TITLE:

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

AUTHOR(S):

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

CITATION:

Komatsu, Hirokazu ...[et al]. (13)C-labeled indolequinone-DTPA-Gd conjugate for NMR probing cytochrome:P450 reductase-mediated one-electron reduction.. Bioorganic & medicinal chemistry letters 2011, 21(2): 790-793

ISSUE DATE:

2011-01-15

URL:

<http://hdl.handle.net/2433/134790>

RIGHT:

© 2010 Elsevier Ltd.; This is not the published version. Please cite only the published version.; 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。

^{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.^{1,2} In particular, the enzyme operates effectively in tumor hypoxia that characterizes various types of solid tumor tissues.³ Under hypoxic conditions, **CYP450** reductase activates certain drugs and molecular probes, which allows the expression of their inherent functions.^{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 ^{13}C NMR/MRI techniques such as multiple-resonance⁶ and hyperpolarized NMR have been developed.⁷ In this association, various types of ^{13}C -labeled compounds have been thitherto reported for application to highly sensitive ^{13}C -NMR analysis of complicated biological systems due to low level of natural ^{13}C abundance.⁸

The present study aims at developing a new class of ^{13}C -NMR probe to monitor **CYP450** reductase-mediated one-electron reduction. We designed and synthesized a

conjugate ($^{13}\text{C-IQ-Gd}$) consisting of a Gd^{3+} -diethylene triamine pentaacetic acid (**DTPA**) complex unit⁹ and a ^{13}C -labeled indolequinone ($^{13}\text{C-IQ}$) unit that undergoes one-electron reduction by **CYP450** reductase (Figure 1). $^{13}\text{C-IQ-Gd}$ alone showed no apparent signal in the ^{13}C -NMR spectrum because of the intramolecular paramagnetic effect of Gd^{3+} ,¹⁰ whereas the $^{13}\text{C-IQ}$ 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 $^{13}\text{C-IQ-Gd}$ 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 aminoindole (**3**). The resulting **3** was converted into indolequinone ($^{13}\text{C-IQ-OH}$) via treatment with LiAlH_4 and Fremy's salt, and was then coupled with DTPA to give $^{13}\text{C-IQ-DTPA}$.¹² Finally, $^{13}\text{C-IQ-DTPA}$ was coordinated with Gd^{3+} to give $^{13}\text{C-IQ-Gd}$.¹³ The NMR probe $^{13}\text{C-IQ-Gd}$ thus obtained was water soluble up to 4

mM. We confirmed that metal coordination resulted in a broadening and weakening in ^{13}C -NMR signal at 57 ppm of the original ^{13}C -IQ-DTPA (Figure 2B) because of the paramagnetic effect of Gd^{3+} .

(Scheme 1)

Upon incubation of ^{13}C -IQ-Gd with CYP450 reductase and its cofactor β -NADPH in hypoxic aqueous solution¹⁴ the reaction was monitored by ^{13}C -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. ^{13}C -IQ-Gd was activated by enzymatic reduction to release the DTPA-Gd unit, resulting in the appearance of a ^{13}C NMR signal along with a decreased extent of intramolecular paramagnetic effect due to separation of the ^{13}C -labeled IQ unit from the DTPA-Gd unit. In contrast, aerobic enzymatic reduction of ^{13}C -IQ-Gd by CYP450 reductase yielded no apparent signal (Figure 2D). These results indicate that the enzymatic reduction of ^{13}C -IQ-Gd occurred in a hypoxia-selective manner, as monitored by ^{13}C -NMR.

(Figure 2)

For further characterization of this enzymatic reduction, we performed a similar enzymatic reaction of $^{13}\text{C-IQ-OH}$ without bearing a **DTPA-Gd** unit that showed a distinct signal at 57 ppm in $^{13}\text{C-NMR}$ (Figure 3A) in contrast to $^{13}\text{C-IQ-Gd}$. After treatment with **CYP450** reductase in the presence of β -NADPH for 1 h in hypoxic phosphate buffer, we observed appearance of a new $^{13}\text{C-NMR}$ signal at 63 ppm (Figure 3B),¹⁵ which is identical with the signal resulted from $^{13}\text{C-IQ-Gd}$ upon similar enzymatic treatment. These results are a strong indication that the $^{13}\text{C-IQ}$ unit was activated by **CYP450** reductase to be released from $^{13}\text{C-IQ-Gd}$ or $^{13}\text{C-IQ-OH}$ 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).¹⁶ In this context, one of the possible reaction products that yielded a characteristic $^{13}\text{C-NMR}$ signal at 63 ppm is a $^{13}\text{C-IQ}$ -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

1. (a) Cheng, J. W. M.; Frishman, W. H.; Aronow, W. S.; *Dm Disease-a-Month* **2010**, 56, 163. (b) Masters, B. S.; Marohnic, C. C.; *Drug Metab. Rev.* **2006**, 38, 209.
2. (a) Tanabe, K.; Zhang, Z.; Ito, T.; Hatta H.; Nishimoto, S. *Org. Biomol. Chem.*, **2007**, 5, 3745.
3. (a) Harris, A. L. *Nat. Rev. Cancer* **2002**, 2, 38. (b) Vaupel, P.; Kallinowski, F.; Okunieff, P. *Cancer Res.* **1989**, 49, 6449.
4. (a) Tanabe, K.; Hirata, N.; Harada, H.; Hiraoka, M.; Nishimoto, S. *ChemBioChem*. **2008**, 9, 426. (b) Komatsu, H.; Harada, H.; Tanabe, K.; Hiraoka, M.; Nishimoto, S.; *Med. Chem. Commun.* **2010**, 1, 50. (c) Tanabe, K.; Harada, H.; Narazaki, M.; Tanaka, K.; Inafuku, K.; Komatsu, H.; Ito, T.; Yamada, H.; Chujo, Y.; Matsuda, T.; Hiraoka, M.; Nishimoto, S. *J. Am. Chem. Soc.* **2009**, 131, 15982.
5. For recent reports on the molecular probe for hypoxia, see: (a) Nakata, E.; Yukimachi, Y.; Kariyazono, H.; Im, S.; Abe, C.; Uto, Y.; Maezawa, H.; Hashimoto, T.; Okamoto, Y.; Hori, H. *Bioorg. Med. Chem.* **2009**, 17, 6952. (b) Zhu, W. P.; Dai, M.; Xu, Y. F.; Qian, X. H. *Bioorg. Med. Chem.* **2008**, 16, 3255. (c) Liu, Y.; Xu, Y. F.; Qian, X. H.; Xiao, Y.; Liu, J. W.; Shen, L. Y.; Li, J. H.; Zhang, Y. X. *Bioorg. Med. Chem. Lett.* **2006**, 16, 1562. (d) Liu, Y.; Xu, Y. F.; Qian, X. H.; Liu, J. W.; Shen, L. Y.; Li, J. H.; Zhang, Y. X. *Bioorg. Med. Chem.* **2006**, 14, 2935. (e) Hodgkiss, R. J.; Middleton, R. W.; Parrick, J.; Rami, H. K.; Wardman, P.; Wilson G. D. *J. Med. Chem.* **1992**, 35, 1920. (f) Hodgkiss, R. J.; Jones, G. W.; Long, A.; Middleton, R. W.; Parrick, J.; Stratford, M. R. L.; Wardman, P.; Wilson, G. D. *J. Med. Chem.* **1991**, 34, 2268. (g) Zhang, S.J.; Hosaka, M.; Yoshihara, T.; Negishi, K.; Iida, Y.; Tobita, S.; Takeuchi T.; *Cancer Res.* **2010**, 70, 4490.
6. Inomata, K.; Ohno, A.; Tochio, H.; Isogai, S.; Tenno, T.; Nakase, I.; Takeuchi, T.; Futaki, S.; Ito, Y.; Hiroaki, H.; Shirakawa, M. *Nature* **2009**, 458, 106. (b) Sakai, T.; Tochio, H.; Tenno, T.; Ito, Y.; Kokubo, T.; Hiroaki, H.; Shirakawa, M. *J. Biomol. NMR* **2006**, 36, 179.
7. Day, S. E.; Kettunen, M. I.; Gallagher, F.A.; Hu, D-E., Lerche, M.; Wolber, J.;

- Golman, K.; Ardenkjaer-Larsen, J. H.; Brindle, K. M. *Nat. Med.* **2007**, *13*, 1382.
8. (a) Boudreau, E.; Pelczar, I.; Borer, P. N.; Heffron, G. J.; LaPlante, S. R.; *Biophys. Chem.* **2004**, *109*, 333. (b) Tang, Y. J.; Martin, H. G.; Myers S.; Rodriguez, S.; Baidoo, E. E. K.; Keasling, J. D. *Mass Spectrom. Rev.* **2009**, *28*, 362. (c) Godin, J. P.; Fay, L. B., Hopfgartner, G. *Mass Spectrom. Rev.* **2007**, *26*, 751. (d) Lagoja, I. M.; Herdewijn, P.; *Synthesis-Stuttgart* **2002**, *3*, 301. (e) Seriannias, B. P. B. *J. Biomol. Struct. Dyn.* **1994**, *11*, 1133.
 9. Idee, J. M.; Port, M.; Robic, C.; Medina, C.; Sabatou, M.; Corot, C. *J. Magn. Reson. Imaging* **2009**, *30*, 1249.
 10. Mizukami, S.; Takikawa, R.; Sugihara, Y.; Hori, Y.; Tochio, H.; Wälchli, M.; Shirakawa, M.; Kikuchi, K. *J. Am. Chem. Soc.* **2008**, *130*, 794.
 11. Naylor, M. A.; Swann, E.; Everett, S. A.; Jaffar, M.; Nolan, J.; Robertson, N.; Lockyer, S. D.; Patel, K. B.; Dennis, M. F.; Stratford, M. R. L.; Wardman, P.; Adams, G. E.; Moody, C. J.; Stratford, I. J. *J. Med. Chem.* **1998**, *41*, 2720.
 12. **¹³C-IQ-DTPA**: Red solid: MP 122–125 °C.; ¹H NMR (300 MHz, CD₃OD) δ 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); ¹³C NMR (67.8 MHz, DMSO-*d*₆) δ 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 C₂₅¹³CH₃₄N₄NaO₁₃⁺ [(M+Na)⁺] 634.2048, found 630.2048.
 13. **¹³C-IQ-Gd**: Orange oil: ESI-MS *m/z* 765.5 (calcd for [M-H][−] 765.1) Purity was confirmed by analytical HPLC.
 14. Bio-reduction by **CYP450** reductase. To establish hypoxia, a solution of NADPH:cytochrome P450 reductase (final concentration: 0.13 μM) and β-NADPH (final concentration: 2 mM) in 5 mM phosphate buffer (pH 7.4) was purged with argon for 10 min at 37 °C. To the resulting solution was added **¹³C-IQ-Gd** (final concentration: 2 mM) and incubated at 37 °C for 1 h. The ¹³C-NMR spectra were then measured (acquisition time: 2048 times). A control aerobic sample solution was incubated and analyzed in a similar manner. Experiment with **¹³C-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 **¹³C-IQ-OH** (2 mM), the signal of reduction product of **¹³C-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.

16. (a) Ferrer, S.; Naughton, D.P.; Threadgill, M.D. *Tetrahedron* **2003**, *59*, 3445. (b) Naylor, M. A.; Swann, E.; Everett, S. A.; Jaffar, M.; Nolan, J.; Robertson, N.; Lockyer, S. D.; Patel, K. B.; Dennis, M. F.; Stratford, M. R.; Wardman, P.; Adams, G. E.; Moody, C. J.; Stratford, I. J. *J. Med. Chem.* **1998**, *41*, 2720.
17. Bio-reduction 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

Supplementary Material is available. ^{13}C -NMR spectra of ^{13}C -IQ-Gd treated by five enzymes.

Scheme 1. Reagents: (a) NaH, $^{13}\text{CH}_3\text{I}$, 64%; (b) HNO_3 , AcOH, 78%; (c) Sn, HCl, EtOH, 76%; (d) LiAlH_4 , THF; (e) Fremy's salt, Me_2CO , NaH_2PO_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}C -IQ-Gd as a probe for one-electron reduction by CYP450 reductase. (B) Chemical Structure of ^{13}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 $\mu\text{g/mL}$) 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 $\mu\text{g/mL}$) in the presence of β -NADPH (2 mM) at 37 $^{\circ}\text{C}$ for 1 h in hypoxic phosphate buffer (pH 7.4, 10% MeCN).

Figure 4. Plausible activation pathways of $^{13}\text{C-IQ-Gd}$ leading to elimination of **DTPA-Gd** unit followed by alkylation of the resulting iminium intermediate.