[Gd(Try-TTDA)]$^{2-}$: Magnetic Resonance based Sensor for the Specific Detection of Cu^{2+} in Living Cells

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Abstract

In this study, we have developed a new contrast agent [Gd(Try-TTDA)]$^{2-}$ that can recognize Cu^{2+} ion in the living cells. Moreover, the Gd^{3+} complex attributes excellent selectivity for Cu^{2+} over a choice of other metal ions. A gradual increases in the relaxivity and signal intensity of *ex vitro* and *in vitro* MR imaging upon Cu^{2+} detection. These results implicate that a new MR based contrast agent [Gd(Try-TTDA)]$^{2-}$ can serve as a Cu^{2+} sensor using relaxometry and MR imaging.

Introduction

Copper plays an important role in various biological processes. An imbalance of Cu (II) ions within human body can cause many diseases including Menkes disease, occipital horn syndrome, and Wilson's disease. The average concentration of copper in the normal blood is 100-150 μg/dL (15.7-23.6 μM) [1]. Magnetic resonance imaging (MRI) is a non-invasive and high spatial resolution technique that can provide 3D images of organisms up to cellular level [2]. Over the past few decades several fluorescence-based sensors for specific detection of heavy metal ions such as mercury, lead, and copper were reported. In contrast, the reported MR-based metal ion sensors are limited. In this study, we designed and synthesized (S)-4-indolyl-3,6,10-tri(carboxymethyl) -3,6,10-triazadodecanedioic acid (Try-TTDA) and its Gd(III) chelate ([Gd(Try-TTDA)]$^{2-}$) in which the metal ion-indole interaction functions as a metal ion sensor.

Methods

The ligand (S)-4-indolyl-3,6,10-tri(carboxymethyl)-3,6,10-triazadodecanedioic acid (Try-TTDA) and its Gd(III) chelate ([Gd(Try-TTDA)]$^{2-}$) was synthesized and characterized by various analytical techniques such as HPLC, ESI-MS, NMR, and nuclear magnetic relaxometer. The interaction between metal ions and side chain indole of [Gd(Try-TTDA)]$^{2-}$ was investigated by quenching of intrinsic tryptophan fluorescence by fluorescence spectroscopy. The HEK 293 cells was incubated with different concentrations of [Gd(Try-TTDA)]$^{2-}$ and copper (II), washed by PBS buffer and scanned by 3.0 T MRI. *Ex vitro* and *In vitro* T_1 weighted MR imaging studies were performed with a 3.0 T MR scanner.

Results and Discussion

L-Tryptophan-based TTDA ligand was synthesized and characterized by NMR and ESI-MS. [Gd(Try-TTDA)]$^{2-}$ identified by ICP-MS and it was obtained by complexation of Gd(III) with Try-TTDA in water at room temperature. The interaction between metal ions and side chain indole of [Gd(Try-TTDA)]$^{2-}$ was investigated by quenching of intrinsic tryptophan fluorescence by fluorescence spectroscopy. The fluorescence intensity of [Gd(Try-TTDA)]$^{2-}$ is quenched almost to zero when ten equivalent of Cu(II) was added. Further, the T_1 relaxivity of [Gd(Try-TTDA)]$^{2-}$ was titrated by variable concentration of M^{n+} at 37.0 ± 0.1 °C and 20 MHz in pH 5.5 acetate buffer solution. The T_1 relaxivity of [Gd(Try-TTDA)]$^{2-}$ was significantly increases, when Cu(II) metal ion added. To demonstrate the potential utility of the new MRI contrast agent, the MR imaging studies were performed by using a 3.0 T MR scanner. The 0.01, 0.05, 1.0, and 2.0 mM concentrations of [Gd(Try-TTDA)]$^{2-}$, respectively, and metal ions in the molar ratio of 1:1 were mixed at room temperature. As shown in Fig. 1, the signal intensity of *ex vitro* T_1-weighted MR images increases as the metal ion interacting with the side chain indole of Gd^{3+} complex. In contrast, the Mg^{2+} ion does not exhibit signal enhancement. It is evident that the interaction tendency follows: Cu (II) > Fe(II) > La(III) > Mg(II). The ability of [Gd(Try-TTDA)]$^{2-}$ for sensing Cu^{2+} ion was further examined by using *in vitro* T_1-weighted MR imaging, as shown in Fig. 2. The 0.5, 1.0, and 2.0 mM concentration of [Gd(Try-TTDA)]$^{2-}$, respectively, and Cu(II) in the 1:1 molar ratio were incubated with HEK293 cells for 4 h at 37.0 ± 0.1 ºC. A significant signal enhancement was observed as the concentration of [Gd(Try-TTDA)]$^{2-}$ and Cu(II) increased.

Conclusion

In summary, [Gd(Try-TTDA)]$^{2-}$ may serve as a smart contrast agent for specific detection of Cu^{2+} in living cells. This contrast agent provides a model candidate for potential MR-based Cu^{2+} imaging to show high selectivity for Cu^{2+} over other metal ions via quenching of intrinsic tryptophan fluorescence, and increases in relaxivity. In addition, the sensing of Cu^{2+} by [Gd(Try-TTDA)]$^{2-}$ was proven by *ex vitro* and *in vitro* T_1-weighted MR imaging.

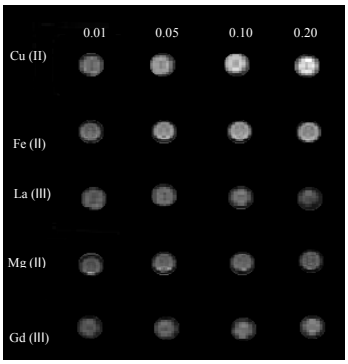

Fig. 1. T_1-weighted images of [Gd(Try-TTDA)]$^{2-}$ and metal ions (molar ratio = 1:1).

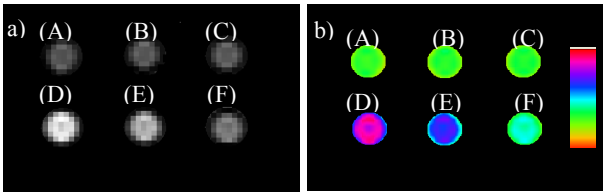

Fig.2. (a) *In vitro* MR images of HEK293 cells for 4 h at 37.0 ± 0.1 ºC. A) Control (cell only); (B) Cells treated with 1.0 mM Gd(III) complex; (C) Cells treated with 1.0 mM Cu(II); (D) Cells treated with 2 mM Cu(II) and 2 mM [Gd(Try-TTDA)]$^{2-}$; (E) Cells treated with 1 mM Cu(II) and 1 mM [Gd(Try-TTDA)]$^{2-}$;(F) Cells treated with 0.5 mM Cu(II) and 0.5 mM [Gd(Try-TTDA)]$^{2-}$.

References

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