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Fe(III)-Templated Gd(III) Self-Assemblies—A New Route toward Macromolecular MRI Contrast Agents[1]

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The Solomon−Bloembergen−Morgan theory predicts that the longitudinal relaxivity of a small Gd(III) complex, r_{1p}, increases upon slowing down its rotational correlation time. This is usually achieved by grafting the complex onto a macromolecule. Previous efforts in designing such macromolecules have concentrated on the synthesis of dendrimeric analogues and covalent or reversible protein conjugates.[2] These studies demonstrated the necessity not only of attaining fast water exchange but also of creating rigid systems in attaining high relaxivity agents.[3]

Surprisingly, there has been little study of supramolecular self-assemblies. These complexes, however, present several advantages, as their syntheses require fewer steps and they can be highly rigid. Although several mixed f block/d block self-assemblies have already been described,[4] the macromolecular MRI contrast agents studied have been only Gd(III) chelates grafted onto tris-bidentate Fe(III) complexes.[5] However, these do not constitute true supramolecular self-assemblies[6] in that, in each case, the Gd(III) complexes were already completely synthesized prior to building the macromolecule.

Herein we describe our approach to Gd(III)-containing supramolecular self-assemblies using Fe(III) as a template. Bis-bidentate ligands were designed comprising two different binding sites, one more selective for Gd(III) and the other for Fe(III). It has previously been shown (Figure 1) that Gd(III) is selective for 1-methyl-2,3-dihydroxypyridinone (HOPO) over 2,3-dihydroxyterephthalamide (TAM) ($pGd_{TREN-trisHOPO} = 19.2$, $pGd_{TREN-trisTAM} < 13$),[7] whereas Fe(III) is selective for TAM over HOPO ($pFe_{TREN-trisHOPO} = 26.8$, $pFe_{TREN-trisTAM} = 34.2$).[8,9] It was therefore predicted that three bis-bidentate ligands, each comprising both one HOPO and one TAM moiety, would coordinate both one Gd(III) and one Fe(III).

In the complexes studied (Figure 2), each ligand contains one TAM moiety (for Fe(III) coordination) linked to either one or two HOPO moieties (for Gd(III) coordination) through a three-carbon linker. This linker has been shown to favor the formation of triple-stranded helicates.[10] In Fe,Gd-**L1**, the three strands are joined by a TREN backbone, while in the other two complexes Fe,Gd-3**L2** and Fe,2Gd-3**L3**, they are left free to self-assemble (or not). All complexes are 3− charged. In Fe,Gd-3**L2**, a small PEG chain was grafted for increased water solubility.

The mixed complexes were synthesized in six, four, and three steps, respectively, starting from previously described building blocks. Formation of the mixed self-assemblies, with the correct ratio of Gd(III) and Fe(III), was confirmed by high-resolution electrospray mass spectrometry. The spectrum of Fe,2Gd-3$\mathbf{L}_C$ (Figure 3), for instance, clearly shows only one product, containing one Fe(III) and two Gd(III).

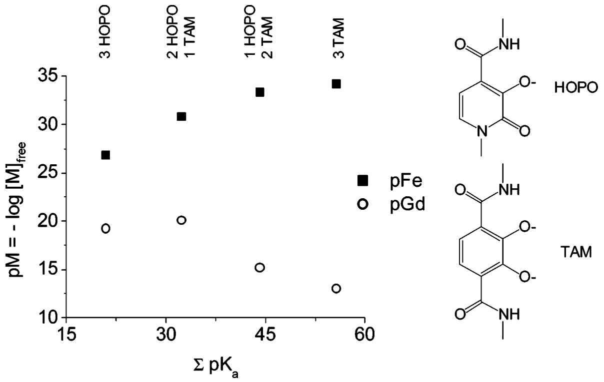

Figure 1. Selectivity of 1-methyl-2,3-dihydroxypyridinone (HOPO) and 2,3-dihydroxyterephthalamide (TAM) for Fe(III) over Gd(III).[7,8]

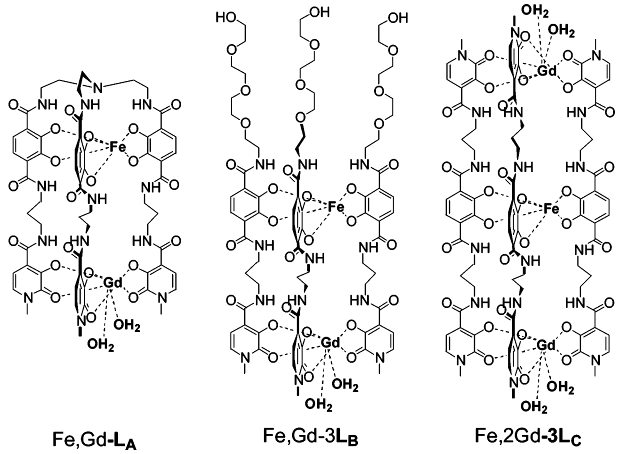

Figure 2. Heteronuclear Fe(III)-templated Gd(III) self-assemblies based on a three-carbon linker.

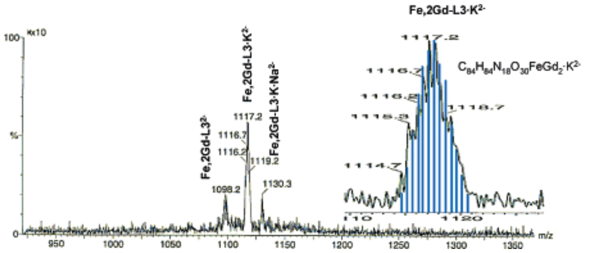

Figure 3. Electrospray mass spectrum of Fe,2Gd-3$\mathbf{L}_C$. The calculated (blue) and experimental (black) isotopic distributions of the molecular ion are shown in the expanded region.

The nuclear magnetic resonance dispersion (NMRD) profiles of the heteronuclear self-assemblies are shown in Figure 4. Since not all complexes bear the same number of Gd(III) ions, the relaxivities

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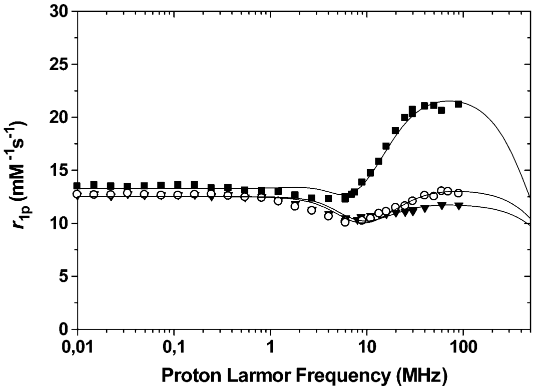

Figure 4. $1/T_1$ NMRD profile at 298 K and pH 7.4 of Fe,Gd-$\mathbf{L}_A$ (▼), Fe,Gd-3$\mathbf{L}_B$ (○), and Fe,2Gd-3$\mathbf{L}_C$ (■). Longitudinal relaxivities are given per Gd(III) ion.

Table 1. Refinement Parameters of the $1/T_1$ NMRD Profiles of Fe,Gd-$\mathbf{L}_A$, Fe,Gd-3$\mathbf{L}_B$, and Fe,2Gd-3$\mathbf{L}_C$

	Fe,Gd-$\mathbf{L}_A$	Fe,Gd-3$\mathbf{L}_B$	Fe,2Gd-3$\mathbf{L}_C$
r_{1p} at 20 MHz (mM^{-1} s^{-1})	11.0	11.5	18.7
q	2	2	2
τ_R (ns)	150	172	312
Δ^2 (s^{-2} × 10^{19})	5.4	6.5	4.9
τ_v (ps)	25.9	22.5	31.7
τ_M (ns)^a	10	10	10
r (Å)	3.06	3.06	3.06
a (Å)^a	4.0	4.0	4.0
D (cm^2 s^{-1} × 10^5)^a	2.24	2.24	2.24

[a] Fixed.

given are per millimolar concentration of Gd(III) for direct comparison. The complexes have high relaxivities, with a maximum centered around 60−100 MHz. The relaxivity of the trinuclear assembly, Fe,2Gd-3$\mathbf{L}_C$, is r_{1p} = 21 mM^{-1} s^{-1} per Gd(III) (or 42 mM^{-1} s^{-1} per molecule) at 90 MHz, an unprecedented high value, especially given the relatively small molecular weight of the assembly. Unlike macromolecules based on commercial contrast agents, the relaxivity of the supramolecules increases significantly at the high Larmor frequencies that are relevant for medical applications. Such a peak at high field was previously observed for medium molecular weight systems, such as the block dendrimer Gd-TREN-bisHOPO-TAM-Asp-Asp$_2$-12OH[11] and hydrophilic dendritic conjugates in which the Gd ion lies at the barycenter of the structure.[12] The high relaxivity in the 2 T field range is characteristic both of an optimally fast water exchange rate and of a relatively long rotational correlation time arising from a rigid structure. The profiles thus strongly suggest that τ_M is still optimal for the heteronuclear complexes and that the two-dimensional self-assemblies can efficiently slow the molecular tumbling.

Details of the refinements of the NMRD profiles of the self-assemblies (Table 1), as compared to the parent mononuclear Gd-TREN-bisHOPO-TAM,[13] indicate that most of the increase in molecular weight is translated into an increase in the rotational correlation time τ_R. The larger trinuclear Fe,2Gd-3$\mathbf{L}_C$ (MW = 2306 g/mol), for instance, is 2.7 times larger than Gd-TREN-bisHOPO-TAM (MW = 831 g/mol), and its rotational correlation time 2.5 times that of the parent complex (τ_R = 312 ps vs 125 ps). This linear relationship between τ_R and the molecular weight is indicative of a highly rigid structure with little internal motion. It has also been observed in highly rigid dendrimers for which the effective increase in τ_R of the Gd(III) complex is higher than that of derivatives of the commercial PAMAM.[11,14] Each Gd(III) centers of each assembly still coordinates two water molecules, which continue rapid exchange with bulk solvent. The electronic relaxation times of the supramolecules are also comparable to that of the parent mononuclear complex. A comparison of the cylindrical architecture of the self-assemblies with the spherical dendrimers or metallostars indicates that the rodlike architectures of the former are still well suited to the attainment of highly rigid, high relaxivity MRI contrast agents. Furthermore, unlike the metallostars, whose relaxivities were measured only under acidic conditions,[5d] the HOPO/TAM assemblies maintain the high relaxivity at physiological pH and when dissolved in human serum.[5]

In summary, the templated self-assembly of Gd(III) hydroxypyridinone and Fe(III) terephthalamide components of a supramolecular structure presents a new class of MRI contrast agents. These compounds utilize the rapid assembly of rigid macromolecules in few synthetic steps. Relaxivity studies indicate that the high-molecular-weight clusters effectively slow the molecular tumbling. This and the fast water exchange produce high relaxivity at the high magnetic fields relevant to future medical applications.

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Supporting Information Available: Detailed experimental procedures and characterization data for the syntheses of the complexes Fe,Gd-$\mathbf{L}_A$, Fe,Gd-3$\mathbf{L}_B$, and Fe,2Gd-3$\mathbf{L}_C$; pH dependence of the selectivity of Fe(III) for TAM over HOPO ligands; inner- and outer-sphere contributions to the NMRD profiles of the assemblies; and MM3 energy minimization of the assemblies. This information is available free of charge via the Internet at http://pubs.acs.org.

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