Synthesis, Kinetic Stability, Relaxometry and X-ray Crystal Structures of Cu(II), Mn(II), and Gd(III) 18-Membered Hexaazamacrocyclic Complexes as Potential MR Contrast Agents

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Introduction

Macrocyclic complexes of gadolinium (Gd) such as DOTA are already clinically used as MR contrast agents. However, the search for new macrocyclic complexes of Gd [1] as well as manganese (Mn) and copper (Cu) continues. The clinical application of any new MR macrocyclic-based contrast agent will depend on a variety of factors, including but not limited to: water solubility, relaxivity, stability (thermodynamic and kinetic), ease of synthesis and derivatization, biodistribution and clearance. We have synthesized the Cu(II), Mn(II) and Gd(III) metal complexes of a previously reported helical eighteen-membered macrocycle (PYAN) that has shown high thermodynamic stability with a variety of metal ions.[2-6] The PYAN macrocycle is composed of two pyridine rings which are connected by diaminoethane linkages. In addition, we report the kinetic stability in human serum, the T1 and T2 NMRD profiles and the X-ray crystal structures of each macrocyclic complex.

Methods

The PYAN macrocycle was synthesized based on modifications of a previous preparation.[2-4] The PYAN metal complexes were prepared from a 1:1 mole ratio of an aqueous solution of the free base of PYAN with an aqueous solution of the appropriate metal salt. Reduction of the solvent yielded crystals of each metal complex. X-ray structure determinations were performed by Crystalytics, Inc. (Lincoln, NE). The T1 and T2 NMRD profiles were obtained on a variable-field T1-T2 analyzer. The appropriate radioactive complexes were formed by incubating 100 microliters of a 1 mM solution of PYAN with 1-3 microliters of the appropriate radioactive metal ion solution (30-100 microcuries) for 1 h. The degree of complexation was determined by developing 10-cm TLC strips in 1:1 methanol:10% ammonium acetate buffer. In this sytem, free metal ions stay at the point of application while PYAN metal complexes move along the strip. The strips were cut into 1 cm segments and the radioactivity counted in a gamma-counter. The radioactive metal complexes (75 microliters) were incubated with 2 mL human serum (Gemini Bio-Products, CA) for up to 8 d. Aliqouts were withdrawn at time 0 (initial), 0.4 h, 1 h, 24 h and then every day for up to 8 d and analyzed by HPLC on a polymer-based size-exclusion column eluted with PBS, pH 7.2.

Results

The reaction of the fully deprotonated PYAN macrocycle with a 1:1 mole ratio of the appropriate non-radioactive metal ion salts yielded single crystalline complexes having elemental compositions corresponding to macrocyclic complex formation. The X-ray crystal structures of the CuPYAN and MnPYAN reveal that the macrocyclic ring is twisted to accomodate the metal ions in a six-coordinate helical fashion. The GdPYAN structure is composed of the macrocyclic complex and nitrate anions. The Gd(III) ion is decacoordinate. The coordination environment is composed of four nitrate oxygen atoms from two nitrate anions and all six of the nitogen atoms belonging to the macrocycle. Qualitatively and quantitatively, the NMRD profiles for the MnPYAN and CuPYAN are characteristic of Mn and Cu complexes which are coordinatively saturated by the ligand and the T1 and T2 relaxivities are dominated by outer-sphere relaxation. The NMRD profile for GdPYAN has contributions arising from both outer-sphere relaxation and inner-sphere dipole-dipole relaxation. The PYAN complexes of Cu-67 and Mn-54 showed excellent stability in human serum with both complexes preserving their integrity and existing in non-protein bound form for up to at least 8 d. The PYAN complex of Gd-153 failed to elute from the size-exclusion column. In 24 h, 100 % of Gd-153 radioactivity was bound to serum proteins.

Discussion

The triflate salts of CuPYAN and MnPYAN readily crystallize from aqueous solvent after volume adjustment. Attempts to crystallize GdPYAN as the triflate salt were unsuccessful and required the nitrate salt for crystallization. The crystal structure of GdPYAN as the nitrate salt shows that the two nitrate ions coordinate in a bidentate fashion to the Gd ion. The structures of the CuPYAN and MnPYAN reflect the rigid, helical nature of the complexes . If used in-vivo, the helicity of the complexes may allow for preferential interaction with DNA or other proteins. Quantitatively, the magnitude of the T1 relaxivities for CuPYAN and MnPYAN are similar to previously reported Mn and Cu complexes which have no inner-sphere water molecules and are coordinatively saturated by the ligand. The outer-sphere relaxation contribution is expected for coordinatively saturated metal ions and indicates that CuPYAN and MnPYAN remain hexa-coordinated to PYAN in solution. Since the outer-sphere relaxivity scales to the number of unpaired electrons for similar macrocyclic complexes, the five unpaired electrons for Mn quantitatively results in higher outer-sphere relaxivity than for the one unpaired electron for Cu. The higher coordination number required by Gd compared to Mn or Cu results in relaxivities that are dominated by inner-sphere relaxation when water molecules displace the nitrate ions. Potentially, up to four water molecules could transiently be bound to the Gd ion. The inner-sphere relaxation mechanism significantly increases both the T1 and T2 relaxivities for GdPYAN. Serum stability measurements are useful for the evaluation of the kinetic stability of metal complexes. The extreme kinetic instability of GdPYAN results from four vacant coordination sites of Gd which are filled by metal-binding groups of serum proteins, thus causing complete dissociation of the complex. The remarkable serum stability of the CuPYAN and MnPYAN complexes results not only from full saturation with six PYAN nitrogens of the Cu and Mn coordination spheres, but may also result from d-electron participation. The participation of the d-electrons in complex formation of transition metals is known to contribute to the stability of such complexes. The structure of the PYAN macrocycle is such that more lipophilic constituents can be added. These modifications could enhance the relaxation times of these complexes and therefore maximize their potential as future contrast agents.

References

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