Journal of Colloid and Interface Science 483 (2016) 165–176

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

journal homepage: www.elsevier.com/locate/jcis

Regular Article

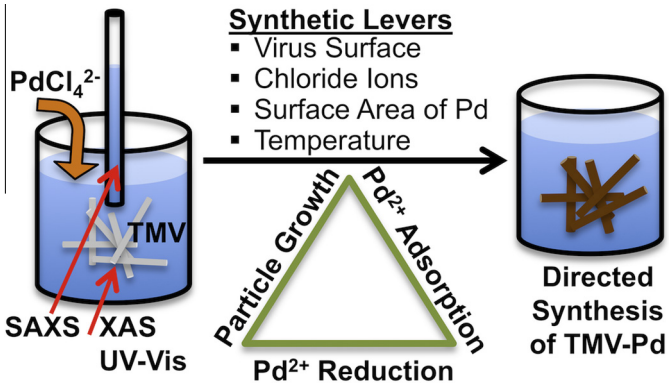
Decoupling and elucidation of surface-driven processes during inorganic mineralization on virus templates

Oluwamayowa O. Adigun [a], Gloria Novikova [a], Erin Lynn Retzlaff-Roberts [a], BongSuk Kim [b], Jeffrey T. Miller [a], L. Sue Loesch-Fries [b], Michael T. Harris [a,*]

[a] 480 Stadium Mall Drive, School of Chemical Engineering, Purdue University, West Lafayette, IN 47907, United States
[b] 915 West State Street, Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907, United States

GRAPHICAL ABSTRACT

Biomineralization during the hydrothermal synthesis of Pd on TMVs.



ARTICLE INFO

Article history:
Received 9 May 2016
Revised 11 July 2016
Accepted 14 July 2016
Available online 15 July 2016

Keywords:
Tobacco mosaic virus
Cysteine
Biomineralization
Adsorption
Reduction
Nanocrystal growth
X-ray absorption spectroscopy
Small angle X-ray scattering

ABSTRACT

There is a lack of fundamental information about the molecular processes governing biomineralization of inorganic materials to produce nanostructures on biological templates. This information is essential for the directed synthesis of high quality nanomaterials via biotemplating. We characterized palladium (Pd) mineralization via the individual adsorption, reduction, and nanocrystal growth processes, which simultaneously occur during the hydrothermal synthesis on the Tobacco mosaic virus (TMV). The adsorption of precursor and reduction of palladium were decoupled through UV–vis Spectroscopy and *in situ* X-ray absorption spectroscopy studies. The role of additional cysteine (Cys) residues, ionic strength, and coating density on the fundamental parameters describing these processes were quantitatively evaluated. Primary nanocrystal growth and structural orientation of Pd nanoparticles was characterized using *in situ* small angle X-ray scattering. The adsorption, reduction of Pd species, and nanocrystal sizes were significantly changed on addition of Cys residues to the amino terminus of the TMV coat protein. Reduction of Pd on an already coated virion was dependent on the Pd surface area, and was hindered by the presence of residual salt. Furthermore, trends in Pd adsorption intensity and capacity suggested that chloride ions affected the adsorption equilibrium. Application of this fundamental approach with

* Corresponding author.
E-mail address: mtharris@purdue.edu (M.T. Harris).

http://dx.doi.org/10.1016/j.jcis.2016.07.028

further optimization of parameters dictating biomineralization would facilitate directed synthesis and scale up of bioinorganic systems.

1. Introduction

Biotemplating has become promising as a means for bottom-up fabrication of nanomaterials via directed growth and hierarchical organization. An increasing range of nanoscale biological objects such as DNA [1–5], RNA [6], microtubules [7–9], viruses [10–18], and assembled protein aggregates [9,19,20], have been employed in the controlled synthesis of inorganic and organic materials under mild and inexpensive conditions. High quality materials resulting from some of these efforts have been applied in varied systems such as chemical and biological sensors [21–23], battery electrodes [12,24–26], nanocircuits [27], memory devices [16], and photovoltaic devices [27]. Particularly, in biotemplating involving inorganic substances, rod-like plant viruses have been used for epitaxial growth of tethered isolated nanoparticles [28,29], solution based nanoparticles [30] and dense surface coatings [31]. These templates are especially attractive due to their availability, inherent stability and malleability through genetic engineering [18,32], click chemistry [10], and phage display [33,34]. The Tobacco mosaic virus (TMV), the virus template employed in this paper, has been genetically engineered in a number of ways to display additional amino acids such as cysteine [5,33], histidine [11], lysine [36], and glutamine [37] on their coat protein via site directed mutagenesis. Each TMV virion consists of 2130 copies of a single coat protein (17.5 kDa), which assemble around RNA into a stiff rod-like particle with a length of 300 nm, outer diameter of 18 nm and inner hollow of 4 nm [35]. The displayed multi-functionalized interfaces on the exterior and interior of the hollow structure serve as viable sites to direct the synthesis of nanoparticles. Previously TMV1Cys (TMV with one extra cysteine) and TMV2Cys (TMV with two extra cysteines) were investigated for increasing the biomineralization of metals such as Au [36], Ag [30,32], Pd [29,32,36–39], and Pt [28].

These studies were performed via conventional electroless deposition involving an external reducing agent. More recently, we have employed the hydrothermal synthesis to form nanowires that are uniform, dense, and monodisperse, as well as tunable along their diameters [17,43]. The quality of nanowires produced with this synthetic method shows the advantages of controlling biomineralization via the molecular interactions on the surface of the biotemplate rather than with an external reducer. Other studies on Ag [31] and Au [32] have also demonstrated the possibility of virus-surface autoreduction of precursor. Nevertheless, controlled synthesis of nanomaterials is still elusive. Yang et al. noticed that under different conditions, nanoparticle coating could be unpredictable. Particles could be completely absent from the surface, mildly decorate the surface, or uniformly coat the virus surface [42], and significant metallization of biotemplates may proceed at low precursor concentration [17,39] instead of at higher concentration [41] as would naturally be expected in chemical reactions. Also, virus surface-mediated mineralization, either in the presence or absence of an external reducer, has resulted in the production of large nanocrystals in the supernatant solution rather than on the virus surface [39,42].

Most synthesis has been performed without consideration of the fundamental processes involved in mineralization. Furthermore, previous attempts to characterize adsorption were performed without knowledge of the existence of surface-mediated reduction. An improper consideration of both chemical reduction and mass transfer driven processes during the experimental procedure (e.g. no stirring) led to inconsistent results [36]. Therefore, it is important to fundamentally characterize the governing molecular processes controlling biomineralization. We have previously shown that at high Pd concentration, reduction is autocatalytically initiated by the virus surface and then proceeds via two first order regimes at the growing Pd interface [41]. Yet the dynamic processes resulting in the different degrees of coating density, uniformity, and primary nanoparticle sizes still remain unclear. In this paper, we further elucidate biomineralization by reframing it within commonly known fundamental molecular processes. After characterizing and confirming these processes, we studied the effects of different system parameters, such as surface identity, ionic strength, and available metal surface for growth and temperature, which may act as synthetic levers for biomineralization. With this approach, directed synthesis will be achievable and scale up of processes will be simpler.

We have identified the adsorption of precursor, reduction of precursor and growth of nanoparticles as the pertinent processes at interplay as the precursor interacts with the surface amino acids. To fully characterize these, we studied the mineralization of Pd onto TMVs using UV–vis spectroscopy along with X-ray absorption spectroscopy (XAS). Statistically relevant information on the nanoparticle size and placement during synthesis was provided by *in situ* small angle X-ray scattering (SAXS) and confirmed by transmission electron microscopy (TEM). Our results indicated the ability to decouple and quantitatively characterize the adsorption and reduction of Pd. Reduction was affected by the addition of exposed Cys to the TMV coat protein surface, the surface area of Pd coating, reaction temperature and chlorine content. The adsorption of precursor was influenced by both the introduction of exposed Cys on the TMV CP and the presence of chlorine anions in solution. Lastly, the addition of Cys to the surface of TMV also had a noticeable influence on the growth and placement of primary Pd nanoparticles during mineralization.

2. Experimental

2.1. Reagents and chemicals

Sodium tetrachloropalladate (II) (99.98%, Sigma Aldrich, St Louis, MO) was used as the Pd precursor. Acrodisc Syringe Filters (Pall life sciences, 13 mm), purchased from Careforde Safety and Scientific (Chicago, IL), were used for filtration of nanoparticles. Poly(methyl methacrylate) (PMMA) plastic cuvettes (VWR Scientific Prod Midwest, Radnor, PA) were used for UV–vis spectra acquisition. Millipore water was used for all experiments. Wild type unmodified TMV (WtTMV) and TMV mutants (TMV1Cys and TMV2Cys) were used in this study. Purification of these TMV variants was performed according to published protocols [33]. Compared to the wtTMV CP, the TMV1Cys CP contains an insertion of a Cys between Ser_1 and Tyr_2 at the amino end of its CP sequence; TMV2Cys contains two sequential Cys at the same position [5,32].

2.2. Ex situ UV–vis characterization

Experiments were performed in a constantly stirred 3.5 mL reactor vessel under controlled temperature conditions. Elevated temperature experiments were conducted below 70 °C to ensure