Intracellular Deprotection Reactions Mediated by Palladium Complexes Equipped with Designed Phosphine Ligands

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Supporting Information

ABSTRACT: Discrete palladium(II) complexes featuring purposely designed phosphine ligands can promote depropargylation and deallylation reactions in cell lysates. These complexes perform better than other palladium sources, which apparently are rapidly deactivated in such hostile complex media. This good balance between reactivity and stability allows the use of these discrete phosphine palladium complexes in living mammalian cells, whereby they can mediate similar transformations. The presence of a phosphine ligand in the coordination sphere of palladium also provides for the introduction of targeting groups, such as hydrophobic phosphonium moieties, which facilitate the accumulation of the complexes in mitochondria.

KEYWORDS: palladium, discrete complexes, bioorthogonal, intracellular catalysis, mitochondrial confinement

The functioning of the cell is dependent on the regulated action of thousands of enzymes, many of which require metal cofactors for their activity. In most of the cases, the metal works either as a Lewis acid or as an electron-transfer center. In recent years, there have been impressive advances toward the development of artificial metalloenzymes capable of achieving transformations that do not occur in nature, with a special emphasis on those promoted by late transition metals. However, translating these metalloprotein catalysts to living settings is far from obvious and has only been proved in isolated cases and in bacterial periplasms.

More success has been attained with discrete, small transition-metal complexes that are capable of crossing cell membranes and, thereby, able to promote intracellular transformations. This is the case for several ruthenium(II) complexes that can mediate the uncaging of allyl-carbamate (alloc) protected amines in living mammalian cells.

Particularly exciting is the possibility of using Pd catalysts in biological contexts, because of the well-known transformative power of palladium in organometallic chemistry. However, the use of palladium complexes in the complex environment of living cells is seriously compromised by many issues, including solubility, stability, biorthogonal reactivity, or toxicity. Therefore, most applications that have been described so far involve the use Pd nanostructures or mesostructures, rather than discrete complexes. Hence, Bradley, Unciti-Broceta, and co-workers have claimed that small Pd(0) microspheres can induce depropargylation reactions or even Suzuki–Miyaura cross-coupling in biological settings, and Chen reported that freshly made palladium nanoparticles can promote similar reactions.

Discrete palladium complexes have also been used, but only sporadically, and with somewhat differing results. Therefore, Chen and co-workers have shown that commercial Pd precursors such as Pd(dba)2 and Pd2(allyl)2Cl2 can cleave propargyloxy groups (prop) in PBS at 37 °C, and even in HeLa cells. However, a recent study by the Weissleder group reveals that these commercial Pd sources perform poorly when using standard tissue culture (HBSS and MEM) as reaction milieu. Remarkably, these authors were able to obtain good yields in alloc-removing reactions by using bis[tris(2-furyl)phosphine]-palladium(II) dichloride as a catalyst, although stability and solubility issues precluded direct use of the complex in living settings. This problem was solved by encapsulating the metal complex within a biocompatible polymer (see Scheme 1).

The above results raise doubts on whether discrete palladium complexes can indeed be used as catalysts in complex biological media and in living cells. Such small-sized palladium complexes are very attractive, because of their well-defined nature, and the possibility of manipulating the ligands to tune solubility, stability, and reactivity properties, and eventually favor cell transport and intracellular targeting.

Herein, we demonstrate that discrete palladium complexes with designed phosphine ligands can promote propargyl and...
alloc cleavage reactions in cell lysates, and even in living mammalian cells. Importantly, appropriate engineering of the ligand allows the mitochondria accumulation of an active palladium catalyst.

Given that previous studies did not clearly establish the in vitro reactivity of different palladium sources to cleave propargyloxy groups, we investigated the catalytic performance of several complexes in PBS and cell lysates.

In addition to standard commercial species, such as [Pd(allyl)Cl]$_2$ (Pd1), Pd(OAc)$_2$ (Pd2), and Pd$_2$(dba)$_3$ (Pd3), we purposely made a series of Pd(II) complexes (Pd4–Pd9) that exhibited designed pyridine, thioether, or phosphine ligands (see Figure 1 and pp S3–S8 in the Supporting Information). In the case of the phosphine-containing derivatives, we built complexes with neutral (Pd6), anionic (Pd7), or positively charged phosphonium tethers (Pd8 and Pd9).

Instead of the typical assay consisting of the cleavage of a propargylic carbamate, we studied the depropargylation of phenol derivative 1. We chose this probe because of its good aqueous solubility, ease of synthesis, and good separation properties by HPLC-MS. Furthermore, in contrast to standard Rhodamine probes, it presents only one propargylic appendage, thereby simplifying the analysis.

The reactions were carried out using a 200 μM solution of substrate 1 and 10 mol % of the Pd sources in either water, PBS buffer, or cell lysates. Reaction conditions: 1 (0.2 mmol, 1 equiv), Pd (0.02 mmol, 0.1 equiv) in 1 mL, 37 °C, 24 h (mean ± s.e.m, n = 2). (c) Pd catalysts used in this work for the study of their catalytic performance in biological compatible media and in living cells. Yields were calculated by RP-HPLC-MS, using coumarin as an internal standard.

Figure 1. (a) Representation of the uncaging reaction of 1, and (b) bar diagram representation of the yields obtained for each catalyst under biological compatible conditions in water, PBS buffer, and cell lysates. Reaction conditions: 1 (0.2 mmol, 1 equiv), Pd (0.02 mmol, 0.1 equiv) in 1 mL, 37 °C, 24 h (mean ± s.e.m, n = 2). (c) Pd catalysts used in this work for the study of their catalytic performance in biological compatible media and in living cells. Yields were calculated by RP-HPLC-MS, using coumarin as an internal standard.
However, complexes Pd6, Pd7, and Pd9 were able to promote the depropargylation reaction in cell lysates. Although the yields were low, their performance is better than that of the other palladium sources (Pd1−Pd5). Possibly, albeit a phosphine ligand is not the best one from a reactivity point of view, the corresponding palladium complexes are more stable and can better resist hostile media such as that present in cell lysates.

We also investigated the ability of these latter phosphine palladium(II) reagents to remove alloc protecting groups, which is a process that likely involves Pd(0) reagents. The uncaging reaction was evaluated in substrate 3, in the absence or presence of added reducing agents (see Figure 2a and the Supporting Information (pp S9−S13)). The reactions were performed using 200 μM solutions of 3 in water, 10 mol % of the palladium complexes, at 37 °C, in the absence or presence of cell lysates (green column) and in cell lysate +0.4 mM of GSH (red column) promoted by catalysts Pd7 and Pd9. [Reaction conditions: 10 (0.2 mmol, 1 equiv), Pd (0.02 mM, 0.1 equiv) in 1 mL, 37 °C, 24 h.] Inset shows Pd7 and Pd9. Yields were calculated by RP-HPLC-MS, using coumarine as an internal standard (mean ± s.e.m., n = 2).

![Figure 2](https://example.com/figure2.png)

**Figure 2.** (a) Uncaging of 3, (b) plot of the yields of reaction obtained with catalysts (i) Pd7 and (ii) Pd9 at 1, 3, 6, and 24 h in only water, or with additives: NaAsc (2 mM) or GSH (0.4 mM) (reaction conditions: 3 (0.2 mmol, 1 equiv), Pd complex (0.02 mM, 0.1 equiv) in 1 mL, 37 °C, 24 h), and (c) uncaging reaction in cell lysates (green column) and in cell lysate +0.4 mM of GSH (red column) promoted by catalysts Pd7 and Pd9. [Reaction conditions: 10 (0.2 mmol, 1 equiv), Pd (0.02 mM, 0.1 equiv) in 1 mL, 37 °C, 24 h.] Inset shows Pd7 and Pd9. Yields were calculated by RP-HPLC-MS, using coumarine as an internal standard (mean ± s.e.m., n = 2).

The above results confirm that phosphine palladium(II) complexes can promote depropargylation and deallylation reactions in water with good yields. Although in cell lysates, the efficiency is considerably lower, we were intrigued to know whether the reactions could be achieved inside living cells, as the intracellular environment does not necessarily parallel that of a cell lysate. A key element to take into account before moving to intracellular reactions is uptake of the potential catalysts. Inductively coupled plasma−mass spectroscopy (ICP-MS) analysis of cellular extracts (Vero cells), obtained after extensive washing and lytic treatment, revealed a higher amount of palladium after the addition of complexes Pd6−Pd9 than of species Pd1−Pd5 (see Figures S12 and S13 in the Supporting Information). Importantly, cell viability tests (MTT in Vero cells, 24 h) demonstrated that the phosphine-containing complexes are essentially nontoxic below 50 μM, and some of them can be used even at higher concentrations (see Figure S14 in the Supporting Information). While substrate 1 was good for in vitro studies, the change in fluorescence after the
reactions is not strong enough for an appropriate monitoring in cellular settings. Therefore, we changed to the propargylated probe 5, which, after uncaging, generates a product 6 that emits at 635 nm when excited in the far-ultraviolet (far-UV) region (see Figure 3a).\textsuperscript{27}

The experiments were carried out by incubation of Vero cells at a final concentration of 50 μM of the probe 5 in culture medium containing 5% fetal bovine serum (FBS-DMEM) for 30 min at 37 °C. The milieu was removed, and cells were washed twice with FBS-DMEM to ensure removal of extracellular probe. Pd reagents (50 μM) were then added in fresh media (0.1% dimethyl sulfoxide (DMSO)), and, after 30 min, cells were imaged by wide-field fluorescence microscopy.

While in the experiments with Pd sources (Pd1–Pd3 and complexes Pd4 and Pd5), we observed almost negligible red emission (Figure S15 in the Supporting Information), with species Pd6–Pd8, equipped with phosphine ligands, we detected strong red emission due to the release of the product 6 inside Vero cells (Figure 3b). The overlap between the absorption of the pyrene unit in Pd9 and that of product 6 precluded the analysis of the reaction with this complex. Overall, these results are consistent with the in vitro studies and confirm that the phosphine ligands are beneficial for observing “in cell” reactivity, likely because the complexes present a good balance between reactivity and stability in the cellular milieu.

Considering the amount of palladium that was present in cells, as measured by ICP-MS of cell extracts after extensive washing steps, and associating the normalized fluorescence intensity with the amount of product, using calibration curves (see the Supporting Information (p S18)), it is possible to estimate whether there is some turnover. Indeed, for Pd7, we calculated an average TON of 10, whereas for Pd8, it is >5, suggesting that catalytic cycles can operate in cellular settings.

The cellular reactions can also be carried out in mammalian cells other than Vero, such as HeLa (see Figure S17 in the Supporting Information). We also checked an inverse protocol in which cells are first treated with the palladium reagents, and the probe is added after 30 min, after the corresponding washing steps. However, in these cases, we did not observe fluorescence, likely because the palladium complexes, over time, are converted to species that are not enough active in the depropargylation process.

We next explored the removal of alloc groups inside living HeLa cells, in this case, using the caged Rhodamine 110 derivative 7, which is essentially nonfluorescent, but emits green light after removing the alloc protecting groups. Initial experiments were carried out by mixing HeLa cells with 7 (50 μM in FBS-DMEM) for 30 min, 2-fold washing with FBS-DMEM, and addition of the Pd complexes. After 30 min at 37 °C, cells were visualized under the microscope. As in the case of the depropargylation reaction, the complexes Pd1–Pd5 raised only very low intracellular fluorescence, however, with species Pd6–Pd8, we could observe strong intracellular green fluorescence (see Figure S18 in the Supporting Information).

Inverting the order of addition, first the palladium complexes, and after 30 min, washing and addition of the probe 7, we detected intracellular fluorescence, but only with complexes Pd8 (see Figure S19 in the Supporting Information) and Pd9 (Figure 4). Similar results were obtained in Vero cells (see Figure S20 in the Supporting Information).

In the case of Pd9, the presence of a pyrene group in the ligand structure allowed a direct monitoring of the species in living cells. As shown in Figure 4, the blue emission of living cells after addition of this complex shows a very good co-localization with the red color of a mitotracker (Figure 4b, panels (i)–(iii)). This was further confirmed by ICP-MS analysis of cells lysed under conditions that allowed the isolation of the mitochondria from the rest of the cytoplasmic mass. As indicated in Figure 4e, Pd9 presents a substantially higher mitochondrial accumulation than the other complexes. Likely, the presence of the pyrene and phosphonium groups in the ligand allows the right combination of charge and...
hydrophobicity to favor a significant mitochondrial accumulation of the complex.

Importantly, in cells treated with Pd9, we observed an efficient transformation of 7 to 8, as deduced from the very strong buildup of green fluorescence, that also accumulates in the mitochondria surroundings (Figure 4c, panels (i)−(iii)). Other phosphine-containing catalysts, such as Pd7 or Pd8, were less active (see Figure 4d, panels (i)−(iii), as well as Figure S21 in the Supporting Information).

Given the positive results with the rhodamine alloc probe (7) and Pd8 and Pd9, using the inverse protocol (palladium complexes added first), we also tested this protocol with the rhodamine derivative having poc instead of alloc protecting groups (Rho-poc).23 The results, which are detailed in the Supporting Information, indicated that, while Pd8 failed to raise significant fluorescence, Pd9 was able to promote the depropargylation. This might be associated with a protective effect of the mitochondria surroundings on the stability of the palladium complex (see Figure S22 in the Supporting Information).

■ CONCLUSIONS

Our results confirm that, while many Pd(0) and Pd(II) sources can effect depropargylation and deallylation reactions in water and PBS, they are much less effective in complex cellular media or living cells. However, palladium complexes featuring designed phosphine ligands can promote the transformations in cell lysates with variable efficiencies, which are dependent on the characteristics of the ligands. Despite this modest activity, these palladium phosphine complexes can be translated to living mammalian cells and promote both depropargylation and alloc cleavage reactions.

Figure 4. (a) Representation of the uncaging reaction. (b) Co-localization of Pd9 with TMRE in HeLa cells, observed 2 h after incubation with the palladium complex: (i) blue light emission of Pd9; (ii) red light emission from the mitochondrial dye TMRE; and (iii) merged image of (i) and (ii). (c) Reaction promoted by Pd9: (i) blue light emission of Pd9, (ii) green light emission from released 8, (iii) merged image of (i) and (ii). (d) Reaction promoted by Pd7: (i) blue channel, (ii) green light emission from released 8, (iii) merged image of (i) and (ii). (e) Graphical representation of the ICP-MS results from mitochondrial isolation of fractioned Vero cells using complexes Pd6−Pd9 (see the Supporting Information for details). (Reaction conditions: Pd complexes (50 μM) were incubated with HeLa cells in DMEM with 5% FBS for 2 h at 37 °C before substrate 7 (50 μM) was added; the cells were incubated for another 30 min and observed under the microscope (scale bar = 20 μm).)
We have also demonstrated that, using a phosphine ligand tethered to phosphonium and hydrophobic pyrene groups, the palladium complex presents a preferential accumulation in mitochondria, wherein it remains active. This type of subcellular targeting might open new opportunities for promoting enhanced and selective biological effects in prodrug activation processes. Our results confirm the viability of engineering well-defined, discrete palladium complexes to promote relevant reactions in cellular milieu. The small-size and ligand tunability of these complexes can offer advantages with respect to other alternatives based on metalloenzymes or nanoconstructs, in terms of simplicity and synthetic access, as well as of controlling properties such as reactivity, targetability, and cellular uptake.

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