S-GalTM, a novel Proton MRI reporter for β-galactosidase

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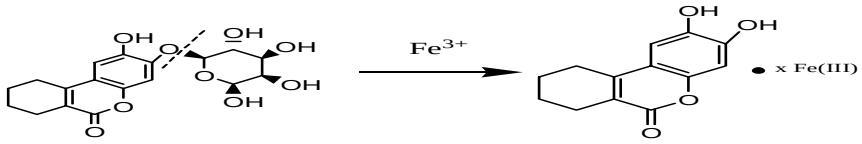
Introduction:

Gene-based therapy has been stimulated by remarkable progress in understanding molecular biology. However, progress and clinical trials would be enhanced by the ability to determine the location, the degree of activity and change in magnitude over time of the expression of the therapeutic genes. The development of non-invasive reporter techniques based on appropriate molecules and imaging modalities may help to assay gene expression. While many nuclear and optical imaging approaches have been presented as gene reporter methods, MR has lagged behind. LacZ, which produces β-galactosidase, has been the primary choice of reporter gene to verify effective transfection in biochemistry for many years, and due to its promiscuous activity many reporter molecules are available for biological and histological analysis. A recent addition to the detective toolkit is S-Gal^{TM}. Upon cleavage by b-galactosidase in the presence of ferric ions (Fe^{3+}, the aglycone chelates iron to produce an intense black stain. In the spirit of multi modality approaches to imaging, it occurred to us that the iron (Fe^{3+}) complex is not only visible, but also paramagnetic. We present data demonstrating this novel approach to detecting gene activity.

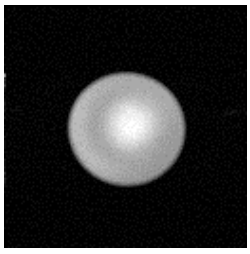
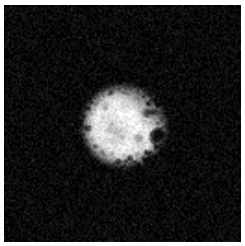
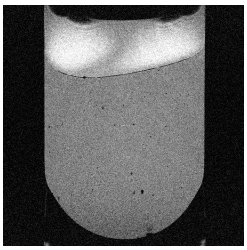
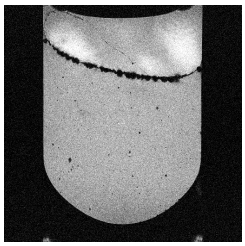


S-Gal^{TM} black complex

Materials and Methods:

S-gal (3,4-cyclohexenoesculetin-β-D-galactopyranoside) and Ferric ammonium citrate were obtained from Sigma. MR images were obtained on a Varian 400 MHz spectrometer equipped with a micro imaging system. In one test for proof of principle, we prepared a 20mm NMR tube containing buffer, ferric ammonium citrate and S-gal. Following a baseline image, β-galactosidase (G5160 from Aspergillus oryzae, Aldrich) was added in situ and the tube reimaged. In a second test for biological relevance, S-Gal and Ferric ammonium citrate were blended with LB agar medium. E.coli and Ecoli induced by IPTG to express β-galactosidase enzyme activity were cultured in 20mm NMR tubes. And distilled water was added on top of the E.coli before imaging.

Results:

 a)
 b)
 c)
 d)

a.) ^{1}H MRI of S-gal and FAC solution, b) β-galactosidase added to "a" (Tr=1000 ms, Te=30 ms, FOV=40 mmx40 mm, matrix=128x128, thk=2 mm)

c.) E.coli d.) E.coli expressing β-galactosidase activity. (Tr=1000 ms, Te=20 ms, FOV= 30 mmx30 mm, matrix=512x512, thk=0.15 mm).

The images demonstrate that S-Gal is sensitive to β-galactosidase, generating a paramagnetic black precipitate revealed as intense T2 contrast. The contrast may be detected from E.coli induced to express β-gal.

Conclusion:

S-Gal^{TM} is commercially available and readily enters cells. Action of β-gal rapidly generates an intense black precipitate which induce strong T2 relaxation and intense MRI contrast. We believe this holds great promise as a novel MRI approach for imaging gene activity and detect gene function.

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