Mn (III) $TPPS_4$: A metallophorphryin used for tumor identification in MRI

From as early as 1948, scientists have studied the potential of porphryins and metalloporphyrins in cancer detection and therapy. For reasons unknown then, these compounds had high affinities for neoplasms (tumors) when injected into mice.[1] In fact, porphyrin concentration was highest in and near necrotic areas (unprogrammed cell death). The potential for metalloporphyrins in tumor identification was further uncovered with the effects of paramagnetic substances on proton relaxation time of surrounding tissues. [2] While X-ray imaging is used for bony, dense structures, Magnetic Resonance Imaging **(**MRI), an application of Nuclear Magnetic Resonance, has been used with soft tissues for decades. The magnetic moments of paramagnetic species such as $MnTPPS_4$ significantly enhance MRI signal in the soft tissues to which it is bound, making it an optimal contrast agent.

Synthesis

$MnTPPS_4$ can be synthesized by heating *meso*-tetra(4-sulfonatophenyl)porphine (H_2TPPS_4) and manganese (II) acetate in acetic acid under reflux for 8 hours.[4] Air bubbling through the reflux solution oxidizes Mn(II) to Mn(III).

$$\mathbf{Mn(II)(CH_3COO)_2 + H_2TPPS_4} \xrightarrow{O_2,\ 115°,\ 8h} \mathbf{Mn(III)TPPS_4 + 2CH_3COOH}$$

The presence of $MnTPPS_4$ and disappearance of H_2TPPS_4 can be confirmed with UV spectroscopy:

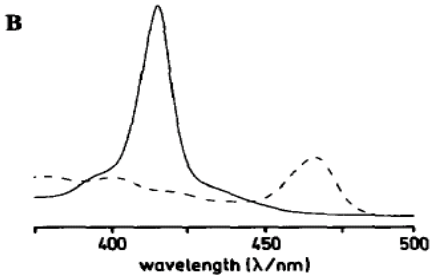

Figure 1: Absorption spectra of equimolar aqueous solutions of H_2TPPS_4 (full line) and $MnTPPS_4$ (dotted line) [2]

Additionally, the absorbance maxima of $Mn(III)TPPS_4$ occur at 465, 561, and 593 nm, while the absorbance maxima of $Mn(II)TPPS_4$ are at 434, 572, and 612 nm.[3]

While the synthesis of $MnTPPS_4$ has been documented since the 1960's, and it is commercially available, it is a very polar molecule and is thus often contaminated with un-identified by-product. Bockhorst and coworkers[4] proposed an optimized purification method: washing the product multiple times with hot acetic acid, then hot ethanol, and dissolving in aqueous Na_2CO_3. The solution is then filtered, acidified with HCl, centrifuged and dried; this procedure is done twice. Finally, drying over KOH in a desiccator gives the desired product, a dark green, metallic solid, at 98% purity.

Tetraphenylporphine sulfonate, $TPPS_4$ can be synthesized by heating *meso*-tetraphenylporphine with concentrated sulfuric acid over a steam bath for 4h.[5] *Meso*-tetraphenylporphine can be prepared by refluxing pyrrole and benzaldehyde in propionic acid.[6]

Structure

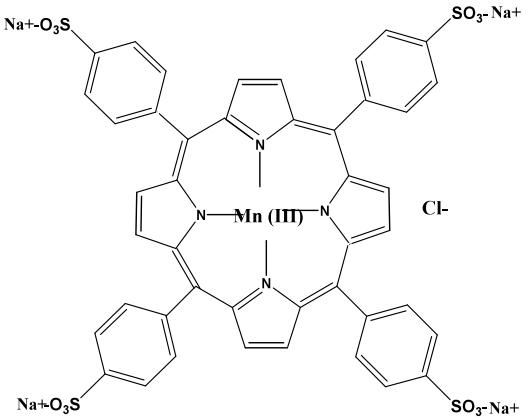

Figure 2: Mn(III)-tetrakis(4-sulfonatophenyl)porphin ($MnTPPS_4$) Mol. Formula: $MnC_{44}H_{24}N_4O_{12}S_4ClNa_4$, MW=1111.3 g/mol *

Tetraphenylsulfonyl is a hydrophilic water-soluble porphryin with a highly developed π-conjugation system. Its Mn(III) complex has been extensively studied as an MRI contrast agent. Mn(III) is a high spin d4 transition metal ion that has four unpaired electrons; it is thus paramagnetic. The geometry of the molecule is square planar, with the Mn(III) chelated by the four nitrogen atoms of a porphine that has four attached sulfonyl groups.

Discussion

Nuclear Magnetic Resonance is the observation of the frequency at which magnetic nuclei in molecules come into resonance with an externally applied electromagnetic field. The signal depends on a variety of factors, including field strength and concentration. It follows that Magnetic Resonance Imaging is essentially a measure of proton density. When the magnetic field varies linearly across an object, the signal intensity is proportional to proton concentration. Tumor identification is possible because of differences in relaxation rates of bulk water of normal tissues and tumors. [3] However, the difference is usually no more than 2-3 fold. T1, longitudinal relaxation time, is the taken by a proton to

[1]Figge, F.; Weiland, G.; Manangiello, L. *Proc. Soc. Exptl. Biol. Med.* **1948**, *68,* 640-641.
[2]Runge, V.M.; Clanton, J.A.; Lukehart, C.M.; Partain, C.L.; James, A.E. Jr. *AJR.* **1983,** *141,* 1209-1215.
[3]Chen, C; Cohen, J.S.; Myers, C.E.; Sohn, M. *FEBS Lett***. 1984**, *168*, 70-74.
[4] Bockhorst, K.; Hoehn-Beriage, M. *Tetrahedron.* **1994**, *50,* 8657.
[5]Srivastava, T.S.; Tsutsui, M. *J. Org. Chem.* **1973**, *38,* 2103.
[6]Adler, A.D.; Longo, F.R.; Finarelli, J.D.; Goldmacher, J.; Assour, J.; Korsakoff, L. *J. Org. Chem.* **1967,** *32*, 476.
*the ligands on the product from the synthesis described are SO_3H instead of SO_3 - Na+

align with the external magnetic field. As referenced by Runge and coworkers,[7] longitudinal relation rate ($^1/_{T1}$) can be described by the following equation:

$$\Delta(^1/_{T1}) = \frac{12\pi^2\gamma^2\varepsilon\mu^2N}{5kT}$$

where ε is the viscosity of the solvent, k is the Boltzman constant, N is the concentration of ions, T is the absolute temperature, and γ is the gyromagnetic ratio for the hydrogen nucleus. The variable of interest is μ, the effective magnetic moment. As the equation shows, spin lattice rate (another name for $^1/_{T1}$) is directly proportional to the square of μ. Paramagnetic species alter the local magnetic field to enhance proton relaxation (spin-lattice T1 and spin-spin relaxation T2 rates) of neighboring nuclei. T2, transverse magnetization time, is also tissue specific; it depends on the exchange of energy and interaction of spins with neighboring nuclei. In essence, T2 measures decay of magnetization perpendicular to the main magnetic field. Through a complex set of equations, it can be determined that paramagnetic species also shortens T2. [7] This is unfavorable, as a decrease in T2 weakens signal.[7] An optimal contrast agent would therefore reduce T1, increasing spin lattice relaxation rate, without significantly compromising T2.

In the presence of an externally applied magnetic field, the magnetic moments of $MnTPPS_4$ that were previously randomly aligned become preferentially aligned with the field.[7] This local magnetic field produced by the paramagnetic species shortens T1 and T2 of surrounding protons in the tumor. This paramagnetic effect decreases with distance:

$$(^1/_{T1}) \propto (^1/_r6)$$

where r is the mean distance from paramagnetic center to the proton.[3]

Thus, an effective paramagnetic contrast agent should include a high spin, metal ion with a large magnetic moment and easily accessible axial coordination sites. Chen and coworkers[3] demonstrated that spin lattice relaxation rate ($^1/_{T1}$) increases linearly with concentration of various metallo-$TPPS_4$ complexes. Of these, the slope is steepest with Mn(III) compared to various other ions and metallo-$TPPS_4$ complexes (Figure 2). It is the most effective in enhancing proton relaxation due to its four unpaired electrons and ability to coordinate axial ligands. While Mn(II) would have five unpaired electrons and theoretically induce an even shorter T1, Mn(II)$TPPS_4$ is not as stable in solution as Mn(III)$TPPS_4$ because it is readily oxidized.

A variety of paramagnetic species have been considered for use as MRI contrast agents – both free and chelated metal ions. Aside from enhancing relaxation rates as determined in vitro, toxicity, stability in human plasma and at various pH, clearance from blood, and tissue distribution must also be taken into account when examining an MRI contrast agent. NMR contrast media can also be classified by method of uptake into body: oral, intravenous, inhalational. Furthermore, blood transport bio distribution and relaxation efficiency are related to differences in binding and aggregation properties of the metalloporphyrin that

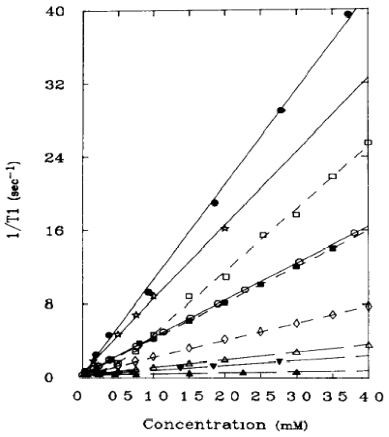

Figure 2: Spin lattice relaxation rate (l/Tr) of water as a function of concentration of metal ion or metallo-$TPPS_4$ complex. Lines are least square fits: (—) Mn, (- - -) Fe, (— —) Cu. Symbols are for experimental data:(•) Mn(III) TPPS, (o) Mn(OAc) $_3$, (✯) $MnC1_2$, (■) Fe(III)$TPPS_4$, (□) $FeCl_3$, (ᴴ) FeEDTA, (x) Cu(II)$TPPS_4$, (B) $CuC1_2$ or $CuSO_4$, (Y) Cu(II)$TPPS_4$-CH_3. [3]

are dose dependent. For example, Fe(III) transforms into low spin dimers at physiological pH. It also aggregates on the surface of bovine serum albumin (mimicking human plasma) 5-7 times more than MnTPPS4; antiferromagnetic coupling between metals ions in the aggregates disables proton relaxation enhancement ability.[8] Also, the dose of metal ions needed for clinical efficiency as a contrast agent is very toxic; chelating the ion significantly lowers toxicity.

Perhaps the most fundamental quality of any tumor identification/contrast agent is the ability to localize in tumors. This selective retention of porphyrins in tumors makes it of special interest in deep tumor detection. Figge and coworkers found that when porphryin was injected into tumor-bearing mice, most of it migrated to the tumor within 24-48 hours. [9] Porphyrin localization was directly observable by the red fluorescence in the tumors that lasted 10-14 days.[9] It has been suggested that porphyrin aggregation is involved with tumor localization.[10] Fiel and coworker also found that porphyrins tend to be localized mostly in soluble and stromal fractions (connective tissue).[10] Mn(III)$TPPS_4$ accumulated in tumors at ratios as high as 1.0:0.3:0.01 (tumor:liver:muscle). In analyzing subcelleular distribution of Mn(III)$TPPS_4$, it was found that accumulation occurs mostly in the extracellular compartments of the tumor, possibly due to mechanism of localization that depends on volume; interstitial space in tumors is large enough to favor retention of large sized porphryins. Finally, Figge and coworkers also noted that while hydrophobic porphyrins would be transported across cell membranes more easily, charged porphyrins (like Mn(III)$TPPS_4$) would remain extracellular. [10]

Conclusion

Mn(III)$TPPS_4$ has been extensively studied for its use as an MRI contrast agent. Its paramagnetism gives it the ability to enhance proton relaxation time and enhance MRI signal, and its porphryin component is useful in tumor detection in that it is selectively retained in those tissues. Of various metallo-$TPPS_4$ compounds studied, it is the most stable in vivo and thus an optimal contrast agent for detection of deep tumors.

[7]Runge, V.M.; Clanton, J.A.; Lukehart, C.M.; Partain, C.L.; James, A.E. Jr. *AJR.* **1983,** *141,* 1209-1215.

[8]Yushmanov, V.E.; Tominaga, T.T.; Borissevitch, I.E.; Imasato, H.; Tabak, M. *MRI.* **1996**, *14,* 255-261.
[9]Kessel, D. *Biochem. Pharm.* **1984,** *33,* 1389-1393.
[10]Fiel R.J.; Button, T.M.; Gilani, S.; Mark, E.H.; Musser, D.A. *Cancer Letters.* **1988**, *40,* 23-32**,**