Hyperpolarized ^{13}C Magnetic Resonance Detection of Carboxypeptidase G2 Activity

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INTRODUCTION

Carboxypeptidase G2 is a bacterial enzyme that is currently employed in a range of cancer chemotherapy strategies, such as gene directed enzyme prodrug therapy (GDEPT), designed to selectively activate non toxic prodrugs into potent cytotoxics in the tumor (1). These therapeutic strategies would benefit from appropriate imaging techniques to assess the biodistribution of CPG2 activity.

We have previously reported the successful hyperpolarisation of several ^{13}C nuclei of 3,5-Difluorobenzoylglutamic acid (3,5-DFBGlu) (2), an in vivo ^{19}F MRS reporter of CPG2 activity (3). Employing Dynamic Nuclear Polarization (DNP) and natural abundance ^{13}C magnetic resonance spectroscopy (MRS) we here report the successful detection of the CPG2-mediated conversion of 3,5-DFBGlu to 3,5-difluorobenzoic acid (3,5-DFBA) and L-glutamic acid (L-Glu) *in vitro*.

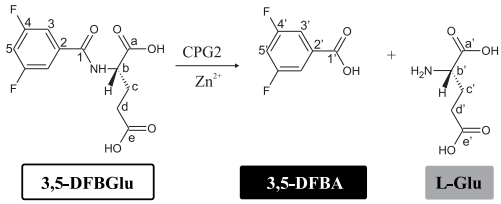

Figure 1. : CPG2-mediated conversion of 3,5-DFBGlu

MATERIAL & METHODS

Dynamic Nuclear Polarisation: 5 mg of 3,5-DFBGlu, 3,5-DFBA or glutamic acid (natural abundance 1% ^{13}C) were separately dissolved in solutions containing 3mg of free radical OX63 with a glassing agent composed of 2:1 DMSO:H_2O and polarized for 3 hours in a HyperSense® DNP at low temperature (1.4 K) with microwave irradiation at 94 GHz. The polarized samples were rapidly dissolved in 4ml of a solution of 100mM TrisHCl, 260 µM $ZnCl_2$, 1 mM EDTA and transferred to 200 µl of 100mM TrisHCl with $ZnCl_2$ (6 mM final concentration) and placed in a 11.7T spectrometer. The pH of the dissolution solution was adjusted with 10M NaOH to provide a final solution with pH = 7.4.

CPG2 activity assay: 3,5-DFBGlu was polarized as described above and transferred to a solution containing 10 units of purified CPG2.

^{13}C MRS: A series of ^{13}C NMR spectra were subsequently acquired every 3 s using a 10° pulse-and-acquire sequence (1 transient, 64k time domain points, a 19kHz spectral width, acquisition time 1.7s, T = 310K).

RESULTS

3,5-DFBGlu, 3,5-DFBA and L-Glu (Figure 1) were successfully hyperpolarized (Figure 2). The ^{13}C T_1 values were calculated from the decay of the hyperpolarized signal over time as described in (4) and are reported in Table 1. Following addition of the polarized 3,5-DFBGlu to the solution of CPG2, several hyperpolarised ^{13}C resonances from 3,5-DFBA and L-Glu were detected as a result of the CPG2 mediated cleavage of 3,5-DFBGlu (Figure 3).

3,5-DFBGlu		3,5-DFBA		L-Glu		ΔΩ (ppm)
$^{13}C_1$	6.5 ± 0.5s	$^{13}C_{1'}$	22.9 ± 1.6s			4.8
$^{13}C_2$	5.7 ± 0.4s	$^{13}C_{2'}$	18.8 ± 1.7s			3.2
$^{13}C_a$	9.3 ± 0.4s			$^{13}C_{a'}$	Not detected	-
$^{13}C_e$	7.5 ± 0.4s			$^{13}C_{e'}$	11.3 ± 0.6s	– 0.4

Table 1. ^{13}C T_1 values and observed chemical shift changes ΔΩ (ppm) on CPG2 mediated cleavage

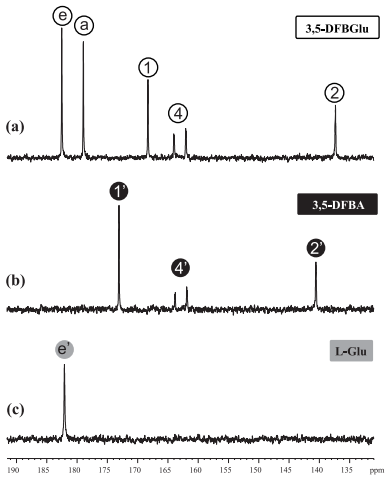

Figure 2. : Hyperpolarised ^{13}C MR spectra of 5mg of (a) 3,5-DFBGlu, (b) 3,5-DFBA and (c) L-Glu. (NS 1, α= 10°, TR =3s)

DISCUSSION AND CONCLUSION

We here report the successful detection of the CPG2-mediated conversion of 3,5-DFBGlu to 3,5-difluorobenzoic acid (3,5-DFBA) and L-glutamic acid (L-Glu) *in vitro*. The relatively long T_1 of the detectable ^{13}C resonances of 3,5-DFBGlu encourage the translation of this approach *in vivo*. With a T_1 over 20 s in the cleaved molecule (3,5-DFBA) and a ~5 ppm change upon cleavage by CPG2, the C_1 of the benzoyl moiety of 3,5-DFBGlu appears to be the best candidate for ^{13}C isotopic enrichment, a required step towards the translation of this approach *in vivo.* This strategy could allow the use of volume coils to gather whole body information on the biodistribution of CPG2.

ACKNOWLEDGEMENTS

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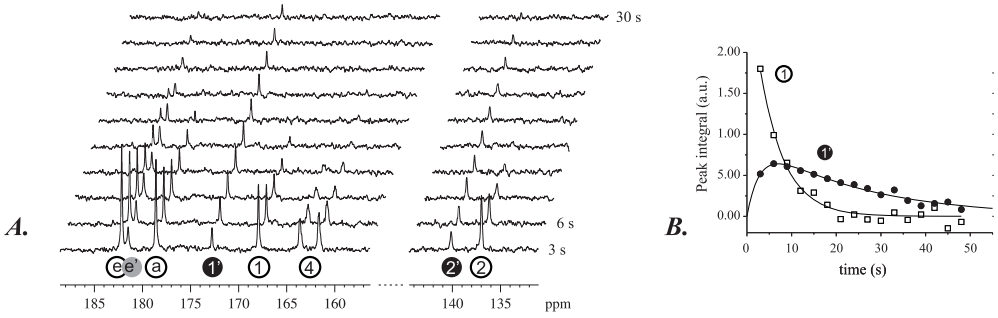

Figure 3. ***A.*** Serial ^{13}C MR spectra recorded every 3s following addition of hyperpolarized 3,5-DFBGlu to 10 units of CPG2. ***B.*** Integrals of the parent peak 1 and its metabolic product 1'.