Tetra-manganese Polyoxometalates: a new candidate contrast agent for MRI

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Introduction

Even though magnetic resonance imaging (MRI) produces images with excellent detail of *in vivo* subjects without the use of exogenous contrast, in some cases, the contrast difference between normal and abnormal tissues is so small that the tissues cannot be distinguished. This problem raises the demand for the development of contrast agents that would make it possible to differentiate between target and background tissues. Manganese chloride ($MnCl_2$) has been studied extensively as an MRI contrast agent. Intracellular accumulation of $MnCl_2$ increases signal intensity due to the T_1-shortening effect of manganese [1]. Its use as a contrast agent in clinical settings has not been feasible due to the cytotoxicity of free Mn^{2+}. Polyoxometalates are a large class of transition metal oxide clusters with readily tuned elemental compositions and diverse geometries. Polyoxometalates (POM) have been used to produce MR contrast [2, 3]. The purpose of this study was to synthesize a new tetramanganese cluster in a POM chelate frame named tetra-manganese Polyoxometalates (Mn_4POM) and to evaluate its application as a contrast agent for MRI. We hypothesized that Mn_4POM can be used as a candidate contrast agent with better MR contrast than free Mn^{2+}.

Materials and Methods

Synthesis of Mn_4POM: $Mn(OAc)_3 \cdot 2H_2O$ (0.1863 g, 0.672 mmol) and $K_{10}[A\text{-}\alpha\text{-}SiW_9O_{34}]$ (0.46g, 0.16 mmol) were dissolved and stirred in 20 mL of 0.5 M NaAc buffer (pH 4.72) at 80 °C for 1.5 h. After cooling down the solution to room temp (pH=4.88), the brown solution was filtered to remove some black precipitate. Slow evaporation of the clear brown-red solution at room temperature resulted in needle-like brown-red crystals. Yield was approximately 0.35 g.

Cytotoxicity assays: MTT assays (Promega, Madison, WI) and G6PD kit (Invitrogen, Carlsbad, CA) were used to determine cell viability. Following 24, 48, 72 h incubation with either $MnCl_2$ (Sigma–Aldrich, St. Louis, MO) or Mn_4POM, cell viability was measured. All measurements were repeated four times for each sample. Data are presented as means ± SDs of the measurements.

MRI: 9L cells were cultured with either $MnCl_2$ or Mn_4POM; the final concentration of Mn^{2+} in the media was 0, 5, 10, or 20 µM. After three days, the cells were collected in a 1.5 ml tube. After an hour of settling by gravity at 4°C, the longitudinal relaxation time (T1) of the cell pellets was measured using a 3T MR scanner (Siemens Medical Solutions, Malvern, PA) [TE = 10 ms, TR = 200, 500, 750, 1000, 1200, 1500, 1800, 2000 ms, respectively, FOV = 128 × 128 mm, NEX = 2, and a slice thickness = 1 mm].

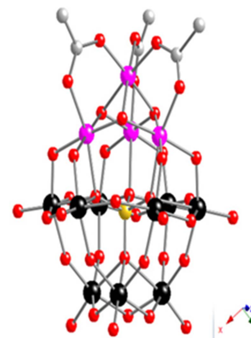

Figure 1. X-ray single crystal structure of Mn_4POM. *red: oxygen (O); pink: manganese (Mn); Black: Tungsten (W); yellow: Silicon (Si); argentine: Carbon (C).*

Results and Discussion

The Mn_4POM was characterized by x-ray single crystal diffraction (Fig. 1). The empty space formed between the Mn^{III}_4 trigonal pyramid and the Silicon-polyanion that could allow small molecules to pass through, thus making this new cluster unique among all the known cubic Mn-containing compounds. One of the key structural features of this compound is the Mn_4 core within its crystal structure, which is strongly paramagnetic and enables MR contrast enhancement to be achieved.

The cell viability of Mn_4POM on both 293T and rat glioma (9L) cells was tested by MTT assay. No significant decrease in cell proliferation was observed when the Mn_4POM concentration is below 30 µM (Fig. 2, left). No difference in release of cytosolic G6PD was found when cells were cultured with up to 20 µM of Mn_4POM (Fig. 2, right); since an increase in G6PD reflects an increase in cytotoxicity, this result indicates minimal cytotoxicity when cells are exposed to low levels of Mn_4POM (less than 20 µM).

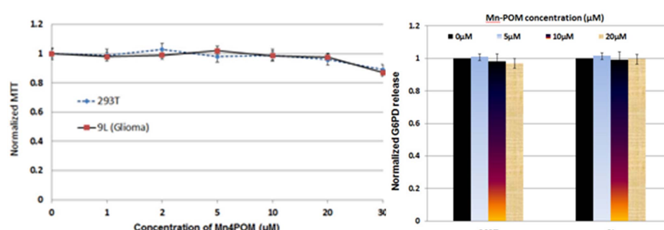

Figure 2. Cytotoxicity graphs: left: MTT assay; right: G6PD assay.

MRI images of cell pellets cultured with different concentrations of the agents are shown in Fig. 3 (top). The percent increase in longitudinal relaxivity (R_1) between cells cultured with different supplementation was calculated. Relative to cells incubated with $MnCl_2$, 9L cells incubated with Mn_4POM showed statistically significant increase in R_1 ($p < 0.01$): a 382.3% net increase in R1 at 10 µM, and a 167.8% net increase in R_1 at 20 µM (Fig. 3, bottom).

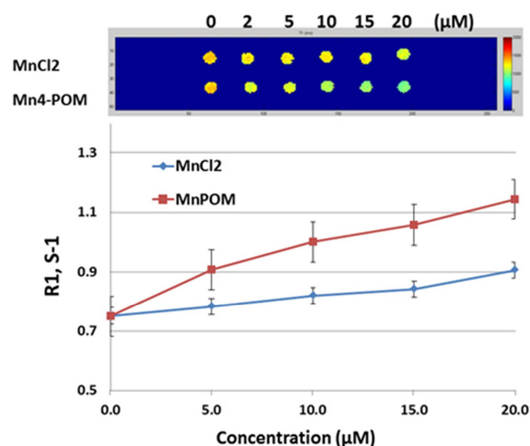

Figure 3. In vitro MRI. 9L cells were incubated with media supplemented with either Mn_4POM or $MnCl_2$ ranging in concentration from 0 to 20 µM for three days. R1 was measured. N = 8. Error bars indicate ±S.E.M.

Conclusion

We have prepared a new tetra-manganese Polyoxometalates(Mn_4POM), capable of producing T_1-weighted MR contrast *in vitro* at low concentrations. Cells cultured with Mn_4POM were able to produce greater contrast than those cultured with free Mn^{2+} *in vitro*, likely due to the Mn_4 core within its crystal structure. Our results suggest that Mn_4POM may also be used for *in vivo* contrast enhancement.

Acknowledgements: This work was supported in part by NIH (NHLBI-HV-10-08).

References

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