Electronic Effects on the Depropargylation Process in the Reaction-based Fluorescent Detection of Palladium Species: Benzocoumarin-based Ratiometric Sensing Systems

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The palladium catalyzed cross-coupling reactions are indispensable in organic synthesis of pharmaceutical compounds, demanding convenient tools for the analysis of residual palladium contents. Propargyl aryl ether-type fluorescent sensing systems that detect Pd(0)/Pd(II)/Pd(IV) species through depropargylation require no additives. We investigated a ratiometric sensing system based on a benzocoumarin dye; thus, three propargyl aryl ethers bearing electronically-different self-immolative linkers were synthesized and their relative response rate toward Pd(0) and Pd(II) were compared. We found that the response rate became faster when the self-immolative linker became more electron-deficient. The best-performing system sensed Pd(0)/Pd(II) species selectively against various other metal species, which is also capable of fluorescent imaging of Pd(0)/Pd(II) species in cells. The work provides us a clue to accelerate the catalytic depropargylation step, which, when combined with a novel fluorophore, would enable us to develop outperforming palladium sensing systems.

Keywords: Fluorescence, Palladium, Depropargylation, Ratiometric, Electronic effect

Introduction

Palladium (Pd) species are widely used as important catalysts in organic synthesis and fuel cells. For example, the modern carbon-carbon cross-coupling reactions are frequently conducted with Pd catalysts in academia as well as in industry. The detection of residual Pd species in pharmaceutical products is crucial for their quality control.^{1,2} Conventionally, inductively coupled plasma mass spectrometer (ICP-MS) has been widely used for analysis of the residual Pd species.^{3,4} ICP-MS needs complex sample preparation steps, well experienced personnel, and high cost, demanding an alternative and efficient analysis method. The fluorescence detection method thus has received much attention in recent years since the pioneering works by Koide and coworkers who developed a fluorescein allyl ether as the first reaction-based fluorescent sensing system for Pd species.⁵ The fluorescein allyl ether undergoes the well-known Pd(0)-catalyzed Tsuji-Trost oxidative insertion that causes deallylation and release the fluorescent fluorescein derivative (Scheme 1). As this sensing scheme detects catalytically active Pd(0) species, additives such as tris(2-furyl)phosphine and sodium borohydride are required to sense Pd(II/IV) species.⁶ The sensing system can be also used to detect Pd(II) or Pt(IV) directly through a catalytic aromatic Claisen rearrangement but at 50 °C in dimethyl sulfoxide (DMSO)pH 10 buffer (1:4).⁷ Our group subsequently devised a different sensing scheme, the depropargylation route (Scheme 1), which required no additives to detect Pd species regardless of their oxidation states.8 This fluorescein

propargyl ether can detect common Pd species, such as $PdCl_2$, $PdCl_2(CH_3CN)_2$, $Pd(OAc)_2$, $(NH_4)_2PdCl_2$, and Na_2PdCl_4 , with high selectivity from other various metal species. The depropargylation process seems to proceed through two routes depending on the Pd species: in the case of Pd(0) species, it proceeds through the allenyl palladium intermediate; in the case of Pd(II) or Pd(IV) species, it proceeds through the hydration route (Scheme 1).⁹ In both cases, the end product is the depropargylated aryl alcohol (ArOH). Therefore, it is possible to develop various fluorescent detection systems for Pd species at different oxidation states, starting from fluorescent ArOH. Indeed, since this work, various aryl propargyl ether-type fluorescent detection systems have been developed by other groups.¹⁰

Recently, we have developed push-pull type benzocoumarin compounds as novel fluorophores. As π -extended coumarin analogues, the linear-shape benzocoumarins have promising photophysical properties for bioimaging applications. Additionally, they are photoand chemo-stable, and show rather environment-insensitive emission behavior in spite of the dipolar nature.^{11–16} In our continuous efforts to explore fluorescent probes,¹⁷ we have reinvestigated the palladium sensing system based on a benzocoumarin dye, which responds to Pd(0)/Pd(II) species with ratiometric fluorescence changes. Compared to the fluorescein-based sensing system that provides turn-on fluorescence response,⁸ this ratiometric sensing system may find usefulness in the quantitative analysis of Pd species in synthetic samples. In this work, to develop an efficient ratiometric sensing system based on the depropargylation

Koide and co-workers, 2007



Scheme 1. Fluorescent detection of Pd species based on the catalytic deallylation and the depropargylation schemes, and supposed intermediates in the depropargylation process.

protocol, we focused on how to accelerate the depropargylation reaction by changing the electron density in the self-immolative linker, *p*-hydroxybenzyl alcohol. The results indicate that the propargylation step proceeds faster as the electron density of the phenyl group in the self-immolative linker decreased.

Results and Discussion

As noted above, the benzocoumarin (BC) dye system has proven to be a versatile dye platform for the development of fluorescent probes. To develop BC-based fluorescent probes with ratiometric signaling capability, we investigated here three propargyl ethers (BC_Pd1, BC_Pd2, and **BC_Pd3**) based on methyl 8-(methylamino)-2-oxo-2*H*benzo[g]chromene-3-carboxylate (BC1) via the carbamate bond (Figure 1). It is well established that, upon depropargylation, the p-hydroxybenzyl group will undergo a fragmentation reaction to release BC1. This chemical conversion will accompany a significant change in the intramolecular charge transfer in the fluorophore and thus an emission spectral change. Thus, we may realize a ratifor ometric sensing system Pd species. The depropargylation step seemed to be dependent on the electron density in the aryl ether moiety, a leaving group. To investigate the electronic effect, in this work we varied the phenyl group in the self-immolative moiety with different electron densities: unsubstituted, mono- and dichlorosubstituted phenyl groups.





Figure 1. Benzocoumarin (BC1) derived, ratiometric sensing systems for Pd species bearing different self-immolative moieties.

Synthesis. The three compounds (**BC_Pd1**, **BC_Pd2**, and **BC_Pd3**) were synthesized from **BC1** by an amide bond formation with the corresponding (propargyloxy)benzyl alcohol, the self-immolative moiety including the reactive propargyl ether. The synthesis was straightforward and the final products were fully characterized by nuclear magnetic resonance and MS analysis.

Photophysical Properties. All the palladium sensing systems displayed their absorption bands with the same maximum wavelength (λ_{abs}) at 350 nm, as they share the common fluorophore. The absorption spectrum of **BC_Pd3** has tailing toward the longer wavelength region. Their emission spectra also mostly overlapped with each other, with the maximum emission peaks in 532–540 nm range. The parent dye **BC1** exhibited an absorption band with λ_{abs} at 450 nm, a significantly longer wavelength from that of the probes; it exhibited the maximum emission wavelength (λ_{em}) at 600 nm, with substantial Stokes shifts of 60–68 nm from the corresponding probe (Figure 2). Thus, the sensing systems were expected to provide ratiometric signal changes in detecting Pd species.

Relative Response Rate. A key concern in this work was to realize faster response in detecting Pd species, from such carbamate-linked propargyl ether type probes. We monitored the time-course of fluorescence changes for BC_Pd1, BC_Pd2, and BC_Pd3, each at 5.0 µM in acetonitrilewater (1/9 by volume), in the presence of an equivalent amount of PdCl₂ as a Pd(II) species. We followed the timedependent changes of the emission peak height ratios between the reaction product **BC1** and probe, I_{600}/I_{530} . In the case of BC_Pd1, the sensing process was slow, as indicated by the slow ratio change (Figure 3(a)). On the other hand, BC_Pd2, in particular, BC_Pd3 exhibited faster response, as indicated by the steeper increase in the ratio value. We also compared the relative response rate of the probes toward (Ph₃P)₄Pd as a Pd(0) species, under otherwise the same conditions (Figure 3(b)). Again, BC_Pd3 exhibited faster response than the other probes, and BC_Pd1 responded little in this case. When we compare the relative rate between the Pd(II) and Pd(0) species, BC_Pd3 responds faster toward the latter species $(I_{600}/I_{530} = 2.0 \text{ vs. } 8.1)$. For the quantitative



Figure 2. Normalized (a) absorption and (b) emission spectra of **BC_Pd1**, **BC_Pd2**, **BC_Pd3** and **BC1**, measured in CH₃CN–water (1/9, by volume) at 25 °C under excitation at the maximum absorption wavelength of each dye. A, absorbance; RFU, relative fluorescence unit.

analysis of residual Pd species, we need to compare the ratio value at a given time with respect to those in a standard curve/data. 18

The fluorescence titration data with the best-performing BC_Pd3 toward the two Pd species revealed its ratiometric detection behavior and the relative response rate (Figure 4). The results suggest that the catalytic depropargylation step (Scheme 1) proceeds faster with Pd(0) species than with Pd(II) species. When we monitored the ratio change of BC_Pd3 (5.0 µM) in the presence of varying concentration of (Ph₃P)₄Pd or PdCl₂ for a fixed reaction time of 2 h, the ratio signal became saturated approximately at two equivalents of the Pd(0) source. From the initial-stage data (Figure 5), the detection limits of BC_Pd3 were determined to be 14 nM and 55 nM for the Pd(0) and Pd(II) species, respectively. It seems that the ratio value is dependent on the Pd source, which suggests that the metal ligands also affect the emission intensity of the probe and its product.



Figure 3. (a) Time-course of fluorescence intensity ratio (I_{600}/I_{530}) changes of **BC_Pd1**, **BC_Pd2**, and **BC_Pd3**, each at 5.0 μ M, upon addition of an equivalent amount of (a) PdCl₂ and (b) Pd(-PPh₃)₄ in CH₃CN–water (1/9) at 25 °C, obtained under excitation at 400 nm, the isosbestic point.



Figure 4. Fluorescence titration of **BC_Pd3** (5 μ M) with (a) (Ph₃)₄Pd and (b) PdCl₂, each at 5.0 μ M in CH₃CN–water (1/9, by volume) at 25 °C. The excitation wavelength was 400 nm.



Figure 5. Fluorescence intensity ratio (I_{600}/I_{525}) of **BC_Pd3** (5.0 μ M) versus increasing concentrations of the (a) Pd(0) and (b) Pd(II) species in CH₃CN–water (1/9). The ratio values were acquired after 2 h incubation at 37 °C. The excitation wavelength was 400 nm.

Finally, we compared the sensing selectivity of **BC_Pd3** toward other metal species. The ratio values (I_{600}/I_{530}), observed in the presence of Cr(II), Ca(II), Co(II), Cu(II), Ni(II), Mg(II), Hg(II), Mn(II), Cd(II), Ag(I), Ba(II), Zn(II),



Figure 6. (a) Fluorescence intensity ratio (I_{600}/I_{530}) changes of **BC_Pd3** (5.0 µM) toward various metal species (1, probe only; 2, Cr(II); 3, Ca(II); 4, Co(II); 5, Cu(II); 6, Ni(II); 7, Mg(II); 8, Hg(II); 9, Mn(II); 10, Cd(II); 11, Ag(I); 12, Ba(II); 13, Zn(II); 14, Pb(II); 15, Pt(II); 16, Fe(III); 17, Au(III); 18, Cr(III); 19, Fe(II); 20, Pt(IV); 21, Rh(III); 22, Pd(0); 23, Pd(II); as chloride salts except Pt(II), for which H₂PtCl₆ was used) in CH₃CN–water (1/9), measured after 2 h at 25 °C.



Figure 7. Fluorescence images of A549 cells incubated with **Pd_BC3** (5.0 μ M) in phosphate buffer saline (PBS) (10 mM, pH 7.4) for 2.0 h; - with Pd(Ph₃)₄ (30 μ M) for 30 min and then with **Pd_BC3** (5.0 μ M) in PBS (10 mM, pH 7.4) for 2.0 h; - with PdCl₂ (30 μ M) for 30 min and then with **Pd_BC3** (5.0 μ M) in PBS (10 mM, pH 7.4) for 2.0 μ M) in PBS (10 mM, pH 7.4) for 2.0 h at 36 °C. (a) The images were obtained under one-photon excitation at 405 nm and collection of emissions through green (450–555 nm) and red (570–750 nm) windows, respectively. The ratiometric images were constructed from the pixel-to-pixel intensity ratio values between the red and green (I_{Red}/I_{Green}) images. The scale bar is 75 μ m. (b) Respective intensity plots of the ratio images.

Pb(II), Pt(II), Fe(III), Cr(III), Fe(II), Pt(IV), and Rh(III) species, each at 10 equivalents, reveal that the probe is highly selective to Pd(0) and Pd(II) species (Figure 6). The other metal species caused slight increase or decrease in the ratio value from the background ratio value ($I_{600}/I_{530} = 0.52$ in the case of the probe only), an inherent feature of the ratiometric sensing system whose emission peak overlaps.

Cellular Imaging of Pd Species. We briefly evaluated the cellular imaging capability of **BC_Pd3**. A549 cells were incubated with Pd(0) or Pd(II) species, each at 30 μ M for 30 min, and then with the probe (5.0 μ M) for 2 h were fluorescently imaged by confocal laser scanning microscopy. The cellular images were obtained by collecting of green (450–555 nm) and red (570–750 nm) fluorescence under excitation at 405 nm. The corresponding ratio ($I_{\text{Red}}/I_{\text{Green}}$) images were significantly higher than that obtained for the cells incubated with probe only (Figure 7). The results

show that **BC_Pd3** can be used to detect Pd(0)/Pd (II) species in biological systems.

Conclusion

We investigated fluorescent detection systems for palladium species at different oxidation states, by following the depropargylation sensing scheme previously reported by us. To develop a ratiometric sensing system, we prepared three (p-propargyloxy)benzyl carbamates based on a dipolar benzocoumarin dye and compared their relative response times depending on the self-immolative linker. The propargyl ether based on o-dichlorophenyl moiety responded to Pd(0) and Pd(II) species faster than the mono-chloro- and non-chloroanalogues. The best-performing system selectively responded to the two palladium species with ratiometric fluorescence signal changes against various other metal species, which is also capable of fluorescent imaging of Pd(0)/Pd(II) species in cells. This work hints us on how to accelerate the catalytic depropargylation step, which, when combined with a novel fluorophore, would lead to outperforming ratiometric probes for biological and analytical applications in future.

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Conflict of Interest. The authors declare no conflict of interest.

Supporting Information. Additional supporting information may be found online in the Supporting Information section at the end of the article.

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