Effect of zinc oxide nanoparticles size/surface curvature on α-synuclein amyloidogenesis

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Abstract

The work focuses on investigating the effect of ZnONP and its varying size (30, 60 and 120 nm) on α-synuclein fibrillation process at physiological conditions. α-Synuclein adsorbs strongly onto the surface of ZnONP, observed using different biophysical techniques like UV-visible spectroscopy, Zeta sizer etc. The ThT assay shows inhibition of α-synuclein fibrillation in presence of different curvature ZnONPs with increased lag time and decreased growth rate. Native α-synuclein has shown to form long unbranched fibrils rich in β-sheet structure, whereas ZnONP interface with larger curvature (120 nm) take the protein off-pathway aggregates faster than any other studied curvature/sizes. Though the secondary and tertiary structure analysis by CD and fluorescence spectroscopy showed that the protein in α-synuclein-ZnONPs aggregate retained the native structure and showed insignificant toxicity towards human neuroblastoma cells (IMR-32). These findings suggest that the inhibitory effect of ZnONP on α-synuclein fibrillation is determined by its surface physicochemical properties, including the size/curvature of nanoparticles. The study offers new insight on the therapeutic potential of ZnONP in combating pathologies related to α-synuclein-mediated amyloidogenesis.

Results

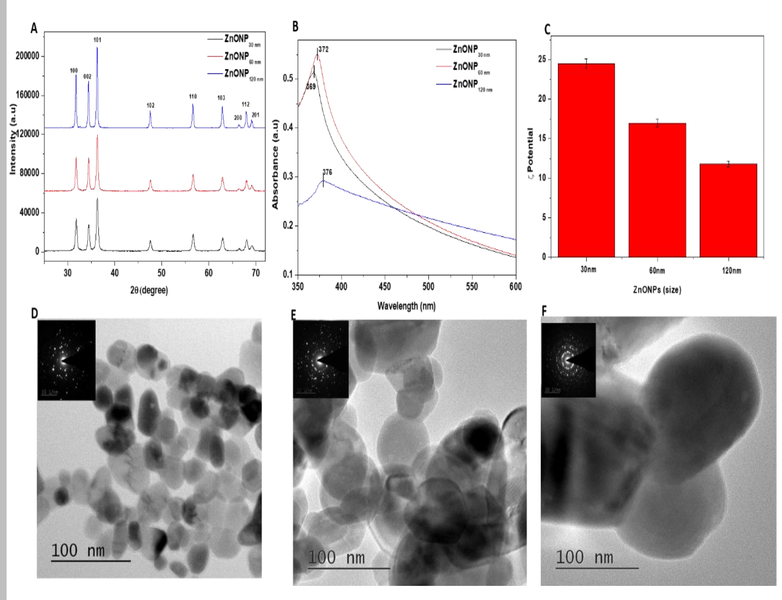

Figure 1. (A) X-ray diffraction pattern, (B) UV-Visible absorption spectra, (C) Zeta potential values and TEM micrographs of ZnONP of different size (D) 30nm, (E) 60nm, (F) 120nm formed from different calcination temperatures. The inset in each TEM image depicts the SAED pattern for each ZnONP.

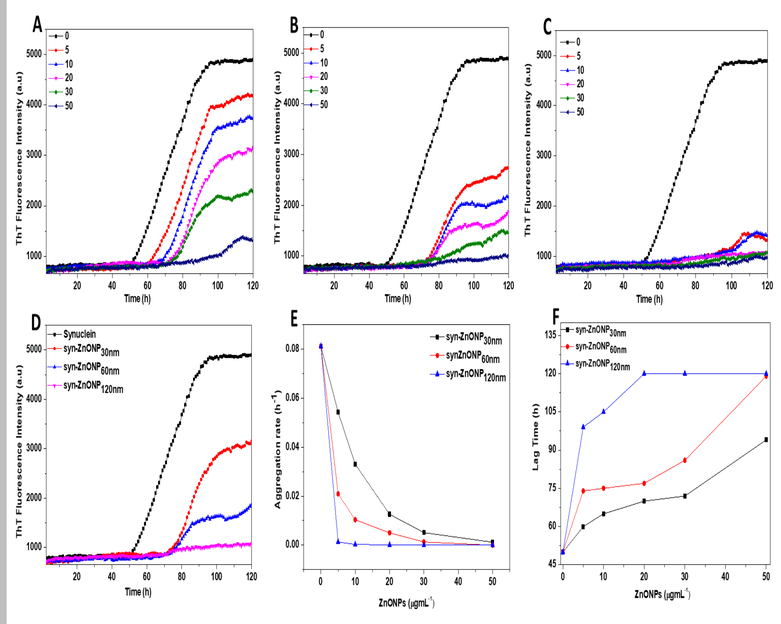

Figure 2. Inhibition of α-synuclein fibrillation measured by ThT assay. Kinetics of amyloid growth in presence of ZnONP (A) $ZnONP_{30nm}$, (B) $ZnONP_{60nm}$, (C)$ZnONP_{120nm}$. (D) The comparative kinetics graph of α-synuclein shows the size dependent inhibition by ZnONP. The comparative graph of (E) aggregation rate and (F) lag time in presence of different size of ZnONP at various concentration.

Results cont....

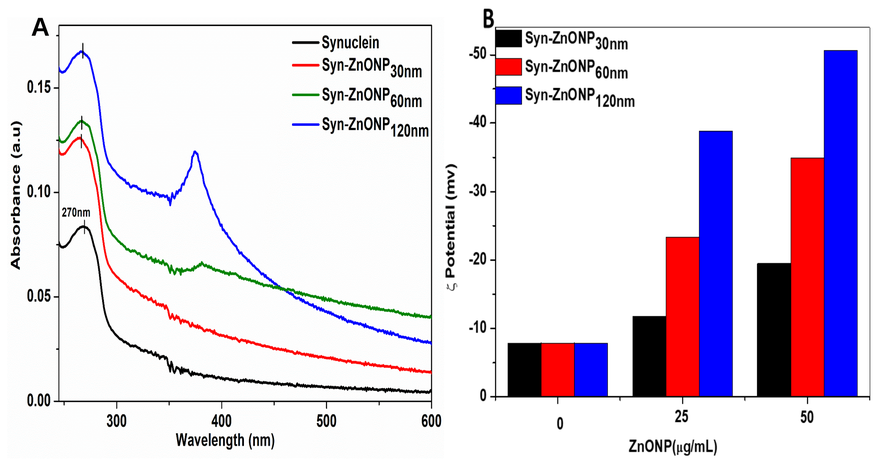

Figure 3. Interaction studies of α-synuclein in absence/presence of ZnONPs (A) UV-Vis absorption spectra (B) Comparative zeta potential analysis.

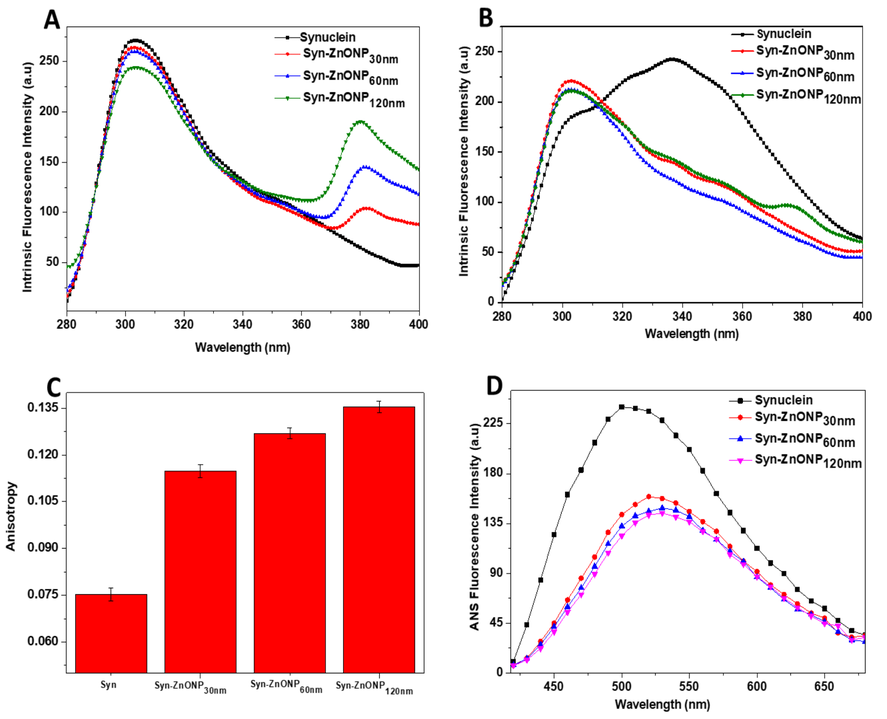

Figure 4. Intrinsic fluorescence spectra of α-synuclein incubated with ZnONP ($ZnONP_{30nm}$, $ZnONP_{60nm}$, and $ZnONP_{120nm}$) for (A) 0h (B) 120h. Measurement of (C) anisotropy and (D) ANS fluorescence of α-synuclein and its conjugates with ZnONP ($ZnONP_{30nm}$, $ZnONP_{60nm,}$ $ZnONP_{120nm}$).

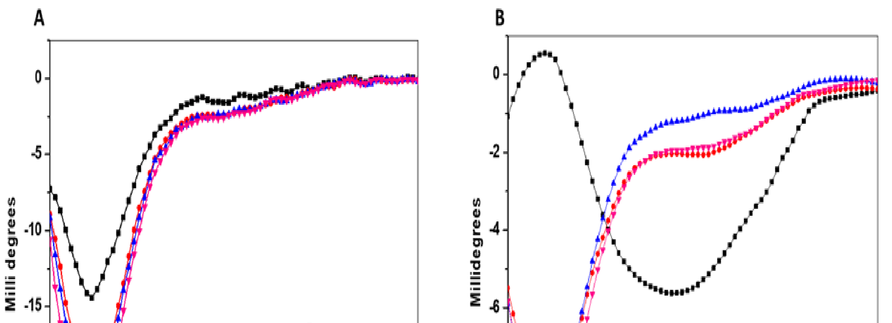

Figure 5. Changes in far-UV CD spectra of α-synuclein incubated with ZnONP ($ZnONP_{30nm}$, $ZnONP_{60nm,}$ $ZnONP_{120nm}$) for (A) 0h and (B) 120h.

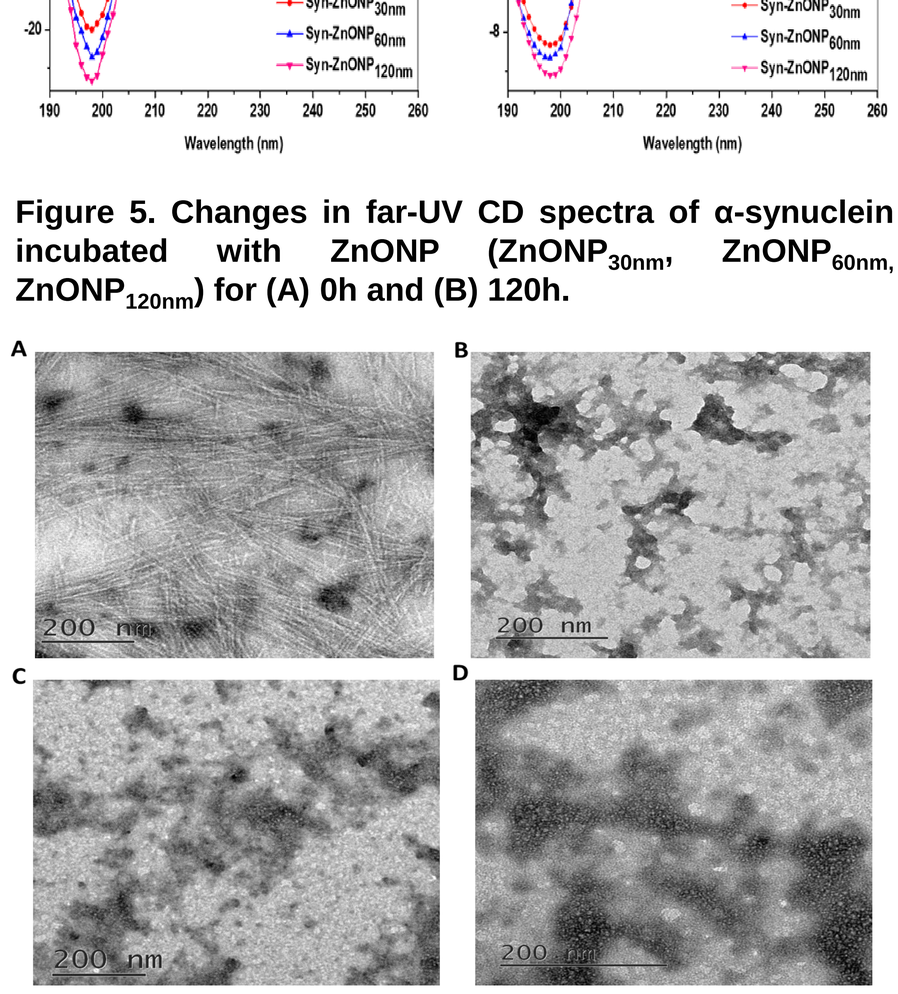

Figure 6. TEM images of (A) α-synuclein without ZnONP interface and α-synuclein incubated with (B) $ZnONP_{30nm}$, (C) $ZnONP_{60nm}$ (D) $ZnONP_{120nm}$ respectively.

Results cont....

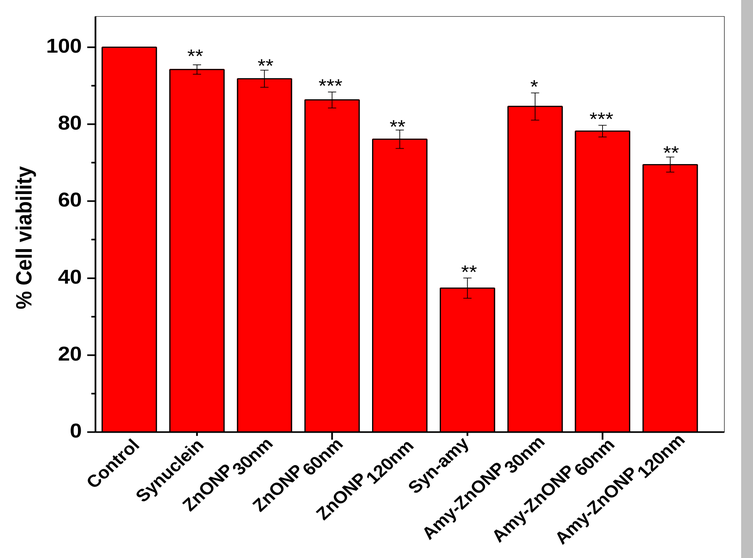

Figure 7. Alamar blue dye reduction assay against neuroblastoma cell line (IMR-32). The results are expressed as percentage viability of cell with respect to control (untreated cells). The data represents the significance, *: P < 0.05, **: P < 0.01, ***: P < 0.001 with respect to the control; obtained from three independent experiments.

Discussion

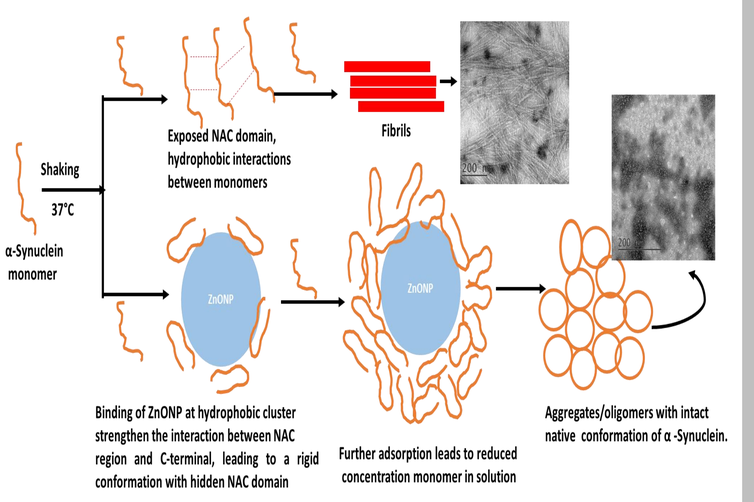

Figure 8. Illustration depicting conformational transition of α-synuclein monomers into amyloid fibrils at 37 ºC under shaking conditions. However, in presence of ZnONPs under similar conditions, protein monomers adsorb onto NP interface ensuring formation of amorphous aggregates (flocs).

Inferences

- Binding of α-synuclein onto ZnONP interfaces avert the conversion of intrinsically unfolded α-synuclein into β-sheet-rich fibrillar assembly, required for nucleation-dependent fibrillation.
- Adsorption of protein monomer on to ZnONP interface render rigidity to protein structure which is leading to inhibition of protein fibrillation.
- High affinity of tyrosine residues in α-synuclein for ZnONP surface results in a stable charge complex formation resulting in disruption of the intermolecular interaction network played by tyrosine involved in synuclein fibrillation.
- Altogether, the data suggests favourable nanoparticle interface interaction with protein monomer that leads to formation of aggregates that are amorphous in nature.

Acknowledgement

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