Amyloid Ion Channels: A Porous Argument or a Thin Excuse?

David Eliezer

Department of Biochemistry and Program in Structural Biology, Weill Medical College of Cornell University, New York, NY 10021

The Journal of General Physiology

Over the past decade, a number of groups have reported that oligomeric intermediates populated during the conversion of proteins from a monomeric disaggregated state into amyloid fibrils can permeabilize lipid bilayers and cell membranes. Some of these studies have presented electrical current traces that suggest the formation of discrete oligomeric membrane ion channels, which exhibit selectivity toward cations that can be blocked by a variety of reagents. In this issue, Sokolov et al. (p. 637) use carefully prepared oligomers of the amyloid-β peptide (Aβ), combined with sensitive current measurements capable of detecting the properties of single ion channels to examine the effects of the oligomers on membrane conductance. They conclude that Aβ oligomers clearly cause an increase in membrane permeability, but convincingly rule out any significant formation of discrete ion channels in their samples. Instead, it appears that the oligomers may interfere with the surface packing of the lipid headgroups and lead to an effective thinning of the membrane, thereby reducing its effectiveness as a conductance barrier.

The membrane-perturbing activity of amyloid oligomers, whether it is due to channel formation or to some other mechanism, would be expected to contribute significantly to amyloid-induced toxicity and may therefore be an appropriate target for the development of new therapeutics. Pinning down the precise mechanism(s) behind oligomer-mediated membrane permeabilization is therefore of paramount importance, as strategies to interfere with this activity will differ greatly depending on our understanding of the underlying mechanism. If oligomeric membrane-inserted ion channels are indeed the culprit, compounds of potential therapeutic value would include those that block the channels or that prevent channel assembly or insertion. If the relevant mechanism of action does not depend on membrane channel insertion but instead involves surface interactions that perturb global membrane structure, design targets might include stabilizing global membrane structure, preventing oligomer–membrane surface interactions, or preventing the formation of soluble oligomers. The work by Sokolov et al. (2006) supports the latter approach, but we are left to muse upon the origin of the apparent discrepancy between their results and previous reports citing discrete channel formation.

Controversy over the toxicity of amyloidogenic proteins is nothing new. Indeed, the relevance of any aspect of protein aggregation to cell death in various amyloid diseases was doubted for years and is still questioned by some (see for example Lee et al., 2004), because the evidence remains indirect. Perhaps the strongest argument in favor of a causative role for aggregation comes from genetic studies demonstrating that specific proteins that form the ordered fibrillar material in disease-associated amyloid are typically responsible for familial forms of the diseases. The different proteins thus implicated do not share any other functional or structural similarities. The only property they have in common is their deposition as amyloid fibrils, suggesting that formation of these fibrils is crucial to these proteins' deleterious effects. In addition, the autosomal dominant nature of many familial forms of these disorders, combined with the successful recapitulation of disease phenotypes upon transgenic introduction of some of the implicated proteins into organisms that do not possess naturally occurring homologues, implies that a gain of function is likely responsible for toxicity in these disorders. Finally, conditions leading to excess levels of even the wild-type versions of some of the relevant proteins are associated with disease, suggesting a mass action effect, consistent with a crucial role for aggregation.

Despite this strong, albeit circumstantial, evidence some continue to doubt the "amyloid hypothesis;" these doubts are fueled in part by considerable evidence that amyloid fibrils themselves, the mature aggregation end product that is observed in diseased tissues, may not be directly involved in causing cell damage and death. Indeed, typical amyloid deposits are absent in certain forms of disease, and in some cases it appears that even when present they do not adversely affect (Tompkins and Hill, 1997) and may even favor (Arrasate et al., 2004) cell survival. These observations, among others, have led to the formation of an alternate amyloid hypothesis, sometimes referred to as the oligomer hypothesis,

Correspondence to David Eliezer: dae2005@med.cornell.edu

Abbreviation used in this paper: Aβ, amyloid-β peptide.

J. Gen. Physiol. © The Rockefeller University Press $8.00
Volume 128 Number 6 December 2006 631–633
http://www.jgp.org/cgi/doi/10.1085/jgp.200609689

which posits that while mature amyloid fibrils may not be toxic, some species formed during the fibril assembly process may be responsible for cell damage. Because fibril assembly is known to involve oligomeric species, these have been proposed as the real culprit (Caughey and Lansbury, 2003; Walsh and Selkoe, 2004). The missing piece of the puzzle, however, remains how these oligomers may act.

How might oligomers be toxic to cells? It is instructive to first consider mechanisms that were proposed for fibril-mediated toxicity. These include (but are by no means limited to) mechanical disruption of cells or tissues, activation of inflammatory responses, improper activation of signaling pathways, overloading of the protein degradation machinery, production of oxidative species through Fenton type reactions, triggering of unfolded protein response pathways, and damage to cell membranes. Of these mechanisms, only the first, mechanical disruption of cells or tissues is necessarily limited to the mature fibrils in amyloid deposits, which, unlike oligomers, occupy volumes of the same order of magnitude as cells. The remaining proposed mechanisms, as well as others, could just as well apply to amyloid oligomers as to amyloid fibrils. Yet the model that has garnered the most attention is that of membrane damage. This may be so because of the common adage that a picture is worth a thousand words. The case for membrane perturbation originally arose in part from early reports that the Aβ peptide was able to perturb calcium homeostasis in neurons (Hardy and Higgins, 1992; Mattson et al., 1992). This was followed shortly by the first reports that Aβ was able to form ion channels in membranes (Arispe et al., 1993). Subsequently, a number of investigators have reported images of amyloid oligomers, formed both in the absence and presence of lipid membranes, that are arranged in toroidal shapes (Lashuel and Lansbury, 2006) and appear to resemble pore structures similar to those formed by oligomeric membrane-lysing toxins such as α-hemolysin (Bhakdi and Tranum-Jensen, 1991; Czajkowsky et al., 1998). Being able to visualize molecular structures with an appearance that is consistent with a putative mechanism of toxicity has generated considerable excitement for the so-called channel hypothesis.

While the idea that polypeptides having arbitrary sequences are capable of forming discrete membrane channel structures may seem farfetched, it is important to note that the same collection of apparently arbitrary proteins already is known to assembly into a common highly ordered structure (O'Nuallain and Wetzel, 2002), namely the mature amyloid fibril. In addition, recent work has suggested that amyloid oligomers are also generic, both structurally (Kayed et al., 2003) and functionally (Bucciantini et al., 2002; Kayed et al., 2004). Nevertheless, there is little evidence at present to directly link the imaged annular structures with membrane channel activity. Indeed, in only a few instances were current records indicative of channel formation and images of channel-like structures recorded on equivalent preparations (Lin et al. 2001; Quist et al. 2005), and in these cases the large number of imaged "channels" appears to be inconsistent with observations in the current traces of single channel events. As pointed out by Sokolov et al., a litmus test for confirming the relevance of single channel observations is that they successfully predict the conductance properties of bulk membranes containing large numbers of channels. These tests have yet to be performed for amyloid ion channels. For example, discrete amyloid ion channels appear be gated by voltage, but the ensemble voltage dependence of currents in bulk samples, which should be predictable from the single channel data, has not been examined. Similarly, currents from individual amyloid channels can be blocked by various reagents, but corresponding blockage of conductance in bulk samples remains to be shown.

Despite these points, there is ample evidence for discrete amyloid-induced current changes across membranes, yet such signals were not observed by Sokolov et al. A potential explanation may involve the notorious dependence of Aβ behavior on experimental conditions. Early on in studies of Aβ aggregation it was noted that different preparations of the peptide exhibited different aggregation kinetics (Soto et al. 1995), and the peptide consequently developed a reputation for being difficult to work with. Some degree of consensus now exists that dissolution of lyophilized Aβ in organic solvents such as DMSO or HFIP results in aggregate-free starting material. This is the approach used by Sokolov et al., who have gone to considerable trouble to develop protocols that lead to uniform and reproducible oligomer preparations (Kayed et al. 2004). In their experiments, the preformed oligomers are added to the solutions bathing the preformed lipid bilayer. On the other hand, dissolution in double-distilled H_2O (ddH_2O) is also commonly used to prepare monomeric Aβ, although this method may not be as effective at disassembling aggregates present in the lyophilized material. In reports that document Aβ ion channel currents, the preparation method typically involves dissolution of the peptide in ddH_2O. Dissolved monomeric peptides are then either added to the solutions bathing preformed lipid bilayers or are sonicated in the presence of lipids to form proteoliposomes, which are then fused into lipid planar bilayers. It is perhaps not surprising that these rather different methods of sample preparations lead to different results. It may well be that channel formation, which is characterized by stable membrane insertion, is facilitated by sonication of protein–lipid mixtures, whereas membrane insertion of aqueously soluble oligomers may be difficult. Furthermore, membrane surfaces are known to nucleate the

aggregation of monomeric Aβ (Yip et al. 2002), but how oligomers formed during lipid-induced aggregation compare to those formed in solution is not known. Despite some similarity between images of annular structures obtained in aqueous solutions and in membranes, it may well be that soluble oligomers are arranged quite differently from channel-forming, membrane-inserted oligomers. Indeed, it is now known that mature amyloid fibrils themselves exist in structurally distinct forms (Petkova et al. 2005) and different forms of amyloid oligomers may therefore also be expected to exist.

Sokolov et al. have shown that soluble Aβ oligomers permeabilize lipid membranes by altering their bulk electrophysiological properties and effectively thinning the insulating barrier they present to conductance. Reconciling their results with previous demonstrations of discrete ion channel formation by Aβ will require careful work to analyze the contribution of discrete channels to bulk conductance, as well as to characterize the relation between the pathways leading to soluble oligomers and to the formation of channels. Both effects may be expected to play an important role in disease, and establishing these roles will require in vivo studies guided by knowledge obtained from biophysical studies of the underlying mechanisms. Intervention efforts targeting the two different effects may require significantly different strategies.

REFERENCES

Arispe, N., E. Rojas, and H.B. Pollard. 1993. Alzheimer disease amyloid β protein forms calcium channels in bilayer membranes: blockade by tromethamine and aluminum. *Proc. Natl. Acad. Sci. USA.* 90:567–571.

Arrasate, M., S. Mitra, E.S. Schweitzer, M.R. Segal, and S. Finkbeiner. 2004. Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death. *Nature.* 431:805–810.

Bhakdi, S., and J. Tranum-Jensen. 1991. α-Toxin of *Staphylococcus aureus. Microbiol. Rev.* 55:733–751.

Bucciantini, M., E. Giannoni, F. Chiti, F. Baroni, L. Formigli, J. Zurdo, N. Taddei, G. Ramponi, C.M. Dobson, and M. Stefani. 2002. Inherent toxicity of aggregates implies a common mechanism for protein misfolding diseases. *Nature.* 416:507–511.

Caughey, B., and P.T. Lansbury. 2003. Protofibrils, pores, fibrils, and neurodegeneration: separating the responsible protein aggregates from the innocent bystanders. *Annu. Rev. Neurosci.* 26:267–298.

Czajkowsky, D.M., S. Sheng, and Z. Shao. 1998. Staphylococcal α-hemolysin can form hexamers in phospholipid bilayers. *J. Mol. Biol.* 276:325–330.

Hardy, J.A., and G.A. Higgins. 1992. Alzheimer's disease: the amyloid cascade hypothesis. *Science.* 256:184–185.

Kayed, R., E. Head, J.L. Thompson, T.M. McIntire, S.C. Milton, C.W. Cotman, and C.G. Glabe. 2003. Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. *Science.* 300:486–489.

Kayed, R., Y. Sokolov, B. Edmonds, T.M. McIntire, S.C. Milton, J.E. Hall, and C.G. Glabe. 2004. Permeabilization of lipid bilayers is a common conformation-dependent activity of soluble amyloid oligomers in protein misfolding diseases. *J. Biol. Chem.* 279: 46363–46366.

Lashuel, H.A., and P.T. Lansbury. 2006. Are amyloid diseases caused by protein aggregates that mimic bacterial pore-forming toxins? *Q. Rev. Biophys.* 39:167–201.

Lee, H.G., G. Casadesus, X. Zhu, A. Takeda, G. Perry, and M.A. Smith. 2004. Challenging the amyloid cascade hypothesis: senile plaques and amyloid-β as protective adaptations to Alzheimer disease. *Ann. N. Y. Acad. Sci.* 1019:1–4.

Lin, H., R. Bhatia, and R. Lal. 2001. Amyloid β protein forms ion channels: implications for Alzheimer's disease pathophysiology. *FASEB J.* 15:2433–2444.

Mattson, M.P., B. Cheng, D. Davis, K. Bryant, I. Lieberburg, and R.E. Rydel. 1992. β-Amyloid peptides destabilize calcium homeostasis and render human cortical neurons vulnerable to excitotoxicity. *J. Neurosci.* 12:376–389.

O'Nuallain, B., and R. Wetzel. 2002. Conformational Abs recognizing a generic amyloid fibril epitope. *Proc. Natl. Acad. Sci. USA.* 99:1485–1490.

Petkova, A.T., R.D. Leapman, Z. Guo, W.M. Yau, M.P. Mattson, and R. Tycko. 2005. Self-propagating, molecular-level polymorphism in Alzheimer's β-amyloid fibrils. *Science.* 307:262–265.

Quist, A., I. Doudevski, H. Lin, R. Azimova, D. Ng, B. Frangione, B. Kagan, J. Ghiso, and R. Lal. 2005. Amyloid ion channels: a common structural link for protein-misfolding disease. *Proc. Natl. Acad. Sci. USA.* 102:10427–10432.

Sokolov, Y., J.A. Kozak, R. Kayed, A. Chanturiya, C. Glabe, and J.E. Hall. 2006. Soluble amyloid oligomers increase bilayer conductance by altering dielectric structure. *J. Gen. Physiol.* 128:637–647.

Soto, C., E.M. Castano, R.A. Kumar, R.C. Beavis, and B. Frangione. 1995. Fibrillogenesis of synthetic amyloid-β peptides is dependent on their initial secondary structure. *Neurosci. Lett.* 200:105–108.

Tompkins, M.M., and W.D. Hill. 1997. Contribution of somal Lewy bodies to neuronal death. *Brain Res.* 775:24–29.

Walsh, D.M., and D.J. Selkoe. 2004. Oligomers on the brain: the emerging role of soluble protein aggregates in neurodegeneration. *Protein Pept. Lett.* 11:213–228.

Yip, C.M., A.A. Darabie, and J. McLaurin. 2002. Aβ42-peptide assembly on lipid bilayers. *J. Mol. Biol.* 318:97–107.