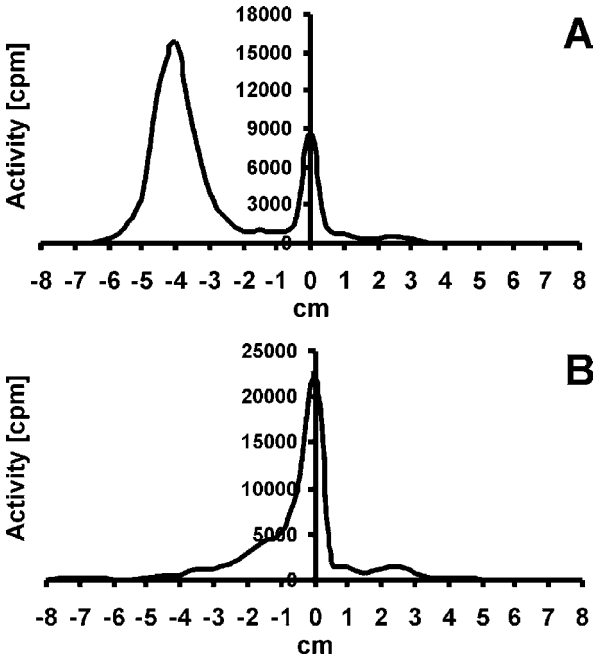

Fig.3. Electrophoretic analysis of ^{211}At-Rh(16-S4-diol) complex (A), and Rh(III)-^{211}At complex (B). Starting point on each chromatogram is the origin, while the directions to the cathode and anode are toward -8 and +8, respectively.

biomolecules by using the ^{211}At-Rh(16-S4-diol) complex. The future investigations will be related to stability studies of the astatide complex and to the possibility of linking the ^{211}At complex to biomolecule.

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