Formation of Amyloid Fibers Triggered by Phosphatidylserine-Containing Membranes†

Hongxia Zhao,‡ Esa K. J. Tuominen,‡ and Paavo K. J. Kinnunen*,‡,§

Helsinki Biophysics and Biomembrane Group, Institute of Biomedicine, P.O. Box 63 (Haartmaninkatu 8), University of Helsinki, FIN-00014 Helsinki, Finland, and Memphys-Center for Biomembrane Physics, Physics Department, University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark

Received May 18, 2004; Revised Manuscript Received June 30, 2004

ABSTRACT: Protein misfolding has been shown to be the direct cause of a number of highly devastating diseases such as Alzheimer's disease, Parkinson's disease, and Creutzfeldt-Jacob syndrome, affecting the aging population globally. The deposition in tissues of amyloid fibrils is a characteristic of all these diseases, and the mechanisms by which these protein aggregates form continue to be intensively investigated. In only a fraction of cases is an underlying mutation responsible, and accordingly, what initiates amyloid formation *in vivo* is the major question that is addressed. In this study, we show that membranes containing phosphatidylserine (PS), a negatively charged phospholipid, induce a rapid formation of fibers by a variety of proteins, viz., lysozyme, insulin, glyceraldehyde-3-phosphate dehydrogenase, myoglobin, transthyretin, cytochrome *c*, histone H1, and α-lactalbumin. Congo red staining of these fibers yields the characteristic light green birefringence of amyloid, and fluorescent lipid tracers further reveal them to include phospholipids. Our results suggest that PS as well as other acidic phospholipids could provide the physiological low-pH environment on cellular membranes, enhancing protein fibril formation *in vivo*. Interestingly, all the proteins mentioned above either are cytotoxic or induce apoptosis. PS−protein interaction could be involved in the mechanism of cytotoxicity of the aggregated protein fibrils, perturbing membrane functions. Importantly, our results suggest that this process induced by acidic phospholipids may provide an unprecedented and generic connection between three current major areas of research: (i) mechanism(s) triggering amyloid formation, (ii) cytotoxicity of amyloidal protein aggregates, and (iii) mechanism(s) of action of cytotoxic proteins.

A number of fatal human diseases, including Alzheimer's systemic amyloidosis, Parkinson's disease, and prion disease, are associated with the deposition of aggregated protein fibrils in organs (*1*). The characteristics of the soluble forms of the proteins involved in amyloidoses are varied, ranging from intact globular proteins to largely unstructured peptides, yet the aggregated forms have many properties in common (*2*), the hallmark being birefringence under a polarizing microscope after Congo red staining. Several proteins have recently been shown to form aggregates that are analogous to the disease-related cytotoxic amyloid deposits, thus suggesting that fibril formation could be a generic property of proper polypeptide chains (*3*, *4*). Likewise, aggregates of proteins that are not connected with any known diseases could be cytotoxic (*5*). However, amyloid formation is not restricted to the proteins associated with diseases, and prion-like aggregates have been demonstrated to convey epigenetic non-Mendelian heritance in yeast (*6*). More recently, Kandel, Lindquist, and co-workers hypothesized that the conversion of a cytoplasmic polyadenylation element binding protein (CPEB)[1] into a self-perpetuating prion-like state would maintain structural changes in synapses, forming the molecular basis of memory (*6*).

The physicochemical basis of amyloid formation remains poorly understood (*1*−*5*). There seems to be a recent consensus about the fact that to aggregate the protein must be partially unfolded (*7*). This can be consistently enhanced in a slightly hydrophobic environment at acidic pH (*8*), and both hydrophobicity and the net charge of the protein seem to be crucial, neutralization of the charges triggering aggregation (*9*). Importantly, the physiological relevance of the low-dielectricity, acidic milieu in accelerating the formation of amyloid fibrils *in vitro* has been questioned and may as such only be effective *in vivo* when the proteins reside in the lysosomes. In this *in vitro* study, we address this question by investigating the potential of acidic phospholipid-containing lipid membranes in providing an environment required for the rapid initiation of amyloid fiber formation.

† This study was supported by the Finnish Academy and Juselius Foundation. Memphys is supported by the Danish National Research Council.

* To whom correspondence should be addressed: Helsinki Biophysics and Biomembrane Group, Institute of Biomedicine, P.O. Box 63 (Haartmaninkatu 8), University of Helsinki, FIN-00014 Helsinki, Finland. Telephone: +358-9-19125400. Fax: +358-9-19125444. E-mail: Paavo.Kinnunen@Helsinki.Fi.

‡ University of Helsinki.

§ University of Southern Denmark.

[1] Abbreviations: brainPS, brain phosphatidylserine; CPEB, cytoplasmic polyadenylation element binding protein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; LUVs, large unilamellar vesicles; NBD-PG, 1-palmitoyl-2-(*N*-4-nitrobenz-2-oxa-1,3-diazol)aminocaproyl-*sn*-glycero-3-phosphoglycerol; POPG, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-*rac*-glycerol; PS, phosphatidylserine; SOPC, 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphocholine.

10.1021/bi049002c CCC: $27.50
Published on Web 07/20/2004

EXPERIMENTAL PROCEDURES

Materials. Brain phosphatidylserine (brainPS), 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (SOPC), 1-palmitoyl-2-(*N*-4-nitrobenz-2-oxa-1,3-diazol)aminocaproyl-*sn*-glycero-3-phosphoglycerol (NBD-PG), and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-*rac*-glycerol (POPG) were from Avanti Polar Lipids (Alabaster, AL). The purity of the these lipids was checked by thin-layer chromatography on silicic acid-coated plates (Merck, Darmstadt, Germany) developed with a chloroform/methanol/water mixture (65:25:4, v/v/v). Examination of the plates after iodine staining and, when appropriate, upon UV illumination revealed no impurities. The concentration of NBD-PG was determined spectrophotometrically in ethanol using a molar absorptivity ϵ_{465} of 21 000. Concentrations of the nonfluorescent lipids were determined gravimetrically with a high-precision electrobalance (Cahn, Cerritos, CA). Cytochrome *c*, lysozyme, α-lactalbumin, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), insulin, myoglobin, transthyretin, Congo red, and thioflavin T were from Sigma. Histone H1 was purified from calf thymus essentially as described previously (*10*). Other chemicals were analytical grade and from standard sources.

Preparation of Large Unilamellar Vesicles. Appropriate amounts of the lipid stock solutions were mixed in chloroform to obtain the desired compositions. The solvent was removed under a stream of nitrogen, and the lipid residue was subsequently maintained under reduced pressure for at least 2 h. The dry lipids were then hydrated at room temperature for 1 h in 20 mM Hepes and 0.1 mM EDTA (pH 7.4). The resulting dispersions were extruded through a single polycarbonate filter (pore size of 100 nm, Millipore, Bedford, MA) using a Liposofast low-pressure homogenizer (Avestin, Ottawa, ON) to produce large unilamellar vesicles (LUVs), with an average diameter between 111 and 117 nm (*11*).

Liposome-Induced Fiber Formation. The indicated proteins [dissolved in 20 mM Hepes and 0.1 mM EDTA (pH 7.4)] were added to SOPC/brainPS (8:2 molar ratio) liposomes in the same buffer at room temperature, to yield final concentrations of 100 μM phospholipids and 0.1 mg/mL protein. These concentrations correspond to protein/phospholipid stoichiometries varying between 6.9:100 (for the largest protein, GAPDH) and 17:100 (for the smallest protein, insulin). Fibers became visible in less than 1 min by phase contrast microscopy (Olympus IX 70, Olympus Optical Co., Tokyo, Japan).

Where indicated, the fibers were incubated for 30 min with 10 μM Congo red [in 20 mM Hepes and 0.1 mM EDTA (pH 7.4)], and their resulting birefringence was observed with the microscope described above, using crossed polarizers in the excitation and emission paths. In the thioflavin T assay, the concentrations of thioflavin T, lipid, and protein in the assay were 25 μM, 100 μM, and 0.1 mg/mL, respectively. All experiments were carried out at room temperature (approximately 24 °C).

Confocal Fluorescence Microscopy. Where stated, trace amounts ($X = 0.02$) of the fluorescent phospholipid analogue NBD-PG were incorporated into liposomes and fibrils were formed as described above. The inverted microscope described above was used with a confocal scanner (Yokogawa, Tokyo, Japan) and a krypton ion laser (Melles Griot, Carlsbad, CA) as a light source, with excitation at 488 nm and a long pass (>510 nm) emission filter set to the appropriate wavelength for monitoring NBD fluorescence. Images were acquired with a B/W CCD camera (C4742-95-12 NRB, Hamamatsu Photonics K.K., Hamamatsu, Japan) interfaced with a computer, and operated by the software (AquaCosmos) provided by the camera manufacturer.

RESULTS AND DISCUSSION

Conditions accelerating rapid amyloid formation *in vitro* require an acidic milieu as well as slightly reduced dielectricity (*3*). However, the biological relevance of these conditions has been questioned. Formation of red fibrous aggregates was originally seen in the course of our studies of the interactions between cytochrome *c* and liposomes (E. K. J. Tuominen and P. K. J. Kinnunen, unpublished observations). These fibers could be formed under a variety of conditions, yet consistently required the presence of an acidic phospholipid, e.g., phosphatidylglycerol, cardiolipin, or phosphatidylserine. In this study, we have extended this observation to a number of proteins, which except for transthyretin have been previously shown to bind to membranes containing acidic phospholipids. Our data thus suggest that membranes containing phosphatidylserine, an acidic phospholipid, may create a surface with the required high local concentration of protons (*12*). More specifically, we show that liposomes composed of phosphatidylserine and phosphatidylcholine (brainPS/SOPC, 8:2 molar ratio) induce the rapid formation of fibrous aggregates by lysozyme, insulin, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), myoglobin, transthyretin, cytochrome *c*, histone H1, and α-lactalbumin, visible by phase contrast microscopy (Figure 1). Similar fibers were formed in the presence of POPG/SOPC (2:8 molar ratio) liposomes (data not shown). Instead, no fibers were seen in the presence of liposomes composed of phosphatidylcholine only (data not shown). Once these structures had been stained with Congo red, the light green birefringence suggested to be characteristic to amyloid was evident (Figure 2). These fibers were also stained with thioflavin T (data not shown).

The proteins mentioned above vary in their size, structure, localization, and function in cells, and some of them are known to form amyloid *in vivo* (Table 1). Common to all these proteins is the presence of cationic residues or cationic amino acid clusters, and except for transthyretin, they have been previously demonstrated to bind to membranes containing acidic phospholipids, with subsequent fusion. The latter could reflect protein aggregation on the surface of liposomes, causing the close opposition of vesicles required for fusion. The binding to acidic phospholipids containing vesicles neutralizes the cationic charges in these proteins. Accordingly, protein−protein interactions in these aggregates would not be counteracted by repulsion due to cationic residues, and protein polymerization can be expected to be facilitated. The content of PS in the liposomes required for fiber formation varied among the different proteins. For histone H1 and lysozyme, an X_{PS} of 0.03 was sufficient. An X_{PS} of 0.05 was needed for insulin, GAPDH, myoglobin, cytochrome *c*, and α-lactalbumin. An X_{PS} of 0.10 was needed for transthyretin. These differences are likely to reflect both the positive surface charge density and the number of cationic amino acid residues present in clusters in these proteins.

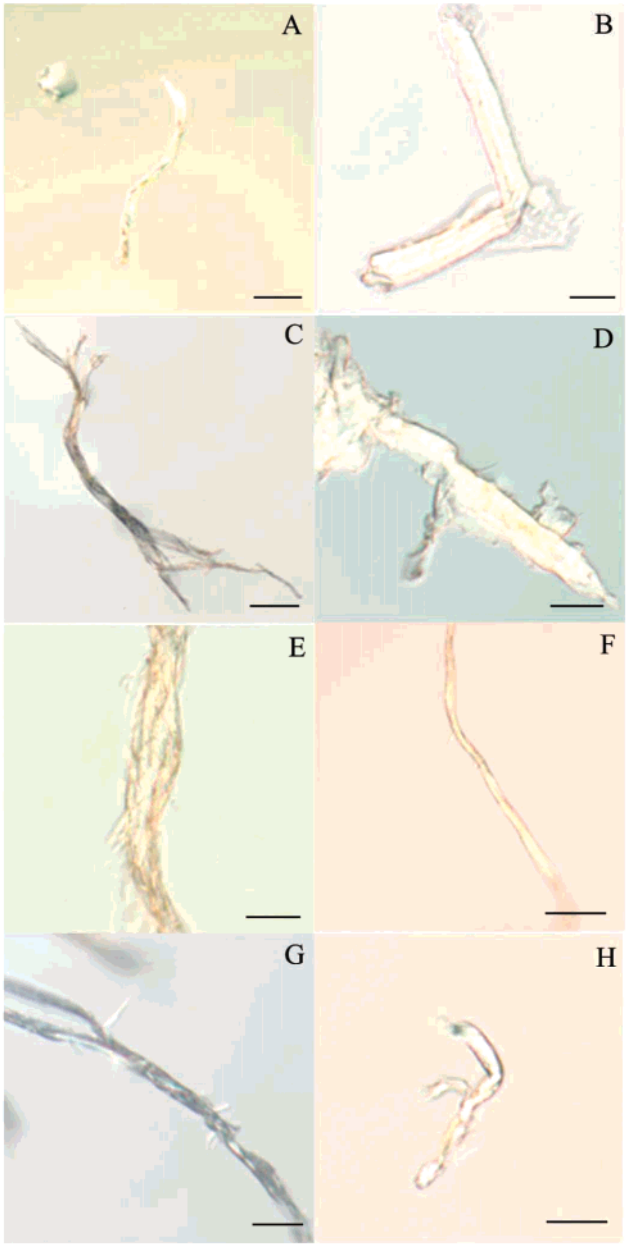

FIGURE 1: Phase contrast microscopy images of the fibers formed after mixing SOPC/brainPS (8:2 molar ratio) liposomes with (A) lysozyme, (B) insulin, (C) GAPDH, (D) myoglobin, (E) transthyretin, (F) cytochrome *c*, (G) histone H1, and (H) α-lactalbumin. The final concentrations of phospholipid and proteins were 100 μM and 0.1 mg/mL, respectively, in 20 mM Hepes and 0.1 mM EDTA (pH 7.4). All experiments were carried out at room temperature (approximately 24 °C). Magnifications were 10× (A, C, and F−H) and 20× (B, D, and E). The scale bars are 5 μm (A−C, G, and H), 10 μm (D and E) in length.

The fibrils formed under these conditions became fluorescent when trace amounts of fluorescent phospholipid analogues were included in the liposomes (Figure 3), thus indicating that phospholipids were present, yet because of the technical challenges involved, the chemical composition and protein:lipid stoichiometries were not determined at this stage. Staining of the fibers by fluorescent phospholipids in a rigorous sense only shows the incorporation of these probes into the fibers. However, in light of the well-documented lipid binding properties of these proteins (except transthyretin), it is likely that the fibers seen by microscopy represent

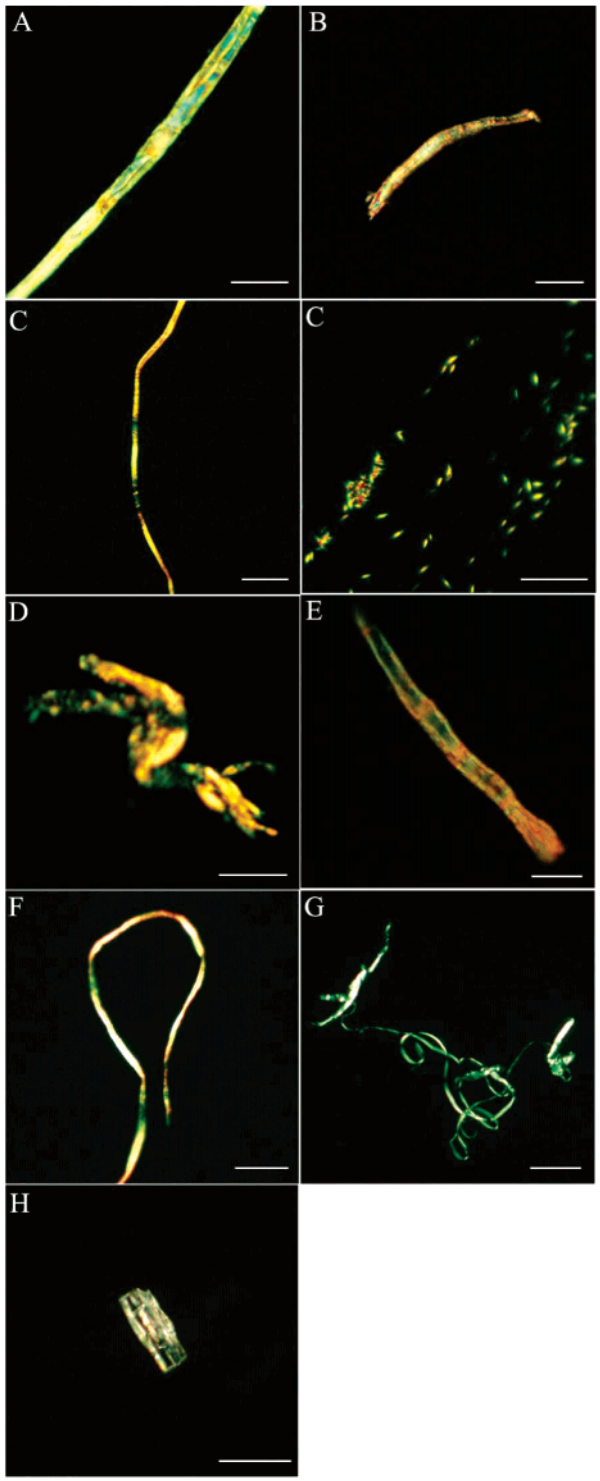

FIGURE 2: Birefringence of protein fibrils induced by phosphatidylserine-containing liposomes, after staining with Congo red (final concentration of 10 μM). The fibrils were formed essentially as described in the legend of Figure 1. Images are for (A) lysozyme, (B) insulin, (C) GAPDH, (D) myoglobin, (E) transthyretin, (F) cytochrome *c*, (G) histone H1, and (H) α-lactalbumin. Magnifications were 20× (A, B, D, E, and H) and 10× (C, F, and G). The scale bars are 5 (A, F, and G), 10 (B, C, and H), and 20 μm (D) in length.

supramolecular lipid−protein structures. Although detailed data are only available for cytochrome *c* and histone H1, we have never obtained any evidence of acidic phospholipids being pulled out from membranes by these proteins (*13*−*17*).

Table 1: Characteristics of the Investigated Proteins

protein	location in cells	binding to acidic phospholipids and induction of liposome fusion	cytotoxicity and/or involvement in apoptosis
lysozyme	extracellular compartment, macrophages	+ (*39*)	+ (*40*)
insulin	extracellular compartment	+ (*41*)	+ (*42*)
GAPDH	cytosol	+ (*43*)	+ (*26*)
myoglobin	extracellular compartment	+ (*44*)	+? (*45*)
transthyretin	extracellular compartment	?	+ (*24*)
cytochrome *c*	mitochondria	+ (*14*)	+ (*46*)
histone H1	nucleus	+ (*34*)	+ (*35*, *47*)
α-lactalbumin	extracellular compartment	+ (*48*)	+ (*49*)

Under the conditions that were used, the liposome-induced fiber formation by these proteins was very rapid, with macroscopic structures becoming visible almost instantaneously after the addition of the indicated proteins to a solution of PS-containing liposomes. Kinetics of fiber formation were not assessed at this stage. We also observed smaller fibrils such as those shown for GAPDH, eventually evolving into more macroscopic, longer fibers (right panel of Figure 2C and Figure 3C). Although there could be trends in the length of the fibers formed by the different proteins, our data at this stage are too limited to claim anything in this regard.

The aggregates formed in the presence of PS-containing liposomes by the proteins that have been investigated appear to be analogous to disease-related amyloid fibrils, suggesting that electrostatic interaction between cationic regions in these proteins with negatively charged lipid membranes could be involved in amyloid formation also *in vivo*, as has been suggested for Aβ peptide, α-synuclein, and prions (*18*−*22*). Enhanced fiber formation has been verified to take place also on negatively charged mica, and it has been suggested that this role of surfaces could be universal for all amyloid-forming proteins (*23*). To this end, tacrine which was involved in the treatment of Alzheimer's disease binds to membranes containing acidic phospholipids (*24*), and it was proposed that this drug could attenuate Aβ−membrane interaction and, consequently, inhibit aggregation and fibril formation by this peptide. Amyloid isolated from patients affected by deposition diseases has a high lipid content (*19*, *25*). In light of these results, it is possible that PS represents the physiological ligand for the anion binding site of transthyretin (*26*−*28*).

There is evidence showing the enhancement by membranes of protein conversion into toxic aggregates (*9*, *25*), as well as demonstrating increased levels of PS in the neural membranes of patients with Alzheimer's disease (*29*). This finding that PS-containing membranes induce the formation of amyloid fibers by a wide range of soluble proteins, independent of their location in extracellular or intracellular compartments, suggests that we are describing a generic property of proteins associating electrostatically to membranes containing acidic phospholipids. Intriguingly, all these proteins have been shown to be cytotoxic or to be involved in the induction of apoptosis (Table 1). Fibrous aggregates within cells have been observed for GAPDH (*30*) and transthyretin (*27*). The relation between fibril formation and cytotoxicity is intriguing yet remains poorly understood (*5*). There seems to be a consensus based on experimental data that "mature" amyloid fibrils are not toxic and that cytotoxicity is due to aggregated fibrillar proteins (*3*, *27*). This view is further supported by the mechanism of action of lytic bacterial toxins, involving their binding to lipid membranes and subsequent aggregation into a pore, permeabilizing the bilayer, as shown for cytolysin (*31*). A somewhat analogous mechanism of membrane permeabilization has been proposed for α-synuclein protofibrils (*32*).

In light of the above, it seems plausible that PS-triggered protein aggregation and fiber formation could be involved in protein misfolding diseases and physiological formation of fibrous proteins, as well as cytotoxicity of these proteins, involving the induction of apoptosis. More specifically, in the presence of such potentially toxic proteins, an altered membrane composition or membrane lipid distribution, such as the exposure of PS on the extracellular plasma membrane leaflet, could result in the formation of toxic aggregates within the membrane and eventually lead to the development of amyloid deposits. To this end, while PS is believed to be exposed in the outer surface of the plasma membrane of apoptotic cells only, increased contents of this phospholipid are found also in the extracellular plasma membrane leaflet of cancer cells and vascular endothelial cells in tumors (*33*, *34*). Studies on the Na^+ channel in axons show PS to be present in the outer surface of neurons and to be required for nerve conduction (*35*). Unfortunately, we could not find quantitative studies addressing the amount and lateral distribution of PS in the outer surface of the plasma membrane. From our studies on Jurkat cells, a human leukemic cell line, we could conclude that the binding of cationic liposomes to the outer surface of these cells involves PS which in some cells appeared to be enriched into microdomains (*36*). We anticipate that fiber formation by the proteins that have been investigated is not specific for PS and PG but is induced by other acidic phospholipids as well. When the wide variety of lipid environments encountered in biomembranes is taken into account, it is obvious that extensive series of further studies are warranted to elucidate the impact of different lipids (e.g., glycosphingolipids, phosphatidylinositol, and cholesterol) on fiber formation by these as well as other relevant proteins. Phospholipids bearing a net negative charge are abundant in various intracellular membranes and would thus be readily available for electrostatic interaction with surface aggregation prone proteins inside the cell. Along these lines, translocation of acidic phospholipid binding proteins from their normal organelle sites could trigger apoptosis, as recently shown for histone H1 (*37*).

Lipid binding properties have been characterized in considerable detail for cytochrome *c* (*13*−*16*) and to a somewhat lesser extent for histone H1 (*13*, *15*, *17*, *38*), including kinetics of membrane association and the impact of surface charge density. For the other proteins of this study, less systematic information is available. Both cytochrome *c* and H1 have been shown to bind rapidly (within mil-

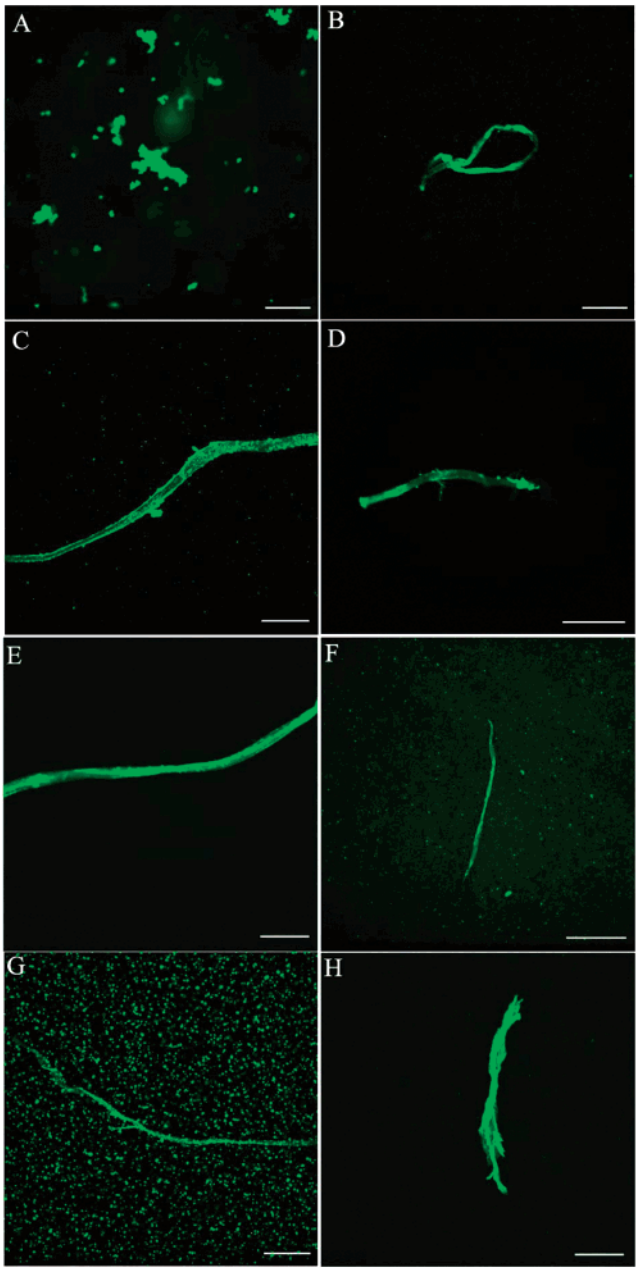

FIGURE 3: Incorporation of the fluorescent phospholipid analogue NBD-PG into the protein fibers. The latter were formed as described in the legend of Figure 1, except that a trace amount ($X = 0.02$) of NBD-PG was additionally included in the liposomes: (A) lysozyme, (B) insulin, (C) GAPDH, (D) myoglobin, (E) transthyretin, (F) cytochrome *c*, (G) histone H1, and (H) α-lactalbumin. Magnifications were 20× (A, B, D, and H) and 10× (C and E−G). The scale bars are 20 (A, D, and F), 10 (B, C, and E), 5 (G), and 9 μm (H) in length.

liseconds) to membranes containing acidic phospholipids (*15*). Notably, the affinity of H1 for acidic phospholipids far exceeds that of cytochrome *c*, the latter protein being quantitatively displaced by H1 from liposomes (*13*). It was further estimated that each H1 binds approximately 20 molecules of acidic phospholipids (*13*). Fluorescence microscopy studies revealed H1 binds to giant PC/PS (9:1 molar ratio) liposomes, with subsequent aggregation of H1 on their surface. Similar behavior of H1 was seen upon its association with human leukemic T cells (*38*). It seems feasible that acidic phospholipid-induced protein fibril formation could involve as an initial step in binding of the protein to the membrane with concomitant conformational changes and subsequent perpetuating self-aggregation in the membrane surface, with further conformational alterations. Surface-associated protein microaggregates could then act as seeds recruiting more protein from solution, resulting in fibril growth. As recently shown for the binding of H1 to cultured human leukemic T cells (*38*), the membrane-associated and -aggregated proteins are likely to cause major rearrangements of membrane components, thus initiating a cascade of disorganization of cellular structures and collapse of the barrier functions of the attacked lipid bilayers, leading to cell death.

ACKNOWLEDGMENT

We thank Ilkka Tuunainen for advice on the polarized microscope and Kristiina Söderholm and Kaija Niva for technical assistance.

REFERENCES

1. Aguzzi, A., and Haass, C. (2003) Games played by rogue proteins in prion disorders and Alzheimer's disease, *Science 302*, 814−818.
2. Kayed, R., Head, E., Thompson, J. L., McIntire, T. M., Milton, S. C., Cotman, C. W., and Glabe, C. G. (2003) Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis, *Science 300*, 486−489.
3. Rochet, J. C., and Lansbury, P. T., Jr (2000) Amyloid fibrillogenesis: themes and variations, *Curr. Opin. Struct. Biol. 10*, 60−68.
4. Stefani, M., and Dobson, C. M. (2003) Protein aggregation and aggregate toxicity: new insight into protein folding, misfolding disease and biological evolution, *J. Mol. Med. 81*, 678−699.
5. Bucciantini, M., Giannoni, E., Chiti, F., Baroni, F., Formigli, L., Zurdo, J., Taddei, N., Ramponi, G., Dobson, C. M., and Stefani, M. (2002) Inherent toxicity of aggregates implies a common mechanism for protein misfolding diseases, *Nature 416*, 507−511.
6. Si, K., Lindquist, S., and Kandel, E. R. (2003) A neuronal isoform of the Aplysia CPEB has prion-like properties, *Cell 115*, 879−891.
7. Kinnunen, P. K. J., Koiv, A., Lehtonen, J. Y., Rytömaa, M., and Mustonen, P. (1994) Lipid dynamics and peripheral interactions of proteins with membrane surfaces, *Chem. Phys. Lipids 73*, 181−207.
8. Whittingham, J. L., Scott, D. J., Chance, K., Wilson, A., Finch, J., Brange, J., and Guy Dodson, G. (2002) Insulin at pH 2: structural analysis of the conditions promoting insulin fibre formation, *J. Mol. Biol. 318*, 479−490.
9. Bokvist, M., Lindstrom, F., Watts, A., and Grobner, G. (2004) Two types of Alzheimer's β-amyloid (1−40) peptide membrane interactions: aggregation preventing transmembrane anchoring *versus* accelerated surface fibril formation, *J. Mol. Biol. 335*, 1039−1049.
10. Cole, R. D. (1989) Purification and analysis of H1 histones, *Methods Enzymol. 170*, 524−532.
11. Wiedmer, S. K., Hautala, J., Holopainen, J. M., Kinnunen, P. K. J., and Riekkola, M.-L. (2001) Study on liposomes by capillary electrophoresis, *Electrophoresis 22*, 1305−1313.
12. Kraayenhof, R., Sterk, G. J., and Wong Fong Sang, H. W. (1993) Probing biomembrane interfacial and pH profiles with a new type of float-like fluorophores positioned at varying distance from the membrane surface, *Biochemistry 32*, 10057−10066.
13. Rytömaa, M., and Kinnunen, P. K. J. (1996) Dissociation of cytochrome *c* from liposomes by histone H1. Comparison with basic peptides, *Biochemistry 35*, 4529−4539.
14. Tuominen, E. K., Zhu, K., Wallace, C. J., Clark-Lewis, I., Craig, D. B., Rytömaa, M., and Kinnunen, P. K. J. (2001) ATP induces a conformational change in lipid-bound cytochrome *c*, *J. Biol. Chem. 276*, 19356−19362.

15. Subramanian, M., Jutila, A., and Kinnunen, P. K. J. (1998) Binding and dissociation of cytochrome *c* to and from membranes containing acidic phospholipids, *Biochemistry 37*, 1394−1402.
16. Rytömaa, M., and Kinnunen, P. K. J. (1994) Evidence for two distinct acidic phospholipid-binding sites in cytochrome *c*, *J. Biol. Chem. 269*, 1770−1774.
17. Koiv, A., Palvimo, J., and Kinnunen, P. K. J. (1995) Evidence for ternary complex formation by histone H1, DNA, and liposomes, *Biochemistry 34*, 8018−8027.
18. Terzi, E., Hoelzemann, G., and Seelig, J. (1994) Alzheimer β-amyloid peptide 25−35: electrostatic interactions with phospholipid membranes, *Biochemistry 33*, 7434−7441.
19. Kim, I.-C., Shirahama, T., and Cohen, A. S. (1967) Lipid content of amyloid fibrils purified by a variety of methods, *Am. J. Pathol. 50*, 869−886.
20. Morillas, M., Swietnicki, W., Gambetti, P., and Surewicz, W. K. (1999) Membrane environment alters the conformational structure of the recombinant human prion protein, *J. Biol. Chem. 274*, 36859−36865.
21. McLaurin, J., Franklin, T., Chakrabartty, A., and Fraser, P. E. (1998) Phosphatidylinositol and inositol involvement in Alzheimer amyloid-β fibril growth and arrest, *J. Mol. Biol. 278*, 183−194.
22. Volles, M. J., Lee, S.-J., Rochet, J.-C., Shtilerman, M. D., Ding, T. T., Kessler, J. C., and Lansbury, P. T., Jr. (2001) Vesicle permeabilization in α-synuclein: implication for the pathogenesis and treatment of Parkinson's disease, *Biochemistry 40*, 7812−7819.
23. Zhu, M., Souillac, P. O., Ionescu-Zanetti, C., Carter, S. A., and Fink, A. L. (2002) Surface-catalyzed amyloid fibril formation, *J. Biol. Chem. 277*, 50914−50922.
24. Lehtonen, J. Y. A., Rytömaa, M., and Kinnunen, P. K. J. (1996) Characteristics of the binding of tacrine to acidic phospholipids, *Biophys. J. 70*, 2185−2194.
25. Lee, E.-N., Lee, S.-Y., Lee, D., Kim, J., and Paik, S. R. (2003) Lipid interaction of α-synuclein during the metal-catalyzed oxidation in the presence of Cu^{2+} and H_2O_2, *J. Neurochem. 84*, 1128−1142.
26. Hammarström, P., Jiang, X., Deechongkit, S., and Kelly, J. W. (2001) Anion shielding of electrostatic repulsion in transthyretin modulates stability and amyloidosis: insight into the chaotrope unfolding dichotomy, *Biochemistry 40*, 11453−11459.
27. Sousa, M. M., and Saraiva, M. J. (2003) Neurodegeneration in familial amyloid polyneuropathy: from pathology to molecular signaling, *Prog. Neurobiol. 71*, 385−400.
28. Chakrabartty, A. (2001) Progress in transthyretin fibrillogenesis research strengthens the amyloid hypothesis, *Proc. Natl. Acad. Sci. U.S.A. 98*, 14757−14759.
29. Wells, K., Farooqui, A. A., Liss, L., and Horrocks, L. A. (1995) Neural membrane phospholipids in Alzheimer disease, *Neurochem. Res. 20*, 1329−1333.
30. Ishitani, R., Tajima, H., Takata, H., Tsuchiya, K., Kuwae, T., Yamada, M., Takahashi, H., Tatton, N. A., and Katsube, N. (2003) Proapoptotic protein glyceraldehyde-3-phosphate dehydrogenase: a possible site of action of antiapoptotic drugs, *Prog. Neuro-Psychopharmacol. Biol. Psychiatry 27*, 291−301.
31. Rossjohn, J., Feil, S. C., McKinstry, W. J., Tweten, R. K., and Parker, M. W. (1997) Structure of a cholesterol-binding, thiol-activated cytolysin and a model of its membrane form, *Cell 89*, 685−692.
32. Volles, M. J., and Lansbury, P. T., Jr. (2002) Vesicle permeabilization by protofibrillar α-synuclein is sensitive to Parkinson's disease-related mutations and occurs by a pore-like mechanism, *Biochemistry 41*, 4595−4602.
33. Utsugi, T., Schroit, A. J., Connor, J., Bucana, C. D., and Fidler, I. J. (1991) Elevated expression of phosphatidylserine in the outer membrane leaflet of human tumor cells and recognition by activated human blood monocytes, *Cancer Res. 51*, 3062−3066.
34. Ran, S., and Thorpe, P. E. (2002) Phosphatidylserine is a marker of tumor vasculature and a potential target for cancer imaging and therapy, *Int. J. Radiat. Oncol. Biol. Phys. 54*, 1479−1484.
35. Cook, A. M., Low, E., and Ishijimi, M. (1972) Effect of phosphatidylserine decarboxylase on neural excitation, *Nature 239*, 150−151.
36. Bose, S., Tuunainen, I., Parry, M., Penate-Medina, O., Mancini, G., and Kinnunen, P. K. J. (2004) Binding of cationic liposomes to apoptotic cells, *Anal. Biochem.* (in press).
37. Konishi, A., Shimizu, S., Hirota, J., Takao, T., Fan, Y., Matsuoka, Y., Zhang, L., Yoneda, Y., Fujii, Y., Skoultchi, A. I., and Tsujimoto, Y. (2003) Involvement of histone H1.2 in apoptosis induced by DNA double-strand breaks, *Cell 114*, 673−688.
38. Zhao, H., Bose, S., Tuominen, E. K. J., and Kinnunen, P. K. J. (2004) Interaction of histone H1 with phospholipids and comparison of its binding to giant liposomes and human leukemic T cells, *Biochemistry* (in press).
39. Kuboi, R., Mawatari, T., and Yoshimoto, M. (2000) Oxidative refolding of lysozyme assisted by negatively charged liposomes: relationship with lysozyme-mediated fusion of liposomes, *J. Biosci. Bioeng. 90*, 14−19.
40. Sava, G., Ceschia, V., and Pacor, S. (1989) Lysozyme and cancer: role of exogenous lysozyme as anticancer agent, *Anticancer Res. 9*, 583−591.
41. Farias, R. N., Vinals, A. L., and Morero, R. D. (1986) Fusion of negatively charged phospholipid vesicles by insulin. Relationship with lipid fluidity, *J. Biol. Chem. 261*, 15508−15512.
42. Porras, A., Zuluaga, S., Valladares, A., Alvarez, A. M., Herrera, B., Fabregat, I., and Benito, M. (2003) Long-term treatment with insulin induces apoptosis in brown adipocytes: Role of oxidative stress, *Endocrinology 144*, 5390−5401.
43. Pitcher, W. H., Keller, S. L., and Huestis, W. H. (2002) Interaction of nominally soluble proteins with phospholipid monolayers at the air−water interface, *Biochim. Biophys. Acta 1564*, 107−113.
44. Bergers, J. J., Van Bloois, L., Barenholz, Y., and Crommelin, D. J. A. (1995) Conformational changes of myoglobin upon interaction with negatively-charged phospholipid vesicles, *J. Liposome Res. 5*, 311−326.
45. D'Agnillo, F., and Alayash, A. I. (2002) A role for the myoglobin redox in the induction of endothelial cell apoptosis, *Free Radical Biol. Med. 33*, 1153−1164.
46. Yang, J., and Liu, X. (1997) Prevention of apoptosis by Bcl-2: release of cytochrome *c* from mitochondria blocked, *Science 275*, 1129−1132.
47. Class, R., Lindman, S., Fassbender, C., Leinenbach, H. P., Rawer, S., Emrich, J. G., Brady, L. W., and Zeppezauer, M. (1996) Histone H1 suppresses tumor growth of leukemia cells in vitro, ex vivo and in an animal model suggesting extracellular functions of histones, *Am. J. Clin. Oncol. 19*, 522−531.
48. Agasoster, A. V., Halskau, O., Fuglebakk, E., Froystein, N. A., Muga, A., Holmsen, H., and Martinez, A. (2003) The interaction of peripheral proteins and membranes studied with α-lactalbumin and phospholipid bilayers of various compositions, *J. Biol. Chem. 278*, 21790−21797.
49. Svanborg, C., Agerstam, H., Aronson, A., Bjerkvig, R., Duringer, C., Fischer, W., Gustafsson, L., Hallgren, O., Leijonhuvud, I., Linse, S., Mossberg, A. K., Nilsson, H., Pettersson, J., and Svensson, M. (2003) HAMLET kills tumor cells by an apoptosis-like mechanism-cellular, molecular, and therapeutic aspects, *Adv. Cancer Res. 88*, 1−29.

BI049002C