Referee's Report on the Ph.D. Thesis

Influence of nanoparticles and polymers on the amyloid fibril formation

by Ing. Monika Holubová

submitted to the Faculty of Science of the Charles University, Department of Physical and Macromolecular Chemistry

The submitted doctoral thesis deals with the influence of various agents on amyloidogenesis, the formation of water-insoluble fibrillar structures (amyloids) from globular proteins. Hen egg white lysozyme (HEWL) was taken as a model protein system and amyloid formation was followed by fluorescence measurements (using Thioflavin T or Nile Red as fluorescent probes) and by transmission electron microscopy. The tested agents included (i) carbon nanoparticles such as single-walled carbon nanotubes, fullerenes, carbon quantum dots (CDs) and nanodiamonds (NDs) and (ii) polysaccharides such as glycogen (GG), mannan, phytoglycogen and various modifications of GG. It was found that while polysaccharides in general accelerated the formation of amyloids, the carbon nanoparticles (except for NDs) acted as inhibitors of the process.

Considering that amyloidogenesis *in vivo* causes various diseases (amyloidoses), the results of the thesis are of high importance. Moreover, choosing nanoparticles for such a testing increases the impact of the work considering that some of those nanoparticles are promising tools for medical diagnostics. It is worth mentioning in this context that the results of the thesis were already published in 3 journals with IF (*Chem. Listy*, *Colloid Polym. Sci.*, *RSC Adv.*) and two other manuscripts have been submitted (both to *Soft Matter*).

The thesis, written in very good English, has 74 pages, contains 35 figures and 4 tables. It consists of reviews about amyloids and their structure and properties and about carbon nanoparticles and polysaccharides which are studied further as agents affecting amyloidogenesis. The review part is followed by an introduction to the used experimental methods and by two chapters summarizing the results, one dealing with carbon nanoparticles and the other with polysaccharides.

Overall, there are basically no substantial reasons to object to the content of the thesis. Should I choose some things which could be improved or corrected, it would be the following minor points:

1) As regards experimental methods, the thesis provides only a general introduction into the principles of these techniques. I was missing a true experimental part, that is, a description of instruments and experimental procedures used for obtaining the results in the thesis.
2) A few comments to the section introducing TEM: Firstly, the author omits any mention of field emission sources. Also, the resolution of current high end TEMs is far better than 0.2-0.5 nm, going down to 0.05 nm for aberration-corrected instruments. I would also tend to disagree with the statement that TEM is a very fast method.

3) Table 4: d*n*/d*c* unit is missing.

Besides the above-mentioned comments, I would like to ask the author several questions during the defense:

1) Is the Nile Red assay as specific as the Thioflavin T assay? The fluorescence response of thioflavin T to amyloids seems to be a quite specific effect caused by intercalation of the dye between β-sheets. Nile Red, on the other hand, is known to be a polarity-sensitive fluorophore so one can easily imagine other conformational changes of proteins which would lead to an increase in Nile Red emission.
2) Quantum dots used for the study are unusually small, having the gyration radius of only 0.69 nm while the typical size of QDs according to the literature is 2–10 nm. Considering their low molar mass of 1.8 kg/mol, the QDs had to scatter very weakly both in LS (zeta sizer) and SAXS. What was the QD concentration for these measurements? What was the hydrodynamic radius of the QDs? How was the density of CDs (1.3 g/cm^3) estimated? Was the molar mass calculated from the size and density consistent with the molar mass obtained from the forward scattering in SAXS?
3) What were the gyration radii of the polysaccharides listed in Table 4? Was the R_g/R_h ratio constant or was there any dependence of the ratio on molar mass or degree of modification?
4) The cinnamoyl-modified GG coded as GG-CIN3 has a bimodal distribution of hydrodynamic radii. Table 4 shows molar masses evaluated separately for both modes. For that calculation, how were the mass fractions of the polysaccharide corresponding to those two populations obtained?

To conclude, my general opinion is that the work of Ing. Monika Holubová described in her thesis represents a significant contribution in the field of biopolymers. I thus fully recommend accepting the submitted thesis for the defense and conferring the Ph.D. degree on the author.

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