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The Positive Influence of the Fullerene Derivative Bonded to Manganese (III) Porphyrin on the Water Protons Relaxation

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Manganese-porphyrin compounds as MRI contrast agents have drawn particular attention due to high relaxivities and unique biodistribution. It has been reported that the charge density of metal center and the steric decompression of substituents, rather than rotational correlation time, were the key factors to determine the relaxivities of manganese (III) porphyrins. In this study, [6, 6]-phenyl-C_{61}-butyric acid ($PC_{61}BA$) was introduced into 5-(4-Aminophenyl)-10, 15, 20- tris (4-sulfonatophenyl) porphyrin (APTSPP) to investigate the influence on water protons relaxation. The obtained $PC_{61}BA$-APTSPP-Mn possesses the relaxivity of 19.2 $mM^{-1}s^{-1}$, greater than that of Mn-APTSPP (11.2 $mM^{-1}s^{-1}$) and clinically used Gd-DTPA (4.1 $mM^{-1}s^{-1}$) at 0.5 T, and even more effective compared with those binding manganese (III) porphyrins to certain macromolecule. It was reasonably speculated that the high relaxivity of $PC_{61}BA$-APTSPP-Mn should ascribe to the charge density variation of Mn^{III} and the steric decompression induced by $PC_{61}BA$. Both fluorescence emission spectra and cyclic voltammetry results verified the presence of electronic communication between $PC_{61}BA$ and APTSPP-Mn. In addition, the hydrodynamic diameter of $PC_{61}BA$-APTSPP-Mn aggregates was much smaller than that of APTSPP-Mn aggreagates, which may contribute to the higher relaxivity by inhibiting the formation of dimers of APTSPP-Mn. Therefore, the introduction of fullerene derivatives is suggested to be a good strategy to improve the relaxivities of manganese (III) porphyrins.

Introduction

The manganese belongs to one of the earliest reported paramagnetic contrast agents for magnetic resonance imaging (MRI)[1-3]. The manganese possesses high spin number, long electronic relaxation time, and labile water exchange, which could shorten spin-lattice relaxation time of water protons just as the case of lanthanides[4-7]. However, differently from lanthanides, Mn is a natural cellular component[8]. Besides, certain Mn complexes have been proved to possess adjunctive antineoplastic activity as powerful superoxide dismutase mimics, peroxynitrite scavengers, and modulators of cellular redox-based signaling pathways[9-11]. Significantly, unlike gadolinium (III) compounds, no obvious relation between Mn and nephrogenic systemic fibrosis (NSF) has been reported yet[12].

Manganese-porphyrin compounds, such as sulfonatoporphyrins, have drawn particular attention among manganese complexes. In addition to high relaxivity, they have been found to be able to specifically accumulate in the tumor site after i.v. administration, thus greatly enhances the image contrast of the tumor *in vivo*[13-17]. As one of the earliest examples of sulfonatoporphyrins, manganese (III) tri-(4-sulfonatophenyl) porphyrin ($TPPS_3$) demonstrated excellent relaxivity (r_1=10.36 $mM^{-1}s^{-1}$ at 20 MHz) among sulfonated porphyrins. In particular, manganese-porphyrin compounds could also be used as p (O_2)-responsive MRI contrast agents based on the redox switch of manganese (II/III)-porphyrin complexes[18].

Fullerene-porphyrin nanostructureshave been extensively investigated in biomedical applications, especially in photodynamic therapy (PDT)[19-24]. Compared with other nanocompounds, the fullerene derivatives are considered to have advantages of easy clearance and functionality due to the flexible structures. In addition, the hydrophobic characteristic of fullerenes could promote the affinity to cell membranes and their aggregates are ready to accumulate in tumor site by the enhanced permeability and retention (EPR) effect. Although the

combination of fullerenes with gadolinium are regarded as future contrast agents[12], the fullerene-porphyrin complexes as contrast agents have not been reported yet.

Herein we reported a water-soluble C_{60}-porphyrin-manganese compound as a MRI contrast agent. It was synthesized by covalently coupling 5-(4-Aminophenyl)-10, 15, 20-tris (4-sulfonatophenyl) porphyrin (APTSPP) to [6, 6]-phenyl-C_{61}-butyric acid ($PC_{61}BA$), followed by coordination with manganese (II) acetate tetrahydrate. It has been reported that the charge density of metal center and the steric decompression were the key factors to determine the relaxivities of manganese (III) porphyrins[25]. The aim of this study is to investigate the influence of $PC_{61}BA$/manganese (III) porphyrin on the water protons relaxation. The results reveal that the introduction of fullerene derivatives to manganese (III) porphyrins is a good strategy to improve the water protons relaxation.

Experimental

Materials and methods

5-(4-Aminophenyl)-10, 15, 20-tris (4-sulfonatophenyl) porphyrin (APTSPP)[26], trisodium salt and [6, 6]-phenyl-C_{61}-butyric acid ($PC_{61}BA$) were synthesized as reported[27], corresponding characterizations were shown in Figure S1-S3 in the Supporting Information (SI).

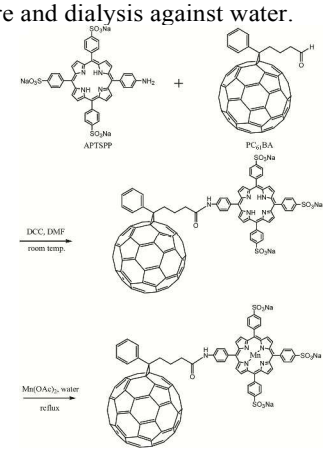
UV-vis absorption spectra were measured on a UNIC UV-4802H spectrometer in aqueous solution at room temperature. Fluorescence emission spectra were obtained on a Fluorolog 3-21. The mass spectral data were collected by matrix-assisted laser desorption/ionization-time-of-flight (MALDI-TOF, AXIMA, Shimadzu, Japan) mass spectrometry using the Biflex II spectrometer (Bruker, Germany) and 4-hydroxyl-R-cyano cinnamic acid as the matrix. Fourier transform infrared (FTIR) spectroscopy measurements were performed using a Nicolet magna-IR750 spectrometer. ^{1}H NMR solution spectra were measured with a Brucker Avance 400 spectrophotometer. The concentrations of Mn ion in the aqueous solutions were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES, ICPE-9000, Shimadzu, Japan). The relaxation times were measured at 0.5 T with NMI20 Analyst instrument (Shanghai Niumag Corporation, Shanghai, China) and 3 T clinical MR systems (Magnetom Trio, Siemens Medical Solutions, Erlangen, Germany). The inversion-recovery method was used to measure T_1. The relaxivities r_1 in pure water and in saline were obtained from the slopes of the relaxation time (T_1) vs the Mn concentration, respectively. Dynamic light scattering (DLS) measurements were carried out with a Malvern Instrument (Zetasizer/nano Series, model ZEN3600). Scanning electron microscope (SEM) imaging was carried out on a JEOL JSM-6701F electron microscope. Cyclic voltammetry (CV) was recorded on an electrochemical workstation (CHI660D). Pt and Ag/AgCl were used as an auxiliary electrode and a reference electrode, respectively, and 0.5 M KNO_3 aqueous solution was served as supporting electrolyte. The scan rate was 100 mVs^{-1}.

Synthesis of APTSPP-Mn

Manganese (II) acetate tetrahydrate (2.6 mg, 10.68 μmol) was added to a solution of APTSPP (10 mg, 10.68 μmol) in deionized water (5 mL). The mixture was refluxed for 24 h and concentrated under reduced pressure, followed by dialysis against distilled water.

Synthesis of $PC_{61}BA$-APTSPP

DCC (50 mg, 0.242 mmol) was added to a solution of APTSPP (50 mg, 0.0534 mmol) and $PC_{61}BA$ (95.69 mg, 0.1068 mmol) in DMF (50 mL) under ice-bath and N_2 atmosphere. The resulting solution was stirred for 3 h and reacted for another 24 h at room temperature, which finally was concentrated under reduced pressure and dialysis against water.



Scheme 1 Illustration of the synthesis of $PC_{61}BA$-APTSPP-Mn.

Synthesis of $PC_{61}BA$-APTSPP-Mn

Manganese (II) acetate tetrahydrate (2.8 mg, 0.0115 mmol) was added into a solution of $PC_{61}BA$-APTSPP (20 mg, 0.0115 mmol) in deionized water (5 mL). The mixture was refluxed for 24 h and concentrated under reduced pressure, followed by dialysis against distilled water.

Results and discussion

Similar to other water-soluble fullerene derivatives[28-30], it is not easy to obtain the molecular ion peak of $PC_{61}BA$-APTSPP-Mn (Figure S4 in SI). However, many fragment ions were observed compared with the MALDI-TOF-MS of APTSPP-Mn (Figure S5). The mass spectral peak at m/z 920 indicated the successful coupling of tris (4-sulfonatophenyl) porphyrin to $PC_{61}BA$, which was further confirmed by the characteristic vibration peaks of $PC_{61}BA$ in Figure S6 and the disappearance of δ=4.36 ppm (s, 2H, amino) in Figure S7. The peak at m/z 1838 should ascribe to 2M ion of APTSPP-Mn, while the strong peak at m/z 1126 is supposed to be [M-C_{60}+2Na]. The UV-vis spectra of APTSPP, APTSPP-Mn and $PC_{61}BA$-APTSPP-Mn (Fig. 1a) were measured in PBS. Both APTSPP-Mn and $PC_{61}BA$-APTSPP-Mn displayed typical B bond (near 468 nm) and Q bond (540~630 nm) absorption of porphyrins[26], and an obvious red shift (from 413 nm to 468 nm) was observed due to the complexing with Mn ion. The absorption of $PC_{61}BA$-APTSPP-Mn at ca. 300 nm is the characteristic absorbance of $PC_{61}BA$. The fluorescence emission spectrum of APTSPP at 646 nm is significantly quenched after chelating Mn ion, while $PC_{61}BA$-APTSPP-Mn exhibited almost negligible fluorescence, indicating the

occurrence of photoinduced intramolecular communication between $PC_{61}BA$ and APTSPP-Mn.

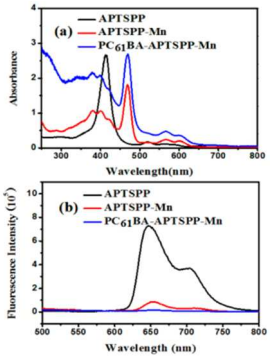


Fig. 1 (a) UV-vis spectra of APTSPP, APTSPP-Mn and $PC_{61}BA$-APTSPP-Mn in PBS, (b) Fluorescence emission spectra of APTSPP, APTSPP-Mn and $PC_{61}BA$-APTSPP-Mn in PBS. λ_{ex} = 413 nm; Concentration: 10^{-5} mmol/L.

The size distributions of APTSPP-Mn and $PC_{61}BA$-APTSPP-Mn in aqueous solution were examined by DLS analysis. The measured size of $PC_{61}BA$-APTSPP-Mn (ca.13 nm) was much smaller than that of APTSPP-Mn (ca.79 nm) as displayed in Fig. 2a. It is speculated that the introduction of $PC_{61}BA$ could effectively hinder the aggregation of APTSPP-Mn. In addition, the measured Zeta potential of $PC_{61}BA$-APTSPP-Mn is -30±0.4 mV, which is more negative than that of APTSPP-Mn (-19.6±0.7 mV). The more electron-deficient property of fullerenes may account for the negative Zeta potential and therefore the smaller size of $PC_{61}BA$-APTSPP-Mn as a result. To further characterize the aggregate size, SEM images were performed (Fig. 2b and 2c). The result reveals that the aggregate size of $PC_{61}BA$-APTSPP-Mn is indeed much smaller than that of APTSPP-Mn, which was reasonably in accordance with the DLS results.

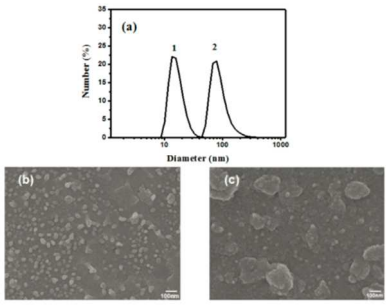


Fig. 2 Size distributions for $PC_{61}BA$-APTSPP-Mn (1#) and APTSPP-Mn (2#) in water at room temperature (a), and SEM micrographs of the aggregation formed by $PC_{61}BA$-APTSPP-Mn (b) and APTSPP-Mn (c). The scale bar represents 100 nm for the SEM.

In vitro Relaxation Study

The relaxivities of $PC_{61}BA$-APTSPP-Mn, as well as T_1-weight imaging *in vitro* were measured at 0.5 T, 37°C, and APTSPP-Mn was used as a control. As shown in Figure 3a and 3b, despite the smaller aggregation size in aqueous solution, the relaxivity of $PC_{61}BA$-APTSPP-Mn is 19.2 $mM^{-1}s^{-1}$, higher than that of APTSPP-Mn (11.3 $mM^{-1}s^{-1}$). This phenomenon is quite different from other MRI contrast agents, since the latter with large aggregation size generally has a longer rotational correlation time and therefore higher relaxivity[31-33]. This abnormality could be explained by the particular relaxation pathways of manganese (III) porphyrins. Taking $(Mn^{III}TPPS)^{3-}$ into an example, the relaxivity of $(Mn^{III}TPPS)^{3-}$ is dominated by the electronic relaxation time, rather than rotational correlation time[34]. In other word, variation of rotational correlation time will not make significant change to the relaxivity of $(Mn^{III}TPPS)^{3-}$. Aime S. et al[11] reported that the introduction of poly-β-CD to $(Mn^{III}TPPS)^{3-}$ can improve the relaxivity of $(Mn^{III}TPPS)^{3-}$ by 35% at most. Herein, $PC_{61}BA$ actually has improved the relaxivity of APTSPP-Mn by 70%. The T_1-weight imaging *in vitro* (Fig. 3a) was highly in accordance with the relaxivity results. The relaxivities of $PC_{61}BA$-APTSPP-Mn at 3.0 T were also evaluated, as shown in Figure S8. The measured relaxivity is 12.2 $mM^{-1}s^{-1}$, still higher than that of APTSPP-Mn (8.2 $mM^{-1}s^{-1}$, Fig S9). All the above results highlight the positive influence of $PC_{61}BA$ on improving the relaxivity of APTSPP-Mn.

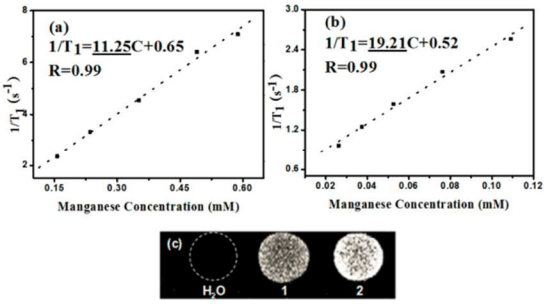


Fig. 3 Linear relationship between T_1 relaxation rates ($1/T_1$) and manganese concentrations for APTSPP-Mn (a), $PC_{61}BA$-APTSPP-Mn (b) in water at 0.5 T and 300 K, T_1-weighted images with D_0=500 ms, at 0.5 T and 300 K (c). (1#) 0.2 mM APTSPP-Mn, (2#) 0.2 mM $PC_{61}BA$-APTSPP-Mn.

To further confirm that rotational motion is not the key factor to influence the relaxivity of APTSPP-Mn, the water protons relaxation was measured in different concentrations of PBS, since high concentration PBS has been proved to induce different aggregation behavior of some MRI contrast agents[31]. DLS characterization was also employed to monitor the size change of APTSPP-Mn aggregates in a series of concentrations of PBS. As shown in Fig. 4a and 4b, the relaxivities of APTSPP-Mn almost keep constant in different concentrations of PBS solution, where APTSPP-Mn may form edge-to-edge structure (J-aggregation)[35]. This once again demonstrated that rotational correlation time is not the determining factor for the relaxivity of manganese (III) porphyrins. Therefore, the $PC_{61}BA$ should improve the relaxivity of APTSPP-Mn by other critical factor rather than the rotational correlation times.

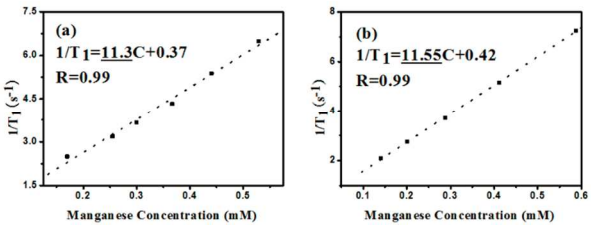


Fig. 4 Linear relationship between T_1 relaxation rates ($1/T_1$) and manganese concentrations for (a) APTSPP-Mn in 1×PBS, (b) APTSPP-Mn in 10×PBS at 0.5 T and 300 K.

Table 1 Number peak values of size distribution measured by DLS for APTSPP-Mn in PBS at different concentrations.

Compounds	Water (nm)	1×PBS (nm)	10×PBS (nm)
APTSPP-Mn	76±12	79±8	247±38

$(Mn^{III}TPPS)^{3-}$ has been well investigated for its anomalous higher relaxivity than other manganese porphyrins[36,37]. For example, Kellar K. E.[38] has systematically studied a series of manganese porphyrins with different relaxivities and stated that the formation of dimers has great influence on the relaxivities of manganese porphyrins. $(Mn^{III}TPPS)^{3-}$ and other porphyrins with high relaxivities are proved to be monomeric in aqueous solution. While those with low relaxivities usually tend to form face-to-face dimmers, as a result, the number of exchangeable water molecules reduces from 2 per porphyrin to 1[38]. The formation of face-to-face dimers can be simply detected by Beer's law as well as comparing the relaxivities before and after addition of deuterated acetone[39]. As shown in Fig. 5, both APTSPP-Mn and $PC_{61}BA$-APTSPP-Mn obey Beer's law in aqueous solution well, and the relaxivities don't display any obvious variation after addition of deuterated acetone (Table 2), indicating that $PC_{61}BA$-APTSPP-Mn is mainly monomeric in aqueous solution as APTSPP-Mn does. Noticeably, the relaxivity change of $PC_{61}BA$-APTSPP-Mn is much smaller than that of APTSPP-Mn after addition of deuterated acetone, which may benefit from the introduction of $PC_{61}BA$ that hinders the aggregation of APTSPP-Mn.

It is proved that the charge density of the metal center and the steric decompression are the key factors to determine the relaxivities of manganese (III) porphyrins such as $(Mn^{III}TPPS)^{3-}$[25]. The high relaxivity of $(Mn^{III}TPPS)^{3-}$ should ascribe to the electronic effect of substituents, which may increase electron density in the central Mn (III) ion and labilize the axial water ligands. The different relaxivities of various manganese (III) porphyrins are closely related to the electronic effect and steric effect of substituents of porphyrins.

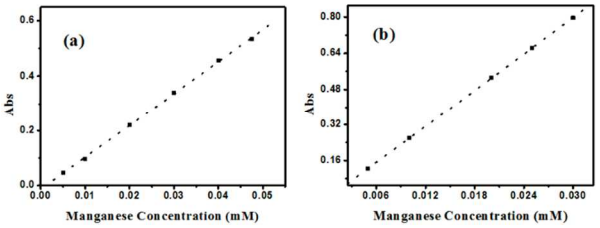


Fig. 5 Beer's law plots of (a) APTSPP-Mn and (b) $PC_{61}BA$-APTSPP-Mn.

Table 2 The varied relaxivity $\triangle r_1$ of APTSPP-Mn and $PC_{61}BA$-APTSPP-Mn after addition of deuterated acetone.

Compounds	50 (μL)	100 (μL)	150 (μL)
APTSPP-Mn (300 μL)	4.4%	7.9%	11.4%
$PC_{61}BA$-APTSPP-Mn (300 μL)	6.2%	7.2%	8.1%

To further explore the influence of $PC_{61}BA$ on the electronic property of APTSPP-Mn, cyclic voltammetry was conducted (Fig. 6). It is revealed that the reduction and oxidation potential of $PC_{61}BA$-APTSPP-Mn are -0.52 V and 1.09 V, respectively; while those of APTSPP-Mn are -0.49 V and 1.12 V, respectively. $PC_{61}BA$-APTSPP-Mn possesses higher reduction potential than APTSPP-Mn. The difference of electrochemical characteristics could be attributed to the formation of conjugate system between $PC_{61}BA$ and APTSPP-Mn, which promotes the intramolecular electron communication among $PC_{61}BA$-APTSPP-Mn. This has been verified by significant fluorescence quenching as shown in Fig. 1b. As a result, the electron density of central Mn (III) ion may be optimized, which lead to labilization of axial water ligands, accelerate the water-exchange and contribute to higher relaxivity as reported for $(Mn^{III}TPPS)^{3-}$ [25].

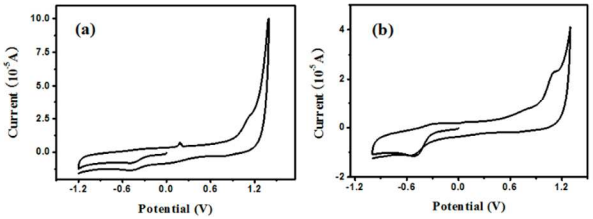


Fig. 6 Cyclic voltammogram of (a) APTSPP-Mn and (b) $PC_{61}BA$-APTSPP-Mn.

Conclusions

A water-soluble $PC_{61}BA$-APTSPP-Mn compound was synthesized and the hybrid exhibited higher relaxivity than that of APTSPP-Mn. As the charge density of metal center and the steric decompression are reported to be the key factors determining the relaxivities of manganese (III) porphyrins, we concluded that the high relaxivity of $PC_{61}BA$-APTSPP-Mn should attribute to the steric effect and the electronic effect induced by $PC_{61}BA$, as confirmed by DLS, fluorescence and CV characteriazations. In a word, the introduction of fullerene derivatives to manganese (III) porphyrins is suggested to be a good alternative to improve the water protons relaxation.

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Notes and references

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Electronic Supplementary Information (ESI) available: More characterization for 5-(4-Aminophenyl)-10, 15, 20-tris(4-sulfonatophenyl) porphyrin (APTSPP), [6, 6]-phenyl-C_{61}-butyric acid ($PC_{61}BA$) and APTSPP-Mn. See DOI: 10.1039/b000000x/

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