Quantum dots induce charge-specific amyloid-like fibrillation of insulin under physiological conditions

Alyona Sukhanova*[a,b], Simon Poly[c], Anton Shemetov[b], Igor Nabiev*[a,b]
[a] European Technological Platform Semiconductor Nanocrystals, Institute of Molecular Medicine, Trinity College Dublin, James's Street, Dublin 8, Ireland; [b] Laboratory of Nano-Bioengineering, Moscow Engineering Physics Institute, 115409 Moscow, Russian Federation; [c] CIC nanoGUNE, Tolosa Hiridibea, 76, 20018 San Sebastian, Spain

ABSTRACT

Agglomeration of some proteins may give rise to aggregates that have been identified as the main cause of amyloid diseases. For example, fibrillation of insulin is related to diabetes mellitus. Quantum dots (QDs) are of special interest as tagging agents for diagnostic and therapeutic studies due to their broad absorption spectra, narrow emission spectra, and high photostability. In this study, PEGylated CdSe/ZnS QDs have been shown to induce the formation of amyloid-like fibrils of human insulin under physiological conditions, this process being dependent on the variation of the surface charge of the nanoparticles (NPs) used. Circular dichroism (CD), protein secondary structure analysis, thioflavin T (ThT) fluorescence assay, and the dynamic light scattering (DLS) technique have been used for comparative analysis of different stages of the fibrillation process. In particular, insulin secondary structure remodelling accompanied by a considerable increase in the rate of amyloid fiber formation have been observed after insulin was mixed with PEGylated QDs. Nanoparticles may significantly influence the rate of protein fibrillation and induce new mechanisms of amyloid diseases, as well as offer opportunities for their treatment.

Keywords: quantum dots, proteopathies, nanoparticles, insulin, amyloidosis, secondary structure.

1. INTRODUCTION

1.1 Nature of amyloid deposition diseases

Proteopathies are diseases related to conformational changes in the proteins structures and characterized by the appearance and accumulation of organized aggregates of misfolded or unfolded proteins in intracellular or extracellular media.[1] It has been demonstrated that these aggregates in their intermediate oligomeric form are very toxic; they contribute to membrane oxidative damage, ion and metal dyshomeostasis, aberrant signal transduction, and mitochondrial dysfunction, the processes provoking cell death.[2]

These aggregates are the result of organized polymerization (so-called amyloid behavior) of proteins or peptides whose secondary structure has been altered by one or more destabilizing events.[3] These events are various, but all of them modify the local environment, triggering a secondary structure shift from α-helix to β-sheet.[1] This conformational change makes the protein molecules to aggregate in an organized way until they stabilize.[4] The aggregation process includes two main stages, which are characterized by the formation of two different types of aggregates: amyloid oligomers and amyloid fibrils.[2, 5] In both cases, nucleation process is an important stage of amyloid derivate formation. There are few possible mechanisms of nucleation. First, this may be unfolding of the native folded structures, mostly α-helixes, and their transition to β-sheet structures followed by the formation of small oligomers.[6] Another way is folding of natively unfolded proteins and peptides yielding β-sheet structures.[7, 8] Finally, this may be the formation of β-rich nuclei directly from native conformation, without passing through a fully unfolded state. The initial stage is the appearance of a locally destabilized unfolded structure, which promotes the formation of native-like aggregates; this leads to conformational rearrangements in the structure of protein monomers and appearance of β-sheets followed by amyloid-like protofibril formation.[9] It has been shown that soluble prefibrillar oligomers may activate important downstream mechanisms leading to cell damage and death.[2] In contrast, large insoluble aggregates in the form of protofibrils and fibrils are less toxic for cells and surrounding tissues.[10]

* nanomedicine.mephi@gmail.com (A.S.) or igor.nabiev@gmail.com (I.N.); www.lnbe.mephi.ru/en/about

Nanosystems in Engineering and Medicine, edited by Sang H. Choi, Jin-Ho Choy, Uhn Lee, Vijay K. Varadan,
Proc. of SPIE Vol. 8548, 85485F · © 2012 SPIE · CCC code: 1605-742/12/$18 · doi: 10.1117/12.946606

Some neurodegenerative diseases, such Alzheimer's (AD) and Parkinson's (PD) diseases and other, have been demonstrated to be proteopathies. Proteopathies have been found not only in various tissues of humans and other animal species, but also in fungi and prokaryotes, such as *Escherichia coli.*[11]

Proteopathies may affect a large variety of native proteins and peptides, irrespective of their structural characteristics or functions *in vivo.* No protein primary structure has been found to be specifically prone to proteopathy-related changes.[12] On the other hand, it has been shown that peptides that are large enough to form abnormal β-sheet secondary structures may provoke proteopathies.[13] Proteopathies involve both extracellular proteins, such as immunoglobulin in heavy-chain amyloidosis, and intracellular proteins, such as α-synuclein in Parkinson's disease.[14]

1.2 Insulin as a precursor of amyloid structures

In the present study, human insulin was used as a model protein to study proteopathy. Human insulin may form amyloid fibers *in vivo* and *in vitro* under certain conditions.[15, 16] The important role of insulin in various biological processes and the presence of this protein in different biological liquids makes it especially important to identify the possible mechanism of its oligomerization and fibrillation.

Insulin is a relatively small hydrosoluble protein consisting of two polypeptide chains linked by two disulphide bridges. It is a hormone secreted by β-cells of the pancreas in response to a rising level of glucose in the blood. Under physiological conditions, insulin molecules associate with each other, forming trimers of protein dimers that can bind two or four Zn^{2+} ions.[17, 18] Vestergaard et al. have performed studies with small-angle X-ray scattering to demonstrate that the formation of an α-helical hexamer is the initial step in the fibrillation of insulin. In this case, the hexamer is a building block for subsequent fibril elongation.[19] On the other hand, data obtained using FTIR spectroscopy and TEM indicate that the secondary structure of nascent insulin fibrils strongly depends on the media composition and other conditions; the resultant fibrils may have either an α-helical or a β-sheet structure.[20] The authors suggested that the insulin fibrillation proceeds as follows: natively folded insulin hexamers dissociate to form monomers, which are in equilibrium with a partially folded conformation that undergoes oligomerization to form the critical nucleus for subsequent fibrillation.[21] This pathway is in good agreement with the recently hypothesized mechanism of fibrillation through a partly unfolded conformation.[9]

The insulin extracellular level is strictly regulated through the control of its synthesis, posttranslational modification, and secretion. Amyloid fibers of insulin have been identified in type II diabetic patients.[22] Insulin molecules have been demonstrated to be prone to refolding and aggregating on arterial walls and membrane surfaces *in vivo*. In addition, aggregation and amyloid-like polymerization of insulin are major drawbacks in the production, storage, and use of insulin preparations.[21, 23]

Nanoparticles (NPs) attract much interest as detection agents for proteopathies due to their capacity for linking with capture molecules and their magnetic and optic properties.[24] It has also been theorized that NPs could influence the fibrillation of amyloidogenic proteins due to their large surface-to-volume ratio, high surface free Gibbs energy, and tunable surface charge. Different types of NPs have been synthesized to study their interaction with small soluble protein aggregates or already formed amyloid fibers in order to maximize their natural clearance after induced disaggregation.[25] It was shown that various NPs act as nucleation centers, thereby contributing to the fibrillation of amyloid-prone protein through increasing its local concentration upon adsorption on the surface of NPs.[26] At the same time, maghemite nanoparticles can inhibit insulin fibrillation.[27]

1.3 Participation of semiconductor nanocrystals in amyloid formation

The purpose of our study was to determine how semiconductor quantum dots (QDs) may influence the structure of human insulin in a solution under physiological conditions. Due to their fluorescence properties, CdSe/ZnS QDs have attracted much interest in various fields of nanomedicine and are used as biosensors for a number of bioanalytical assays, as well as for *in vivo* targeting in animal.[28-30] Recent data indicate that CdTe QDs covered with N-acetyl-L-cysteine[25] or thioglycolic acid (TGA)[31] inhibit the fibrillation of Aβ(1-40); CdSe/ZnS QDs covered by dihydrolipoic acid and conjugated with the Aβ-peptides inhibit Aβ(1-42) fibrillation.[32]

Our study have demonstrated that CdSe/ZnS QDs covered with specific PEG derivatives promote the fibrillation of human insulin under physiological conditions. We have demonstrated an important role of the QD surface charge in the destabilization of the insulin protein structure and in insulin fibrillation. Our results confirm that QDs influence the structural properties of human insulin and its behavior in solutions. PEGylated QDs with a high negative or positive charge have no strong effect on insulin fibrillation. In contrast, slightly negative or neutral QDs bearing hydroxyl groups at their surface may strongly accelerate insulin fibrillation.

2. RESULTS AND DISCUSSION

2.1 Interaction of CdSe/ZnS QDs with human insulin *in vitro*

The dynamic light scattering (DLS) technique was used to show that CdSe/ZnS QDs coated with a modified PEG-OH polymer (QD-PEG-OH) were stable during their incubation in a sodium phosphate buffer solution (pH 7) at a temperature of 37°C for more than a week. Under the same experimental conditions, a solution of human insulin displayed no signs of aggregation during a week of incubation. QDs coated with the modified PEG-COOH (QD-PEG-COOH) or a mixture of PEG-OH and PEG-NH_2 polymers (QD-PEG-NH_2/PEG-OH) were found to be sufficiently stable for DLS analysis. The stability of QDs alone or in a mixture with insulin in a solution was monitored by recording their UV–Vis and fluorescence spectra.

Incubation of QD-PEG-OH with human recombinant insulin led to binding of insulin with NPs: the DLS spectra of a mixture of QDs with insulin recorded immediately after their mixing showed that the particles formed were larger than those in solutions of QD-PEG-OH or insulin alone. The UV–Vis and fluorescence spectra confirmed that QDs were stable in a solution for more than a week when mixed with human insulin under physiological conditions. Thus, incubation of human insulin with QDs covered with PEG-OH at pH 7 and 37°C promoted the aggregation process with a dynamic increase in the size of aggregates resulting in the appearance of micrometer-sized aggregates after 24 h of incubation. In contrast, incubation of QD-PEG-COOH or QD-PEG-NH_2/PEG-OH with insulin under the same conditions did not lead to any signs of insulin fibrillation.

The time course of the aggregation of human insulin in the presence of QD-PEG-OH at pH 7 and 37°C was studied using DLS analysis (Figure 1). The size distribution data showed that the particle size in the mixture of QD-PEG-OH with insulin increased rapidly during incubation. The process of insulin fibrillation in the presence of QD-PEG-OH can be divided into several stages. The first stage or early kinetics (Figure 1A) is the formation of large aggregates, partially folded intermediates of fibrillation with sizes of 200–400 nm. Further incubation (late kinetics) for as long as 24 h leads to the appearance of aggregates with sizes of 50 nm, 200 nm, and 1 μm in diameter (Figure 1B). The appearance of small (50 nm) particles suggests the formation of nuclei and, later, large fibrils (particles larger than 1 μm). The observed mechanism agrees with a recently described pathway of fibrillation of globular proteins,[9] with oligomers of partly unfolded protein appearing originally, followed by structural rearrangements to form β-sheet structures and fibrils. It is known that human insulin may form fibrils with a definite structure, the so-called amyloid fibrils, but only under extreme experimental conditions, namely, long-term incubation of insulin solutions at pH 2 and 60–70°C.[20, 21, 33] Insulin fibrillation in the presence of QDs shown in Figure 1 occurred under physiological conditions, which had never been observed for this protein before. We further analyzed the specific structural features of aggregates formed by insulin in the presence of QD-PEG-OH under physiological conditions.

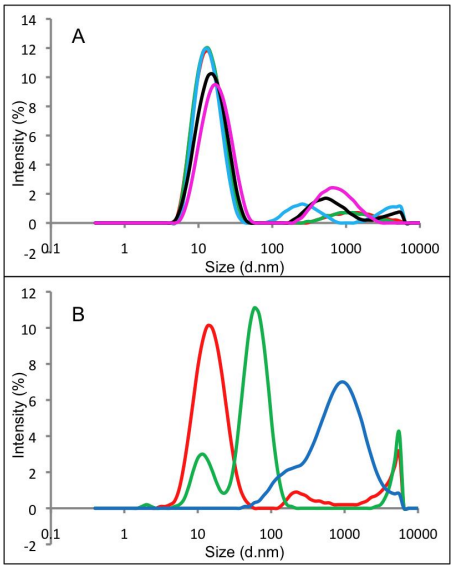

Figure 1. Changes in the particle size distribution in a mixture of recombinant human insulin and CdSe/ZnS QD-PEG-OH during (**A**) 30 min and (**B**) 24 h of incubation. Human insulin (2 mg/ml) was incubated in the presence of 3.44 μM QDs in 10 mM sodium phosphate buffer (pH 7) at 37ºC.

A. DLS curves recorded after 0 (red), 5 (green), 10 (blue), 20 (black), and 30 (magenta) min of coincubation of insulin and QDs.

B. DLS curves recorded after 0 (red), 12 (green), and 24 (blue) h of coincubation of insulin and QDs.

2.2 QD-induced insulin aggregation is structurally similar to amyloid fibrillation phenomena

Structural analysis of aggregations formed during the incubation of human insulin with QD-PEG-OH at pH 7 and 37°C showed the appearance of the structural features of amyloid protein fibrillogenesis. Indeed, addition of QD-PEG-OH to a human insulin solution induced a change in the far UV circular dichroism (CD) spectra of the protein. The insulin secondary structure transition from its native form rich in α-helix to a β-sheet was evidenced by the loss of intensity of the 222-nm band (Figure 2A). The solution of QD-PEG-OH alone did not exhibit any CD activity in the far UV region (Figure 2B). The solution of human insulin alone during its incubation under the same experimental conditions had a stable CD spectra corresponding to the native α-helix-rich form of the protein (Figure 2B). These data indicate that the aggregation of insulin induced by QD-PEG-OH involves a change in the secondary structure similar to that occurring during amyloid fibrillation[21, 33] and confirm the results of DLS suggesting the appearance of partly unfolded intermediates and fibril formation at the final stages of incubation. In order to further investigate the influence of QDs on the aggregation state of human insulin, the time course of insulin fibrillation in the presence of QD-PEG-OH was monitored using the amyloid-specific dye ThT.

ThT and its derivatives are currently used as specific tagging agents of amyloid fibrils for *in vitro* research.[34] ThT is a fluorescent dye whose absorbance and emission spectra change upon interaction with a cross-β structure with a shift of the maximum of absorption from 350 nm to 438 nm and a shift of its fluorescence emission maximum from 450 to 482 nm.[34] In our study, the ThT fluorescence assay showed that insulin aggregation induced by QD-PEG-OH exhibited characteristics of amyloid fibrillation. At 37°C, no fibrils were formed under the standard conditions (2 mg/ml of insulin) at pH 7 during 30 h in the absence of QDs (Figure 3): there was no ThT fluorescence signal at 482 nm; hence, insulin incubated alone did not form amyloid fibrils. In presence of QD-PEG-OH, fibrils were formed as soon as after 4–6 h of incubation at neutral pH: one may observe a continuous increase in the ThT fluorescence signal at 482 nm throughout the incubation period, which reflects the formation of amyloid-like fibrils in the mixture of insulin and QD-PEG-OH. Thus, the data indicate that fibril structures form during coincubation of insulin with QD-PEG-OH.

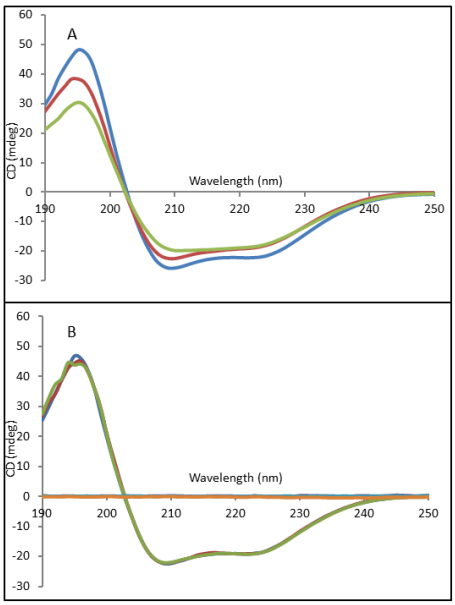

Figure 2. Changes of the insulin secondary structure in the presence of CdSe/ZnS QD-PEG-OH. Circular dichroism spectra of human insulin (2 mg/ml) were recorded in the (**A**) presence or (**B**) absence of 3.44 μM of QDs. QDs were also incubated alone (control), in which case there was no pronounced CD signal (the baselines in Panel B). All solutions were incubated at 37ºC for 24 h in a sodium phosphate buffer solution (pH 7) at 37°C. The CD spectra were recorded after 0 (blue), 12 (red), and 24 (green) h of incubation.

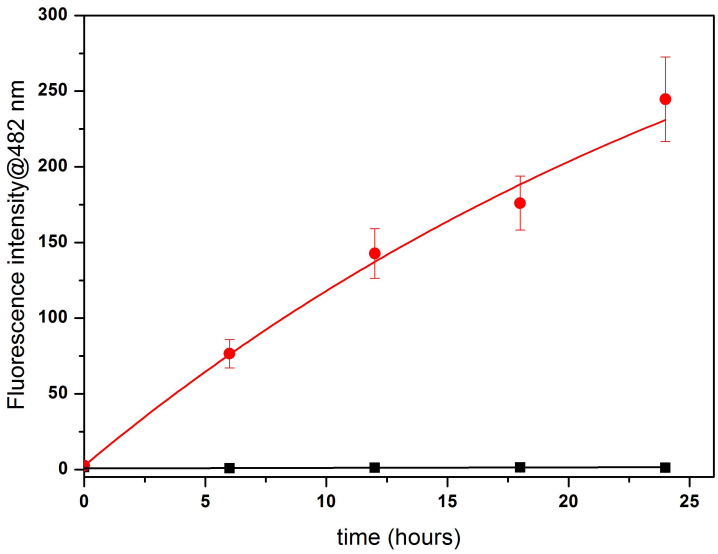

Figure 3. Fibrillation kinetics of insulin at 37ºC monitored using the ThT-based fluorescence assay. Human recombinant insulin (2 mg/ml) was incubated in the absence (black) or in the presence (red) of 3.44 μM CdSe/ZnS QD-PEG-OH in a 10 mM sodium phosphate buffer solution (pH 7) at 37°C for 24 h.

2.3 Effects of the QD surface charge and size on QD-induced insulin fibrillation

We used QDs coated with modified PEG-COOH, PEG-NH_2, or PEG-OH polymers to compare their effects on QD-induced fibrillation. We incubated the QDs bearing –COOH, –NH_2, or –OH groups or a mixture of these functional

groups on their surfaces with insulin samples at pH 7 and 37°C for 24 h and analyzed the role of the charge on the surface of QDs in insulin fibrillation. We demonstrated that only slightly negatively charged QD-PEG-OH induced insulin aggregation. Indeed, Figure 4 shows that QD-PEG-COOH (Figure 4B) or QD-PEG-NH_2/PEG-OH (Figure 4C) nanoparticles did not change the insulin secondary structure, whereas QD-PEG-OH induced significant conformational changes in the insulin secondary structure (Figure 4A), as described previously.

To explain these results we estimated the charges of different QDs used to induce insulin fibrillation. The zeta potential measured at pH 7 and 37°C was greater than –17.4 mV for QD-PEG-OH, –80 mV for QD-PEG-COOH, and +15.7 mV for QD-PEG-NH_2/PEG-OH.

Association of these data demonstrates that nanoparticles with a small negative surface charge caused the strongest induction of the fibrillation of human insulin. This finding agrees with the results of an earlier study indicating that gold nanoparticles with a small negative charge at their surface could induce insulin fibrillation; in that study, the nanoparticle negative charge was hypothesized to be the main factor inducing protein fibrillation.[35]

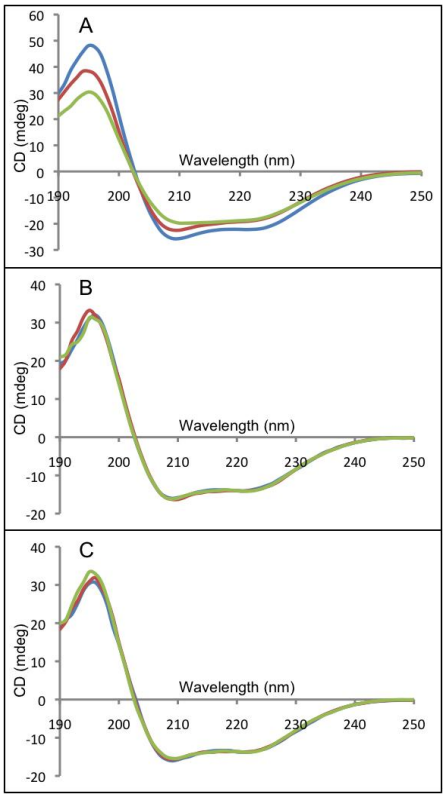

Figure 4. Changes of the human insulin secondary structure in the presence of CdSe/ZnS QDs with different surface charges. Circular dichroism spectra of human insulin (2 mg/ml) were recorded in the presence of 3.44 μM of CdSe/ZnS QDs coated with (**A**) PEG-OH, (**B**) PEG-COOH, and (**C**) QD-PEG-NH_2/PEG-OH. Reaction mixtures buffered with 10 mM sodium phosphate (pH 7) were incubated for 24 h at 37ºC. CD spectra were recorded after 0 (blue), 12 (red), and 24 (green) h of incubation.

We used QDs of different sizes coated with the same -OH functional groups in order to vary the curvature of the charged surface interacting with human insulin in the solution. We synthesized CdSe/ZnS QDs with core diameters of 2.5, 3.3, and 4.8 nm and the emission peaks at wavelengths of 530, 570, and 610 nm (QDs-530, QDs-570, and QDs-610), respectively. All these QDs were solubilized in water and covered with modified PEG-OH polymer. The hydrodynamic radii and zeta potentials of these QDs were analyzed using the DLS technique in order to ensure the purity of the QD solution and estimate the individual charge at the surface of each type of QDs. Comparative analysis of the ability of

these QDs to induce insulin fibrillation at pH 7 and 37°C showed that only QDs-570 could induce secondary structure changes during 3 days of incubation (Figure 5B). In contrast, QDs-530 and QDs-610 (Figures 5A, 5C) did not induce any observable changes in the insulin CD spectra during a 3-day incubation at pH 7 and a temperature of 37°C and, hence any changes of the secondary structure of insulin. The insignificant difference between the spectra shown in Figure 5C can be attributed to the noise created by the absorption spectra of QDs-610.

Analysis of the effect of charge distribution at the surface of PEG-OH-covered QDs on the induction of insulin fibrillation leads to the conclusion that the induction of insulin fibrillation process observed in this study was not directly related to the value of the charge on the QD surface. Instead, it depended on the surface curvature of the QD surface interacting with the protein determining a specific charge distribution over this surface and, hence, a specific spatial pattern of these charges interacting with individual insulin molecules.

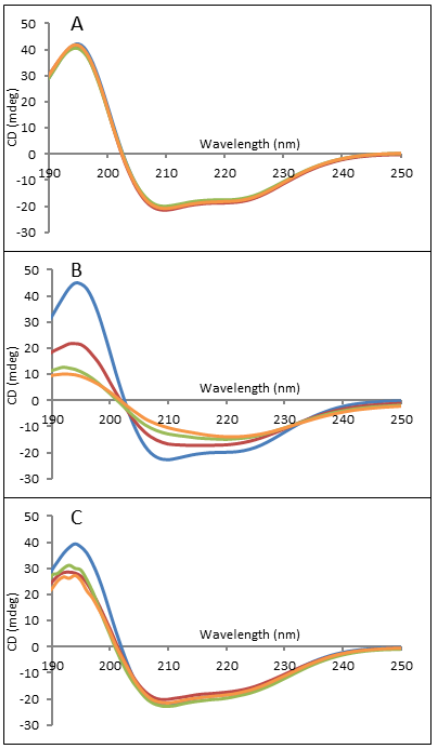

Figure 5. Changes of the insulin secondary structure in the presence of PEG-OH-coated CdSe/ZnS QDs of different diameters. The circular dichroism spectra of human insulin (2 mg/ml) were recorded in the presence of 3.44 μM of QDs with core diameters of (**A**) 2.5 nm, (**B**)3.3 nm, and (**C**) 4.8 nm. All solutions were incubated for 3 days in a 10 mM sodium phosphate buffer (pH 7) at 37ºC; the CD spectra were recorded after 0 (blue), 1 (red), 2 (green), and 3 (orange) days of incubation.

3. CONCLUSIONS

Our study of the effect of CdSe/ZnS QDs coated with PEG derivatives on recombinant human insulin demonstrates their ability to induce the protein amyloid-like fibrillation. Our results show that this QD-induced effect depends not only on the QD charge, but also on the curvature of QDs and, hence, the size of the charged surface capable of interacting with the protein molecule. This finding explains different results obtained in other studies on the effect of different classes of nanoparticles on amyloid-prone proteins, which may seem contradictory only because the charge distribution was not taken into account.[32, 36] In order to demonstrate the protein specificity of different possible spatial patterns of charges, further studies on the effect of PEG-covered QDs on different amyloid-prone and non-amyloid-prone proteins are required. If their results confirm our hypothesis that a specific surface charge pattern affects the amyloidogenicity of native proteins under physiological conditions, then it will be possible to limit the risks entailed by the use of *in vivo*

nanoparticles and develop anti-amyloid nanoparticles for treatment of amyloid-related diseases. These nanoparticles with a specific pattern of charge at their surface would create the *in vivo* conditions under which an amyloid-prone protein, e.g., insulin or tau protein, that has already been unfolded can be refold into its native form. Development of these therapeutic nanoparticles will require long, comprehensive research on all the possible interactions between nanoparticles and proteins *in vivo*. This stage could be speed up by the development of a lab-on-a-chip and the use of an algorithm for simulating the charge distribution at the surface of nanoparticles and their possible interaction with proteins whose structures are available from databases.

4. ACKNOWLEDGEMENTS

This study was supported by the Ministry of Education and Science of the Russian Federation (grant no. 11.G34.31.0050) and by the European Commission through the FP7 Cooperation Program (grant no. NMP-2009-4.0-3-246479 NAMDIATREAM). The authors thank Vladimir Ushakov for the help in preparation of the manuscript.

REFERENCES

[1] Dobson, C.M., "Protein misfolding, evolution and disease," Trends Biochem. Sci. 24, 329-332 (1999).

[2] Glabe, C.G., "Common mechanisms of amyloid oligomer pathogenesis in degenerative disease," Neurobiol. Aging 27, 570-575 (2006).

[3] Moore, R.A., Hayes, S.F., Fischer, E.R. and Priola, S.A., "Amyloid formation via supramolecular peptide assemblies," Biochemistry 46, 7079-7087 (2007).

[4] Go, N., "The consistency principle in protein structure and pathways of folding," Adv. Biophys. 18, 149-64 (1984).

[5] Chiti, F. and Dobson, C.M., "Protein misfolding, functional amyloid, and human disease," Annu. Rev. Biochem. 75, 333-366 (2006).

[6] Nguyen, J., Baldwin, M.A., Cohen, F.E. and Prusiner, S.B., "Prion protein peptides induce alpha-helix to beta-sheet conformational transitions," Biochemistry 34, 4186-4192 (1995).

[7] Abelein, A., Bolognesi, B., Dobson, C.M., Graslund, A. and Lendel, C., "Hydrophobicity and conformational change as mechanistic determinants for nonspecific modulators of amyloid beta self-assembly," Biochemistry 51, 126-137 (2012).

[8] Pawar, A.P., Dubay, K.F., Zurdo, J., Chiti, F., Vendruscolo, M. and Dobson, C.M., "Prediction of "aggregation-prone" and "aggregation-susceptible" regions in proteins associated with neurodegenerative diseases," J. Mol. Biol. 350, 379-392 (2005).

[9] Chiti, F. and Dobson, C.M., "Amyloid formation by globular proteins under native conditions," Nat. Chem. Biol. 5, 15-22 (2009).

[10] DeMarco, M.L. and Daggett, V., "From conversion to aggregation: protofibril formation of the prion protein," Proc. Natl. Acad. Sci. USA 101, 2293-2298 (2004).

[11] de Groot, N.S., Sabate, R. and Ventura, S., "Amyloids in bacterial inclusion bodies," Trends Biochem. Sci. 34, 408-416 (2009).

[12] Pastor, M.T., Esteras-Chopo, A. and Serrano, L., "Hacking the code of amyloid formation: the amyloid stretch hypothesis," Prion 1, 9-14 (2007).

[13] Zhang, S., Iwata, K., Lachenmann, M.J., Peng, J.W., Li, S., Stimson, E.R., Lu, Y., Felix, A.M., Maggio, J.E. and Lee, J.P., "The Alzheimer's peptide a beta adopts a collapsed coil structure in water," J. Struct. Biol. 130, 130-141 (2000).

[14] Giasson, B.I., Forman, M.S., Higuchi, M., Golbe, L.I., Graves, C.L., Kotzbauer, P.T., Trojanowski, J.Q. and Lee, V.M., "Initiation and synergistic fibrillization of tau and alpha-synuclein," Science 300, 636-640 (2003).

[15] Bucciantini, M., Giannoni, E., Chiti, F., Baroni, F., Formigli, L., Zurdo, J., Taddei, N., Ramponi, G., Dobson, C.M. and Stefani, M., "Inherent toxicity of aggregates implies a common mechanism for protein misfolding diseases," Nature 416, 507-511 (2002).

[16] Jimenez, J.L., Nettleton, E.J., Bouchard, M., Robinson, C.V., Dobson, C.M. and Saibil, H.R., "The protofilament structure of insulin amyloid fibrils," Proc. Natl. Acad. Sci. USA 99, 9196-9201 (2002).

[17] Chang, X., Jorgensen, A.M., Bardrum, P. and Led, J.J., "Solution structures of the R6 human insulin hexamer," Biochemistry 36, 9409-9422 (1997).

[18] Xu, Y., Yan, Y., Seeman, D., Sun, L. and Dubin, P.L., "Multimerization and aggregation of native-state insulin: effect of zinc," Langmuir 28, 579-86 (2012).

[19] Vestergaard, B., Groenning, M., Roessle, M., Kastrup, J.S., van de Weert, M., Flink, J.M., Frokjaer, S., Gajhede, M. and Svergun, D.I., "A helical structural nucleus is the primary elongating unit of insulin amyloid fibrils," PLoS Biol. 5, e134 (2007).

[20] Nielsen, L., Frokjaer, S., Carpenter, J.F. and Brange, J., "Studies of the structure of insulin fibrils by Fourier transform infrared (FTIR) spectroscopy and electron microscopy" J. Pharm. Sci. 90, 29-37 (2001).

[21] Nielsen, L., Khurana, R., Coats, A., Frokjaer, S., Brange, J., Vyas, S., Uversky, V.N. and Fink, A.L., "Effect of environmental factors on the kinetics of insulin fibril formation: elucidation of the molecular mechanism," Biochemistry 40, 6036-6046 (2001).

[22] Pease, L.F., 3rd, Sorci, M., Guha, S., Tsai, D.H., Zachariah, M.R., Tarlov, M.J. and Belfort, G., "Probing the nucleus model for oligomer formation during insulin amyloid fibrillogenesis," Biophys. J. 99, 3979-3985 (2010).

[23] Sluzky, V., Tamada, J.A., Klibanov, A.M. and Langer, R., "Kinetics of insulin aggregation in aqueous solutions upon agitation in the presence of hydrophobic surfaces," Proc. Natl. Acad. Sci. USA, 88, 9377-9381 (1991).

[24] Georganopoulou, D.G., Chang, L., Nam, J.M., Thaxton, C.S., Mufson, E.J., Klein, W.L. and Mirkin, C.A., "Nanoparticle-based detection in cerebral spinal fluid of a soluble pathogenic biomarker for Alzheimer's disease," Proc. Natl. Acad. Sci. USA 102, 2273-2276 (2005).

[25] Xiao, L., Zhao, D., Chan, W.H., Choi, M.M. and Li, H.W., "Inhibition of beta 1-40 amyloid fibrillation with N-acetyl-L-cysteine capped quantum dots," Biomaterials 31, 91-98 (2010).

[26] Linse, S., Cabaleiro-Lago, C., Xue, W.F., Lynch, I., Lindman, S., Thulin, E., Radford, S.E. and Dawson, K.A., "Nucleation of protein fibrillation by nanoparticles," Proc. Natl. Acad. Sci. USA 104, 8691-8696 (2007).

[27] Skaat, H., Sorci, M., Belfort, G. and Margel, S., "Effect of maghemite nanoparticles on insulin amyloid fibril formation: selective labeling, kinetics, and fibril removal by a magnetic field," J. Biomed. Mater. Res. A 91, 342-351 (2009).

[28] Yu, W.W., Chang, E., Drezek, R. and Colvin, V.L., "Water-soluble quantum dots for biomedical applications," Biochem. Biophys. Res. Commun. 348, 781-786 (2006).

[29] Medintz, I.L., Uyeda, H.T., Goldman, E.R. and Mattoussi, H., "Quantum dot bioconjugates for imaging, labelling and sensing," Nat. Mater. 4, 435-446 (2005).

[30] Shemetov, A.A., Nabiev, I. and Sukhanova, A., "Molecular interaction of proteins and peptides with nanoparticles," ACS Nano 6, 4585-4602 (2012).

[31] Yoo, S.I., Yang, M., Brender, J.R., Subramanian, V., Sun, K., Joo, N.E., Jeong, S.H., Ramamoorthy, A. and Kotov, N.A., "Inhibition of amyloid peptide fibrillation by inorganic nanoparticles: functional similarities with proteins," Angew. Chem. Int. Ed. 50, 5110-5115 (2011).

[32] Thakur, G., Micic, M., Yang, Y., Li, W., Movia, D., Giordani, S., Zhang, H. and Leblanc, R.M., "Conjugated quantum dots inhibit the amyloid beta (1-42) fibrillation process," Int. J. Alzheimers Dis. 2011, 502386 (2011).

[33] Bouchard, M., Zurdo, J., Nettleton, E.J., Dobson, C.M. and Robinson, C.V., "Formation of insulin amyloid fibrils followed by FTIR simultaneously with CD and electron microscopy," Protein Sci. 9, 1960-1967 (2000).

[34] LeVine, H., 3rd, "Thioflavine T interaction with synthetic Alzheimer's disease beta-amyloid peptides: detection of amyloid aggregation in solution," Protein Sci. 2, 404-410 (1993).

[35] Wagner, S.C., Roskamp, M., Pallerla, M., Araghi, R.R., Schlecht, S. and Koksch, B., "Nanoparticle-induced folding and fibril formation of coiled-coil-based model peptides," Small 6, 1321-1328 (2010).

[36] Wu, W.H., Sun, X., Yu, Y.P., Hu, J., Zhao, L., Liu, Q., Zhao, Y.F. and Li, Y.M., "TiO2 nanoparticles promote beta-amyloid fibrillation in vitro," Biochem. Biophys. Res. Commun. 373, 315-318 (2008).