Channel Formation by a Neurotoxic Prion Protein Fragment*

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Prions cause neurodegenerative disease in animals and humans. Recently it was shown that a 21-residue fragment of the prion protein (106–126) could be toxic to cultured neurons. We report here that this peptide forms ion-permeable channels in planar lipid bilayer membranes. These channels are freely permeable to common physiological ions, and their formation is significantly enhanced by “aging” and/or low pH. We suggest that channel formation is the cytotoxic mechanism of action of amyloidogenic peptides found in prion-related encephalopathies and other amyloidoses. The channels reported here are large enough and nonselective enough to mediate cell death through discharge of cellular membrane potential, changes in ionic homeostasis, and specifically, influx of calcium, perhaps triggering apoptosis.

Prions are proteinaceous infectious agents that cause transmissible and genetic neurodegenerative diseases, such as scrapie and bovine spongiform encephalopathy in animals and kuru and Creutzfeldt-Jakob disease in humans (for review see Ref. 1). The cellular form of the prion protein (PrPc), 1 which does not cause disease, can be converted into a pathogenic form (PrPSc), and this transition appears to take place without any covalent modifications to the molecule (2). Regions of the prion protein that are predicted to be a-helical actually form b-sheet when synthesized and aggregate into amyloid fibrils similar to those found in prion induced encephalopathies (2). Recent studies have implicated channel formation as a possible pathogenic mechanism of other amyloidoses, such as Alzheimer’s disease (3, 4) and type II diabetes mellitus (5). Previous studies have shown that a 21-amino acid fragment of the prion protein (PrP 106–126; Fig. 1) could be toxic when chronically exposed to primary rat hippocampal cultures at micromolar concentrations (6). Other peptides from the prion protein were not found to be neurotoxic. Amyloid deposition is a frequent but not universal feature of prion-related encephalopathies (1). A major question in prion induced diseases and other amyloidoses has been whether amyloid plays a role in cell death. Recent studies have suggested that the full-length b-amyloid peptide (AB) from Alzheimer’s disease or the neurotoxic fragment AB (25–35) could form ion-permeable channels in lipid bilayer membranes (3, 4). Additionally, we have recently shown that the amyloidogenic peptide amylin, found in the islets of Langherans of patients with type II diabetes mellitus, can also form ion permeable channels at cytotoxic concentrations (5). We therefore set out to examine the effects of the neurotoxic fragment of the prion protein on planar bilayer membranes.

MATERIALS AND METHODS

PrP 106–126 (>95% purity) was purchased from Bachem Bioscience Inc. (King of Prussia, PA), and azolectin (soybean phosphatide extract, granulated, 45% phosphocholine content) was purchased from Avanti Polar Lipids, Inc. (Birmingham, AL). Only a single peak was observed on high pressure liquid chromatography analysis of the peptide in three solvent systems. Lyophilized PrP 106–126 was dissolved in deionized water at a concentration of 2 mg/ml, distributed into 25-ml aliquots, and stored at −20 °C. The peptide was thawed before addition to the lipid bilayer membranes and never frozen again.

Planar Lipid Membrane Experiments—Solvent-free and painted planar lipid bilayer membranes were formed as described previously (3, 7, 8). Usually, after the initial incorporation of PrP 106–126, free peptide in the aqueous solution was washed out. Membranes used in experiments were stable and had conductances of less than 10 pS up to voltages of ±100 mV for a period of at least 10 min prior to peptide addition.

Recording Equipment—Voltage clamp conditions were employed and contact with the aqueous phases was made using Ag/AgCl electrodes with agar salt bridges. Electrode asymmetry was always less than 1 mV. Membrane verification was performed by monitoring membrane capacitance and resistance. Data were digitized and stored on VHS tape and played back for later analysis. An Axopatch 1C amplifier with head stage CV-3B was used for measuring membrane current. For data acquisition, a digital tape recorder and video cassette recorder allowed recording of large amounts of data. A storage oscilloscope was used for monitoring membrane capacitance and single-channel recordings. The cis-solution (peptide containing) was taken as the virtual ground, and the sign of the membrane voltage corresponded to the trans-side of the membrane.

RESULTS AND DISCUSSION

At neutral pH, PrP 106–126 induced a conductance in solvent containing membranes when added to concentrations greater than or equal to 20 μM (Fig. 2A). The induced conductance was voltage-independent (Fig. 2B) and was due to the formation of ion-permeable channels (Fig. 3A). Several single channel conductances were observed, including the most common conductances of 20, 40, and 60 pS (Fig. 3B). In a 10-fold gradient of sodium chloride across the membrane, the reversal potential was 15 millivolts (dilute side positive), indicating that the channels were permeable both to sodium and chloride (PNa/PCl approximately equals 2.5). Other selectivity measurements

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1 The abbreviations used are: PrPc, cellular form of the prion protein; PrPSc, pathogenic form of the prion protein PrP, prion protein; pS, picosiemens.

FIG. 1. The amino acid sequence of PrP 106–126. Positively charged residues are shown in boldface type. The peptide has a net positive charge of 2 at pH greater than 7 and a net positive charge of 3 at pH less than 6 due to the ionization of histidine around pH 6.5. The rest of the peptide is remarkably hydrophobic.
Prion Channels

Aging of the prion peptide dramatically enhanced its channel formation activity. Thus, PrP 106–126 aged for 9 days at room temperature in 100 mM NaCl could increase membrane conductance at concentrations as low as 0.1 μM, indicating an activity increase of approximately 200-fold. For peptide aged for 3 days, activity was increased by approximately 20-fold. Single channels were also formed by this aged prion peptide, although there were some slight differences in the distribution of single channel conductances observed (Fig. 4).

Channel activity could also be enhanced by acidic pH. At pH 4 indicated that virtually all common physiologic ions were permeable through the channel in the sequence Ca2+ > Na+ > K+ > Li+ > Rb+ > Cs+ > Cl−.

FIG. 3. Single channel currents induced by PrP 106–126 at neutral pH. A, current due to single channels in a membrane induced by PrP 106–126 at a final concentration of 20 μM is shown. Salt solutions contain 100 mM NaCl, 1 mM MgCl2, 5 mM HEPES adjusted to pH 7.5. The membrane was formed from azolectin. The membrane voltage was held constant at +50 mV throughout this recording. Note that several different sizes of current jumps can be seen in this recording reflecting the heterogeneity of channels induced by PrP 106–126. This may reflect different states of aggregation of the peptide and/or different conformations within the membrane. B, histogram of prion 106–126 single channel conductances at pH 7.5. A histogram of single channel conductances induced by PrP 106–126 in 100 mM NaCl, pH 7.5 is shown. Note the three prominent peaks at approximately 20, 40, and 60 pS. The 40 and 60 pS channel could potentially represent aggregates of the 20 pS channels.
4.5 to 5.0, PrP 106–126 could induce channel activity at a concentration of 1 μM (Fig. 5). Single channel conductances of 20, 40, and 60 pS were observed most frequently (Fig. 5B). These conductances indicate that the 10 pS channel or even a lower conductance channel might be the unitary channel from which larger aggregates are formed.

Channels were irreversibly associated with the membrane. Extensive washing out of the aqueous compartment containing peptide had no effect on the peptide-induced conductance.

The channels reported here are clearly due to the PrP 106–126 itself. Under the conditions of our experiments, channels were not observed in the absence of added prion peptides. The channels formed rapidly upon peptide addition to the chamber and did so at concentrations comparable with those required for neurotoxicity (6). Furthermore, the concentrations of PrP 106–126 used are comparable with the concentrations needed for channel formation by other peptide channel formers (3–5, 7–9). We suggest that channel formation accounts for the neurotoxicity of PrP 106–126 and may account for the neuronal loss observed in prion-related encephalopathies. The relative lack of ionic selectivity of these channels suggests that they would impose a substantial leakage permeability upon the cell membrane. Neurons in particular would be highly vulnerable to a leakage conductance of this kind, because they must maintain a relatively tight plasma membrane to preserve electrical signaling along that membrane. Thus, inward leakage of sodium and calcium as well as outward leakage of potassium would place a severe metabolic load on the cell and also subject it to potential ionic toxicity. Furthermore, these ionic disturbances might potentially trigger apoptosis in cells. Alternately, this leakage conductance could lead to depolarization, which might trigger disturbances via influxes of calcium through voltage-dependent calcium channels or NMDA receptors (10, 11). Calcium influx might also be triggered through influx of sodium followed by sodium/calcium exchange. The voltage independence of the conductance induced by these channels suggests that they would be permanently and stably open in the membrane. The reversibility of channel formation means that these channels would continue to impose a metabolic strain on cells once inserted.

The enhancement of activity seen in an acid environment suggests that aggregation of the peptide contributes to channel formation. A similar conclusion may be inferred from our results with aging of prion peptides. It is unclear whether this aggregation in vivo might occur in an acidic endosomal compartment. Acid pH has also been shown to convert PrP 106–126 from α-helical to β-sheet conformation (12). This might promote peptide aggregation, amyloid formation, and channel formation. It has also been observed that certain peptides from the prion proteins, such as PrP 109–122, can induce the α-helix to β-sheet transition in other peptides from the prion protein (13). This might be a model for the conversion of PrPc to PrPsc in vivo. It has been noted that PrPsc aggregates on its own, and it has been suggested that PrPc is converted to PrPsc most likely in the endosomal pathway (15).

A significant implication of our findings is that there may be a close relationship between amyloid formation, cytotoxicity, and channel formation. We suggest that the β-sheet structures...
assumed by amyloid forming peptides may also be conducive to channel formation under the appropriate conditions. This may lead to disruptions of ionic homeostasis and even to cell death. The fact that small β-sheet-forming peptides from three different amyloid producing diseases can be cytotoxic and form similar ion permeable channels at cytotoxic concentrations, strongly suggests that channel formation may be an important cause of cytotoxicity in amyloidoses. Further studies will be required to verify this hypothesis. Screening the channels formed for drugs capable of blocking them might provide a means of searching for candidate therapies to ameliorate the damage done in these amyloidoses.

REFERENCES
1. Prusiner, S., and de Armond, J., (1994) Annu. Rev. Neurosci. 17, 311–339
2. Gasset, M., Baldwin, M. A., Lloyd, D. H., Gabbiel, J.-M., Holtzman, D., Cohen, F., Fletterick, R., and Prusiner, B. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 10940–10944
3. Arispe, N., Rojas, E., and Pollard, M. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 567–571
4. Mirzabekov, T., Lin, M.-C., Yuan, W. L., Marshall, P., Carman, M. Tomaselli, K., Lieberburg, I., and Kagan, B. L. (1994) Biochem. Biophys. Res. Commun. 202, 1142–1148
5. Mirzabekov, T., Lin, M.-C., and Kagan, B. L., (1996) J. Biol. Chem. 271, 1988–1992
6. Forloni, G., Angeretti, N., Chiesa, R., Monzani, E., Salmona, M., Bugiani, O., and Tagliavini, F., (1993) Nature 362, 543–546
7. Kagan, B. L., Baldwin, R. L., Munoz, D., and Wisnieski, B. (1992) Science 255, 1427–1429
8. Kagan, B. L., and Sokolov, Y. (1994) Methods Enzymol. 235, 699–713
9. Kagan, B. L., Selsted, M. D., Ganz, T., and Lehrer, R. I. (1996) Proc. Natl. Acad. Sci. U. S. A. 87, 210–214
10. Farthrop, D. J., Bodeis, A. R., and Davies, D. S. (1991) Arch. Toxicol. 65, 437–444
11. Orrenius, S., McCabe, M. J., Jr., and Nicotein, P. (1992) Toxicol. Lett. 64/65, 357–364
12. De Gioia, L., Selvaggini, C., Ghibaudi, E., Diemede, L., Bugiani, O., Florloni, Gianluigi, Tagliavinis, F., and Salmona, M. (1994) J. Biol. Chem. 269, 7859–7862
13. Nguyen, J., Baldwin, M. A., Cohen, E., and Prusiner, S. (1995) Biochemistry 34, 4186–4192
14. Laszlo, L., Lowe, J., Self, T., Kenward, N., Landon, M., McBride, T., Farquhar, C., McConnel, I., Brown, J., Hope, J., and Mayers, J. (1992) J. Pathol. 166, 335–341
15. Pike, C. S., Walencwicz, A. S., Glabe, C. G., and Cotman, C. W. (1991) Brain Res. 563, 311–314
