Novel "Smart" 1H MRI Contrast Agents for Assessing LacZ Gene Expression

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Introduction

The application of reporter genes to study gene expression and regulation in biological systems is common practice. Among the widely used reporter proteins, β-gal (*lacZ*) is recognized as the most attractive reporter gene, and its introduction has become a standard means of assaying clonal insertion, transcriptional activation, protein expression, and protein interaction. Many colorimetric substrates are available commercially, but *in vivo* assays would be more powerful. Recently, Weissleder *et al*.[1] presented a near infrared *in vivo* approach based on DDAOG, Meade *et al*.[2] reported a proton MRI approach using EgadMe, and Mason *et al*.[3,4] presented both proton and ^{19}F NMR methods using S-gal™ and fluorophenol β-D-galactosides. S-gal™ was effective, but the molecule was designed for histology and can be optimized for *in vivo* MRI applications. We now present analogs of S-gal™ further demonstrating this fundamentally novel mechanism of "smart" ^{1}H MRI contrast agent, whereby the paramagnetic material is not generated until β-gal acts on the substrates (here **AZD-3** or **AZD-5)** in the presence of Fe^{3+} ions to generate a precipitate (Figure 1).

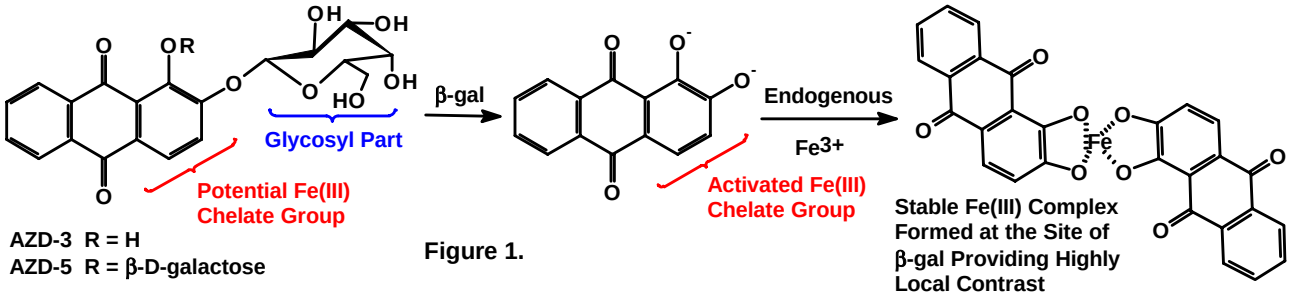


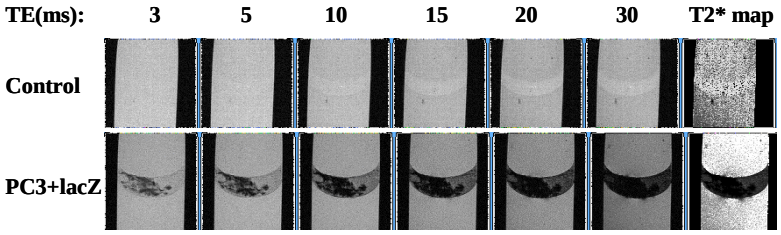
Figure 1.

Materials and Methods

AZD-3 and **AZD-5** were stereoselectively synthesized and characterized in our lab. MR images were obtained using a Varian Unity INOVA 400 NMR spectrometer with gradient echo imaging: TR=100ms, Flip angle=10°, TE=multiple values 3-30ms, Matrix=256×128, FOV=48×24mm. As an example 10^6 PC3-LacZ or wild type cells were layered in agarose ferric ammonium citrate (2.5 μg/mL) and AZD**-5** (1.5 μg/mL).

Results

A series of tests in solution and cultured tumor cells proved the principle. Both **AZD-3** and **AZD-5** were cleaved effectively by β-gal generating an intense black precipitate, which provides strong T_2* relaxation and intense Fe(III)-based ^{1}H MRI contrast (Figure 2).



Conclusion

These results provide further evidence for the broad specificity of β-gal to cleave diverse substrates. The black paramagnetic precipitate is analogous to that formed using commercial S-gal™ and demonstrates the potential for derivatizing the substrate to optimize the MR active molecule. Here, ferric ions were added. However, it is noteworthy that tumor cells, as compared with their normal counterparts, frequently exhibit increased uptake and utilization of iron and thus endogenous ferric ions may suffice for *in vivo* applications. We believe, this novel "smart" Fe(III)-based ^{1}H MRI contrast agent mechanism holds great promise as a fundamentally different ^{1}H MRI platform for *in vivo* assessing *lacZ* gene activity.

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References

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