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Topical Review

Amyloid Peptide Channels

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Abstract. At least 16 distinct clinical syndromes including Alzheimer's disease (AD), Parkinson's disease (PD), rheumatoid arthritis, type II diabetes mellitus (DM), and spongiform encephelopathies (prion diseases), are characterized by the deposition of amorphous, Congo red-staining deposits known as amyloid. These "misfolded" proteins adopt β-sheet structures and aggregate spontaneously into similar extended fibrils despite their widely divergent primary sequences. Many, if not all, of these peptides are capable of forming ion-permeable channels in vitro and possibly in vivo. Common channel properties include irreversible, spontaneous insertion into membranes, relatively large, heterogeneous single-channel conductances, inhibition of channel formation by Congo red, and blockade of inserted channels by Zn^{2+}. Physiologic effects of amyloid, including Ca^{2+} dysregulation, membrane depolarization, mitochondrial dysfunction, inhibition of long-term potentiation (LTP), and cytotoxicity, suggest that channel formation in plasma and intracellular membranes may play a key role in the pathophysiology of the amyloidoses.

Key words: Alzheimer's — Amyloid deposits — Prion — Pores — Lipid bilayers — Aβ channels

Introduction

In 1854 Rudolph Virchow employed the term "amyloid" to describe amorphous "starch like" deposits in tissues that stained with iodine, believing them to be carbohydrate in nature. Subsequent investigations revealed that these proteinaceous deposits stained intensely with the dye Congo red, and exhibited apple-green birefringence in polarized light. Electron microscopy (EM) revealed fibrils 80–100 Angstroms in width and of indeterminate length (*see* Sipe & Cohen, 2002, for review). At least 16 different clinical syndromes are characterized by amyloid deposits. Although all amyloid deposits contain glycosaminoglycans and the pentraxin amyloid P, the major amyloid protein is unique to each disease state. Amyloid proteins exhibit no amino acid sequence homology, but do share the physical chemical properties that lead to the characteristic appearance of amyloid fibrils. Specifically, they are rich in β-pleated sheet with the axis of the peptides oriented perpendicularly to the fibril axis. While it is striking that such biochemically diverse sequences can adopt similar conformations, the role of amyloid proteins in the pathogenesis of disease remains unclear.

Several widespread illnesses such as Alzheimer's disease (AD), type II diabetes mellitus (DM) and the spongiform encephelopathies (prion diseases, PrD) are amyloidoses (*see* Falk, Commenzo & Skinner, 1997, for a review of clinical syndromes) (Table 1). A substantial body of recent work suggests that the Alzheimer amyloid peptide, Aβ, plays a central role in the etiology of AD (*see* Selkoe, 2002, for review). The mechanism by which Aβ causes illness, however, has remained unclear, as Aβ possesses no enzymatic or highly specific receptor binding activity. (*see* Merlini & Berlotti, 2003, for a review of amyloidoses.)

In 1993 Arispe et al. (1993a,b) made the remarkable discovery that Aβ could form ion-permeable channels in lipid bilayer membranes (BLMs). These channels were relatively large, heterodisperse, non-specifically cation selective, Ca^{2+} permeable and sensitive to blockade by Zn^{2+} (Arispe, Pollard & Rojas, 1996). They proposed that channel formation might be the mechanism by which Aβ kills neurons, the primary target cell in AD. They hypothesized that membrane depolarization and/or Ca^{2+} influx might be responsible for toxicity, and they calculated that a single Aβ channel of 4 nS could cause a significant leak in the plasma membrane of a typical neuron, resulting in a change in internal [Na] of 10 μM/s.

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Table 1. Amyloid diseases and proteins

Disease	Protein	Abbreviation
Alzheimer's disease, Down's Syndrome (Trisomy 21)	Amyloid precursor protein	Aβ
Heredity cerebral angiopathy (Dutch) Creutzfeld-Jacob Gerstmann-Straussler syndrome (GSS) Scrapie (sheep) Bovine spongiform encephalopathy	Prion protein	PrP^{c}/PrP^{sc}
Type II Diabetes Mellitus	Islet Amyloid Polypeptide (amylin)	IAPP
Dialysis-associated amyloidosis	Beta-2-microglobulin	B2M
Senile cardiac amyloidosis	Atrial natriuretic factor	ANF
Familial amyloid polyneuropathy	Transthyretin	TTR
Reactive amyloidosis Familial Mediterranean Fever	Serum amyloid A	SAA
Familial amyloid polyneuropathy (Finnish)	Gelsolin	Agel
Macroglobulinemia	Gamma-1 heavy chain	AH
Multiple myeloma	Ig-lambda, Ig-Kappa	AL
Familial polyneuropathy-Iowa (Irish)	Apolipoprotein A1	ApoA1
Hereditary cerebral myopathy-Iceland	Cystatin C	Acys
Nonneuropathic hereditary amyloid with renal disease	Fibrinogen Alpha	AFibA
Nonneuropathic hereditary amyloid with renal disease	Lysozyme	Alys

For technical reasons, it has not been possible to detect Aβ channels directly in AD brain cells, but channels have been observed in vitro from a number of other amyloid peptides, including islet amyloid polypeptide (IAPP or amylin; Mirzabekov, Lin & Kagan, 1996), a neurotoxic fragment of the prion protein (PrP106–126; Lin, Mirzabekov & Kagan, 1997), β2-microglobulin (β2M; Hirakura & Kagan, 2001), serum amyloid A (SAA; Hirakura et al., 2001), atrial natriuretic factor (ANF, Kourie et al., 2001a), polyglutamine (PG; Monoi et al., 2000; Hirakura et al., 2000), transthyretin (TTR; Hirakura et al., 2001), alpha synuclein (AS; Lashuel et al., 2002; Azimova & Kagan, 2003), calcitonin (Stipani et al., 2001) and lysozyme (ring structures; Malisauskas et al., 2003). The observance of channels amongst such a diverse variety of peptide sequences suggests a deep underlying similarity in the physical chemical structures involved. As we discuss below, the channels formed by these amyloid peptides are very different from the ion channels usually found in excitable tissues, but they share a number of common characteristics that would be expected to render them toxic to their target tissues. While channel-forming toxins are well known throughout various phyla (e.g., Schein, Kagan & Finkelstein, 1978; Kagan et al., 1981; Kagan, 1983), these channel-forming "autotoxins" may represent a novel form of disease pathogenesis.

Aβ

Aβ channels were the first amyloid channels to be reported (Arispe, Rojas & Pollard, 1993a) and have been characterized most completely. The initial report described channels in lipid bilayers which were voltage independent and relatively cation selective ($(P(K^+) = 11\ P(Cl^-))$). The lifetime of these channels was quite long, in the range of minutes to hours. Channels of several different conductances were observed, but all were susceptible to block by tromethamine ($tris^+$) and aluminum (Al^{3+}). Channels were permeable to Ca^{2+} as well. Further work from this group showed that extremely large conductance channels could sometimes be observed (up to 5 nS, Arispe, Pollard & Rojas, 1993b), and that a 4 nS channel could cause the intracellular $[Na^+]$ to change by as much as 10 μM/s. They suggested that ionic leakages of Na^+, K^+, and Ca^{2+} could disrupt membrane potential and cellular homeostasis in a matter of seconds. They proposed channel formation as the molecular mechanism of Aβ cytotoxicty.

Some laboratories had difficulty at first in observing channel activity of Aβ 1–40 or 1–42 (e.g., Mirzabekov et al., 1994). Similar difficulties with the reproducibility of Aβ's toxic effects had been noted in the literature. The channel hypothesis did not attract significant support at that time, perhaps due to the difficulties with reproducibility. This difficulty plagued all studies of Aβ peptide, and was eventually traced to the irreproducible aggregation of the Aβ peptide (Pike et al., 1993). Aggregation of amyloid peptides is a complex process beyond the scope of this review. Although extended insoluble fibrils are the hallmark of amyloid deposits, there is now evidence to suggest that smaller aggregates may be responsible for the toxic properties of amyloid peptides (Caughey & Lansbury, 2003). Aggregation is a nucleation-dependent process, and the variable presence of nuclei may have accounted for the lack of reproducibility in earlier studies (Harper & Lansbury, 1997). More recent toxicity studies clearly establish that monomers and fibrils are non-toxic,

while some intermediate states of aggregation/oligomerization are toxic (Janson et al., 1999; Caughey & Lansbury, 2003).

Despite these difficulties, it has been shown that Aβ can form channels in planar lipid bilayers, liposomes, neurons, oocytes, and fibroblasts, and that channel formation is tightly linked to toxicity (Fraser et al., 1997). Aβ has also been shown to interfere with long-term potentiation (LTP) (Walsh et al., 2002), a model for memory. There is evidence that only channel-forming versions of Aβ can inhibit this process (Chen et al., 2000). Since memory is one of the earliest functions disrupted in AD, this suggests that the early pathology of AD might be mediated by Aβ channels.

Eventually it was discovered that not only did the aggregation state of Aβ peptides determine their toxicity and channel-forming abilities, but that this state was highly variable and sensitive to parameters such as pH, solvent exposure, concentration, and presence of nucleation agents (Pike et al., 1993, Harper & Lansbury, 1997, Hirakura et al., 1999). It thus became clear that factors that enhanced aggregation, such as acidic pH, also enhanced toxicity and channel formation. Agents, such as Congo red, which inhibit aggregation, can block toxicity and inhibit channel formation (Ingrosso et al., 1995; Hirakura et al., 1999). More strikingly, Hirakura, Lin & Kagan (1999) showed that single-channel conductance depended on aggregation state. Exposure to organic solvents, which tend to monomerize Aβ peptides, lowered the median single-channel conductance. Acidic pH, which increases aggregation, shifted the distribution to larger single-channel conductances. These results implied that heterodisperse Aβ channels could be formed by oligomers of variable number.

Although Aβ 1–40 and 1–42 are the species found in amyloid deposits in vivo, other Aβ peptides have been shown to form ion channels. Mirzabekov et al. (1994) described Aβ 25–35 channels that were voltage-dependent and relatively poorly selective amongst common physiologic ions. Since Aβ 25–35 was cytotoxic to neurons and other cells, it was considered at the time a good model for Aβ peptide toxicity even though it is never found in vivo. Using variants of Aβ 25–35, Lin (1996) was able to show that the channel-forming ability of Aβ 25–35 was necessary, but not sufficient for cytotoxicty. Thus, all peptides that killed cells also formed channels, but at least 2 channel-forming Aβ 25–35 variants did not kill cells. Aβ 25–35 channel activity is enhanced by negatively charged lipids and decreased by cholesterol. Channels are irreversibly associated with the membrane and show a variety of single-channel conductances. Cation selectivity is moderate, and the channels exhibit voltage-dependent opening and closing, which is markedly different from the voltage-independent Aβ 1–40 and 1–42 channels (Lin & Kagan, 2002). Aβ variants shorter than 10 residues did not form channels, suggesting a minimum membrane-spanning length of β-sheet consistent with known channel structures such as staphylococcal alpha-toxin and anthrax toxin (Song et al., 1996; Petosa et al., 1997). However, recently a much shorter variant, Aβ 31–35 has been reported to form channels (Qi & Qiao, 2001).

Aβ 1–40 and 1–42 have also been observed to induce cation-selective currents in rat cortical neurons (Furukawa, Abe & Akaike, 1994; Weiss, Pike & Cotman, 1994), hNT cells (Sanderson, Butler & Ingram, 1997), and in patches from gonadotropin-releasing hormone (GnRH) secreting neurons (Kawahara et al., 1997). Aβ 1–40 and 1–42 channels seem to be indistinguishable in terms of their observable channel properties. (Arispe et al. 1993a,b; Hirakura et al., 1999). Aβ1–40 and 1–42 have also been shown to transport Ca^{2+} into liposomes and to kill fibroblasts (Rhee, Quist & Lal, 1998; Lin, Zhu & Lal, 1999; Zhu, Lin & Lal, 2000). The killing can be inhibited by Aβ antibodies, trometh$amine^{+}$, or Zn^{2+}, but not by antioxidants, consistent with a channel-mediated mechanism of cell death. It has also been demonstrated that plasma membrane cholesterol content (and therefore fluidity) controls the cytotoxicty of Aβ 1–40 and 1–42 (Arispe & Doh, 2002), adding further support to this notion that channel activity is critical for cytotoxicty. Aβ 1–42 can also directly induce cytochrome c release from mitochondia, which could be related to its channel-forming activity, alone or in combination with other mitochondrial proteins (Kim et al., 2002).

Kourie has described four distinct channel phenotypes formed by Aβ 1–40 in lipid bilayers (Kourie et al., 2001b). These include a ''bursting'' fast cation channel (63 pS), a ''spikey'' fast cation channel (63 pS), a medium-conductance channel (275 pS), and an inactivating large-conductance channel (0–589 pS). These channel types can be reproducibly distinguished by their kinetic behavior, ion selectivity, and current-voltage relationships. Given the various single-channel conductances reported by Arispe et al. (1993a,b, 1996) and Hirakura et al. (1999), we can safely say that a wide range of Aβ 1–40 channel phenotypes exists. This diversity is consistent with the rapid and variable aggregation of Aβ 1–40 into oligomers of varying sizes. Several structural models for Aβ channels have been proposed (Durell et al., 1994) and interconversion of these models could also account for the heterogeneity of single-channel conductances observed. Recent evidence using pore-blocking peptides favors a model with a β-hairpin lining the pore and a pair of histidine residues that bind Zn^{2+} at the mouth of the pore (Arispe, 2004).

PrP106–126 Channels

Prions are proteinaceous infectious agents that cause neurodegenerative diseases such as Creutzfeld-Jacob in humans, "mad cow" disease in bovines, and scrapie in sheep. These diseases are characterized by spongiform degeneration of brain tissue and deposition of amyloid consisting primarily of fragments of the prion protein (PrP). A key step in the conversion of the normal non-toxic cellular prion protein PrP^{c} to the toxic, infectious scrapie prion (PrP^{sc}) appears to be the transition of α-helical regions of PrP to β-sheet (Pan et al., 1993). Forloni et al. (1993) showed that PrP106–126 was neurotoxic. This peptide is predicted to be α-helical, but in fact forms β-sheet when synthesized and aggregates into amyloid fibrils (Gasset et al., 1992). Lin et al. (1997) showed that PrP106–126 was able to form ion-permeable channels in BLMs. The channels were irreversibly associated with the membrane, had relatively long lifetimes (seconds to minutes), and exhibited a wide range of single-channel conductances (10–400 pS in 0.1 M NaCl). The channels were somewhat non-selective, showing permeability to Na^+, K^+, Cl^- and Ca^{2+} ($P_{Na^+}/P_{Cl^-} = 2.5$). "Aging" of the peptide by letting it stand in solution at room temperature for hours to days (which promotes aggregation and enhances neurotoxicity) enhanced channel activity by as much as 2 orders of magnitude. Acidic pH (4.5) also enhanced channel activity by 50-fold and resulted in a shift of the distribution of single-channel conductances to higher conductance levels. Acidic pH is known to promote the conversion of PrP 106–126 from α-helix to β-sheet (De Gioia et al., 1994).

Although Manunta et al. (2000) were unable to replicate PrP106–126 channel activity or neurotoxicity (perhaps due to differences in PrP106–126 aggregation state), Kourie and Culverson (2000) demonstrated several distinct cation channels formed by PrP106–126 in BLMs. These included: 1) A TEA-sensitive channel with fast kinetics (140 pS); 2) a dithiodipyridin-sensitive channel with slow kinetics (40 pS); 3) a large channel with 5 subconductance levels (900–1500 pS).

Kourie and co-workers went on to show that the antimalarial agent quinacrine could block PrP106–126 channels by reducing mean current through the open channel (Farrelly et al., 2003). They also demonstrated that Cu^{2+} could modulate PrP106–126 channel properties, most likely by binding to M_{109} and H_{111} at the channel mouth, initiating a form of "fast channel block". (Kourie et al., 2003)

Most recently this group has made the noteworthy discovery that a larger PrP fragment, PrP82–146, which forms amyloid fibrils in the brains of patients with Gerstman–Straussler-Scheinker disease (GSS), can form ion channels. They went on to show that scrambling the sequence of the 127–146 region did not affect channel formation, whereas scrambling the sequence of the 106–126 region inhibited channel activity, thus demonstrating the critical nature of the 106–126 region in channel formation. Channel properties of these larger peptides were quite similar to those of PrP106–126. The antibiotic rifampicin, previously shown to decrease aggregation and toxicity of Aβ peptides, was shown to decrease but not eliminate channel activity when added prior to channel formation.

It is intriguing that in one familial form of prion disease, where no amyloid is formed, a mutant form of PrP becomes a transmembrane protein, perhaps causing damage by channel formation (Hegde, Mastrianni & Scott 1998).

Taken together, these studies strongly suggest that channel formation plays a role in the neurotoxicity of prion diseases and support the idea that channel inhibitors or blockers could be used as therapeutic agents.

Islet Amyloid Polypeptide

Islet amyloid polypeptide (IAPP, amylin) is a 37 amino-acid hormone that forms amyloid deposits in the islets of Langerhans of patients with type-II (adult onset) diabetes mellitus. IAPP is believed to have a role counter-regulatory to that of insulin. IAPP is toxic to insulin-producing β-cells and IAPP amyloid deposits correlate inversely with β-cell mass in type-II diabetes (Westermark and Villander, 1978; Lorenzo et al., 1994).

Exposure of IAPP to lipid membranes induces an α-helix to β-sheet transition in IAPP structure (McLean & Balasubramanian, 1992). Mirzabekov et al. (1996) reported that human IAPP could form ion channels in lipid bilayers at cytotoxic concentrations, whereas the non-toxic and non-amyloidogenic rat IAPP (rats do not get diabetes), which differs at 5 amino acids, did not form channels. The IAPP channels could insert into the membrane at positive or negative voltages (relative to the IAPP side, which was taken as ground). Once inserted, channels rapidly opened at negative voltages and rapidly inactivated at positive voltages, with the inactivation fading over the course of minutes. IAPP channels were irreversibly associated with the membrane. The open channel showed a linear *I-V* relation and a unique single-channel conductance of 7.5 pS in 10 mM KCl.

Channel activity was inversely proportional to salt concentration, suggesting that negative surface charge on the membrane played a role in binding the positively charged (net charge = +5) IAPP. This was confirmed by experiments with membranes of varying lipid composition, which showed that channel activity increased with increasing surface

charge and decreased with decreasing membrane fluidity.

Janson et al. (1999) reported that large hIAPP fibrils were not cytotoxic, whereas smaller hIAPP aggregates were cytotoxic. They also showed that these smaller aggregates, but not fibrils, could disrupt BLMs. Light scattering showed these aggregates to contain approximately 25–6,000 IAPP molecules. Anguiano, Nowack & Lansbury (2002) reported that "protofibrillar" IAPP could permeabilize liposomes to Ca^{2+}, while not allowing fura-2 (MW 832) or FITC-Dextran (MW 4,400) to cross the membrane. It has also been reported that IAPP disrupts Ca^{2+} homeostasis (Kawahara et al., 2000). Hirakura et al. (2000) demonstrated that IAPP channel formation, like Aβ1–42 and PrP106–126, could be inhibited by Congo red prior to membrane insertion, and reversibly blocked by Zn^{2+} after channel formation. The consistency of these results indicates that these three amyloid peptides likely are acting in a very similar mechanistic manner.

Atrial Natriuretic Peptide

C-type natriuretic peptide (CNP), atrial natriuretic peptide (ANP), and brain-derived natriuretic peptide (BNP) comprise a family of hormones involved in the regulation of ion and fluid balance. ANP amyloid deposits are found in a large majority of hearts of the elderly, and are thought to play a role in atrial fibrillation and other cardiac disorders (McCarthy and Kasper, 1998). Kourie described channels formed in lipid bilayers from human ANP (1–28) (Kourie et al., 2002), C-type natriuretic peptide 22 and OaC-type natriuretic peptide (18–39) from platypus (Kourie, 1999). ANP channels were cation selective but heterogeneous and could be divided into 3 groups:

1) A Ba^{2+}-sensitive channel with fast kinetics and 3 modes (spike, burst, and open; 68 pS).
2) A large-conductance channel with some subconductance states and time-dependent inactivation (273 pS).
3) A transiently activated channel (160 pS).

CNP channels exhibited weak cation selectivity, large single-channel conductance (546 pS) and high open probability. Channel properties of all these peptides were felt to be consistent with the pathologic effects of these peptides in animal models and human disease. Although these peptides have a known receptor and cGMP-mediated effects on cellular ion-transport systems, the ability of these peptides to directly form ion channels may have physiologic relevance. For instance, it was suggested that ANP channels would tend to hyperpolarize muscle membrane and prevent muscle contraction. The large-conductance channel was postulated to play a role in ANP-induced changes in membrane potential and electrolyte homeostasis.

Serum Amyloid A

Serum amyloid A (SAA) comprises a family of apolipoproteins whose acute phase isoforms increase as much as 1000-fold during states of inflammation or infection. The N-terminal 76 amino acids of SAA are found as amyloid AA fibril deposits in liver, kidney and spleen during chronic inflammatory diseases such as rheumatoid arthritis and chronic infections such as tuberculosis. SAA has also been implicated in atherosclerosis, AD, and cancer (Sipe, 2000).

Hirakura et al. (2002) reported that an acute phase isoform, SAAp, could form ion channels in BLMs at physiologic concentrations. Single-channel conductances were heterogeneous, ranging from 10–1000 pS. The channels were voltage-independent and modestly cation-selective, but were permeable to Ca^{2+} and Cl^{-} as well as monovalent cations. Preincubation with Congo red inhibited channel formation, but had no effect on established channels. Zn^{2+} was able to block reversibly most of the SAA-induced current at a concentration of 100 μM. This group also reported that the acute phase isoform SAA1 could lyse bacterial cells when expressed in *E. coli*. Expression of the constitutive isoform SAA4 did not lyse cells. Sequence comparison showed that the acute phase isoform SAA1 differed from the constitutive phase isoforms SAA4 at 50% of residues, and had significantly greater hydrophobicity in the N-terminal region. These results were reminiscent of the microbial killing properties of other channel-forming toxins such as colicins (Schein et al., 1978), yeast killer toxins (Kagan, 1983), defensins (Kagan et al., 1990), protegins (Sokolov et al., 1999), magainins (Berkowitz, Berms & Zasloff, 1990) and perforins (Liu, Persechini & Young, 1995). It was proposed that SAA's channel activity might play a role in AA amyloidosis pathology and in host defense by SAA during states of infection.

Wang et al. (2003) used electron microscopy (EM) to show that murine SAA2.2 exists as a hexamer with the subunits arranged in a ring surrounding a central channel. The approximate observed pore diameter of 25 Angstroms is consistent with the electrophysiologic findings of large-conductance channels by Hirakura et al. (2002), although it is unclear why other oligomers of SAA were not observed. Perhaps the hexamer has different conformations indistinguishable by EM, corresponding to different conductance levels of the channel.

Alpha-synuclein

Alpha-synuclein (AS) is a synaptic protein that is the precursor of the peptide NAC (AS66–95) found in the amyloid deposits in Lewy bodies in AD and Parkinson's disease (PD). Mutations in AS can lead to familial PD (Baptista, Cookson & Miller, 2004) and suggest that AS may play a role in PD pathogenesis similar to the role of Aβ in AD pathogenesis. Furthermore, NAC is found in the amyloid deposits of AD, suggesting that amyloid deposition may be a pathogenic link between the syndromes of AD and PD, which commonly overlap. For example, AD patients often have motor abnormalities, and PD patients frequently suffer from dementia. Other intermediate syndromes (e.g., dementia with Lewy body disease) suggest that AS may be damaging to neurons other than the dopaminergic cells targeted in PD (McKeith et al., 2004). Volles and Lansbury (2002) showed that AS could permeabilize liposomes in a graded fashion to solutes of varying molecular weight, suggesting a "pore-like" mechanism. Mutations in AS (A30P and A53T) linked to familial PD and known to accelerate the formation of oligomers (protofibrils) enhanced the permeabilizing activity. Lashuel et al. (2002) showed that AS could form annular, pore-like oligomers as viewed by electron microscopy and that PD mutations accelerated the formation of these annular structures. Lashuel et al. (2003) also showed that the disease promoting "arctic" mutation of Aβ also led to formation of annular structures. Taken together, these data indicate that aggregates smaller than fibrils may have a pathogenic role in AD and PD and mutations that cause disease may enhance the likelihood of annular structures, which can permeabilize lipid membranes. Consistent with these findings, Azimova and Kagan (2003) have reported that NAC can form ion-permeable channels in BLMs, and that like other amyloid channels, NAC is inhibited by Congo red, blocked by Zn^{2+} and shows a multiplicity of single-channel conductances. NAC channels were very long-lived and poorly selective amongst physiologic ions.

Beta-2-Microglobulin

Beta-2-Microglobulin (β2M) is a 99-residue immune-system peptide belonging to the MHC Class I complex, which presents antigens to T cells. Since β2M is filtered by the kidney, β2M plasma levels rise 50–100fold during renal failure. Patients with chronic renal failure on hemodialysis usually experience "dialysis-associated amyloidosis" (DAA), in which amyloid deposits of β2M appear in joints, bone and other organs (Drueke, 1998). Renal transplantation appears to lower β2 M levels, prevents amyloid deposit growth, and reduces clinical symptoms such as joint swelling. β2M can induce Ca^{2+} efflux from calvariae, collagenase production, and bone resorption (Brinckehoff et al., 1989; Moe & Sprague, 1992; Peterson & Kang, 1994).

Hirakura and Kagan (2001) demonstrated that β2M could form channels in BLMs at physiologic concentrations. A variety of single-channel conductances were observed from 0.5–120 pS, with a modal conductance of 90 pS (0.1 M KCl). Channel formation was inhibited by preincubation with Congo red, and existing channels could be blocked by Zn^{2+}. Channel lifetime was generally long (seconds). β2M channels were irreversibly associated with the membrane, and showed slight rectification with currents at (trans) positive voltages being larger than those at negative voltages. β2M channels were virtually non-selective between cations and anions ($P_{K^+} = P_{Cl^-}$). Acidic pH enhanced channel activity, suggesting that the uremic/acidotic state in renal failure might enhance the tendency of β2M to form channels in vivo.

Although little is known about the cellular and molecular mechanisms of pathology in DAA, the similarity of β2M channels to other amyloid channels suggests that β2M might be toxic to target cells in joint, bone and other organs. β2M plasma levels rise during infection and inflammation, although not as dramatically as serum amyloid A (SAA, *see* above). This suggests that β2M might play a role in host defense against pathogens.

Polyglutamine

Huntington's (HD) and other "triplet repeat" diseases are caused by an expansion in the genome of the codon CAG coding for glutamine. The expression of tracts of polyglutamine (PG) longer than 37 appears to be responsible for a toxic "gain of function" in the protein huntingtin, the gene product of the autosomal dominant gene causing this neurodegenerative disease. PG length is inversely correlated with age of disease onset (*see* Li & Li, 2004, for review). Classic Huntington's disease is characterized by neuronal destruction in cortex and striatum, but recent evidence implies that neuronal dysfunction such as impaired long-term potentiation (LTP) probably occurs earlier. Formation of amyloid-like intraneuronal aggregates containing mutant huntingtin correlated with disease progression in transgenic mice (RG/2) expressing an expanded PG tract. Toxicity of mutant huntingtin appears to be proportional to PG repeat length. A PC12 cell line expressing PG of length 150 shows increased vulnerability to apoptosis without huntingtin aggregation, indicating that aggregates may not be necessary for cellular dysfunction.

Hirakura et al. (2000) reported channel formation by PG (average MW = 6,000 = 50 residues) in BLMs. Channels were long-lived, relatively

Table 2. Electrophysiologic properties of amyloid peptides

Peptide	Voltage dependence	Single-channel conductance	Ion selectivity (permeability ratio)	Blockade by zinc	Inhibition by Congo Red	Reference
Aβ25–35	Dependent	10–400 pS	Cation (P_K/P_{Cl} = 1.6)	+	+	Mirzabekov et al. (1994)
Aβ1–40	Independent	10–2000 pS	Cation (P_K/P_{Cl} = 1.8)	+	N.D.	Hirakura et al. (1999)
Aβ1–40	Independent	50–4000 pS	Cation (P_K/P_{Cl} = 11.1)	+	N.D.	Arispe et al. (1993a,b, 1996)
Aβ1–42	Independent	10–2000 pS	Cation (P_K/P_{Cl} = 1.8)	+	+	Hirakura et al. (1999)
CT105 (C-terminal fragment of Amyloid Precursor Protein (APP)	Independent	120 pS	Cation	+	+	Kim et al. (1999)
Islet Amyloid Polypeptide (Amylin)	Dependent	7.5 pS	Cation (P_K/P_{Cl} = 1.9)	+	+	Mirzabekov et al. (1996)
PrP106–126	Independent	10–400 pS	Cation (P_K/P_{Cl} = 2.5)	+	+	Lin et al. (1997)
PrP106–126	Independent	140,900, 1444 pS	Cation (P_K/P_{Cl} > 10)	N.D.	N.D.	Kourie and Culverson (2000)
PrP 82–146	Independent		Cation	N.D.	N.D.	Bahadi et al. (2003)
Serum Amyloid A	Independent	10–1000 pS	Cation (P_K/P_{Cl} = 2.9)	+	+	Hirakura et al. (2002)
C-type Natiuretic Peptide	Independent	21,63 pS	Cation (P_K/P_{Cl} > 10)	+	+	Kourie (1999)
B2-Microglobulin	Independent	0.5–120 pS	Non-selective	+	+	Hirakura and Kagan (2001)
Transthyretin	Independent	Variable	Cation (Variable)	+	+	Hirakura et al. (2001)
Polyglutamine	Independent	19–220 pS	Non-selective	–	–	Hirakura et al. (2000)
Polyglutamine	Independent	17 pS	Cation	N.D.	N.D.	Monoi et al. (2000)
NAC (Alpha-Synuclein 65–95)	Independent	10–300 pS		+	+	Azimova and Kagan (2003)

[a]Channels open at negative voltages and close at positive voltages.
[b]Channels close at positive voltages.
N.D., not determined.

non-selective, and heterogeneous with single-channel conductances ranging from 19–220 pS in 0.1 M KCl. Acidic pH enhanced channel formation. Congo red did not inhibit channel formation, and Zn^{2+} did not block channels. The latter two findings distinguish PG from other amyloid channels and may reflect an underlying structural difference in the channels. Huntington's disease is not a classical amyloid disease, and the aggregates of huntingtin observed in neurons are not classical amyloid deposits.

Monoi et al. (2000) reported that PG40 could form cation-selective channels in BLMs. The channel had long lifetimes and a conductance of 17 pS in 1 molal CsCl. PG29 did not form channels, consistent with the finding that PG tracts < 37 do not cause disease in HD. They also proposed a model, the mu-helix for the PG induced channel. The minimum polypeptide length for a membrane-spanning mu-helix is 37 residues, in agreement with the clinical data.

Panov et al. (2002) reported that Huntington's disease mitochondria have decreased membrane potential and depolarize at lower Ca^{2+} levels than control mitochondria. A similar defect was noted in brain mitochondria for transgenic mice expressing *huntingtin* with a pathogenic PG tract. They also used electron microscopy to identify mutant huntingtin on mitochondrial membranes and showed that the mitochondrial defect could be mimicked by a fusion protein with a long PG repeat. These results implicate PG in mitochondrial damage seen in patients and model animals prior to clinical/behavioral/ structural effects. These results are consistent with channel formation by PG in the mitochondrial membrane, leading to decreased membrane potential.

Summary

Channel formation has been described for at least 8 different classes of amyloid peptides and proteins (Table 2). Treatments such as aging and acidic pH, which enhance amyloid formation, also enhance toxicity and channel formation. Inhibitors of amyloid aggregation, such as Congo red, block channel formation and toxicity. The Aβ channel blocker Zn^{2+} also inhibits Aβ toxicity to fibroblasts. Pore-like structures can be observed in EM, and toxic mutants form these structures more readily. The common physiologic properties of amyloid channels (irreversiblity, non-selectivity, large conductance, long lifetimes, heterogeneity of conductance size) suggest they would act as toxic leaks in plasma and/or intracellular membranes, causing disruption of membrane potential and energetic balance. Aβ peptides have also been observed to inhibit LTP. The channel hypothesis provides a molecular mechanism for amyloid pathogenesis and is consistent with recent observations that smaller amyloid aggregates (protofibrils) are likely responsible for amyloid toxicity. Inhibitors of amyloid formation or channel blockers may provide new therapeutic approaches to amyloid diseases and CAG triplet-repeat diseases.

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