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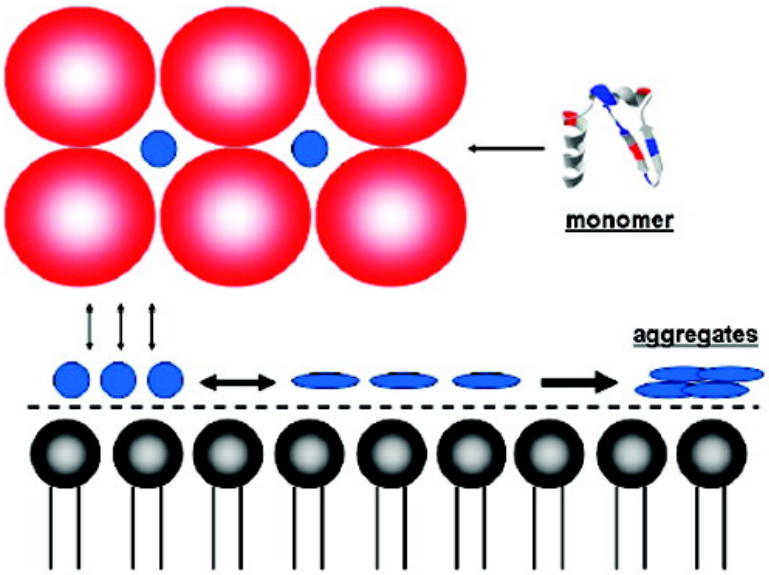

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Misfolding of Amyloidogenic Proteins at Membrane Surfaces: The Impact of Macromolecular Crowding

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Amyloidogenic disorders are characterized by the pathological conversion of proteins into macromolecular assemblies with toxic consequences.[1] In general, the fate of specific polypeptides in situ is strictly controlled by their immediate biochemical environment, folding into either nontoxic structures, or misfolded disease causing products. Nascent amyloidogenic proteins are released in vivo into an environment which is congested by other large biomolecules.[2] This confined environment, induces a phenomenon referred to as "macromolecular crowding" and has major thermodynamic and kinetic consequences on the folding behavior of these proteins.[3] Macromolecular crowding can stimulate protein aggregation as the reduced free volume favors compact protein states, and the reduced activity of water induces a decreased protein solubility, which favors the self-association of amyloidogenic proteins.[3a] However, folding/misfolding of proteins is controlled not only by a three-dimensional aqueous environment crowded by inert macromolecules but also, to a critical extent, by interactions with chaperone interfaces or membranes, which can be described as two-dimensional crowding surfaces.[4] The lipid bilayers of the plasma membrane and organelles can also mediate pathological effects of amyloidogenic proteins, including synuclein, IAPP, and Aβ, which is involved in Alzheimer's disease (AD),[1c,4a,5] and whose oligomerization process can be initiated intracellularly as shown by Selkoe's lab and others.[6]

Various target lipid membranes have been shown to induce an electrostatically driven surface crowding (enrichment) of Aβ-protein, which is followed by accelerated misfolding into toxic aggregates at rates significantly higher than in a membrane-free environment.[4,5,7] The aggregation pathway seems to be fundamentally different for surface adsorbed Aβ as compared to the situation in solution. However, to this date, all of these studies have been carried out under in vitro conditions, using only isolated target molecules in diluted aqueous environments, and thereby neglecting a key feature of molecular crowding—the confined space. This fact does not only change folding and aggregation patterns in an aqueous 3D folding space[3b,4a] but can also effect the 2D crowding at membrane surfaces, influencing both protein association and membrane-mediated folding and aggregation. As schematically visualized in Figure 1, we used concentrated solutions of Ficoll 70 (red spheres), a large inert crowding polymer, and Aβ_{1-40} (blue spheres) as an amyloidogenic model system to study the impact of macromolecular crowding on the process of membrane-surface-induced protein misfolding. This has provided us with general insights into how molecular crowding effects influence protein folding on two-dimensional templates such as membranes or chaperone interfaces within the cell.

Figure 2 presents representative, time-dependent circular dichroism (CD) experiments, where 50 μM Aβ is interacting at a 60/1 lipid to protein molar ratio with membranes composed of the neutral DMPC and the acidic DMPG lipids at a 2:1 molar ratio, prepared as small unilamellar vesicles (Supporting Information). The presence of a two-dimensional charged membrane surface accelerated the

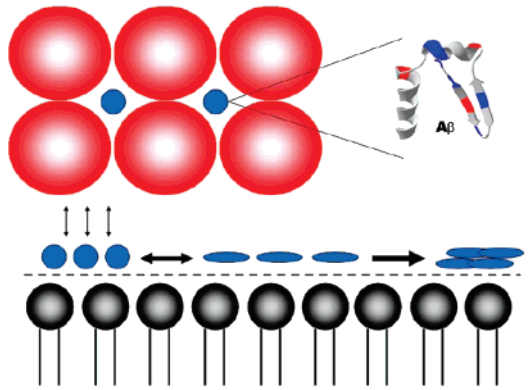

Figure 1. A schematic representation of the impact of macromolecular crowding on membrane-mediated aggregation of amyloidogenic proteins (left to right): (i) diffusion controlled electrostatically driven protein association onto membrane surfaces; (ii) membrane-induced temporal structural changes; (iii) protein aggregation above the critical concentration barrier.

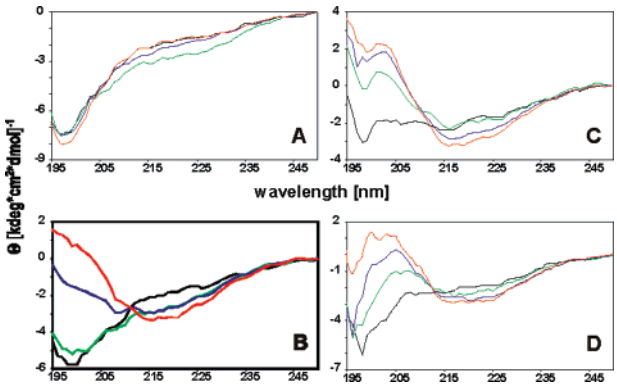

Figure 2. Time-dependent CD spectra of 50 μM Aβ_{1-40} at 301 K in buffer (A). Added to 3 mM DMPC/DMPG (2:1 molar ratio) without Ficoll 70 present (B); 200 g/L Ficoll 70 (C); 350 g/L Ficoll 70 (D). Day 0 (black); day 1 (green); day 6 (blue); day 21 (red).

transition of Aβ from a mainly random structure into a predominantly β-sheet state under all crowding conditions, compared to the membrane-free case (Figure 2a). Supplementary CD experiments in the absence of lipid membranes but with varying amounts of Ficoll present (Figure S1) revealed only minor structural changes without any pronounced time dependence, during the 3 weeks of aggregation trials, which is the opposite effect to that seen in the presence of membranes. In all cases, the populations of β-like structures were higher if charged membranes were present (Figure 3).

However, the response to the amount of crowding agent in the presence of membranes is not linear (Figures 2 and 3). An initial increase in crowding agent concentration leads to elevated levels of membrane surface association due to a change in the equilibrium

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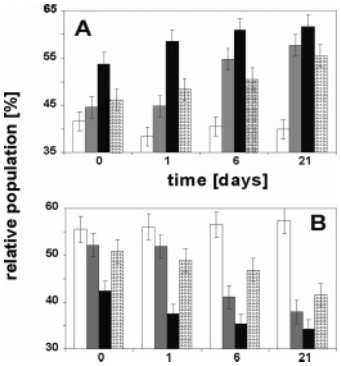

Figure 3. Time dependence of the fraction of secondary structures upon deconvolution of the CD spectra in Figure 2: (A) β-like features, (B) unordered structure: Aβ_{1-40} without membranes and Ficoll 70 (white); added to membranes without Ficoll 70 (gray), 200 g/L Ficoll 70 (black) and 350 g/L Ficoll 70 (shaded).

with the free Aβ. At higher concentrations, the increased viscosity induces a reduction in the diffusion rates, resulting in a reduced rate of Aβ-membrane binding events compared to the less crowded case. This is clearly seen in both membrane samples with crowding agent present (200 and 350 g/L). First, no lag time for a conformational transition to β-sheet structures is observed for these samples, while in the crowding-free case, only minor changes can be seen up to the sixth day, indicating a lag time. The fastest and most pronounced structural changes are found for Aβ_{1-40} when membranes and 200 g/L Ficoll 70 were present. Upon an increase of the Ficoll 70 concentration to 350 g/L, the temporal conformational changes were slower than for the medium crowded sample but faster than for the protein in a crowding-free environment. These temporal changes of β-structures (β-strand and β-turn together) are displayed in Figure 3A for Aβ_{1-40} under all crowding conditions and in Figure 3B for unordered fractions. The most significant changes in the relative populations are visible if the protein is in a medium-crowded environment.

The main goal of the work presented here is to address the problem, how the presence of a 3D crowded medium affects Aβ's ability to change structure on membrane surfaces which per se function as natural 2D crowding and folding templates. This is apparently a mode of action which many other systems, including antimicrobial peptides or interfacial enzymes, have in common with Aβ.[5,7,8]

Clearly, the presence of lipid membranes with a negative surface potential accelerates the aggregation of Aβ_{1-40} under crowding conditions in a similar way as membrane-associated Aβ in a crowding agent free environment, as observed previously.[7] However, the effect of crowding on membrane-induced structural changes of Aβ appears to be concentration dependent. In all cases, rates of aggregation are enhanced, although at a greater concentration of crowding agents, the rates are not as high. This can be explained by a volume exclusion effect in crowded media, which will force Aβ toward the membrane surface simply by increasing the effective concentration of Aβ monomers in solution and their activity. Therefore, the effective membrane surface concentration will increase and induce accelerated aggregation as seen in Figure 2. The associated exclusion of lag time might also correlate to the same effect since Schilling et al. showed that very high concentrations of Aβ (as present at our membrane surfaces) can aggregate without lag time.[9] However, crowding has not only the effect of excluded volume but also, at high concentrations, the viscosity of the medium will play a significant role.[3a] Therefore, at high concentrations of Ficoll 70 (42cP viscosity at 350 g/L), the aggregation process is becoming mainly diffusion limited, as visible in a slower aggregation process.[2b,3a] Most likely, even the affinity of Aβ toward the membrane surface is reduced.

Finally, our results provide a strong indication that crowding can have a severe impact on the structural behavior of proteins and peptides interacting with membrane interfaces, with consequences not only for protein aggregation but also for the activity of peripheral proteins, which play a key role in many cellular processes.

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Supporting Information Available: Experimental procedures and CD spectra and analysis for membrane-free aggregation experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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