13rd International Electronic Conference on Synthetic Organic Chemistry (ECSOC-13), 1-30 November 2009.
http://www.mdpi.org/ecsoc-13/ & http://www.usc.es/congresos/ecsoc/13/

[c006]

Synthesis and characterization of Gd-C_4-thyroxin-DTPA, a potential new MRI contrast agent. Study of its non covalent interaction with human serum albumin.

C. Henoumont, L. Vander Elst, S. Laurent, R.N. Muller

University of Mons, Department of General, Organic and Biomedical Chemistry, Laboratory of NMR and Molecular Imaging, Mendeleev Building, 19 Avenue Maistriau, 7000 Mons, Belgium.

Abstract :

The synthesis and the physicochemical characterization of a new contrast agent for magnetic resonance imaging (MRI), Gd-C_4-thyroxin-DTPA, which has a high affinity for human serum albumin (HSA), are reported. The results show that this chelate is characterized by a relatively high relaxivity, which increases moreover with the concentration. This reflects an aggregation of the molecules in solution. It is also characterized by a better stability versus the transmetallation with the zinc ion than the parent compound, the Gd-DTPA (Magnevist®, Bayer Healthcare). The study of its interaction with human serum albumin was performed by the proton relaxometry technique, which has revealed a relatively high affinity (Ka of the order of 10000 M^{-1}, with 2 binding sites). Finally, competition experiments with ibuprofen and salicylate, of which the binding sites on HSA are known, were performed by the NMR diffusometry method. The results suggest that the chelate shares one of the binding site of ibuprofen.

Introduction :

As compared to other imaging techniques, magnetic resonance imaging (MRI) has better spatial and temporal resolutions but its sensibility is relatively weak. In most cases, the use of contrast agents is required. Most of them are gadolinium complexes, which have the property of increasing the signal of the pathological zones, improving the image contrast. Very efficient contrast agents, i.e. contrast agents with a high proton relaxivity (defined as the increase of the water proton relaxation rate induced by 1 mmole per liter of contrast agent), are thus needed. This can be obtained for example with molecules interacting non-covalently with endogenous macromolecules, like human serum albumin (HSA).
The synthesis and the physicochemical characterization of a potential MRI contrast agent which has a high affinity for HSA, Gd-C_4-thyroxin-DTPA (figure 1), are reported. The study of its interaction with albumin was performed by proton relaxometry [1] and NMR diffusometry [2].

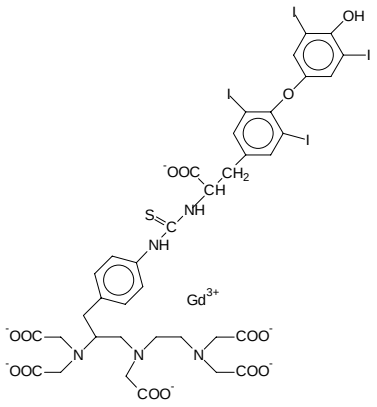

Figure 1 : Chemical structure of Gd-C_4-thyroxin-DTPA.

Results and discussion :

Gd-C_4-thyroxin-DTPA was first characterized in water by the measurement of its NMRD (Nuclear Magnetic Resonance Dispersion) profile on different solutions of increasing concentration. The observed relaxivity of the chelate increases with the concentration, particularly between 20 and 60 MHz : at 20 MHz, the relaxivity increases from 9.3 s^{-1} mM^{-1} for a concentration of 0.5 mM in the gadolinium chelate, to 28.7 s^{-1} mM^{-1} for a concentration of 1.65 mM. This can be explained by the formation of aggregates between the molecules in solution.

The gadolinium ion is very toxic, and it is thus important to verify the kinetic inertia of the chelate towards the exchange with endogenous ions. The stability of the complex was tested in the presence of zinc ions. In the blood, this ion is indeed the most likely to challenge the gadolinium ion because of a similar ionic radius and a quite large concentration. The results show a markedly higher stability of Gd-C_4-thyroxin-DTPA in comparison with the parent compound Gd-DTPA (Magnevist®, Bayer Healthcare).

The affinity of Gd-C_4-thyroxin-DTPA for HSA was evaluated by proton relaxometry. This method consists in measuring the water proton relaxation rate on different solutions of HSA 4% with increasing concentrations in the contrast agent. For example, when the concentration of the gadolinium chelate is of 0.5 mM, an increase of the relaxation rate equal to 380 % was observed in the presence of HSA. The use of a model where all the binding sites are considered to be identical and independent allows to estimate the association constant and the number of binding sites. In the case of Gd-C_4-thyroxin-DTPA, an association constant of the order of 10000 M^{-1} with 2 binding sites was obtained, which reveals a relatively high affinity of this chelate for HSA.

Competition experiments with ibuprofen (which has its strong binding site on the Suddlow site II of HSA) and with salicylate (which has its strong binding site on the Suddlow site I of HSA) were performed by NMR diffusometry in order to evaluate the binding site of Gd-C_4-thyroxin-DTPA on HSA. In these experiments, the diffusion coefficient of ibuprofen (10 mM) or salicylate (10 mM) was measured in the presence of the europium complex (2 mM) and of HSA 4%. The results show that the presence of the europium chelate increases strongly the diffusion coefficient of ibuprofen (by 19%) but has little effect on the diffusion coefficient of salicylate (3%). This suggests thus that the chelate shares one of the binding sites of ibuprofen.

General experimental procedure :

C_4-thyroxin-DTPA was synthesized by reaction between isothiocyanatebenzyl-DTPA (Macrocyclics, Dallas, USA) and the amine function of thyroxin (Sigma, Bornem, Belgium) (figure 2).

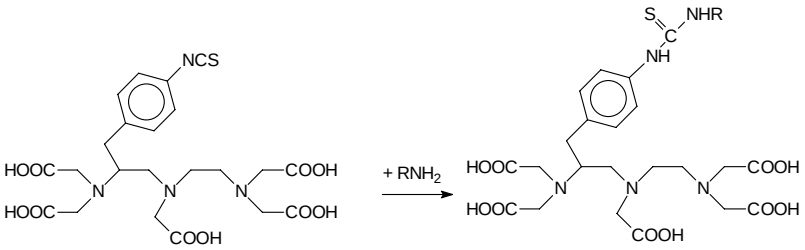

Figure 2 : Synthesis of C_4-thyroxin-DTPA.

The complexes are obtained by reaction of the ligand with gadolinium chloride for the measurement by proton relaxometry, and with europium chloride for the measurement by NMR diffusometry (because of the very broad signals that the gadolinium ion induces in an NMR spectrum).
The NMRD (nuclear magnetic resonance dispersion) profiles were recorded on a fast field cycling relaxometer (Stelar, Mede, Italy) working between 0.24 mT and 0.24 T. The additional relaxation rates at 0.47 T, 1.41 T, 7.05 T and 11.75 T were measured on Minispec mq-20 and mq-60 (Bruker, Karlsruhe, Germany), and on high resolution spectrometers AMX300 and AVANCEII-500 (Bruker).
The measurement by proton relaxometry were performed at 0.47 T and 310 K on a Minispec mq-20 (Bruker) and the measurement by NMR diffusometry were performed at 4.7 T and 310 K on an AVANCE-200 spectrometer (Bruker). In this last case, the sequence used is PGSE (pulsed field gradient spin echo) with values of δ and Δ of 1 and 4 ms respectively. The temperature is maintained at 310 K by circulation of water in the gradient coil (water bath HAAKE UWK 45).

References :

[1] R.N. Muller, B. Radüchel, S. Laurent, J. Platzek, C. Piérart, P. Mareski, L. Vander Elst, Eur. J. Inorg. Chem. (1999) 1949-1955.
[2] L.H. Lucas, C.K. Larive, Concepts Magn. Res. Part A 20A(1) (2004) 24-41.