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Membrane perturbation effects of peptides derived from the N-termini of unprocessed prion proteins

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Abstract

Peptides derived from the unprocessed N-termini of mouse and bovine prion proteins (mPrPp and bPrPp, respectively), comprising hydrophobic signal sequences followed by charged domains (KKRPKP), function as cell-penetrating peptides (CPPs) with live cells, concomitantly causing toxicity. Using steady-state fluorescence techniques, including calcein leakage and polarization of a membrane probe (diphenylhexatriene, DPH), as well as circular dichroism, we studied the membrane interactions of the peptides with large unilamellar phospholipid vesicles (LUVs), generally with a 30% negative surface charged density, comparing the effects with those of the CPP penetratin (pAntp) and the pore-forming peptide melittin. The prion peptides caused significant calcein leakage from LUVs concomitant with increased membrane ordering. Fluorescence correlation spectroscopy (FCS) studies of either rhodamine-entrapping (REVs) or rhodamine-labeled (RLVs) vesicles, showed that addition of the prion peptides resulted in significant release of rhodamine from the REVs without affecting the overall integrity of the RLVs. The membrane leakage effects due to the peptides had the following order of potency: melittin > mPrPp > bPrPp > pAntp. The membrane perturbation effects of the N-terminal prion peptides suggest that they form transient pores (similar to melittin) causing toxicity in parallel with their cellular trafficking.

Keywords: Prion protein; Phospholipid vesicle; Membrane perturbation; Fluorescence; FCS; CD

1. Introduction

Prion diseases, also known as transmissible spongiform encephalopathies, are fatal neurological disorders of humans and animals that appear in sporadic, familial and infectiously acquired forms. These disorders are caused by conversion of a normal neuronal glycoprotein (PrP^C) into an infectious, conformationally altered isoform (PrP^{Sc}) [1–3].

The conversion of PrP^C into PrP^{Sc} occurs via a post-translational process [4]. PrP^C is monomeric and readily digested by proteinase K, whereas PrP^{Sc} forms insoluble aggregates and shows a high resistance to proteolytic digestion [5]. The characteristics of the two forms of the prion protein (PrP) can be related to their differences in secondary structure. PrP^C adopts a predominantly α-helical structure in its globular C-terminal half, and its N-terminus is largely unstructured [6], whereas PrP^{Sc} has a large content of β-sheet secondary structure [7].

Abbreviations: PrP, prion protein; PrP^C, cellular isoform of PrP; PrP^{Sc}, scrapie isoform of PrP; mPrPp, peptide with sequence corresponding to the N-terminus of the mouse prion protein, residues 1–28; bPrPp, peptide with sequence corresponding to the N-terminus of the bovine prion protein, residues 1–30; NLS, nuclear localization sequence; CPP, cell-penetrating peptide; pAntp, penetratin, Antennapedia homeodomain-derived CPP; ER, endoplasmic reticulum; DHPC, 1,2-dihexanoyl-*sn*-glycero-3-phosphatidylcholine; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; POPG, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol; POPG/POPC, [30/70] notation refers to vesicles with 30 mol% POPG content; SUVs, small unilamellar vesicles; LUVs, large unilamellar vesicles; Rh, rhodamine; REVs, Rh-entrapping LUVs; RLVs, Rh-labeled LUVs; FCS, fluorescence correlation spectroscopy; CD, circular dichroism; DPH, diphenylhexatriene, membrane bound fluorescence probe; P/L, total peptide-to-phospholipid molar ratio

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PrP^C is unusual in that it can adopt multiple membrane topologies during biogenesis at the ER membrane [8–12]. Most PrP^C molecules are fully translocated into the lumen of the ER; this form, denoted ^{Sec}PrP, is eventually attached to the outer leaflet of the plasma membrane through a C-terminal glycosylphosphatidylinositol (GPI) anchor. Some PrP^C molecules assume transmembrane orientations. These forms, designated ^{Ctm}PrP and ^{Ntm}PrP, span the ER lipid bilayer once, either with the C- or N-terminus, respectively, on the lumenal side of the ER.

The signal sequence of ^{Sec}PrP is normally cleaved by a signal peptidase that acts in the lumen of the ER. However, in a recent study, it has been shown that in neuronal cells, the ^{Sec}PrP form retains its N-terminal signal sequence following its exit from the ER and during its trafficking to the cell surface [11]. It has also been reported that ^{Ctm}PrP is unprocessed and contains an uncleaved N-terminal signal peptide [13]. The signal sequence appears to have an unusual role in prion proteins in that it has two separate functions, both targeting and topogenesis [13–15]. It has also been suggested that ^{Ctm}PrP is associated with the neurodegeneration observed in prion disease [9]. Another variant of PrP discussed as a neurotoxic intermediate is cytosolic PrP, ^{Cyt}Pr, which also sometimes retains the signal peptide [16–18]. Hence, even if the situations where unprocessed prion proteins appear may be relatively rare, these situations may be important for a pathological process.

Previously, we have investigated certain properties of the N-termini of the unprocessed mouse (residues 1–28) and bovine (residues 1–30) PrPs, denoted mPrPp [19] and bPrPp [20], respectively. These sequences (Table 1) comprise the signal peptide (residues 1–22 for mPrPp, residues 1–24 for bPrPp) and an identical and highly positively charged, NLS-like, sequence (residues 23–28 for mPrPp, residues 25–30 for bPrPp). The mPrPp and bPrPp sequences are similar to those of certain chimeric cell-penetrating peptides (CPPs), and we found that mPrPp and bPrPp can indeed function as CPPs ([21], and Magzoub et al., unpublished results). CPPs are able to translocate into various cells, carrying a conjugated hydrophilic macromolecular 'cargo' [21,22]. Recent observations on CPP entry into cells emphasize the role of heparan sulphate as a mediator of raft-dependent macropinocytosis, a particular form of endocytosis [23,24]. Cellular heparan sulphate has also been shown to interact with prion proteins [25], and PrP^{Sc} incorporation into CHO cells requires glycosaminoglycan expression [26]. The interaction of various forms of PrP with model membranes have also been investigated [27,28].

In the present study, we have studied the interactions of mPrPp and bPrPp with large unilamellar phospholipids vesicles (LUVs) of varying surface charge densities, using fluorescence and CD spectroscopic methods. The membrane interaction effects of the two prion peptides were compared with those of two well-characterized peptides: the CPP penetratin (pAntp), and the pore-forming peptide melittin (Table 1). The steady-state fluorescence studies were complemented by fluorescence correlation spectroscopy (FCS) studies. FCS gives information on the translational diffusion of fluorescent particles with high specificity [29].

2. Materials and methods

2.1. Materials

The prion peptides and pAntp were produced by Neosystem Laboratoire, Strasbourg. Peptides were used as purchased. The identity and purity were controlled by amino acid, mass spectral and HPLC analyses. Peptides were of Immunograde quality (purity estimated at ~80%). In each case, peptides from more than one batch were used. Melittin from the venom of honeybee (*Apis mellifera*) was obtained from Sigma. 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC), and 1-palmitoyl-2-oleoyl-phosphatidylglycerol (POPG), were purchased from Avanti Polar Lipids, Alabaster, of the best quality, and were used without further purification. Diphenylhexatriene (DPH) was obtained from Sigma. Rh (tetramethylrhodamine-5,6-isothiocyanate) and Rh-PE (6-tetramethylrhodamine-1,2-dihexadecanoyl-3-phosphoethanolamine) were purchased from Molecular Probes, The Netherlands. Calcein, a fluorescein derivative, was also purchased from Molecular Probes (product no. C-481).

2.2. Determination of peptide concentrations

After weighing on a microbalance, the peptide concentrations in the stock solutions were determined by light absorption on a CARY 4 Spectrophotometer, using quartz cuvettes with a 1-cm light path. All spectra were baseline corrected. Molar absorptivities of 5690 and 1280 M^{-1} cm^{-1}, at 280 nm, for tryptophan and tyrosine, respectively, were applied.

2.3. Sample preparations

2.3.1. Preparation of LUVs

Large unilamellar vesicles were prepared by initially dissolving the phospholipids at the desired concentration (with the chosen POPG/POPC molar ratio) in a chloroform/ethanol mixture, to ensure complete mixing of the components, and then removing the solvent by placing the sample in a high vacuum for 3 h. The dried lipids were dispersed in 50 mM potassium phosphate buffer (pH 7.4). The dispersion was run through a freeze–thaw cycle five times and then passed through two polycarbonate filters (0.1 μm pore size) 20 times in an Avanti manual extruder.

2.3.2. Preparation of REVs and RLVs

To prepare the Rh-entrapping LUVs (REVs), prior to extrusion, the dried lipid film (prepared as described above) was dispersed in 50 mM potassium

Table 1
The origin and amino acid sequences of the four peptides studied[a]

Peptide	Origin	Sequence	Net charge	Hydrophobicity[b]
pAntp	Homeodomain (*Drosophila*)	RQIKIWFQNRRMKWKK	+7	−1.7
mPrPp	N-terminus of mouse prion protein (residues 1 to 28)	MANLGYWLLALFVTMWTDVGLC KKRPKP	+3	0.3
bPrPp	N-terminus of bovine prion protein (residues 1 to 30)	MVKSKIGSWILVLFVAMWSDVGLCKKRPKP	+5	0.4
Melittin	Honeybee venom (*Apis mellifera*)	GIGAVLKVLTTGLPALISWIKRKRQQ	+5	0.3

[a] Given are also the net charges of the peptides, as well as their average hydrophobicities. The charged residues are underlined.
[b] The average hydrophobicity of the peptides is calculated using the scale devised by Kyte and Doolittle [60].