

Review

The role of lipid–protein interactions in amyloid-type protein fibril formation

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Abstract

Structural transition of polypeptide chains into the β -sheet state followed by amyloid fibril formation is the key characteristic of a number of the so-called conformational diseases. The multistep process of protein fibrillization can be modulated by a variety of factors, in particular by lipid–protein interactions. A wealth of experimental evidence provides support to the notion that amyloid fibril assembly and the toxicity of pre-fibrillar aggregates are closely related and are both intimately membrane associated phenomena. The present review summarizes the principal factors responsible for the enhancement of fibril formation in a membrane environment, viz. (i) structural transformation of polypeptide chain into a partially folded conformation, (ii) increase of the local concentration of a protein upon its membrane binding, (iii) aggregation-favoring orientation of the bound protein, and (iv) variation in the depth of bilayer penetration affecting the nucleation propensity of the membrane associated protein. The molecular mechanisms of membrane-mediated protein fibrillization are discussed. Importantly, the toxicity of lipid-induced pre-fibrillar aggregates is likely to have presented a very strong negative selection pressure in the evolution of amino acid sequences.

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Keywords: Protein–lipid interactions; Membrane-induced protein aggregation; Amyloid fibrils

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Abbreviations: CL, cardiolipin; DG, diacylglycerol; Chol, cholesterol; IAPP, islet amyloid polypeptide; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIP, PI 4-phosphate; PIP₂, PI 4,5-P₂; PS, phosphatidylserine; SM, sphingomyelin

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1. Introduction

Lipid–protein interactions play a key role in a wide variety of cellular processes, including signal transduction, intracellular transport, enzyme catalysis, energy conversion in the cell, antimicrobial defense, and control of membrane fusion (Lee, 2004; Palsdottir and Hunte, 2004; Dowhan et al., 2004; Kinnunen et al., 1994). Critical for all these functions are unique structural features of the protein molecules representing an ensemble of rapidly interconverting conformational substates highly sensitive to various environmental factors. Lipid bilayer, the basic structural element of biological membranes, is commonly regarded as a two-dimensional liquid providing a variety of environments, which can affect protein structure and dynamics via both specific and non-specific interactions. Accordingly, these interactions are controlled not only by general physicochemical characteristics of a membrane, such as its phase state, bilayer curvature and elasticity, surface charge, and degree of hydration, but also by the exact chemical nature of membrane lipids, extent of acyl chain unsaturation, conformation and dynamics of lipid headgroups and acyl chains, and protein–lipid selectivity arising from factors such as the hydrophobic matching at the protein–lipid interface (Jensen and Mouritsen, 2004). Importantly, these factors can exert influence not only on protein conformation, but also on its oligomerization state, a number of studies providing evidence for substantial enhancement of protein and peptide aggregation in a membrane environment (Han and Tamm, 2000; Fernandes et al., 2003; Paquet et al., 2001).

During the last decade, one specific type of protein aggregates has drawn particular interest due to its involvement in the pathogenesis of the so-called conformational diseases. More specifically, these aggregates are featured by the presence of specific filamentous structures, amyloid fibrils, having a core cross- β -sheet structure in which polypeptide chains are oriented in such a way that the β -strands run perpendicularly to the long axis of the fibril, while the β -sheets propagate in its direction (Serpell, 2000; Sunde and Blake, 1998). Mature fibrils have diameters of 4–13 nm and usually contain 2–6 laterally associated or twisted protofilaments, each 2–5 nm in diameter (Jimenez et al., 1999;

Khurana et al., 2003). Interestingly, the ability to form fibrils is not limited to proteins associated with conformational diseases. There are good grounds for believing that amyloid-forming propensity is a generic property of polypeptide chain since a number of disease-unrelated proteins and peptides have been demonstrated to form fibrils (Dobson, 2004; Srisailam et al., 2002).

Several lines of evidence suggest that the formation by the A β peptide of amyloid fibers and their cytotoxic action are membrane associated processes (Yip et al., 2001, 2002; Fernandez and Berry, 2003; Bokvist et al., 2004; Thirumalai et al., 2003; Stefani, 2004). Lipid bilayer may act as an effective catalyst of fibrillogenesis, providing a generic environment where protein molecules adopt conformation and orientation promoting their assembly into protofibrillar and fibrillar structures (Thirumalai et al., 2003; Stefani, 2004; Sparr et al., 2004; Zhao et al., 2004). Cell membrane is further thought to be the direct target mediating amyloid-induced cell death. Amyloid formation has been reported to induce membrane permeabilization resulting from alterations in bilayer structure and/or uptake of lipids into the forming fiber (Sparr et al., 2004; Zhao et al., 2004, 2005; Lin et al., 2001; Michikawa et al., 2001). It has also been hypothesized that extraction of membrane lipids by the forming amyloid (Sparr et al., 2004; Zhao et al., 2004) may be the direct cause for membrane permeabilization and cell death (Zhao et al., 2005).

2. Membrane-induced protein fibrillization

Vast majority of studies on membrane-mediated fibrillogenesis have been undertaken with model systems including amyloidogenic peptides or proteins and lipid vesicles of varying composition (Bokvist et al., 2004; Sparr et al., 2004; Sharp et al., 2002; Lindström et al., 2002; Terzi et al., 1997). An important conclusion reached is that the fibrillogenic properties of membrane-bound proteins are largely determined by the chemical nature of membrane lipids and the mode of protein–lipid interactions. Several studies have demonstrated anionic phospholipids to represent the main membrane component responsible for the enhancement of fibril formation, as shown for α -synuclein (Zhu et al., 2003; Jo et al., 2000, 2004; Narayanan and Scarlata, 2001;

Table 1
Involvement of membranes in fibril formation by amyloidogenic proteins and peptides

Disease	Protein or peptide determinant	Membrane system	Ref.
Parkinson's disease, Lewy body variant of Alzheimer's disease, multiple system atrophy	α -Synuclein	PA/PC, PG/PC, PS/PC, PG/PE vesicles Planar PC/PS bilayers PC, PA, PS, PI vesicles Brain membrane fractions Synaptosomal membranes	Zhu et al. (2003) Jo et al. (2000) Necula et al. (2003) Lee et al. (2002) Jo et al. (2004)
Alzheimer's disease	A β peptide	Total brain lipid bilayers PC/PG vesicles PC/ganglioside vesicles PA, PS, PI, PIP, PIP ₂ , CL, PC, PE, SM, Chol, DG, gangliosides, sonicated lipid suspensions	Yip et al. (2001) Bokvist et al. (2004) Choo-Smith et al. (1997) Chauhan et al. (2000)
Type II diabetes	Islet amyloid polypeptide	PG/PC vesicles, liposomes from pancreas lipids PC, PS/PC vesicles, rat insulinoma tumor cells	Knight and Miranker (2004) Sparr et al. (2004)
Alzheimer's disease	Tau	PS vesicles	Chirita et al. (2003)
Spongiform encephalopathies	Prion protein	PG, PC, PC/Chol/SM vesicles	Kazlauskaitė et al. (2003)
Familial polyneuropathy, systemic amyloidosis	Transthyretin	PC/PS, PC/PG vesicles	Zhao et al. (2004)
Systemic amyloidosis	Lysozyme	PC/PS, PC/PG vesicles	Zhao et al. (2004)
Thyroid carcinoma	Calcitonin	PC, PC/Chol, PC/PS, PC/ganglioside vesicles	Wang et al. (2005)

Necula et al., 2003; Lee et al., 2002), A β peptide (Yip et al., 2001; Bokvist et al., 2004; Choo-Smith et al., 1997; Chauhan et al., 2000), islet amyloid polypeptide (Sparr et al., 2004; Knight and Miranker, 2004; Padrick and Miranker, 2002), tau (Chirita et al., 2003), lysozyme, transthyretin, cytochrome *c*, insulin, myoglobin, and endostatin (Zhao et al., 2004). The extent of fibrillization of A β _{1–40} increases upon association with PA, PS, PI, PIP, PIP₂, and CL, in contrast to the neutral (cholesterol, cerebrosides, and diacylglycerol) and zwitterionic lipids (PC, PE, and SM), and anionic lipids lacking phosphate groups (Knight and Miranker, 2004). Moreover, the enhancement of fibrillization correlates with the degree of phosphorylation of PI, in the order PIP₂ > PIP > PI, thus suggesting that phosphate groups are essential for fibril formation. However, other studies provided evidence for selective binding of A β _{1–40} peptide to gangliosides and the acceleration of fibril formation in the presence of ganglioside-containing vesicles (Choo-Smith et al., 1997; Matsuzaki and Horikiri, 1999). The formation of β -structure in A β _{1–42} induced by PIs was found to result from interactions of the peptide with both the PI phosphate group and inositol ring (McLaurin et al., 2000). As summarized in Table 1, the induction of fibril formation *in vitro* has been reported not only for

membranes containing anionic phospholipids, but also for bilayers composed of mixtures of the phospholipids (PC and PE) with gangliosides or cholesterol, and the so-called 'raft' consisting of sphingomyelin and cholesterol. Accordingly, it does not seem feasible to attribute the ability to promote protein fibrillization to a certain class of lipids. Nevertheless, the chemical nature of the bilayer components is likely to have a significant role in determining the mode and extent of the membrane binding of these proteins, as well as in the adoption of aggregation-prone protein conformation. Yet, the molecular details of lipid–protein interactions resulting in the transformation of polypeptide chain into fibrillar structures may differ considerably, depending on the structural characteristics of the proteins or peptides in question.

Cumulatively, the available data on membrane-assisted fibrillogenesis reveals protein assembly into regular fibrillar structures to involve several distinct steps, i.e. (i) the initial binding of the protein to the lipid bilayer. This step is commonly (but not necessarily) driven by electrostatic attraction and is followed by (ii) a structural transformation of the bound protein into a highly aggregation-prone partially folded conformation. Subsequently, (iii) oligomerization of the membrane-bound proteins occurs, yielding (iv) fiber formation and

growth due to the association of monomers to the protein oligomers. A number of studies indicate substantial acceleration of fiber growth in a membrane environment compared to that in solution, as reported for α -synuclein (Necula et al., 2003; Jo et al., 2004), IAPP (Knight and Miranker, 2004), and A β peptide (Choo-Smith et al., 1997). Another essential feature of membrane-induced fibrillization involves lipid inclusion into the forming fiber, observed for α -synuclein (Lee et al., 2002), IAPP (Sparr et al., 2004), transthyretin, lysozyme, cytochrome *c*, endostatin, and insulin, for instance (Zhao et al., 2004).

To illustrate possible reasons for the observed changes in the aggregation pathway and kinetic parameters of fibrillogenesis in membranes we will briefly outline the different mechanistic and kinetic models proposed to describe fiber formation in solution.

3. Molecular mechanisms and kinetics of fibril formation

Protein fibrillization is an extremely complex process, commonly involving kinetic competition between formation of amorphous aggregates and fibrillar species, a variety of intermediates, multiple conformational states, and a number of filamentous forms (Wang, 2005; Dima and Thirumalai, 2002; Bitan et al., 2001). Several models have been proposed to quantitatively describe this process (Come et al., 1993; Naiki and Nakakuki, 1996; Lomakin et al., 1996, 1997; Naiki and Gejyo, 1999; Serio et al., 2000; Kelly, 2000; Soto and Saborio, 2001; Pallitto and Murphy, 2001; Hall and Minton, 2004). The experimentally observed sigmoidal profile of fibrillogenesis kinetics is usually interpreted in terms of the two basic models—nucleation-dependent polymerization (NDP) (Shen et al., 1993) or diffusion-limited aggregation (DLA) (Tomski and Murphy, 1992). Kinetic data are in general consistent with the NDP model, in which protein assembly into fibrillar structures is treated as proceeding via two phases, the initial slow nucleation (or lag phase) and the subsequent fast elongation (growth). Nucleation phase involves monomer association into a critical oligomeric nucleus, being the highest energy state and thus the thermodynamically unfavorable species along the polymerization pathway. Once a nucleus has formed, its further elongation via the attachment of additional monomers becomes energetically favorable, thereby resulting in exponential fibril growth. Three essential features of the nucleation-dependent protein polymerization are worthy of mention, viz. (i) the existence of lag phase (or nucleus formation as a rate-limiting step), (ii) exponential decrease of lag time with increasing monomer concentration, and (iii) substantial

increase of the reaction rate upon the addition of pre-formed fibrils (seeding effect).

The DLA model was proposed to explain the kinetics of self-association of the A β peptide (Tomski and Murphy, 1992). It was hypothesized that peptide monomers spontaneously convert to octamers, which then stack into fibrils. Further fibril elongation was assumed to occur through diffusion-limited end-to-end association of the shorter fibrils. This model offers the advantage of allowing for the time-dependence of fibril length, yet it also postulates complete conversion of monomers to oligomers, which is not the case.

A detailed kinetic model developed by Lomakin et al. (1996) assumes a rapid and reversible equilibration between A β monomers and micelles, with the generation of nuclei from micelles and fibril growth by irreversible binding of monomers to their ends. This model permits quantitation of both fibril concentration and length as a function of time. To account for the AFM and EM data suggesting the existence of two types of A β linear aggregates (3–4 nm diameter filaments, also referred to as protofilaments or protofibrils, and 8–10 nm diameter fibrils), Pallitto and Murphy forwarded a kinetic model involving irreversible partitioning of the unfolded peptide between two pathways, the former producing stable monomers and dimers, and the latter generating an unstable assembly-competent intermediate (Pallitto and Murphy, 2001). Further steps include cooperative association of the intermediate states into a multimeric nucleus, elongation of the nucleus into filaments, aggregation of filaments into fibrils, and fibril elongation via end-to-end association. Importantly, this model not only takes into account the time-dependent changes in the mass concentration and length of fibrils, but also explicitly includes both filaments and fibrils as distinct aggregated forms.

Other mechanisms suggested to describe fibril formation by amyloidogenic polypeptides are referred to as template assembly (TA), monomer-directed conversion (MDC), and nucleated conformational conversion (NCC) (Kelly, 2000). These models consider two structurally distinct peptide states, a soluble (S) state and an assembly-competent (A) state. In the TA model, it is assumed that filaments and fibrils grow via the attachment of the S-state monomeric species to the A-state nucleus, with monomer structural transition into the A state as the rate-determining step (Cohen and Prusiner, 1998). The MDC model implies the formation of reversible AS dimers in promoting the rate-limiting conversion of the S-state monomers into the assembly-competent form (Kelly, 2000). Results from studies on amyloid fibril formation by the yeast prion protein Sup35 were interpreted in terms of the NCC

model that invokes oligomeric intermediates associating into a nucleus where rate-determining conformational conversion takes place (Serio et al., 2000).

To what extent the above models are appropriate for the description of membrane-mediated fibril formation remains to be elucidated. Despite the lack of detailed knowledge on the fibrillogenesis pathway(s) in membrane systems, the existing data comply with a nucleation-dependent process. More specifically, the kinetics of IAPP fibrillization in the presence of vesicles composed of zwitterionic (PC) and anionic (PG) phospholipids was demonstrated to exhibit a sigmoidal profile, with an initial lag phase of relatively slow fiber nucleation and a rapid elongation phase, during which the remainder of the soluble peptide was converted into fibers (Knight and Miranker, 2004). Another noteworthy finding of this study is that there exists surface charge density corresponding to 70 mol% PG, where the rate of fibrillogenesis is maximal, suggesting that the rate of fiber formation is limited by the self-assembly of peptide at the lipid–water interface. Further evidence in favor of nucleation-dependent fibrillization of α -synuclein induced by anionic phospholipids (PS, PA, PI), micelles of anionic detergents (alkyl sulfates containing 12–20 carbons), and fatty acids (arachidonic acid) was obtained by Necula et al. (2003). All the above amphiphiles share an anionic headgroup and an alkyl chain as principal structural features. It was assumed that the key role of alkyl chain is to support micellization, i.e. to ensure surface area sufficient for effective nucleation, while the anionic headgroups are required to promote the accumulation of the protein into the interfacial region, its conformational changes, and subsequent aggregation in the membrane (Necula et al., 2003; Chirita et al., 2003). However, for proteins adopting a polymerization-prone conformation while partially inserted into the lipid bilayer, the alkyl chain length dependent dimensions of the hydrophobic core may be of significance to fibril formation as well (Chirita et al., 2003). Although the above considerations are far from being comprehensive, they demonstrate the complexity and multiplicity of factors which may contribute to the nucleation of fibrils and the rate of their growth in membrane systems.

4. Membrane-related factors promoting protein fibrillization

4.1. Protein conformational changes

Accumulating evidence lends support to the hypothesis that structural transformation of a polypeptide chain into a partially folded conformation is a critical prereq-

uisite for fibril formation (Uversky and Fink, 2004). The native structures of fibril-forming proteins and peptides vary considerably and may be folded or unfolded, rich in β -sheet, α -helix, or contain both α -helical and β -sheet regions (Fink, 2005; Munishkina et al., 2004; Johansson, 2003; Srisailam et al., 2002). The tendency to aggregate into insoluble fibrillar structures has been found even for α -helical proteins such as cytochrome *c*₅₅₂ (Pertinhez et al., 2001), myoglobin (Fandrich et al., 2001), lung-surfactant protein C (Johansson, 2001). The conditions causing partial unfolding of natively folded proteins and partial folding of natively unfolded proteins and peptides render them amenable to polymerization into similar cross- β -structure that is postulated to be a universal energetic minimum for aggregated proteins (Dobson, 2001). In vitro fibrillization-favoring conditions are created by acidic pH, elevated temperatures, and adding organic solvents or denaturants (Zerovnik et al., 2002; Xiong et al., 2001; Hurshman et al., 2004; Ahmad et al., 2004). In vivo abnormal partial unfolding or folding may result from destabilization of the protein structure upon mutation, oxidation, heat stress, or changes in pH and the dielectric properties of the protein's local environment (Zerovnik, 2002; Trzesniewska et al., 2004; Gamblin et al., 2000). Evidently, protein–membrane interactions may play an important role in modulating conformational transitions of both membrane and soluble proteins, including amyloidogenic ones (Zhao et al., 2004, 2005). An increasing number of studies provides support to the idea that lipid bilayers can lower the activation energy barrier for protein unfolding. Partial unfolding in a membrane environment has been reported for cytochrome *c* (Muga et al., 1991; Pinheiro et al., 1997), phospholipase A₂ (Tatulian et al., 1997), bacterial toxins (Butko et al., 1997; Muga et al., 1993), acetylcholinesterase (Shin et al., 1997), pheromone-binding protein (Wojtasek and Leal, 1999), and recombinant human prion protein (Morillas et al., 1999). Conformational transitions are usually interpreted as originating from the lowered interfacial pH, being a consequence of proton accumulation in the vicinity of negatively charged membrane surface (Träuble, 1977). The higher charge state of the protein at lower pH may enhance side chain charge repulsion on the protein surface and thereby give rise to a more open structure with exposed, aggregation-prone hydrophobic patches. Neutralization of the protein surface charges by anionic lipid headgroups may be essential for nucleation (Chiti et al., 2003). However, the role of lipids as a structure-forming environment is not restricted to electrostatic phenomena and other bilayer characteristics such as the thickness of the hydrocarbon region determining the extent of hydrophobic mismatch

or lateral packing pressure may have an impact on the protein structure as well (Bowie, 2004). Partial unfolding is thought to be a critical step in the fibrillogenesis of globular proteins (Uversky and Fink, 2004). Under physiological conditions the structure of the latter involves a relatively tightly folded conformation, which is the dominating species in solution, and multiple partially unfolded conformations. In the membrane environment partially unfolded conformations become energetically favorable. Two such conformations, referred to as the molten globule and pre-molten globule, are currently considered to represent the most likely fibrillogenic intermediates (Uversky and Fink, 2004). The molten globule is featured by the lack of well-defined tertiary structure, high content of secondary structure, native-like topology and hydrodynamic radius close to that of the native protein, while pre-molten globule is a much less compact state with low content of secondary structure (Ptitsyn, 1995). Substantial evidence suggests that the fibrillogenic intermediates are structurally closer to the pre-molten globule state (Uversky and Fink, 2004). Self-association of this species is thought to be driven by hydrophobic interactions between non-polar side chains (Fink, 1998).

The so-called natively unfolded proteins and peptides exhibit a different conformational behaviour and partial folding was shown to be a key step in the fibrillization of natively unfolded α -synuclein (Uversky et al., 2001a), islet amyloid polypeptide (Kayed et al., 1999) and protomyosin α (Pavlov et al., 2002). The association of α -synuclein with anionic surfaces was followed by stabilization of helical secondary structure (Davidson et al., 1998; Eliezer et al., 2001), thus suggesting that in this case fibrillization proceeds after a transition of a partially folded intermediate from α -helix to β -sheet (Fezoui et al., 2000). It was shown that the A β peptide undergoes a conformational transition from a random coil to a β -sheet upon binding to ganglioside-containing membranes (Matsuzaki and Horikiri, 1999; Yanagisawa et al., 1995). Importantly, zwitterionic, anionic phospholipids and the oligosaccharide moiety of the ganglioside were unable to induce similar transitions (Choo-Smith and Surewicz, 1997). The mechanism of ganglioside-mediated A β fibrillization was proposed to involve the self-assembly of a glycolipid-bound peptide on the membrane surface into β -sheet-rich microaggregates, which then act as nuclei recruiting more peptide molecules from the solution and thus giving rise to fibril formation by the β -sheet augmentation mechanism. Ganglioside-rich domains in neural membranes may serve in vivo as templates promoting the self-association of A β peptides and the formation of mature amyloid plaque (Yanagisawa et

al., 1995). Another example is provided by the prion protein PrP^C, whose post-translational refolding into a β -sheet-rich abnormal conformer PrP^{Sc} and subsequent polymerization were found to depend on the proportion of sphingomyelin and cholesterol and the content of ganglioside GM1 in membrane (Naslavsky et al., 1999). The above studies reveal a key role for membranes in the induction of fibrillogenesis by a stabilization of partially folded and highly flexible aggregation-prone protein conformations.

4.2. Protein accumulation at lipid–water interface

Another membrane-related phenomenon that is thought to be essential for the modulation of fibrillization rate involves an increase in the local concentration of the protein in the membrane. Accordingly, protein adsorption to oppositely charged surfaces may result in an increase in its local concentration by up to two orders of magnitude. Anionic surfaces have been shown to promote protein aggregation and fibrillization at low protein concentrations in bulk solution (Zhu et al., 2002) and it has been proposed that the high local concentration of protein on the anionic surface overcomes the energy barrier for nucleation (King et al., 1999; Wilson and Binder, 1997). Similar mechanisms have been suggested for anionic micelles (Chirita et al., 2003) and other polyanions, such as nucleic acids, polyglutamate, and microtubules (Goedert et al., 1996; Friedhoff et al., 1998; Hasegawa et al., 1997; Ackmann et al., 2000; Goers et al., 2003). Likewise, increase in the protein's local concentration may arise from its preferential association to lipid domains of certain composition, as suggested for the specific association of the prion protein with cholesterol and sphingomyelin containing membranes promoting aggregation and polymerization by local accumulation of PrP^{Sc} (Naslavsky et al., 1999). However, one should bear in mind that in the case of diffusion-controlled reactions such as protein aggregation and fibril elongation, slower diffusion of the aggregated species in a membrane environment may result in decreased rates of fibril elongation and nucleation.

4.3. Orienting effect of membranes

The assembly processes occurring in a membrane environment can be promoted by particular disposition of the aggregating species relative to a lipid–water interface. Non-uniform charge distribution and dipole moments of polypeptide chain are thought to account for specific orientation of proteins or peptides when associated with charged membranes (van Klompenburg et al.,

1997). One example pointing to the importance of orienting effect for fiber formation process is provided by the recent study on the fibrillization of IAPP induced by DOPC/DOPG membranes (Knight and Miranker, 2004). It was suggested that upon binding to the negatively charged bilayer IAPP transforms into a non-fibrillar intermediate state, in which the charge of the cationic N-terminus is neutralized by the anionic lipid headgroup, thus promoting peptide clustering at high protein:lipid ratios, when the peptide adopts orientation normal to the membrane surface. The authors concluded that the free energy contribution of the lipid bilayer to the transition state of fiber formation is determined by increasing the local concentration and the concomitant alignment of the peptide molecules on the surface.

4.4. Modulation of the protein nucleation propensity

The role of membranes in fibrillogenesis may involve also variations in the proportion of bilayer-buried and exposed regions of the protein molecule. Along these lines changes in the depth of bilayer penetration may be essential for the ability a protein to act as a nucleus in fibril formation. A number of amyloid-forming proteins including α -synuclein, A β peptide, prion protein, and apolipoprotein C-III were found to insert into lipid bilayers (Pillot et al., 1996, 1997; Volles et al., 2001; Lins et al., 2002; Ege and Lee, 2004) and this penetration was suggested to contribute to lipid-mediated aggregation and fibrillization of these proteins (Lee et al., 2002; Choo-Smith et al., 1997; Perrin et al., 2001; Cole et al., 2002; Uversky et al., 2001b). SDS/PAGE revealed that wild type α -synuclein bound to synaptosomal membranes can seed the aggregation of cytosolic α -synuclein (Lee et al., 2002). These data were interpreted as indicating that a protein partially inserted into a lipid bilayer may act as an anchor for site-directed fibril assembly. Using environmentally sensitive probes it was demonstrated that wt α -synuclein exhibits predominant binding to the lipid headgroups, while the non-fibrillogenic mutant A53T inserts into the membrane interior to a greater extent so that the hydrophobic parts are buried within the bilayer. This masking of the hydrophobic, β -sheet promoting region was proposed to inhibit the self-assembly of the mutant protein into fibrils, while the superficial location of wt α -synuclein in membranes appears to favor seeding of fibril formation. The authors hypothesized that equilibrium between membrane-bound and cytosolic α -synuclein may be crucial for the physiological function of α -synuclein because any significant shift in the equilibrium due to missense mutations or changes in membrane fluidity

may lead to abnormal protein aggregation and Lewy body formation. This example emphasizes the significance of parameters such as membrane free volume in regulating the extent of bilayer penetration of the protein, and, as a consequence, in modulating protein self-association and fibril-formation. Another example is provided by the effect of cholesterol on the fibrillogenesis of A β _{1–40} with the insertion of this peptide into bilayers made of brain lipids being inversely correlated with the cholesterol content and subsequent surface fibrillization (Yip et al., 2001).

Altering the composition or chemical modification of membrane lipids (for instance, due to oxidative damage) could bring about the changes in protein structure and bilayer location that could promote pathological aggregation and fibrillization.

4.5. Membrane as a template for protein aggregation

The mechanisms of surface-induced fibrillization may be fundamentally different from that occurring in bulk solution because of the restrictions imposed by the dimensionality of the system and the physicochemical and dynamic properties of the surface (Zhu et al., 2002, 2004; Renault et al., 1999). This was demonstrated for the lipid-induced polymerization of actin (Renault et al., 1999). More specifically, it was found that chemically identical lipid surfaces behave differently when present as monolayers or liposomal bilayers. Accordingly, linear actin polymers were formed on the surface of PC/stearylamine liposomes, while only dotlike structures with many branching point defects were induced by monolayers of these lipids. The different behavior of bilayer and monolayer was explained by the differences in dynamic features of these systems. To this end, because of surface tension thermal fluctuations on the scale of filament length are orders of magnitude smaller in amplitude for a monolayer than for a bilayer. Another illustrative example is provided by the fibrillization of recombinant immunoglobulin light chain variable domain on the surface of native mica (Zhu et al., 2002). In addition to a significant acceleration of fibril formation on the surface, two novel types of fibril growth on the surface were found, a bidirectional linear assembly of specific oligomers into protofibrils, which has not been observed in solution, and a linear growth from preformed amorphous protein aggregates lacking long-range order. It was hypothesized that amorphous aggregates may act as nuclei for fibril formation. This view is corroborated by a number of studies in which fibrils were observed to grow from amorphous aggregates (Kazlauskaitė et al.,

2003; Raffin et al., 1999; Lundberg et al., 1997; Blackley et al., 2000). In particular, kinetic analyses of amyloid formation by the prion peptide revealed three kinetically relevant species: monomer, amorphous aggregate, and amyloid (Lundberg et al., 1997). A dual role of the amorphous aggregates has been suggested, implicating release of monomers into solution and providing initiation sites for fibril growth. Large amorphous aggregates concentrated around POPG vesicles have been observed for the β -sheet-rich isoform of the prion protein (Kazlauskaitė et al., 2003). Fibril formation by the linear association of oligomeric species on a mica surface and radial growth of protofibrils from a common core have been reported for A β 40 (Blackley et al., 2000). Thus, a surface may not only concentrate a protein in amorphous deposits serving as reservoir for fibril formation but also provides specific mechanisms of fibrillization, as exemplified above by the bidirectional linear elongation of fibrils through attachment of oligomeric building blocks. Obviously, chemical nature and structural state of membrane constituents along with collective properties of lipid bilayer may play essential role in determining the pathways and kinetics of membrane-enhanced fibrillogenesis.

5. Concluding remarks

Although the mechanisms underlying protein fibrillization have been extensively investigated during the past decade we are only beginning to understand the phenomenon of membrane-mediated fibrillogenesis on the molecular level. The purpose of this brief review is to emphasize general principles governing protein aggregating and amyloidogenic behavior in membranes. While our survey is far from being exhaustive of all reports offering insight into the fibrillogenesis modulating roles of lipids, the work covered provides very strong support to the view that lipid–protein interactions may be involved in vivo in initiating and modulating amyloid fibril formation. In this process, lipids seem to act as anti-chaperones facilitating protein transition into partially folded states and their subsequent assembly into protofibrillar and fibrillar structures. The changes in composition and physicochemical properties of lipid bilayer associated with aging or other physiological or pathological processes may increase membrane binding of specific proteins thereby triggering amyloidogenesis in vivo. In this way, the proteins could be targeted for degradation or adverse amyloid deposition. In this context, it is tempting to speculate that coupled evolution of protein sequences and their membrane environment involved negative selection against sequence changes

which increased the tendency of polypeptide chains to aggregate in a manner producing cytotoxic protofibrils.

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