C-labeled indolequinone-DTPA-Gd conjugate for NMR probing cytochrome:P450 reductase-mediated one-electron reduction.

Komatsu, Hirokazu; Tanabe, Kazuhito; Nishimoto, Sei-ichi

Bioorganic & medicinal chemistry letters (2011), 21(2): 790-793

© 2010 Elsevier Ltd.; この論文は出版社版ではありません。引用の際には出版社版をご確認ご利用ください。This is not the published version. Please cite only the published version.
$^{13}$C-labeled indolequinone-DTPA-Gd conjugate for NMR probing
cytochrome:P450 reductase-mediated one-electron reduction

Hirokazu Komatsu, Kazuhito Tanabe,* and Sei-ichi Nishimoto*

Department of Energy and Hydrocarbon Chemistry, Graduate School of Engineering,
Kyoto University, Katsura Campus, Nishikyo-ku, Kyoto 615-8510, Japan

*corresponding author

Phone: +81-75-383-2505 FAX: +81-75-383-2504
e-mail: tanabeka@scl.kyoto-u.ac.jp, nishimot@scl.kyoto-u.ac.jp
Abstract

We designed and synthesized a new class of $^{13}$C-labeled NMR probe, $^{13}$C-IQ-Gd, to monitor one-electron reductions by cytochrome:P450 (CYP450) reductase under hypoxic conditions. $^{13}$C-IQ-Gd consisted of a Gd$^{3+}$-diethylene triamine pentaacetic acid (DTPA) complex unit and an indolequinone ($^{13}$C-IQ) unit bearing a $^{13}$C-labeled methoxy group. The $^{13}$C-NMR signal of $^{13}$C-IQ-Gd was suppressed because of the intramolecular paramagnetic effect of Gd$^{3+}$, whereas enzymatic reduction mediated by CYP450 reductase under hypoxic conditions yielded an intensified $^{13}$C-NMR signal due to enzymatic activation of the IQ unit followed by release of the DTPA-Gd unit from $^{13}$C-IQ-Gd. This $^{13}$C-NMR spectral change allowed the monitoring of CYP450 reductase-mediated one-electron reduction.

Keywords

$^{13}$C-NMR probe, Gd-complex, Paramagnetic effect, Cytochrome:P450 reductase, Hypoxia
Cytochrome P450 (CYP450) reductase catalyzes one-electron reduction of various substrates with the aid of cofactors such as β-NADPH. CYP450 reductase has been identified to play key functions in liver detoxification, biological synthesis of steroid hormones, metabolism of fatty acids and drug activation in malignant tumor cells.\textsuperscript{1,2} In particular, the enzyme operates effectively in tumor hypoxia that characterizes various types of solid tumor tissues.\textsuperscript{3} Under hypoxic conditions, CYP450 reductase activates certain drugs and molecular probes, which allows the expression of their inherent functions.\textsuperscript{4,5} In this context, one-electron reduction mediated by CYP450 reductase has attracted considerable attention for designing hypoxia-targeting drugs and probes.

Recently, highly sensitive and sophisticated \textsuperscript{13}C NMR/MRI techniques such as multiple-resonance\textsuperscript{6} and hyperpolarized NMR have been developed.\textsuperscript{7} In this association, various types of \textsuperscript{13}C-labeled compounds have been thitherto reported for application to highly sensitive \textsuperscript{13}C-NMR analysis of complicated biological systems due to low level of natural \textsuperscript{13}C abundance.\textsuperscript{8}

The present study aims at developing a new class of \textsuperscript{13}C-NMR probe to monitor CYP450 reductase-mediated one-electron reduction. We designed and synthesized a
conjugate ($^{13}$C-IQ-Gd) consisting of a Gd$^{3+}$-diethylene triamine pentaacetic acid (DTPA) complex unit$^9$ and a $^{13}$C-labeled indolequinone ($^{13}$C-IQ) unit that undergoes one-electron reduction by CYP450 reductase (Figure 1). $^{13}$C-IQ-Gd alone showed no apparent signal in the $^{13}$C-NMR spectrum because of the intramolecular paramagnetic effect of Gd$^{3+}$,$^{10}$ whereas the $^{13}$C-IQ unit was activated upon treatment with one-electron reducing CYP450 reductase to be separated from the Gd-DTPA unit and thereby showed intense $^{13}$C-NMR signal.

(Figure 1)

The synthesis of $^{13}$C-IQ-Gd is outlined in Scheme 1. The hydroxyl group of 1 was methylated by means of $^{13}$C-labeled methyl iodide, and following nitration and reduction gave aminooindole (3). The resulting 3 was converted into indolequinone ($^{13}$C-IQ-OH) via treatment with LiAlH$_4$ and Fremy’s salt, and was then coupled with DTPA to give $^{13}$C-IQ-DTPA.$^{12}$ Finally, $^{13}$C-IQ-DTPA was coordinated with Gd$^{3+}$ to give $^{13}$C-IQ-Gd.$^{13}$ The NMR probe $^{13}$C-IQ-Gd thus obtained was water soluble up to 4
mM. We confirmed that metal coordination resulted in a broadening and weakening in 13C-NMR signal at 57 ppm of the original 13C-IQ-DTPA (Figure 2B) because of the paramagnetic effect of Gd3+.

(Scheme 1)

Upon incubation of 13C-IQ-Gd with CYP450 reductase and its cofactor β-NADPH in hypoxic aqueous solution the reaction was monitored by 13C-NMR. As shown in Figure 2C, a new signal appeared at 63 ppm after the enzymatic treatment for 1 h. This response is consistent with the present molecular design. 13C-IQ-Gd was activated by enzymatic reduction to release the DTPA-Gd unit, resulting in the appearance of a 13C NMR signal along with a decreased extent of intramolecular paramagnetic effect due to separation of the 13C-labeled IQ unit from the DTPA-Gd unit. In contrast, aerobic enzymatic reduction of 13C-IQ-Gd by CYP450 reductase yielded no apparent signal (Figure 2D). These results indicate that the enzymatic reduction of 13C-IQ-Gd occurred in a hypoxia-selective manner, as monitored by 13C-NMR.
For further characterization of this enzymatic reduction, we performed a similar enzymatic reaction of $^{13}$C-IQ-OH without bearing a DTPA-Gd unit that showed a distinct signal at 57 ppm in $^{13}$C-NMR (Figure 3A) in contrast to $^{13}$C-IQ-Gd. After treatment with CYP450 reductase in the presence of $\beta$-NADPH for 1 h in hypoxic phosphate buffer, we observed appearance of a new $^{13}$C-NMR signal at 63 ppm (Figure 3B), which is identical with the signal resulted from $^{13}$C-IQ-Gd upon similar enzymatic treatment. These results are a strong indication that the $^{13}$C-IQ unit was activated by CYP450 reductase to be released from $^{13}$C-IQ-Gd or $^{13}$C-IQ-OH during the enzymatic reaction. Recent studies on the activation mechanism of IQ derivatives under reduction conditions demonstrated that IQ was converted to an electrophilic intermediate 4, which was trapped by ambient nucleophiles (Figure 4). In this context, one of the possible reaction products that yielded a characteristic $^{13}$C-NMR signal at 63 ppm is a $^{13}$C-IQ-protein adduct. We therefore attempted to identify the reaction
products. However, the product characterization was unsuccessful, because purification of the reaction product could not be achieved.

(Figure 3)

(Figure 4)

An attempt was also made to feature the selective activation of $^{13}$C-IQ-Gd by CYP450 reductase.$^{17}$ We compared enzymatic activity toward $^{13}$C-IQ-Gd of CYP450 reductase with five other oxidases and reductases such as glucose dehydrogenase, alcohol dehydrogenase, peroxidase, nitrate reductase, and flavin reductase. In contrast to CYP450 reductase (see Figure 2C), incubation of $^{13}$C-IQ-Gd with the other enzymes failed to give signal in $^{13}$C NMR (Figure S1). Thus, it is most likely that $^{13}$C-IQ-Gd undergoes one-electron reduction exclusively by CYP450 reductase.

In summary, we designed and synthesized a new class of $^{13}$C-labeled NMR probe for monitoring of enzymatic one-electron reduction by CYP450 reductase. The reduction of $^{13}$C-IQ-Gd, which consisted of a $^{13}$C-labeled IQ unit and a DTPA-Gd unit, was
monitored by $^{13}$C-NMR. Hypoxia-selective activation of $^{13}$C-IQ-Gd induced appearance of a $^{13}$C-NMR signal of the $^{13}$C-methoxy group in the IQ unit, which was attributable to a decreased extent of the intramolecular paramagnetic effect of Gd$^{3+}$. In view of these properties, $^{13}$C-IQ-Gd is a promising candidate for an NMR probe for the presence of CYP450 reductase.

**Acknowledgement**

This work is partly supported by the Innovative Techno-Hub for Integrated Medical Bio-imaging Project of the Special Coordination Funds for Promoting Science and Technology, from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan.
References and notes


12. $^{13}$C-IQ-DTPA: Red solid: MP 122–125 °C.; $^1$H NMR (300 MHz, CD$_3$OD) $\delta$ 1.95 (s, 8H), 2.24 (s, 3H), 3.30–3.35 (10H), 3.76 (d, $J = 150$ Hz, 3H), 3.85 (s, 3H), 4.66 (s, 2H), 5.64 (s, 1H); $^{13}$C NMR (67.8 MHz, DMSO-$d_6$) $\delta$ 9.3, 32.0, 54–58 (strong), 106.5, 120.6, 122.3, 127.6, 137.3, 159.2, 170.8, 172.6, 173.6, 176.6, 177.5, 178.1; FABMS: m/e 634 [(M+Na)$^+$]; HRMS calcd for C$_{25}$H$_{31}$N$_4$NaO$_{13}$ $^+$ [(M+Na)$^+$] 634.2048, found 630.2048.

13. $^{13}$C-IQ-Gd: Orange oil: ESI-MS m/z 765.5 (calcd for [M-H]$^-$ 765.1) Purity was confirmed by analytical HPLC.

14. Bioreduction by CYP450 reductase. To establish hypoxia, a solution of NADPH:cytochrome P450 reductase (final concentration: 0.13 μM) and $\beta$-NADPH (final concentration: 2 mM) in 5 mM phosphate buffer (pH 7.4) was purged with argon for 10 min at 37 °C. The resulting solution was added $^{13}$C-IQ-Gd (final concentration: 2 mM) and incubated at 37 °C for 1 h. The $^{13}$C-NMR spectra were then measured (acquisition time: 2048 times). A control aerobic sample solution was incubated and analyzed in a similar manner. Experiment with $^{13}$C-IQ-OH was conducted in a similar manner in the presence of MeCN (final concentration: 10%).

15. Although a small amount of CYP450 reductase (0.13 μM) was used for the reduction of $^{13}$C-IQ-OH (2 mM), the signal of reduction product of $^{13}$C-IQ-OH
was distinctly observed in the NMR spectra as shown in Figure 3. This result strongly indicates that the catalytic reduction of indolequinone derivatives by CYP450 reductase occurred in the present system.


17. Bioreduction by other enzymes. Five Enzymes (Glucose dehydrogenase (from microorganism) PQQ- 0.1 mg/mL, alcohol dehydrogenase (from yeast) 0.25 mg/mL, peroxidase (from horseradish) 0.1 mg/mL, nitrate reductase (cytochrome) 0.005 unit, and flavin reductase (recombinant) 1 μg/mL) were used in this study.

**Supplementary Material**

Supplemental Material is available. $^{13}$C-NMR spectra of $^{13}$C-IQ-Gd treated by five enzymes.
Scheme 1. Reagents: (a) NaH, $^{13}$CH$_3$I, 64%; (b) HNO$_3$, AcOH, 78%; (c) Sn, HCl, EtOH, 76%; (d) LiAlH$_4$, THF; (e) Fremy’s salt, Me$_2$CO, NaH$_2$PO$_4$, water, 77% in 2 steps; (f) DTPA, TATU, DMAP, DIPEA, DMF, 77%; (g) GdCl$_3$, NaHCO$_3$, water, 94%.
Figure 1. (A) Molecular design of $^{13}\text{C-IQ-Gd}$ as a probe for one-electron reduction by CYP450 reductase. (B) Chemical Structure of $^{13}\text{C-IQ-Gd}$. 
Figure 2. $^{13}$C-NMR spectra of (A) $^{13}$C-IQ-DTPA alone and (B, C, D) $^{13}$C-IQ-Gd upon treatment with CYP450 reductase (10 µg/mL) in the presence of β-NADPH (2 mM) at 37 °C in phosphate buffer (pH 7.4): (B) before treatment with reductase; (C) treated under hypoxic conditions for 1 h; (D) treated under aerobic conditions for 1 h.
Figure 3. $^{13}$C-NMR spectra of $^{13}$C-IQ-OH (2 mM) (A) before and (B) after treatment with CYP450 reductase (10 µg/mL) in the presence of β-NADPH (2 mM) at 37 °C for 1 h in hypoxic phosphate buffer (pH 7.4, 10% MeCN).
Figure 4. Plausible activation pathways of $^{13}$C-IQ-Gd leading to elimination of DTPA-Gd unit followed by alkylation of the resulting iminium intermediate.