Hybrid bioinorganic insulin amyloid fibrils

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Supplementary information

Materials and methods

Materials - all the chemicals including bovine insulin were purchased from Sigma-Aldrich, and used without further purification

Transmission Electron Microscopy – A 20 µl aliquot of mineralized insulin fibril was placed on a copper grid with carbon treatment. Samples were viewed by a Tecnai G2 20 UT microscope working at 200 kV, Energy-dispersive X-ray (EDX) analysis was performed with an EDAX detector with calibration.

Atomic Force Microscope – A 20 µl aliquot of mineralized insulin fibril was placed on a silicon wafer with the size of 1 cm * 1cm by nitrogen blowing. Samples were viewed by a Veeco Dimension 3000 microscope.

Insulin fibril formation Bovine insulin was dissolved in 2M guanidine hydrochloride and was dialyzed against three rounds of 25 mM HCl at 4 ºC. A stock solution containing 0.1mM bovine insulin in 25 mM HCl was prepared by diluting the dialyzed sample with 25 mM HCl. The reaction mixture was then placed in a hot plate and was kept at 65 ºC for 20 h without agitation, which resulted in the formation of amyloid fibrils as determined by AFM.

Potassium hexachloroplatinate functionalized insulin fibril The as prepared solution of amyloid fibrils (0.1 mM) was incubated at room temperature with 0.1 M water soluble hexachloroplatinic acid for 10 minutes. The pH value was kept at about 1. Excess hexachloroplatinic acid was removed by dialysis at 4 ºC against three rounds of water, followed by addition of an aqueous solution of potassium chloride. This resulted in the formation of a weakly yellow precipitate. The formation of hexachloroplatinic acid coated fibril was demonstrated by TEM analysis. Be cautious, hexachloroplatinic acid is toxic.

Calcium apatite mineralized insulin fibril. Similar to the protocol described above, a 0.1 mM amyloid fibrils solution was incubated with 0.5 M potassium phosphate for 10 minutes. The pH value of the solution was monitored and kept within 9.5-10. Excess phosphate salt was removed by dialysis. During the addition of phosphate, a
precipitation gradually occurs, resulting in the formation of a separated layer. The resulting solution was then incubated for 10 minutes at room temperature with a 0.5 mM calcium chloride aqueous solution, which resulted in the calcification of the amyloid fibrils, which was demonstrated by TEM analysis.

**Figure legends**

*Figure S1* AFM image of dilute insulin fibril (Scale bar 1μm). The length is ranged from 1μm to 4 μm.

*Figure S2* AFM image of intermediate product from anionic phosphate decorated (left) and fully functionalized insulin fibril (right). Compared with the pristine amyloid fibril, these fibrils have a larger diameter (about 8 nm and 15 nm respectively).