A Novel Approach in the Development of 19F NMR Reporter to Assess LacZ Gene Expression

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Introduction

The *lacZ* gene encoding β-gal 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. Therefore, noninvasive *in vivo* detection of *lacZ* transgene expression and regulation would be of considerable value in many ongoing and future clinical cancer gene therapy trials. 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. Recently, Mason *et al.* have presented both proton and ^{19}F NMR methods using S-gal™[3], AZD-3[4] and fluorinated phenolic β-D-galactosides.[5,6] A problem with the ^{19}F NMR approach was that product aglycones were not trapped at site of activity, but washed out and hence difficult to detect *in vivo*. Iron chelation therapy as novel strategies using some chemical chelators capturing iron in tumor has been applied to clinically treat some cancers. We now explore this new class of ^{19}F NMR reporter (**LCD-1**) exploiting enzyme-activated Fe-chelation formation to specifically retain the ^{19}F NMR reporter, aiming to increase signal-to-noise and potentially reducing the requisite doses of reporter molecule (**Figure 1)**.

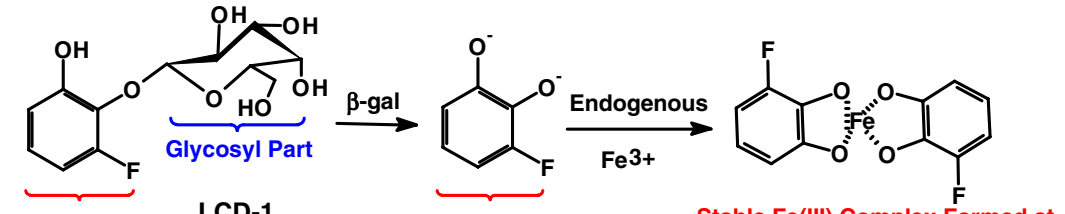


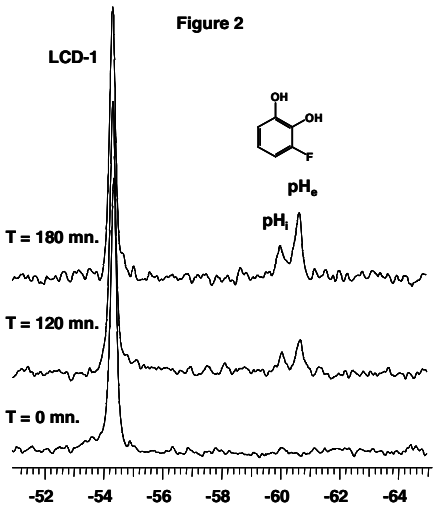
Figure 1.

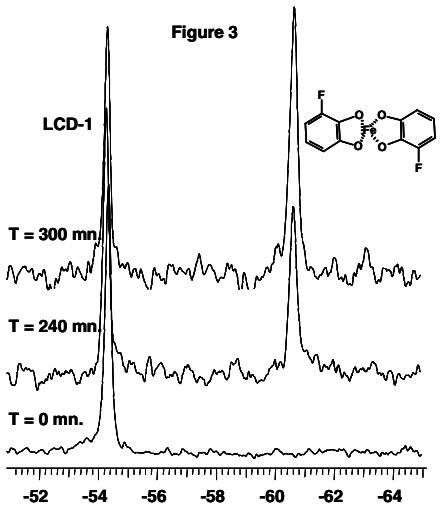
Materials and Methods

LCD-1 was stereo- and regio-selectively synthesized and characterized in our lab. ^{19}F NMR spectra were obtained using a Varian Unity INOVA 400 NMR spectrometer with a dilute solution of sodium trifluoroacetate (NaTFA) in a capillary as external standard (ppm) at 37°C.

Results

LCD-1 was stable in solution and gave a single sharp ^{19}F NMR signal. Addition of β-gal gave effective cleavage (215 μmol/min per unit β-gal). Addition of **LCD-1** to MCF7-*lacZ* cells caused rapid cleavage (**Figure 2**, 8 μmol/min per million cells) generating two new signals, which we attribute to intra and extra cellular compartments. These provide an indication of transmembrane pH gradient. When ferric ammonium citrate (FAC) was included, cells generated a purple solution indicative of Fe-complex formation (**Figure 3).** Now only a single resonance was observed, which we believe represents the trapped complex.





Conclusion

The reporter molecule specially accumulating, targeting or delivering to tumor cells is a great challenge for *in vivo* application with good sensitivity, specificity and spatial localization. These preliminary data demonstrate the feasibility of a novel approach to detecting β-gal activity, *i.e.*, generating complexes to trap the released reporter molecule products. Reassuringly, the product signals remain narrow and detectable, and we believe this fundamentally different approach shows promise for developing *in vivo* ^{19}F NMR platforms to gene reporter molecules.

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References

[1] Tung *et al. Cancer Res.*, **2004**, 64, 1579-1583. [2] Louie et *al. Nature Biotechnol*, **2000**, 18, 321-325. [3] Cui et *al. ISMRM*, Kyoto, *#1712,* **2004.** [4] Yu *et al. 13th ISMRM*, *#0167,* Florida, May, **2005.** [5] Yu et *al. Curr. Med. Chem.*, **2005**, 12, 819-848. [6] Yu et *al. Bioconjugate Chem.*, **2004**, 15, 1334-1341.