Materials Science and Engineering C 48 (2015) 103–111

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

# Formation of platinum-coated templates of insulin nanowires used in reducing 4-nitrophenol

Kiersten M. Batzli [a], Brian J. Love [a,b,c,*]

[a] Department of Materials Science and Engineering, University of Michigan, 2300 Hayward St, Ann Arbor, MI 48109, United States
[b] Macromolecular Science and Engineering Research Center, University of Michigan, 2300 Hayward St, Ann Arbor, MI 48109, United States
[c] Department of Biomedical Engineering and Biologic and Materials Sciences (Dentistry), University of Michigan, 2300 Hayward St, Ann Arbor, MI 48109, United States

ARTICLE INFO

*Article history:*
Received 31 July 2014
Received in revised form 10 October 2014
Accepted 22 November 2014
Available online 24 November 2014

*Keywords:*
Insulin fibrils
Nanowires
Self-assembly
4-Nitrophenol

ABSTRACT

Modern technology demands ever smaller and more efficient nanoparticles, wires and networks. The natural tendency for amyloid proteins to form fibrillar structures is leveraged in creating high aspect ratio, nano-sized protein fibers as scaffolds for metallized nanowires. The morphology of fibrils is influenced by induced strain during denaturing and early aggregation and subsequent fibril deposition with platinum leads to controlled catalyst surfaces based on the initial protein precipitate. Here we have created insulin fibrils with varying morphologies produced in the presence of heat and strain and investigated their metallization with platinum by TEM. The catalytic activity of the metal-coated protein fibrils was resolved by tracking the reaction kinetics of the conversion of 4-nitrophenol to 4-aminophenol in the presence of the produced nanowires using UV–Vis spectroscopy. The effects of fibril morphology and temperature on the pseudo-first-order kinetics of conversion are investigated. Conversion to 4-aminophenol occurs on the order of minutes and is independent of temperature in the range tested (7 to 20 °C). Two regimes of conversion are identified, an early higher rate, followed by a slower later rate.



## 1. Introduction

The controlled production and subsequent deployment of metallic nanostructures, such as nanowires and nano-networks, offer new pathways to produce more efficient and more active chemical catalysts, electrode materials and sensors. These structures have high aspect ratios, surface/volume ratios and few defects and lattice boundaries [58]. They are often constructed by the reduction or electroless deposition of colloidal suspensions of metal complexes onto polymeric nanofiber precursors [26]. More controlled nanostructure production hinges on the robust production of the polymeric nanofibers onto which these colloids are templated.

Amyloid proteins aggregate into a variety of morphologies, including fibrillar structures with high aspect ratios. Their reproducible shapes and structures offer potential use as deposition templates for inorganic structures. Due to the widespread clinical relevance of amyloid aggregation in the progression of neurodegenerative and other diseases, their aggregation behavior has been extensively studied commonly through the ThT assay [4] and prior research has focused on understanding the conditions and pathways for aggregation of these proteins, as well as methods to inhibit or modify aggregation after onset [3,53]. Other recent studies have demonstrated using these natural fibril formations as high aspect ratio templates for creating metallic electrodes and catalysts [14,23,29,35,45,50,58]. These studies focus on the metallic plating of fibrils formed in conditions known to cause aggregation (e.g. low pH and temperatures above 55 °C). Thus far, the ability to metallize proteins into nanowires and nano-structured networks has been shown [12] and integration studies are forthcoming to insert these bio-inorganic hybrid structures into working devices [2,52]. However, the range of network structures and characteristics available by tuning of the fibril-forming parameters has not yet been documented.

Ultrathin nanowires have been synthesized in a variety of ways, including on carbon black nanotubes [59], DNA [22], yeast derivatives [48] and more recently through amyloid protein templating [14,23,29,35,45,50,57,58]. Amyloid proteins reliably form thin and long fibrils in specific environmental conditions [10,13,54] including high temperature [17,31,42,44], altered pH [1,9,24,43,44], elevated pressure [16,31] and the presence of salts or urea [6,7,18,25,44,49]. Agitation [38,39,49], shear [6,7,20,21,28,40] or the presence of other surface modulators can also affect the amyloid fibril structure and raise the aggregation rate, though the mechanisms of influence are not well understood. The effects of shear and agitation on protein aggregation are highly debated. Some studies attribute aggregation of proteins not only to shear forces but rather to the effects of surface–liquid interactions of proteins resulting from various methods of agitation and shear [5,8,32,34,55]. Other studies have shown shear and agitation to effectively induce aggregation in proteins which are prone to fibrillation, including β-lactoglobulin [21,28], glucagon protein [15], amyloid-β [20,27] and insulin [6,7,44,49]. It

* Corresponding author at: 2300 Hayward St, H.H. Dow Building, University of Michigan, Ann Arbor, MI 48109, United States.
*E-mail address:* bjlove@umich.edu (B.J. Love).

http://dx.doi.org/10.1016/j.msec.2014.11.056
0928-4931/

is thought that shear in polymer and protein solutions can orient the macromolecules in the direction of shear and that the driving force for orientation can be enough to favor misfolded chain conformations and aggregation.

Thermally-induced protein aggregation is common in many proteins, including insulin. However, while fibril formation is initiated immediately upon exposure to elevated temperature, the formation of superfibrillar structures may take hours or days, though the rate depends on the incubation temperature [31,41,42]. The present work reports on the rapid creation and subsequent metallization of the various fibrillar insulin networks thermally nucleated in the presence of variable applied strain as a method of varying fibril morphology.

Insulin amyloid fibers have been used extensively in templating of nanowires and nanowire networks, though α-synuclein has also been used by Padalkar et al. to create Cd and Pb nanowires [45]. Insulin fibers are highly stable after formation and amino acids expressed on the insulin fibril can interact with inorganic substrates, making the amyloid precipitates ideal for the subsequent templating. The produced metallic nanowires can be integrated into sensors, high surface area electrodes or used as a catalytic surface to speed reactions, such as converting 4-nitrophenol to 4-aminophenol. 4-Nitrophenol is used in producing pharmaceuticals, fungicides, insecticides and rubbers and is found as a residue associated with explosives production and testing. 4-nitrophenol is relatively toxic with a low $LD_{50}$ level, and its conversion to 4-aminophenol yields a product that is less lethal to surrounding species and potentially more biodegradable in the environment. 4-Aminophenol is also used in the production of pharmaceuticals, corrosion inhibitors and anticorrosion lubricants [51]. The reduction of 4-nitrophenol by $NaBH_4$ in the presence of noble metal nanoparticles as catalysts is efficient in producing 4-aminophenol [46,47]. Thus, the catalytic reduction of 4-nitrophenol with an excess of $NaBH_4$ was used as a model system to evaluate the catalytic activity of the platinum coated (Pt-coated) insulin fibers.

The production of insulin fibers and their templating to form Pt-coated catalyst nanowires to convert 4-nitrophenol to 4-aminophenol is documented here. We also present our determination of amyloid attributes arising from the controlled formation experiments. The influence of fibril morphology on the produced nanowires and their catalytic response is also reported on, as is the effect of temperature during 4-nitrophenol conversion. If the morphologies of nanostructured materials are more controlled these templated materials might be more effective catalysts.

## 2. Materials and methods

### 2.1. Insulin solution

Insulin solutions were prepared by dissolving the appropriate amount of lyophilized porcine insulin (Sigma Aldrich) in a 0.01 M HCl solution (pH 2) for a final concentration of 5 mg/mL. The solution is briefly mixed and stored at 4 °C. Samples are used within 24 h of preparation to minimize degradation.

### 2.2. Fibril production

Insulin fibrils were produced using a combined heating and straining protocol. Using a TA ARES Series oscillatory rheometer with 25 mm parallel plates, aliquots of insulin solution were strained during heating in an air oven. The parallel plate geometry creates a direct relationship between displacement and strain within the sample compared with a Couette geometry which has different strain conditions comparing the axial and radial zones of the specimen geometry. The parallel plate geometry also allows for easy sample extraction post-processing with minimal turbulence to the strained fluid after the heating and straining protocol.

All experiments were conducted with a working gap size between 0.2 and 0.5 mm. Samples are loaded at room temperature followed by heating to 65 °C at a controlled ramp rate of 10 °C/min with applied strain of 1%, 10% or 100% and 10 rad/s frequency for 15 min. Here, strain is expressed as a dimensionless percentage and defined as the ratio between the applied rotational displacement and the gap described by the equation:

$$strain = \frac{deformation}{gap\ size}.$$

Following heating and straining, the insulin solution was stored at room temperature for 24 h before metallizing.

A subset of unstrained fibrils were produced for comparison. These include fibrils that were heated without strain using the same heating protocols and for the same length of time and fibrils that were made by seeding an aliquot of non-aggregated insulin solution with a small amount of strained fibrils (1% strain). Both of these fibril solutions were stored at room temperature and otherwise treated according to the same metallizing protocols as the strained fibrils.

### 2.3. Metallizing protocol

Fibrils were metallized using a method adapted from Zhang et al. [58]. The protein solution was diluted to its final concentration of 0.2 mM and mixed with 2.5 mM aged Pt(IV)Cl salts in a 1:5 molar ratio and stirred using a magnetic stir bar for 24 h. The platinum-fibril solution is then reduced by adding cold $NaBH_4$ dropwise to the chilled solution with 40 μL added every 5 min to achieve a final $NaBH_4$ concentration of 2.5 μM. The solution is chilled and mixed for 12 h before testing.

### 2.4. Fibril characterization

Samples of insulin fibrils and Pt-coated fibrils were prepared for characterization by placing a drop of the solution onto an aluminum foil substrate and allowing to air-dry at room temperature. Measurements were made using a Phillips Scanning Electron Microscope (XL30) with attached EDAX for energy dispersive spectra characterization.

### 2.5. UV–Vis spectroscopy probing of catalytic activity

The catalytic activity of the Pt-coated fibrils was measured by tracking the catalysis of 4-nitrophenol to 4-aminophenol by the presence of Pt-coated fibrils and an excess of $NaBH_4$ based on work by Chang et al. [11]. The transformation of 4-aminophenol from 4-nitrophenol, illustrated in Fig. 1, occurs by electron transfer on the catalytic surface of the Pt-coated fibril using $NaBH_4$.

The formation of 4-aminophenol from 4-nitrophenol can be easily tracked using UV–Visible absorption spectroscopy. Cuvettes were prepared from 1.5 mL ultrapure $H_2O$ and 250 μL of 4-nitrophenol mixed with $NaBH_4$ (1.5 4-NP:1 $NaBH_4$). UV–Vis spectra were obtained from a

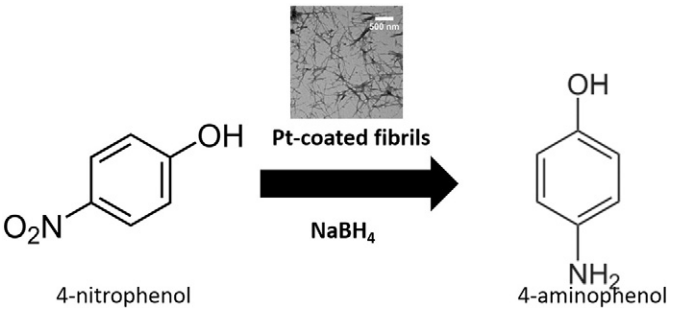


**Fig. 1.** Schematic illustrating the reduction of 4-nitrophenol to 4-aminophenol by Pt-coated fibrils in an excess of $NaBH_4$.