Is $\text{Pd}_2(\text{DBA})_3$ a Feasible Precursor for the Synthesis of Pd Nanoparticles?

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Received: April 14, 2009; Revised Manuscript Received: May 23, 2009

The organometallic reagent tris(dibenzylideneacetone) dipalladium(0) [$\text{Pd}_2(\text{DBA})_3$] has been used as a reagent for synthesis of hexagonal microstructures ranging from $0.5–10 \mu\text{m}$. We have shown elsewhere that $\text{Pd}_2(\text{DBA})_3$ spontaneously forms hexagonal and cubic microstructures. The results obtained herein confirm that microstructures formed by spontaneous aggregation of $\text{Pd}_2(\text{DBA})_3$ produce the same morphology and carbonaceous chemical composition as those produced with a process that has been called RNA-mediation by pyridyl-modified RNA (pmRNA). Synthesis products from both methods of preparation were analyzed using a combination of scanning transmission electron microscopy (STEM), electron energy loss spectroscopy, energy dispersive X-ray spectroscopy, and selected area electron diffraction. Indexing of electron diffraction patterns from hexagonal molecular crystals and metallic Pd nanoparticles combined with STEM Z-contrast imaging and X-ray dot maps were used to positively differentiate the two materials. On the basis of these results, we conclude that the observed microparticles with hexagonal morphology are composed of $\text{Pd}_2(\text{DBA})_3$. Surfaceants such as pmRNA sequence Pd017 or Triton X-100 stabilize suspensions of spontaneously formed crystalline aggregates of $\text{Pd}_2(\text{DBA})_3$ in aqueous mixtures of tetrahydrofuran and $\text{H}_2\text{O}$, but they are not required for their formation. Crystalline Pd metal nanoparticle composites that range from 50 to 200 nm in diameter, that are observed in both spontaneous and RNA-mediated preparations, are an impurity that arises during the synthesis of $\text{Pd}_2(\text{DBA})_3$ by chemical reduction. There is no evidence for a competing decomposition process that forms crystalline Pd metal nanoparticles on the time scale of the experiments.

Introduction

Tris(dibenzylideneacetone) dipalladium(0), $\text{Pd}_2(\text{DBA})_3$, is a relatively stable complex of zerovalent Pd, in which each Pd center is coordinated by three $\eta^2$-alkene ligands.1 The decomposition of $\text{Pd}_2(\text{DBA})_3$ to Pd nanoparticles in the solid state occurs on the time scale of months and is quite slow compared to other zerovalent Pd solids such as $\text{Pd}(\text{PPh}_3)_4$. However, the solution chemistry of $\text{Pd}_2(\text{DBA})_3$ is complex due to ligand exchange reactions including oxidative addition leading to catalytic cycles during which Pd changes its redox state. Colloidal Pd often forms as a byproduct of redox cycling. On the other hand, in the absence of ligands that undergo oxidative addition in solution, $\text{Pd}_2(\text{DBA})_3$ crystallizes in CH$_2$Cl$_2$, CHCl$_3$, benzene, and toluene to yield crystals of $\text{Pd}_2(\text{DBA})_3$(solvent).2–4 Thus, there is a solvent- and ligand-dependent competition between formation of metallic Pd nanoparticles and molecular crystals of $\text{Pd}_2(\text{DBA})_3$.

The coordination chemistry of Pd(II) and Pd(0) has been extensively studied to understand the role played by both oxidation states in Pd catalysis.5 Studies of the displacement reactions of $\text{Pd}_2(\text{DBA})_3$ revealed that it is susceptible to oxidative addition of propenyl chloride, which is useful for its application as a heterogeneous catalyst. However, given that reductive elimination does not return the Pd atom to the original $\text{Pd}_2(\text{DBA})_3$ adduct, the catalytic species is the Pd atom itself rather than $\text{Pd}_2(\text{DBA})_3$. The Pd(0) species that forms during the cycle can aggregate to form colloidal particles known as Pd black.6 Formation of Pd black is not rapid compared to catalysis under most conditions, and it is dependent on the ligation strength of the Pd(0) coordination sphere. Pd is stabilized by soft ligands such as PPh$_3$ and the $\eta^2$-coordinated alkenes of DBA as well as many $\pi$-systems including quinones, alkynes, cyclopentadiene, etc. Nitrogenous ligands such as bidentate ligands bipyridine and o-phenanthroline and pyridine have played a more limited role in the coordination chemistry of Pd(0). Primary amines such as hexadecyl amine have had application as a stabilizer in nanoparticle synthesis following hydrogenation reactions.16 Systematic study of these ligands leads to the concept that Pd acts as the donor (nucleophile) in these adducts and the $\pi^*$-acceptor orbitals of the ligand (electrophile) are key to stability of the complex. The “inverse electron demand” of Pd(0) leads to a trend in ligation strength that is the reverse of that normally observed where the ligand acts as the nucleophile and the metal is the electrophile. In this type of bonding Pd(0) acts as an electron donor to $\pi^*$ orbitals of the coordinating ligand. Unlike more conventional $\pi$-backbonding, there is little stabilization arising from $\sigma$ donation to Pd(0). Pyridine is a poor $\pi^*$ acceptor, which may explain why no homoepctic pyridine adduct of Pd(0) has been reported. The occurrence of pyridine in Pd(0) coordination chemistry is rare.17 Pyridine is known to play a role in certain catalytic reactions that may be dependent upon ligation to Pd(II) adducts.18,19 Although nitrogenous ligands are capable of stabilizing Pd nanoparticles, they do not displace $\eta^2$-coordinating ligands such as DBA unless the olefin is hydrogenated.16 An understanding of ligand coordination strength to Pd is of key importance for catalyst design. Not only must the relative ligation strength of substrate and product be tuned for catalytic turnover, but the...
stability in solution must be considered since formation of colloidal Pd removes catalytic Pd from solution.

There has been an interest in Pd nanoparticle catalysts as an alternative to homogeneous catalysis by Pd complexes.20–25 Aside from potential for novel reactivity, heterogeneous catalysis by Pd has the potential to mitigate the competition between redox cycling of catalytic Pd complexes and formation of noncatalytic Pd black. This strategy turns the catalytic foie of catalysis into a friend by harnessing well-defined nanoparticle structures as catalytic sites. Heterogeneous Pd nanoparticle catalysts have been found to have activity in hydrogenation reactions and, more recently, in C–C bond formation.20,21 The synthesis of Pd nanoparticles26 and nanoparticle clusters, also called giant nanoparticles,27 has been demonstrated starting from Pd(II) compounds such as Pd(OAc)2, Pd(acac)2, and Na2PdCl4. The discovery Pd particles of well-defined stoichiometry28 has led to an interest in the synthesis of particles of controlled size.29 Cubo-octahedral and icosaehedral Pd particles are formed that appear to follow the relationship of Chini’s series where the number of atoms around a central Pd atom, \(N = \frac{1}{\sqrt{3}}(10m^3 + 15m^2 + 11m + 3)\) is 13, 55, 147, 309, 561, etc., for \(m = 1, 2, 3, 4, 5\), etc., defines successive coordination spheres.27–31 Shape control has been introduced into the synthesis of Pt and Pd nanoparticles by control of surfactant and reducing conditions starting from Pd(II) and Pt(II) precursors.24–34 In all cases the particles are formed by chemical reduction. When Pd2(DBA)3 is used as starting material, H2 gas is used to hydrogenate the olefinic bonds of the DBA ligand in order to release Pd(0).16 Pd nanoparticle clusters of 50–200 nm diameter, observed in preparations of Pd2(DBA)3, appear to be impurities present from the synthesis of Pd3(DBA)4,35,36 which is accomplished by reduction of Pd(II) complexes in the presence of excess DBA.3

Spontaneous formation of Pd nanoparticles from Pd2(DBA)3, in the absence of a reducing agent appears to be sufficiently slow that heating or other input of energy is required even to make composite materials. Nanoparticle synthesis methods using Pd2(DBA)3, as the precursor including sonochemical decomposition37 and thermal annealing38 have been reported. These conditions for nanoparticle formation involve elevated temperature as a necessary step to break the \(\eta^2\)-bonds that stabilize zerovalent Pd in Pd2(DBA)3. However, neither of these methods is sufficient to create pure Pd nanoparticles, but rather they produce carbon fibers that contain Pd nanoparticles. The strong affinity of Pd(0) for unsaturated carbon bonds was recognized by Ito et al. in studies of olefinic groups that were capable of displacing DBA ligands.9 This reactivity, noted above as the inverse electron demand of Pd, apparently extends to carbon nanotubes, which present a novel templating agent for the creation of Pd wires capable of hydrogen sorption based on reaction with Pd2(DBA)3.39 Given these considerations, the report of pyridyl-modified ribonucleic acid (pmRNA) mediation of Pd–Pd bond formation at room temperature is surprising.40–42

When Pd2(DBA)3 is added to a solvent such as tetrahydrofuran (THF) or THF/H2O mixtures, micrometer-sized structures form spontaneously.35,36 These structures have hexagonal plate, rod, and star morphologies that may arise from the nearly trigonal symmetry of molecular Pd2(DBA)3 crystals observed in THF. The triclinic unit cells of Pd2(DBA)3 crystals in solvents such as CHCl3 and CH3Cl2 contain a cocrysallized solvent molecule.2,3 The lattice parameters are sufficiently close to a trigonal unit cell that these cocrysalized solvent molecules may be responsible for a distortion of the lattice. While there is no X-ray crystal structure of Pd2(DBA)3 in THF, the hexagonal selected area electron diffraction (SAED) spot pattern observed from Pd2(DBA)3 hexagonal microcrystals at short times (i.e., prior to beam damage) is consistent with a trigonal unit cell that contains only Pd2(DBA)3 and no solvent molecule.43 Hence, the observed hexagonal-shaped organic crystal could be a pseudohexagonal habit of the trigonal Pd2(DBA)3 unit cell. The pseudohexagonal form of microcrystalline Pd2(DBA)3, formed in THF is difficult to study by electron diffraction due to the fact that these hexagonal organic crystals are only transiently stable under a 200 kV electron beam.35 After less than one minute in the electron beam, the sample begins to lose crystallinity due to the formation of small (2–4 nm) Pd nanoparticles throughout the microcrystal. The formation of Pd nanoparticles35 inside a larger structure provide an indication that the hexagonal microplatelets are composed of Pd2(DBA)3, rather than metallic Pd.

Pd2(DBA)3 microcrystals of both cubic and hexagonal morphology35,36 are remarkably similar to the forms observed for metallic Pd nanocrystals, but the length scale of the Pd2(DBA)3 microcrystals is at least 10 times larger in all cases and can be up to 1000 times larger in specific cases.26,28 Given the morphological similarity but difference in length scale, it is of interest to compare Pd nanocrystals to microcrystals with the hexagonal and cubic structures which were reported to be mediated by pmRNA in a sequence-dependent manner using Pd2(DBA)3 as the starting material.41 The hexagonal Pd nanoparticles observed on the 10–100 nm length scale24,26,43,44, bear a stunning similarity to the morphology of Pd2(DBA)3 microcrystals on the 0.5–10 μm length scale. Cubic Pd nanoparticles have also been reported.28,45 These are also observed on a length scale of 2–4 nm,46 which is ~500 times smaller than those reported for products pmRNA-mediated.41 Larger Pd nanoparticles have been observed, but these are multiply twinned clusters of Pd61 particles, which is the most commonly observed type of cuboctahedral particle.28 The morphological similarities of different shapes, hexagons, and cubes on different length scales suggests that the chemical composition and structure of the micrometer-sized Pd2(DBA)3 particles should be carefully verified. In previous studies, we established that the micrometer-sized Pd2(DBA)3 particles of hexagonal and cubic morphology are essentially identical to those reported as products pmRNA mediated.36 In the following, we study particles produced from Pd2(DBA)3, with and without pmRNA to compare two hypotheses that have been advanced to explain the observation of micrometer-sized particles derived from Pd2(DBA)3 in THF/H2O suspensions.

The hypothesis of pmRNA-mediated metal–metal bond formation40–42 states that pmRNA strips away at least one of the DBA ligands and presents the face of a Pd crystal that grows in a controlled morphology. The inspiration for this type of controlled growth can be found in biominalization of all kinds.47 This type of process is observed in the interaction of nucleic acids during the growth of CdS quantum dots48–51 and Ag nanoclusters.52 In the structures studied here,35,36,40–42,53 a pyridyl modification is carried on every uridine of the RNA sequence Pd017 as a 5-(4-pyridylmethyl) carboxamide group. Ag nanoclusters.52 In the structures studied here,35,36,40–42,53 a pyridyl modification is carried on every uridine of the RNA sequence Pd017 as a 5-(4-pyridylmethyl) carboxamide group. The Pd017 sequence contains 12 uridines in its variable region.40 Thus, the metalation and templating mechanism would necessarily involve binding interactions of the pyridyl groups with Pd(0) atoms from Pd2(DBA)3. The proposed mechanism is unusual since all reported syntheses of Pd nanoparticles use reduction of Pd(II)20–26,28,32,33 and the formation of Pd black from Pd(0) in organic reaction mixtures has not led to the isolation of nanoparticles. Moreover, the pyridine side chain in
pmRNA is an unusual choice as a chemical mediator because of its weak coordination to Pd(0).\textsuperscript{12–15,54}

The alternative hypothesis is that the hexagonal particles are formed spontaneously as microcrystals of Pd\textsubscript{2}(DBA)\textsubscript{3} in THF/H\textsubscript{2}O solutions and that pmRNA plays the role of a surfactant when it is present. Stabilization of particles of Pd\textsubscript{2}(DBA)\textsubscript{3} in aqueous solution has precedent using the detergent Triton X-100 as a stabilizer.\textsuperscript{55} Thus, pmRNA may act to slow precipitation, thereby providing metastable suspensions of Pd\textsubscript{2}(DBA)\textsubscript{3} under some conditions. The use of surface-attached pmRNA has also been reported, and particles of both cubic and hexagonal morphology on surfaces have been observed using TEM.\textsuperscript{42} Atomic force microscopy (AFM) has also provided evidence on the nanometer scale for hexagonal particles on etched surfaces that present a grid of surface attached pmRNA molecules.\textsuperscript{53} However, control experiments reveal that hexagonal and cubic particles are formed on surfaces even without pmRNA.\textsuperscript{35,36} Our previous reports\textsuperscript{35,36} have established the identity of Pd\textsubscript{2}(DBA)\textsubscript{3} particles spontaneously formed in THF/H\textsubscript{2}O solutions and on surfaces. By use of transmission electron microscopy/selected area energy diffraction (TEM/SAED), scanning tunneling microscope/electron microscopy (STM/EEL), and scanning electron microscopy/electron diffraction spectrometry (SEM/EDS) methods, we determined the morphology, chemical composition, and structure of the hexagonal Pd\textsubscript{2}(DBA)\textsubscript{3} microcrystals (0.5–10 μm) and smaller Pd impurities that are clusters of 2–4 nm nanoparticles with a total diameter of 50–200 nm. Herein, we present further supporting evidence for the hypothesis that mediation by pmRNA Pd017 does not significantly alter the composition of the spontaneously formed Pd\textsubscript{2}(DBA)\textsubscript{3} microcrystals either in solution or on surfaces. The present study represents the first direct comparison of microparticles produced with and without added pmRNA Pd017.

Methods

Sample Preparation. The Pd\textsubscript{2}(DBA)\textsubscript{3} reagent powder (STREM, Lot#A4805106) was used as received and unpurified. 14.65 mg were dissolved in 2 mL of THF (from Fischer, distilled prior to use) to produce 8 mM solutions of Pd\textsubscript{2}(DBA)\textsubscript{3}. This solution was added to variable amounts of water and THF to create 400 μM Pd\textsubscript{2}(DBA)\textsubscript{3} solutions and resulted in suspensions of Pd\textsubscript{2}(DBA)\textsubscript{3} in binary solutions with ranging from 30 vol % THF/H\textsubscript{2}O to 100 vol % THF. When pmRNA was dissolved in the suspensions, diethylpyrocarbonate (DEPC) water was used. These solutions were then cast and allowed to dry in ambient atmosphere onto either indium tin oxide (ITO, Delta Technolo-

gies), lacey carbon TEM grids (Ted Pella Part No. 01883), Formvar-coated TEM grids (Ted Pella Part No. 01820), or bare Au. More in-depth sample preparation and observations of the solution turbidity and precipitate formation after adding Pd\textsubscript{2}(DBA)\textsubscript{3} was previously reported.\textsuperscript{35}

RNA Pd017 was made by in vitro transcription from PCR-generated or synthesized DNA templates as described previously.\textsuperscript{41} For production of modified RNA, 5-(4-pyridylmethyl)carboxamide UTP, analyzed by liquid chromatography-tandem mass spectrometry,\textsuperscript{56} was substituted for unmodified UTP in standard transcription reactions. RNA concentrations were estimated by absorbance at 260 nm using calculated extinction coefficients.

Surface attachment of RNA to ITO was carried out by modification of the ITO surface based on the following protocol, which is illustrated in Figure 1. ITO slides were first hydroxylated, by immersion in a 10 mM NaOH solution overnight at 60 °C (2 mL/2 slides; NaOH solution was made using enzyme-free water). Then, they were rinsed with enzyme-free water, N\textsubscript{2} dried, and UVO cleaned for 5 min. The slides were immersed in a 1% solution of 3-aminopropyltrimethoxysilane (3-APS) in anhydrous toluene (total volume: 2 mL/2 slides) as shown in Figure 1A. The reaction was carried out at 60 °C, in air, for 30 min. The slides were then rinsed with toluene and absolute ethanol and cured overnight at 120 °C. The heterobifunctional cross-linking agent SSMCC (4-(N-maleimidomethyl)cyclohexane-1-carboxylic 3-sulfo-N-hydroxysuccinimide ester) was coupled to the amino groups presented on the surface using 2 mg/mL of SSMCC in 0.05 M KP buffer at pH 7.2 by soaking for 1 h at ambient temperature (Figure 1B). The maleimide-activated slides were then rinsed and immersed in the solution (1 μM pmRNA; 0.05 M KP buffer; pH 7.2) for 3 h (Figure 1C). Subsequently, the slides were rinsed thoroughly, first with buffer and then with water, and dried in N\textsubscript{2}. To create a surface-attached pmRNA, 5′-poly(ethylene)glycol-thioester-linked-pmRNA was added to the maleimide-coated ITO surface (Figure 1D). The Michael addition is the coupling reaction that binds pmRNA to ITO (Figure 1E). The preparation of 5′-poly(ethylene)glycol-thioester-linked-pmRNA shown in Figure 1F has been discussed elsewhere.\textsuperscript{42} The surfaces were hydrated with a thin layer of water, treated with 50 vol % THF/H\textsubscript{2}O to 100% vol THF solution, and allowed to evaporate to dryness following the previous protocol.\textsuperscript{42}

SEM and EDS. A Hitachi S-3200N, equipped with a Robinson backscattered electron detector (BSE) (for atomic number contrast imaging) and Oxford Link ISIS EDS (for quantitative chemical analysis and X-ray dot maps), was
employed in this study. The operating voltage for imaging and chemical analysis was 20 kV. To eliminate sample charging, the variable pressure of the sample chamber was set to 60 mbar. Digital image acquisition of BSE micrographs and quantitative chemical analysis was accomplished by using the Oxford Link ISIS software.

Low-Voltage STEM. A Hitachi S5500 low-voltage STEM (Pleasanton, CA) equipped with a cold cathode field emission electron source was capable of 0.4 nm resolution in secondary electron mode when operated at 30 kV. This in-lens field-emission STEM was equipped with secondary and backscatter electron detectors with the ability to mix both signals and capture images in a magnification range of 100–2,000,000×. The microscope was also equipped with a bright field and an adjustable dark field detector allowing a range of collection angles to be captured for diffraction or Z-contrast imaging. Chemical analysis in the microscope was performed with an Oxford INCA (Oxfordshire, UK) energy dispersive X-ray spectrometer attached to the LV-STEM sample chamber. The X-ray dot maps and spectrum reports were generated using the INCA software.

TEM and SAED. Phase contrast imaging with the HRTEM and EELS analysis was performed with a JEOL 2010F transmission electron microscope operated at 200 kV (λ = 2.51 pm) equipped with a high-resolution pole piece (URP2F) used to capture lattice fringe micrographs.57 Column alignment was performed with the first condenser lens in TEM probe mode, spot size set at 5 (~4.48 V) and the condenser mini lens set at α = 3 (~3.93 V). Conventional brightfield (BF) captured images were taken with a Gatan Imaging Filter (GIF) coupled to the microscope using Digital Micrograph 3.3.1. Digital image acquisition parameters included a scan rate of 0.5 s and a final image resolution of 1024 × 1024 pixels. All HRTEM images were acquired at the instruments optimum contrast transfer function (termed Scherzer focus).58 Obtaining this imaging condition by utilizing the live FFT of the real image ensured theoretical requirements for micrograph contrast interpretation could be applied.

SAED was used to document the long and short-range atomic ordering of the features of interest. The resulting SAED patterns were acquired at camera lengths of 60, 80, and 100 cm and then recorded on Kodak Electron image film (SO). The microscope was also equipped with a high-resolution pole piece (URP2F) used to capture lattice fringe micrographs.57 Column alignment was performed with the first condenser lens in TEM probe mode, spot size set at 5 (~4.48 V) and the condenser mini lens set at α = 3 (~3.93 V). Conventional brightfield (BF) captured images were taken with a Gatan Imaging Filter (GIF) coupled to the microscope using Digital Micrograph 3.3.1. Digital image acquisition parameters included a scan rate of 0.5 s and a final image resolution of 1024 × 1024 pixels. All HRTEM images were acquired at the instruments optimum contrast transfer function (termed Scherzer focus).58 Obtaining this imaging condition by utilizing the live FFT of the real image ensured theoretical requirements for micrograph contrast interpretation could be applied.

The formation of hexagonal microcrystals was compared with and without surface-attached pmRNA (see Methods). Initially, we compared the appearance of the hexagonal microplatelets by optical microscopy (OM). Then, HRTEM and SEM methods were employed to determine the composition of the hexagonal microplatelets when pmRNA is present. These results are compared to previous results showing the morphological similarity to microcrystals of Pd2(DBA)3. In all cases, beam damage alters the crystals, which is consistent with their assignment as organic rather than metallic microcrystals. All of the methods agree that the composition of the pmRNA-mediated particles is the same as the spontaneously formed particles.

Hexagonal Microstructures Formed on Surfaces with and without pmRNA. By use of OM we compare the morphology of hexagonal platelets prepared in pmRNA-free solutions and in the presence of surface-attached pyridyl-modified-RNA Pd017 using the methods shown in Figure 1. Figure 2 shows comparison of hexagonal platelets, observed by OM, formed on two different material surfaces with and without pmRNA present. Figure 2A shows an ITO substrate prepared with surface attached pmRNA Pd017 as described in the Methods section.41,42 Hexagons, with lateral dimensions on the micrometer scale, were observed on the pmRNA modified surface after Pd2(DBA)3 was added in a THF solution. Figure 2B shows the results of an experiment in which the linker molecule SSMCC was attached to the surface. Figure 2C shows relatively small hexagons formed on an ITO surface with H2O added. Figure 2D shows hexagonal platelets formed on a bare hydrophobic gold substrate, without surface attached pmRNA Pd017. The platelets in Figure 2D have the same morphology and same average size as those platelets prepared in the presence of pmRNA, as observed in Figure 2A.

Structure and Composition of pmRNA-Mediated Pd2(DBA)3 Microstructures. TEM grids with microstructures created by incubation of Pd2(DBA)3 with pmRNA P017 were prepared according to the solution protocols described elsewhere.40–42 Morphology, elemental composition, and crystallographic orientation of the hexagons of several samples were studied in a
200 kV TEM with EELS and SAED. Figure 3 shows one example of a bright field (BF) micrograph and corresponding diffraction pattern (inset) acquired to determine the atomic ordering of the hexagonal sample. Lack of thickness fringes in the BF micrograph and diffuse rings in the diffraction patterns from the morphologically hexagonal material provide evidence that the particles were neither single crystal nor polycrystalline Pd metal. The type of diffuse SAED shown in Figure 3 is consistent with a predominantly carbon composition as observed in samples prepared without pmRNA Pd017. In general, diffuse patterns of this type arise from beam damage. We have further shown that extreme beam damage can even lead to the formation of small (<5 nm) Pd nanoparticles inside the hexagonal microcrystals.

In contrast to our findings, a recent report of a SAED pattern, reproduced in Figure 4, is indicative of a polycrystalline metal lattice with a face-centered cubic unit cell. However, no accompanying TEM image was presented to permit identification of the structure that gave rise to the SAED pattern. Therefore, it is not possible to determine the morphology of the structure that gave rise to the diffraction pattern in Figure 4. Moreover, this SAED pattern does not agree with that of Figure 5, which shows the only other published SAED evidence by these authors in support of the assignment of the structure. Lack of thickness fringes in the BF micrograph and diffuse rings in the diffraction patterns from the morphologically hexagonal material provide evidence that the particles were neither single crystal nor polycrystalline Pd metal. The type of diffuse SAED shown in Figure 3 is consistent with a predominantly carbon composition as observed in samples prepared without pmRNA Pd017. In general, diffuse patterns of this type arise from beam damage. We have further shown that extreme beam damage can even lead to the formation of small (<5 nm) Pd nanoparticles inside the hexagonal microcrystals.

Figure 3. BF TEM micrograph showing the morphology of hexagonal microstructures resulting from synthesis using pmRNA Pd017 incubation with Pd$_2$(DBA)$_3$.

Figure 4. Reproduction of SAED data presented in Eletter attached to Ref. 40. The original figure caption is “Electron diffraction from hexagonal particles synthesized in the presence of the cycle 8 of the RNA pool of sequences.”

sample such as demonstrated by the lattice fringes of Figure 5A would not produce a spot pattern, since spot patterns are indicative of a single crystal. However, the reported beam damage agrees with our report of beam damage of organic crystals of Pd$_2$(DBA)$_3$. The beam damage is also consistent with the observation of diffuse scattering (vide supra) since the electron beam would cause a breakdown of the initially formed crystalline Pd$_2$(DBA)$_3$.

The hexagons often form in clusters when deposited from solution. The pmRNA Pd017-incubated clusters were further analyzed with 200 kV STEM Z-contrast imaging, EELS, and EDS. The secondary electron (SE) and Z-contrast (composition dependent contrast) STEM micrographs of parts A and D of Figure 6 show clusters of hexagons with edge lengths on the micrometer scale and are an example of representative features found during STEM analysis of multiple regions on the TEM grid. At higher magnification, the light contrast of the hexagonal feature in the Z-contrast micrograph of Figure 6C is consistent with the carbon-rich composition of the hexagonal molecular crystals. Core-loss EELS spectra from the hexagonal particles were analyzed and the hexagons were found to be composed of an average of 92.5 (% C and 6.40 (% Pd. These data confirm that the composition of hexagonal particles produced using pmRNA Pd017 using the reported methods are mostly carbon as observed previously for hexagonal particles produced with pmRNA Pd017.

The SAED and EELS data collected from the pmRNA Pd017-incubated samples indicate that the hexagonal microstructures are not crystalline Pd metal. The only evidence of Pd metal was the relatively sparse ~200 nm nanoparticle clusters that are a spontaneous breakdown product of Pd$_2$(DBA)$_3$, as shown in both the Z-contrast and diffraction contrast micrographs of parts E and F of Figure 6, respectively.

### Structure and Composition of Spontaneously Formed Pd$_2$(DBA)$_3$ Microstructures

The spontaneous formation of hexagonal microstructures was also observed in binary solutions of THF/H$_2$O without pmRNA incubation. A low-magnification SEM backscattered electron (BSE) micrograph of a TEM grid (corner of Cu grid square top left) prepared with features created in a 50 vol % THF/H$_2$O binary mixture is shown in Figure 7A. Three micrometer-sized clusters of hexagons are seen; two are suspended by the lacy carbon film, and one cluster is located on the Cu grid bar. Figure 7B is a higher magnification BSE micrograph of a hexagonal cluster located on a different area of the TEM grid. The contrast of the hexagons denotes a low Z material, when compared to the contrast of the
observed to form spontaneously from Pd2(DBA)3 and in the presence of the pmRNA sequence known as Pd034. The two SAED patterns that have been presented as evidence for assignment of these cubic particles as metallic Pd are presented as an overlay of SAED patterns shown in Figure 8. The overlay shows a significant discrepancy between the spot and ring pattern in parts A and B of Figure 8, obtained from the center and edge of a cube-shaped particle, respectively. The camera length (L) is usually calibrated with a metal standard. Diffraction pattern indexing typically includes calculating the relativistic corrected electron wavelength (λ) (λ = 0.0418 Å @ 80 kV and λ = 0.0251 Å @ 200 kV), measuring distances (R) from the (000) spot to the (hkl) reflections, and finding angles between the lattice planes. Since Rd = Lλ, the accuracy of L, λ, and R is critical for determining the lattice spacing (d) of atomic planes in the material. For a cubic metal, d spacings can be used to calculate the lattice parameter (a) using the relationship a = d√(h² + k² + l²), where h, k, and l are the Miller indices. However, there was no reported calibration of L, calculation of λ, or indexed values of R, which complicates interpretation of the d spacings of the cube-shaped particles. Therefore, to understand the significance of the data, we proceeded by assuming that L = 40 cm as reported in the figure caption. While this uncalibrated value would not be appropriate for an accurate determination of the lattice constant, it is sufficient for the purpose of comparison of the d spacings in the spot and ring pattern. Since both SAED patterns were acquired with the same L and λ, the lattice plane reflections should match exactly if they originated from the same Pd lattice. However, the indexed (111) plane reflections of the spot pattern (red boxes) exhibit a ~20% difference in d spacing when compared to the (111) reflection of the ring pattern. Moreover, the (200) ring and (220) spot reflections were incorrectly indexed to have the same d spacing. Such mismatches between the reported ring and spot diffraction data indicate these patterns cannot have originated from the same material. The lack of representative HRTEM micrographs and EDS data further hampers the assignment of the cube-shaped particles.

A Critical Examination of the Assignment of Cubic Particles as Metallic Pd. Cubic-shaped particles have been observed to form spontaneously from Pd2(DBA)3 and in the Cu grid, and indicates these hexagons were not composed of high atomic number material like Pd. Quantitative EDS analysis of 5 representative hexagons was performed and the resulting average atomic percentage of carbon and palladium was >90 at % and <10 at %, respectively. Indexed diffraction patterns as well as microanalysis results on these spontaneously formed hexagonal microstructures have been presented elsewhere. These data confirm that the chemical composition of the hexagons is the same within experimental error, whether they are incubated with pmRNA Pd017 or in the absence of pmRNA.

Figure 6. (A) STEM SE micrograph showing a representative example of clustered particles of hexagonal morphology, which resulted from synthesis using pmRNA Pd017 incubation with Pd2(DBA)3. (B) A higher magnification STEM SE micrograph of the hexagonal feature at the tip of the arrow in A. (C) The contrast of this STEM Z-contrast micrograph, of the exact region of part B, shows low contrast which results from the carbonaceous (low Z) composition of the hexagon. (D) A lower magnification STEM Z-contrast micrographs of another hexagonal cluster shows a bright contrast region, confirmed to be Pd rich by EDS analysis. (E) High-magnification BF STEM micrograph showing the morphology of the Pd-rich region indicated by the arrow in part D. The contrast of this micrograph shows smaller grains within the nanoparticle disflect the electrons differently. (F) This STEM Z-contrast micrograph of the exact region in part E also shows the nanoparticle is composed of yet smaller nanoparticles which have agglomerated. The low contrast feature in the bottom right of the image is the edge of a carbonaceous hexagon to which the nanoparticle was either attached or embedded.

Figure 7. (A and B) BSE SEM micrographs of hexagonal microstructures formed spontaneously without RNA in a 50 vol% THF/H2O binary solution containing Pd2(DBA)3. These hexagonal clusters are representative of the features sampled for quantitative EDS analysis. The hexagonal shape and carbon composition of these particles matches the morphology and composition of samples created with RNA.

Figure 8. Electron diffraction obtained from a single cubic palladium containing particle (A) near the edge and (B) in the center of the particle. Accelerating voltage, 200 kV; camera length, 40 cm. This figure is an overlay of the Figure 2.5 (p. 54) of ref 60.
Structure and Composition of the Decomposition Product of Pd$_2$(DBA)$_3$. We have studied the polycrystalline Pd nanoparticles observed in various solution preparations of Pd$_2$(DBA)$_3$. We have noted that these 50–200 nm Pd nanoparticles can be observed in the reagent powder as purchased from STREM. In all of the samples that were prepared without pmRNA, using Pd$_2$(DBA)$_3$ as a reagent crystalline Pd nanoparticles were found to coexist with the hexagonal molecular crystals. X-ray dot maps were used to observe the occurrence of Pd metal on a TEM grid prepared with 8 mM Pd$_2$(DBA)$_3$/THF solution. Figure 9A shows a BSE image of an area on the grid which contained a hexagonal particle (white arrow). The X-ray dot map of Pd L$_\alpha$ X-rays (Figure 9B) proves the presence of Pd metal in the 100% THF/Pd$_2$(DBA)$_3$ solution. However, no X-ray diffraction signal was detected from the hexagon feature (white arrow). On the other hand, the C K$_\alpha$ X-ray map does show the hexagonal feature (white arrow) composed mostly of carbonaceous material.

The morphology of the Pd nanoparticles shown in the BF TEM micrograph of Figure 10A is markedly different from the faceted hexagonal structures shown in Figures 6, 7, and 9. The atomic ordering of the Pd metal nanostructures analyzed with SAED and indexed diffraction patterns, like the one shown in Figure 10B, proved the presence of Pd metal—the metal bond length was 0.275 nm, which matches the published value for Pd metal. The HRTEM BF micrographs of parts C and D of Figure 10 show lattice fringes from a 50–200 nm Pd metal discoid that is composed of smaller nanoparticles clustered together. The presence of these lattice fringes provides more evidence of the polycrystalline nature of the Pd nanoparticle impurities. The common occurrence of Pd metal nanoparticles in samples incubated with and without pmRNA indicates that the metallic nanoparticles were present a priori and impurities from synthesis or degradation during storage of the reagent Pd$_2$(DBA)$_3$ was the source.

Conclusions

There are two competing aggregation processes that occur in solutions of Pd$_2$(DBA)$_3$. Hexagonal microcrystals of Pd$_2$(DBA)$_3$ form spontaneously in THF/H$_2$O and on surfaces provided the THF volume fraction is greater than 50%. As the H$_2$O percentage increases in THF/H$_2$O binary mixture, Pd$_2$(DBA)$_3$ is destabilized and it forms aggregates that sometimes have a tendency to cluster. In this work we observed clusters of hexagons (Figures 6 and 7) with similar carbon composition and dimensions to those obtained in solution samples incubated with pmRNA Pd017. In all cases studied, i.e., even in the presence of pmRNA, the composition of hexagons of aggregated Pd$_2$(DBA)$_3$ was determined to be >90 atom % carbon by both EELS and EDS. Under appropriate growth conditions, Pd$_2$(DBA)$_3$ will form molecular crystals with a pseudohexagonal habit in this solvent system, but other crystalline habits are also observed to form spontaneously depending on the solvent conditions. A second much slower process leads to formation of Pd nanoparticles from the decomposition of Pd$_2$(DBA)$_3$, in an electron. This observation agrees with the thermal stability of Pd$_2$(DBA)$_3$ and with its known coordination chemistry.

A major conclusion of the present work is that the spontaneous aggregation of Pd$_2$(DBA)$_3$ is the major product observed in the mixtures of THF/H$_2$O studied, even in the presence of surfactants such as pmRNA. We have attempted to compare the two methods using conditions that are as close as possible to those reported in the pmRNA studies. However, it has not been possible to reproduce the reported solution conditions of 5% THF/H$_2$O using pmRNA-free solvent. Solubility of Pd$_2$(DBA)$_3$ is an issue as acknowledged in a recent erratum, which states that ~50% THF, rather than 5% THF, was used in experiments designed to deposit hexagonal Pd$_2$(DBA)$_3$ microcrystals on pmRNA. Here we have also compared Pd$_2$(DBA)$_3$ microcrystals made on surfaces using those protocols. In the surface studies, there was no significant difference in morphology of samples formed spontaneously and those with added pmRNA.
Pd₃(DBA)$_3$: Precursor for Pd Nanoparticles?

We have addressed the metal–metal bonding hypothesis by direct comparison and found that the hexagonal microcrystals are not metallic Pd. Our findings are corroborated by the erratum to Ref. 53, which states that the authors could “not be certain” that the particles were metallic Pd.$^{62}$ The data presented here resolve the uncertainty. As determined by EDS, EELS, and SAED, the hexagonal nanoparticles are not metallic Pd in any of the solution or surface preparation methods studied. On the basis of the data above and on the erratum, the ring pattern in Figure 4 is most readily explained as a SAED pattern of one of the Pd impurities found in the reagent powder as evidenced by comparison with the Pd impurity particle in Figure 10A that gives rise to a ring pattern in Figure 10B, which matches that of Figure 4.

The reproducible pseudohexagonal form of Pd₃(DBA)$_3$ results from evaporation of THF/H$_2$O solutions provides a method for deposition of a Pd-containing material on a surface with a controlled geometry. Although no essential role can be identified for pmRNA Pd017 in the formation of the hexagonal Pd₃(DBA)$_3$ particles, it could act as a detergent that would stabilize the formation of a growing aggregate in a suspension. The role of wetting and surface structure appears to play a significant role in determining the size and distribution of Pd₂(DBA)$_3$ hexagonal microcrystals. If the growth is slowed, Pd₃(DBA)$_3$ aggregates will remain longer using Pd₂(DBA)$_3$. We thank Dr. Marta Cerruti formerly of the W.M. Keck Center for preparation of pmRNA Pd017 in solution and Dr. Russell of Appalachian State University for donating Americas Inc.'s electron microscopy division for use of a W.M. Keck Center for preparation of pmRNA Pd017 in solution and surface studies. We also thank Hitachi High Technology Americas Inc.'s electron microscopy division for use of a HD2300 equipped with a Gatan, Inc. Enfina EELS spectrometer and Dr. Russell of Appalachian State University for donating access to a state-of-the-art low kV STEM/EDS for nanomaterial analysis.

Acknowledgment. The authors would like to thank Drs. Gugliotti, Feldheim, and Eaton, formerly of the W.M. Keck Center for Evolutionary Materials Synthesis, for guidance in the synthesis of pmRNA Pd017 and preparation of samples using Pd₃(DBA)$_3$. We thank Dr. Marta Cerruti formerly of the W.M. Keck Center for preparation of pmRNA Pd017 in solution and surface studies. We also thank Hitachi High Technology Americas Inc.'s electron microscopy division for use of a HD2300 equipped with a Gatan, Inc. Enfina EELS spectrometer and Dr. Russell of Appalachian State University for donating access to a state-of-the-art low kV STEM/EDS for nanomaterial analysis.

References and Notes


