Ligand-Stabilized Pd Nanoparticle Catalysts for ^{1}H and ^{13}C Hyperpolarization In Water

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Abstract: Parahydrogen-induced polarization (PHIP) is a hyperpolarization technique in magnetic resonance utilizing the attachment of singlet spin isomer of hydrogen to target molecules capable of improving observable magnetization several orders of magnitude. The ability of heterogeneous catalysts to produce biologically relevant compounds with large signal enhancements in water facilitates medical application. This work presents Pt and Pd nanoparticle catalyst systems achieving high polarization (1.2% for both ^{1}H and ^{13}C) in aqueous media due to control of surface-ligand interactions during PHIP.

Motivation

Despite the profound advantages of nuclear magnetic resonance (NMR) in disciplines such as chemistry and medicine, its inherently low sensitivity due to the constraints of thermal polarization at liquid water temperatures has led to several hyperpolarization techniques to increase observable signals. The advent of methods such as dynamic nuclear polarization (DNP)(1), parahydrogen-induced polarization (PHIP)(2)(3), and signal amplification by reversible exchange (SABRE)(4) has led to enhancements of detectable magnetization by several orders of magnitude(5). While dissolution DNP has been demonstrated in biomedical applications, current DNP methods are limited by long polarization times and costly equipment. SABRE and PHIP techniques utilize the *para* nuclear spin isomer, parahydrogen (para-H_2). Para-H_2 is generated by passing hydrogen gas over a catalyst bed at low temperature and can be enriched to as high as 99.82% at 20 K(6). The subsequent polarization enhancement of para-H_2 is then obtained by hydrogenation of unsaturated precursors of biological probes of interest during PHIP, or non-hydrogenative coordination via SABRE.

Current PHIP methods predominantly use homogeneous transition metal catalysts such as $[Rh(I)(nbd)_2]^+[BF_4]^-$ and its derivatives(7), which cannot be readily removed from the mixture and which raise toxicity concerns in medical translation. To overcome this challenge, aqueous heterogeneous platinum and palladium catalysts are presented here which are stabilized for aqueous suspension and PHIP hydrogenation by the amino acids L-cysteine (LCys) and its derivative N-acetyl-L-Cysteine (NAC). Unlike homogeneous catalysts, heterogeneous catalysts have the potential to be immobilized on support material allowing for quick filtration of the catalyst away from the hyperpolarized molecules in solution, a necessary requirement for potential delivery of hyperpolarized contrast agents in a clinical setting. Furthermore, these ligands allow control of pairwise diffusion of para-H_2 during PHIP reactions, a necessary component of preserving signal enhancement (Fig 1). This allows optimization of metal surface environments on heterogeneous systems to reach polarization levels exceeding their reported homogeneous analogues in aqueous PHIP.

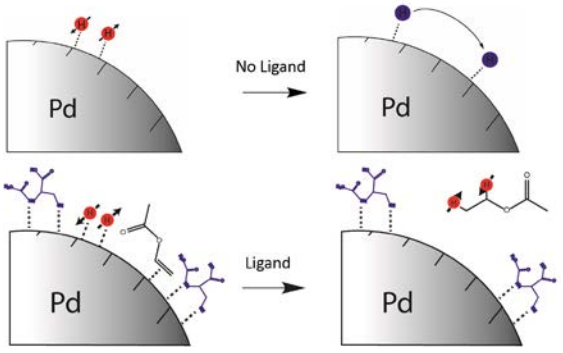

Fig. 1: Mechanism of para-H_2 addition on Pd nanoparticle surface with and without N-acetyl-L-cysteine (NAC) ligand. Without ligand, coordination hydrogen spin between atoms is lost, but ligand coordination works to preserve coordinated pairwise addition.

To overcome the relatively short T_1 relaxation of most organic protons in solution (typically 1-5 seconds), experiments to generate hyperpolarized ^{13}C spectra are also performed. Through cycling magnetic field strength during PHIP reactions from earth field to <0.1 μT using a μ-metal chamber,

hyperpolarization from attached para-H_2 can be transfered to adjacent heteronuclei with longer-lived T_1 relaxation and observable signal enhancement such as ^{13}C and ^{15}N. This not only allows longer timeframes to separate catalyst and acquire images in biological application, but better sensitivity against living tissue with abundant background water signal. To demonstrate the potential for biological applications, the acetate precursor ethyl acetate is hyperpolarized from vinyl acetate, a compound previously shown in PHIP side arm hydrogenation investigations(8). Acetate is an established metabolite probe for *in vivo* hyperpolarization studies(9). The amino acid propargylglycine (PraH) is also hydrogenated with para-H_2 into hyperpolarized allylglycine without chemical protection on the amine, and ^{1}H polarization is observed(10). As often homogeneous PHIP catalysts are reported to be unstable in the presence of amine groups, this is the first demonstration of aqueous PHIP of an unprotected amino acid by a heterogeneous catalyst.

Materials and Methods

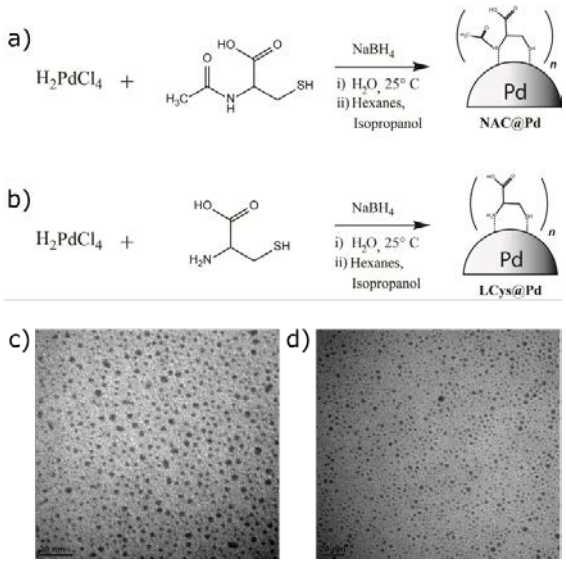

Fig. 2: Synthesis summaries of a) NAC@Pd and b) LCys@Pd nanoparticles. TEM images of c) LCys@Pd and d) NAC@Pd.

Figure 2(10) shows the synthesis of palladium particles using NAC (NAC@Pd) and LCys (LCys@Pd) as ligands. For both systems, the precursor H_2PdCl_4 was dissolved in water and reduced by $NaBH_4$ in the presence of ligand at a molar ratio of 1:1 in open air. This synthesis was initially used for LCys@Pt systems to achieve high ^{1}H polarization of 2-hydroxyethyl acrylate (HEA). Solvent fractionation of NAC@Pd and LCys@Pd using isopropanol and hexanes followed by centrifugation and repeated washes with ethanol yielded nanoparticle products able to achieve higher polarization than bulk mixtures. TEM images of LCys@Pd and NAC@Pd prepared in water reveal average particle sizes of 2.5 ± 0.8 nm and 2.4 ± 0.6 nm, respectively (Fig 2c-d)(10).

PHIP experiments were performed by suspending nanoparticles and substrate in degassed D_2O and heating to 80°C before pressurization with para-H_2 to 6.5 bar. The sample was shaken in transit to the magnet and inserted for ^{1}H experiments whereas for ^{13}C a field cycling step in and out of a 3-concentric ring µ-metal chamber was included before scanning. The data was acquired after a single 45°$_x$ (^{1}H) or 90°$_x$ (^{13}C) proton-decoupled pulse, and the enhancement calculated by comparison to thermal spectra. Concentration experiments showed optimal polarization at 10 mg/mL for all particles.

Results

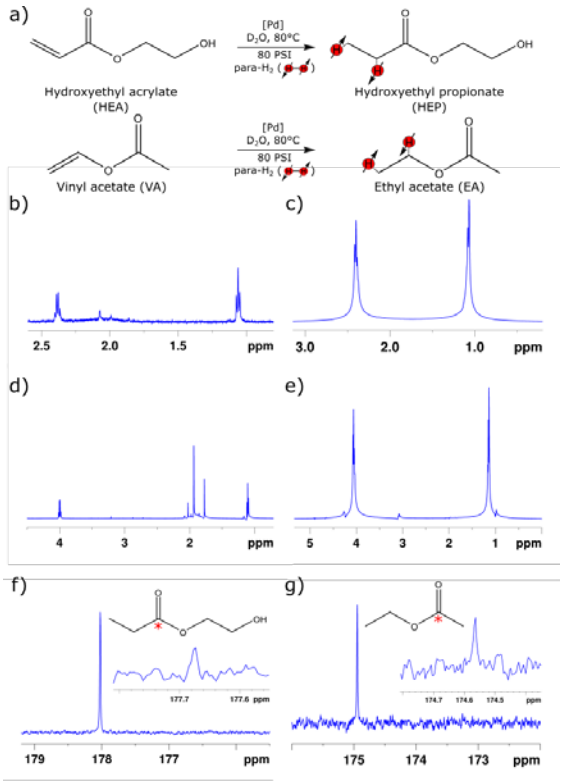

Fig. 3: a) Reaction mechanism of vinyl acetate and HEA hydrogenation and transfer of polarization to ^{13}C. Thermal ^{1}H spectra of b) HEP and d) ethyl acetate, as well as their hyperpolarized spectra (c) and e), respectively. ^{13}C hyperpolarized spectra following zero field transfer for f) HEA and g) ethyl acetate products are also shown. Thermal ^{13}C spectra were acquired after 256 scans and overlayed. Spectra shown in magnitude mode.

^{1}H PHIP experiments involving hydrogenation of 2-hydroxyethyl acrylate (HEA) into hydroxyethyl propionate (HEP), a water-soluble standard within the PHIP community utilized in angiography studies, were performed with all nanoparticle systems. Pt nanoparticles using the tripeptide glutathione and unfractionated LCys as ligands, yielded 0.3% and 0.7% ^{1}H polarization in the earliest work in aqueous heterogeneous PHIP(11)(12). Since then, LCys@Pd and NAC@Pd with optimized purification procedures are shown here to achieve 0.9% and 1.2% ^{1}H polarization with HEP, respectively(10). This is nearly double the previously highest reported value, and comparable to the analogous homogeneous Rh catalyst in water. Furthermore, while recent field cycling approaches have yielded ^{13}C hyperpolarization in water, similar measurements of hyperpolarized ethyl acetate from vinyl acetate using NAC@Pd have yielded a 20-fold improvement over other works (P_{13C} = 1.2%)(13). The resulting ^{1}H and ^{13}C spectra for HEP and ethyl acetate using NAC@Pd are shown in Figure 3.

Propargylglycine was also hydrogenated using NAC@Pd into allylglycine, its single hydrogenation alkene product, and thermal spectra are taken for comparison. Within the time regime of our experiments, only major allylglycine product peaks from single hydrogenations are observed (Fig 4). ^{1}H polarization of 0.9% is obtained(10).

Discussion

In heterogeneous PHIP, pairwise addition of para-H_2 is thought to be preserved by ligand density, which limits diffusion across the particle surface. Significant diffusion would otherwise lead to loss of para-H_2 spin order. Thus, ligands are designed not only for control of particle dispersity and solubility, but also for their ability to restrict H_2 diffusion (and catalytic exchange between hydrogen molecules) during hydrogenation reactions while preserving spin order. Consistent synthesis procedures also showed higher NAC@Pd ligand density 1.6x than that of LCys@Pd despite similar particle size (40%wt versus 25%wt), which cannot be explained by the increase in molecular weight of NAC compared to LCys (163.19 versus 121.16 g/mol, respectively). This suggests that NAC is better able to stack on the particle surface, and that this efficient stacking contributes to the ability of NAC to improve pairwise H_2 addition.

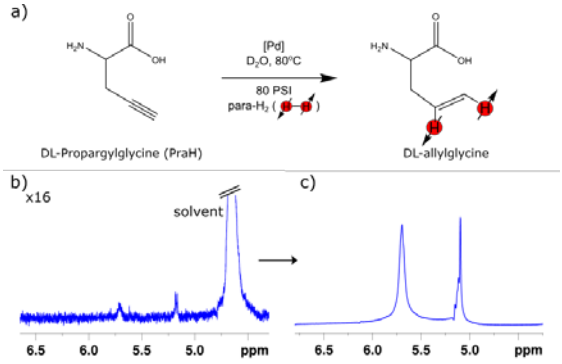

Fig. 4: a) Reaction mechanism of DL-propargylglycine into DL-allylglycine. b) Hyperpolarized ^{1}H spectrum after hydrogenation, and c) thermal ^{1}H spectrum.

Recently, work by Zacharias *et al* demonstrates metabolic imaging of hyperpolarized aqueous diethyl succinate hyperpolarized in water(14). Homogeneous Rh(dppb)(nbd)BF_4 catalyst was dissolved and used to hydrogenate diethyl fumarate (20 mM) before injection into live mice using an optimized polarizer and deuterated compounds for spin preservation. Comparison with our system indicates our current catalyst shows only 18-fold concentration and 2-fold polarization away from current *in vivo* investigations. Considering that 1.1 mM is typical for most physiological conditions, the catalyst systems proposed here may be sufficient for *in vivo* use. Since transit time to the magnet was recorded at 9 seconds from pressurization to spectra acquisition, it is possible that with a well-optimized polarizer nearly two full T_1 relaxation cycles(12) could be avoided, leading to potential polarizations ≥ 10%.

As advances in PHIP are made towards broader investigation of biologically relevant substrates, one barrier is that unprotected functional groups have been shown to hinder polarization due to unfavorable interactions with PHIP catalysts(7,15). Because of this problem and widespread interest in hyperpolarized peptides, protecting groups for amines and carboxylates are often investigated to create product analogs of varying biological activity and solubility. Strategies proposed to remove the protecting group by cleavage after para-H_2 addition(8) are untenable for a clinically relevant technique. In this work, aqueous parahydrogenation of unprotected DL-propargylglycine is investigated as a substrate for NAC@Pd to demonstrate the advantage in using a heterogeneous system. Propargylglycine (PraH) is a naturally occurring, water-soluble amino acid found in certain fungal systems with an unsaturate

triple bond(16). ^{1}H PHIP experiments using Boc-protected PraH in methanol-d_4 and homogeneous Rh(dppb)(COD)BF_4 catalyst yielded polarization values of P_{1H} = 0.4%, comparable to ~1.2% when adjusting from 50% para-H_2 enrichment to ~100%(17). PraH has recently been incorporated into sunflower typsin inhibitor along with O-propargyl-L-tyrosine in mixed solvent system to yield P_{1H} = 0.14% (expected 0.42% with ~100% para-H_2) without loss of activity(18), demonstrating PraH's potential in biological investigations.

Figure 4 shows hyperpolarized ^{1}H peaks from DL-allylglycine, yielding a polarization of P_{1H} = 0.9%(10). However, PraH's triple bond has the potential for two parahydrogenations(14), allowing further enhancement under higher conversion regimes, though only characteristic I_z–S_z peaks for allylglycine are observed here. Both investigations of propyne in gas-phase heterogeneous PHIP(19) and O-propargyl-L-tyrosine in solution PHIP(18) report only single hydrogenation product enhancement. Magnetic field cycling experiments were attempted on PraH, though no ^{13}C enhancement was observed. This can be explained due to allylglycine's dense proton environment on adjacent carbons to the hydrogenation site which likely interfere with the observed magnetization after transfer. The relatively large distance to the ester carbon often chosen in ^{13}C transfer requires zero field draw speeds longer than para-H_2 relaxation and is unlikely to be observed.

Summary

In conclusion, this work demonstrates novel water-soluble palladium nanoparticles as catalysts in aqueous heterogeneous PHIP, capable of transferring polarization to ^{13}C on biologically relevant compounds. Both ^{13}C and ^{1}H polarizations are unprecedented in aqueous heterogeneous reactions, ^{13}C by up to two orders of magnitude compared to the previous state-of-the-art experiments. High ^{13}C enhancement on compounds of biological interest represents a crucial advancement in heterogeneous PHIP application. Similarly, robust polarization of unprotected peptides and other nitrogenous compounds widens the breadth of potential PHIP labels to meet the future needs of diagnostic imaging.

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