Permeabilization of Lipid Bilayers Is a Common Conformation-dependent Activity of Soluble Amyloid Oligomers in Protein Misfolding Diseases

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Amyloid fibrilization is multistep process involving soluble oligomeric intermediates, including spherical oligomers and protofibrils. Amyloid oligomers have a common, generic structure, and they are intrinsically toxic to cells, even when formed from non-disease related proteins, which implies they also share a common mechanism of pathogenesis and toxicity. Here we report that soluble oligomers from several types of amyloids specifically increase lipid bilayer conductance regardless of the sequence, while fibrils and soluble low molecular weight species have no effect. The increase in membrane conductance occurs without any evidence of discrete channel or pore formation or ion selectivity. The conductance is dependent on the concentration of oligomers and can be reversed by anti-oligomer antibody. These results indicate that soluble oligomers from many types of amyloidogenic proteins and peptides increase membrane conductance in a conformation-specific fashion and suggest that this may represent the common primary mechanism of pathogenesis in amyloid-related degenerative diseases.

Soluble amyloid oligomers are a common intermediate in the pathway for amyloid fibril formation and have been implicated as the primary toxic species of amyloids related to neurodegenerative disease (1–6). More recent reports indicate that soluble amyloid oligomers are intrinsically toxic even when they are formed from proteins that are not normally related to degenerative disease (3), and the toxic activity of soluble oligomers may be related to a common generic structure that they share (6). Although the primary mechanism of amyloid toxicity is not clear, the fact that different amyloids reside in either the cytosolic or extracellular compartments and the observation that cytosolic amyloid aggregates are toxic when applied externally to cells (6, 7) points to the cell plasma membrane as a potential primary target of amyloid pathogenesis. Indeed, there are many reports of membrane perturbations caused by amyloids like Aβ (8), but it isn’t clear whether these effects are specific to soluble oligomers or whether they are common to other types of amyloids. Here we report that homogeneous populations of spherical amyloid oligomers and protofibrils increase the conductivity of membranes by a non-channel mechanism. This effect is observed for all soluble oligomers tested regardless of protein sequence and is not observed for amyloid fibrils or soluble low molecular weight species, suggesting that the increase in membrane conductivity may be a primary common mechanism of amyloid oligomer pathogenesis.

**MATERIALS AND METHODS**

**Pep tide Synthesis—**Pep tide synthesis: Aβ peptides, prion 106–126, and IAPP were synthesized by fluoren-9-ylmethoxy carbonyl chemistry using a continuous flow semiautomatic instrument as described previously (9). The purity was checked by analytical reverse phase-high performance liquid chromatography and by electrospray mass spectrometry. Polyglutamine KKQ40KK was a gift from Dr. Ronald Wetzel, and α-synuclein was a gift from Dr. Ralf Langen.

**Preparation of Oligomers and Fibers—**Lyophilized peptides and proteins were resuspended in 50% acetonitrile in water and re-lyophilized. Soluble oligomers were prepared by dissolving 1.0 mg of peptide or protein in 400 μl of hexafluoropropanol for 10–20 min at room temperature. 100 μl of the resulting seedless solution was added to 900 μl of double distilled H2O in a siliconized Eppendorf tube. After 10–20 min incubation at room temperature, the samples were centrifuged for 15 min at 14,000 × g, and the supernatant fraction (pH 2.8–3.5) was transferred to a new siliconized tube and subjected to a gentle stream of N2 for 5–10 min to evaporate the hexafluoropropanol. The samples were then stirred at 500 r.p.m. using a Teflon-coated micro stir bar for 24–48 h at 22 °C. Aliquots (10 μl) were taken at 6–12-h intervals for observation by atomic force microscopy, electron microscopy (EM), and size exclusion chromatography (SEC). The time at which the oligomers are most populated depends on several factors, such as speed of stirring and the concentration, so it is important to check their size and homogeneity with SEC. The maximum occurs between 6 h to 4 days. For prion 106–126 the samples were heated at 65 °C for 30 min, and poly(Q) was heated for 2 h at 37 °C before stirring. These conditions were empirically determined to provide homogeneous populations of oligomers. In the case of IAPP, oligomer formation should be monitored as early as possible. The purity of IAPP is important, because the presence of reduced IAPP promotes fibril formation leading to short-lived oligomers.

Fibrils were prepared under three different conditions, water (pH 3.8–4.2), 10 mM Tris (pH 7.4), and 50 mM Tris, 100 mM NaCl (pH 7.4), each containing 0.02% sodium azide. The final peptide or protein concentration was 0.3–0.5 mg/ml. The samples were prepared as described above for oligomers but stirred at room temperature for 6–9 days. Fibril formation was monitored by thioflavin T fluorescence and UV light scattering. Once fibril formation was complete, the solutions were centrifuged at 14,000 × g for 20 min, the fibril pellet was washed three times with the doubly distilled water and then re-resuspended in the desired buffer. The morphology was verified by negative stain EM.

**Bilayers—**Bilayers were formed at room temperature by the union of two monolayers formed from a mixture (1:1 by weight) of phosphatidylincholine and phosphatidylserine, phosphatidylethanolamine, and cho...
**Membrane Permeabilization by Amyloid Oligomers**

**FIG. 1. Characterization of oligomers and fibrils.** A, morphologies of soluble spherical oligomers and fibrils. EM images of spherical oligomer preparations (top row) and fibrils (bottom row). Panel A, Aβ42-soluble spherical oligomers. Panel B, α-synuclein-soluble spherical oligomers. Panel C, IAPP-soluble spherical oligomers. Panel D, poly(Q)-soluble spherical oligomers, (poly(Q) spherical oligomers tend to cluster together). Panel E, prion 106–126-soluble spherical oligomers. Panel F, Aβ42 fibrils. Panel G, α-synuclein fibrils. Panel H, IAPP fibrils. Panel I, poly(Q) fibrils. Panel J, prion 106–126-insoluble fibrils. B, size exclusion chromatography of Aβ42 oligomers. Soluble oligomers elute at ~15 min, while the void and included volumes of the column are ~8 and 30 min, respectively. Integration of the peak areas indicate that ~90% of the peptide runs as oligomers with an apparent molecular mass of ~90–110 kDa based on calibration with globular protein size standards.

**FIG. 2. Effect of Aβ42 oligomeric intermediates on membrane conductance.** A, bilayer currents recorded in response to voltage ramps from −100 to +100 mV at a rate of 60 mV/s. Only spherical oligomers increased the conductance of the bilayer in a reproducible concentration-dependent fashion. Aβ42 oligomers increases conductance in a concentration-dependent fashion. Curves are labeled with Aβ42 concentration in micromolar. Panels B and C show currents recorded at a voltage of +150 mV. B, sequential addition of soluble low molecular weight Aβ42 (arrows A–C) has no effect on conductivity and does not prevent the conductance increase elicited by oligomers (arrow D). Arrow A, 1 μM; arrow B, 2 μM; arrow C, 4 μM; arrow D, 2 μM oligomers in the presence of 4 μM soluble low molecular weight. C, sequential addition of Aβ42 fibrils (arrows A–C) has no effect on conductivity and does not block the effect from oligomers (arrow D). Arrow A, 1 μM; arrow B, 2 μM; arrow C, 4 μM; arrow D, 2 μM oligomers in the presence of 4 μM fibrils.

**Thioflavin T Fluorescence—**Thioflavin T fluorescence was determined as described (12). The fluorescence emission spectrum was measured using a Spex Fluorolog-2 spectrophotometer.

**Size Exclusion Chromatography—**SEC was performed using a Hewlett Packard 1050 liquid chromatograph and a 0.78 × 30 cm Tosoh Haas G3000SWxl column in 100 mM NaCl, 50 mM Tris, pH 7.4, at a flow of 0.4 ml/min. The eluate was monitored by UV absorbance at 230 nm.

**RESULTS AND DISCUSSION**

Investigating the conformational basis of soluble amyloid oligomer toxicity requires the preparation homogenous and relatively stable populations of conformationally distinct soluble monomers, oligomeric intermediates and fibrils. Homogeneous samples of low molecular weight species (monomer or dimer), spherical oligomers, and fibrils were prepared from Aβ40 and Aβ42 (Alzheimer disease) α-synuclein (Parkinson disease), IAPP (Type II diabetes), polyglutamine (KKQ40KK) (Huntington disease), and prion (106–126) H1 (Prion diseases), using the procedures we have described previously (6). Although the conditions that promote the formation of spherical oligomers are remarkably similar for the different proteins and...
peptides, the conditions were optimized for each peptide or protein to obtain the most homogenous samples possible (see “Materials and Methods”). All preparations were visualized by electron microscopy (Fig. 1A). The soluble oligomer samples contain a homogenous population of spherical particles with an average diameter of 3–5 nm and are free of detectable mature amyloid fibrils. The fibrillar samples contain predominately long mature amyloid fibrils with very few, if any, spherical oligomers. The homogeneity was analyzed by size exclusion chromatography (Fig. 1B). Oligomeric Aβ42 elutes as a symmetrical peak of ~90–110 kDa and contains less than 10% low molecular weight monomer or dimeric species. The soluble low molecular weight samples contain a peak that elutes at the position expected for monomer or dimer and contains less than 1% of high molecular weight species (13).

We examined the effect of these relatively homogeneous samples on membrane conductivity using planar lipid bilayers (10). Spherical Aβ42 oligomers specifically increase the conductance of the bilayer (Fig. 2A). The increase in conductivity is approximately proportional to the concentration of oligomers. No increase in conductance was observed for low molecular weight Aβ species (monomer or dimer) (Fig. 2B) or fibrils (Fig. 2C). Moreover, the prior addition of soluble low molecular weight Aβ or Aβ fibrils does not prevent the conductivity increase caused by the subsequent addition of spherical Aβ oligomers (Fig. 2, B and C). Aβ40 spherical oligomers also exhibited the same conductivity increase when added to lipid bilayers (supplementary Fig. 1).

The increase in membrane conductance in response to oligomer addition occurs in the absence of any evidence of discrete ion channel or pore formation (Fig. 3A). The high sensitivity recording indicates that there is little change in the noise level in the current trace as the current increases from 0 to ~100 pA after oligomer addition. Previous studies of the interaction of Aβ with membranes suggested that Aβ forms pores or channels in membranes (8, 14, 15). The increase in conductivity reported here is unique and distinct from previous reports of pore or channel formation by Aβ. In particular, we do not find discrete unitary conductance changes or evidence of open and closed states that are characteristic of ion channels. The conductance induced by soluble oligomers is not inhibited by Tris ions or Congo red that have been reported to inhibit Aβ channels (15, 16). We examined the ion selectivity by measuring the bimodal reversal potential. The observed reversal potentials are zero, indicating that the conductance is not ion selective (Fig. 3B). The conductance we observe for soluble Aβ oligomers is ~100-fold greater than previously reported for similar concentrations of Aβ that were not conformationally characterized (14, 15). Rather than forming pores or channels, soluble oligomers appear to enhance the ability of ions to move through the lipid bilayer on their own. Although the explanation for these differences is not entirely clear, the conditions used in the previous reports of amyloid Aβ ion channels seem unlikely to include significant amounts of soluble amyloid oligomers.

Since preincubation of soluble oligomers with oligomer-specific antibody inhibits the toxicity of all types of soluble oligomers in cell culture (6), we tested the effect of this antibody on membrane conductance. The increase in conductivity caused by Aβ spherical oligomers is rapidly reversed (Fig. 3C) upon the addition of a conformation-dependent antibody that is specific for amyloid oligomers (6) but is not reversed by nontarget control antibodies (data not shown). The fact that anti-oligomer antibody can reverse the conductivity increase suggests the oligomeric Aβ does not undergo a

![Figure 3](http://www.jbc.org/)

**FIG. 3. Characterization of the oligomer-induced conductance increase.** A, high sensitivity recording of the oligomer-induced membrane conductivity increase. The current was recorded at a membrane potential of 150 mV. The bottom trace was recorded prior to the addition of Aβ42 oligomers. The upper traces show 10-s current traces recorded in a 2-min period after the addition of 0.1 μM Aβ42 oligomers. There is no significant change in the noise of the current trace as the current increases from zero to ~100 pA. B, reversal potentials. Bilayer currents were recorded as described in the legend to Fig. 2A, with 1.0 μM Aβ42 oligomers. Line 1, 10 mM KCl cis; 10 mM KCl trans. Line 2, 10 mM KCl cis; 100 mM KCl trans. The reversal potential is zero, indicating there is no preference for the conductivity of potassium or chloride ions. C, oligomer-induced membrane conductivity is rapidly reversed by anti-oligomer antibody. Arrow A, 0.5 μM Aβ42 oligomer addition; arrow B, 4 μM anti-oligomer antibody addition. The conductivity is reversed after addition of anti-oligomer antibody but not by nontarget control antibodies (data not shown).

![Figure 4](http://www.jbc.org/)

**FIG. 4. Spherical oligomers from other types of amyloids increase membrane conductivity in a concentration dependent fashion.** Bilayer currents recorded in response to voltage ramps from −100 to +100 mV at a rate of 60 mV/s. Curves are labeled with oligomer concentration in micromolar. A, α-synuclein oligomers. B, IAPP oligomers. C, poly(Q) oligomers. D, prion 106–126 oligomers.
conformation change to a form that is not recognized by the antibody upon membrane interaction and that the oligomers are only peripherally associated with the bilayer rather than stably inserted into the hydrocarbon core. Anti-oligomer antibody appears to remove Aβ from its association with the membrane, because the infrared amide I signal at 1627 cm\(^{-1}\) that is specific for membrane-associated Aβ drops precipitously after anti-oligomer antibody addition.\(^2\)

Since other amyloidogenic proteins also form spherical oligomers that have a common conformation-dependent structure and are also cytotoxic (6), we tested whether they have similar effects on membrane conductivity. Spherical oligomers from α-synuclein, IAPP, poly(Q), and prion 106–126 also specifically increase bilayer conductivity in a concentration-dependent fashion (Fig. 4, A–D). As observed for Aβ, low molecular weight species and fibrils from these amyloid-forming proteins and peptides have no effect on membrane conductivity when tested at concentrations up to 8 \(\mu\)M (supplementary Fig. 2). The increase in conductivity caused by α-synuclein, IAPP, poly(Q), and prion 106–126 oligomers was also reversed by addition of anti-oligomer antibody (supplementary Fig. 3). Preincubation of soluble oligomers with anti-oligomer antibody also prevents the increase in membrane conductance when they are applied to lipid bilayers (data not shown).

Using homogeneous populations of low molecular weight species, spherical oligomers, and fibrils, we found that all types of spherical oligomers examined specifically induce dramatic increases in the conductivity of membranes. Since the monomeric or low molecular weight species and amyloid fibrils do not induce a conductivity change, this effect is specific for the particular conformation associated with spherical oligomers. We have recently shown that the soluble oligomers share a common structure that is associated with cellular toxicity, suggesting that soluble oligomers share a common primary mechanism of pathogenesis (6). This predicts that oligomers would also share a common target, and since some types of amyloid oligomers are cytotoxic, while others are extracellular, the common target must be accessible to both the cytosolic and extracellular compartments. The plasma membrane of the cell is one of the few targets that is accessible to both compartments. We demonstrate that the increase in membrane conductivity induced by spherical oligomers is also a common property shared by all oligomers tested. This suggests that the increase in membrane permeability caused by spherical oligomers may represent the common primary mechanism of pathogenesis.

The different effects of low molecular weight Aβ and oligomeric Aβ in increasing membrane conductance is correlated with the different effects on membrane structure that have been reported as determined by small angle x-ray diffraction (17). Monomeric or low molecular weight Aβ produced a broad increase in electron density in the center of the bilayer accom-

\(^2\)P. Axelsen, personal communication.

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