INTERACTIONS BETWEEN THE CONSERVED HYDROPHOBIC REGION OF THE PRION PROTEIN AND DODECYLPHOSPHOCHOLINE MICELLES

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The three dimensional structure of PrP110-136, a peptide encompassing the conserved hydrophobic region (CHR) of the human prion protein, has been determined at high resolution in dodecylphosphocholine (DPC) micelles by NMR. The results support the conclusion that the CmPrP, a transmembrane form of the prion protein, adopts a different conformation than the reported structures of the normal prion protein determined in solution. Paramagnetic relaxation enhancement studies with gadolinium-DTPA indicated that the CHR peptide is not inserted symmetrically in the micelle, thus suggesting the presence of a guanidium-phosphate ion pair involving the side-chain of the terminal arginine and the detergent headgroup. Titration of DPC into a solution of PrP110-136 revealed the presence of a surface bound species. In addition, paramagnetic probes located the surface-bound peptide somewhere below the micelle-water interface when using the inserted helix as a positional reference. This localization of the unknown population would allow a similar ion pair interaction.

A number of neurodegenerative diseases, such as Alzheimer’s Disease, Huntington’s Disease, and prion diseases are associated with the presence of amyloid deposits in brain tissues resulting from the misfolding of a specific protein. Some prion diseases have a unique feature that sets them apart; they are transmissible, and are therefore given the name of transmissible spongiform encephalopathies. The infectious agent is a misfolded form of the protein associated with the disease, the prion protein. Prion diseases, including Creutzfeldt-Jakob Disease and Gerstmann-Sträusler-Scheinker syndrome (GSS) are classified in three categories: sporadic (with no known environmental sources), familial (associated with mutations of the prion protein) and transmitted (from known environmental sources)1.

The prion protein is a cell surface glycoprotein anchored to the membrane via a glycosylphosphatidylinositol anchor. The mature protein in humans contains 208 amino acids. The N-terminal half of the protein is unstructured in solution and contains eight octarepeat regions and a highly conserved region (residues 112-128) referred to as the conserved hydrophobic region (CHR). The other half of the polypeptide is a folded globular domain composed of a short β-sheet and three α-helices in which the last two helices are linked via a disulfide bridge2. Although a wealth of information has been assembled to shed light on the involvement of the prion protein with prion diseases, little is known about the biological function of the normally folded, cellular form of the prion protein, PrPC3. Recently, this protein has been shown to be essential for the long-term integrity of peripheral myelin sheaths4.

Prion diseases are associated with a conformational change from the normal cellular form PrPC, which is mainly α-helical5,6, to a β-rich conformation, denoted PrPSc, with all the characteristics of amyloidal material. The latter is highly insoluble and resistant to degradation by proteinase K. While amyloid deposits are observed in neuronal tissues for all prion diseases, it is not clear whether they are responsible for pathogenicity. Nevertheless, understanding how prions undergo conformational changes may help in understanding the mechanism of infectivity (transmissibility).

A number of studies have focused their attention on the possible effects of mutations on the stability of
the folded domain of PrP<sup>C</sup> and its sensitivity to conversion to PrP<sup>Sc</sup><sup>7</sup>. Chesebro and coworkers have shown that the deletion of residues between residues 108 and 124 impacts PrP<sup>Sc</sup> formation<sup>8</sup>. Furthermore, the presence of the CHR domain is required for the conversion of recombinant PrP<sup>C</sup> to PrP<sup>Sc</sup> in the presence of phospholipid bicelles<sup>9</sup>. The authors of the latter study suggested that electrostatic interactions between the bilayer surface and the protein play an important role in the conversion of PrP<sup>C</sup>. Work from Wang and co-workers strengthens this suggestion. They showed that infectious prions were obtained from mixing recombinant PrP with total liver RNA and 1-palmitoyl-2-oleoyl-phosphatidylglycerol (POPG) lipid molecules<sup>10</sup>. Thus, it is reasonable to consider whether membrane-surface interactions with PrP may lower the energy barrier of PrP<sup>C</sup> to PrP<sup>Sc</sup> and facilitate this spontaneous conversion.

In addition to membrane-surface interactions, the presence of a transmembrane form of the prion protein, denoted C<sup>mp</sup>PrP, has been associated with certain types of TSE diseases. The C<sup>mp</sup>PrP form was discovered during studies of PrP translocation in the endoplasmic reticulum (ER)<sup>11,12</sup>. This form results from an incomplete translocation of the polypeptide in which residues ~112-135 span the membrane bilayer. Normally, C<sup>mp</sup>PrP is present in small amounts (less than 2%) and is probably disposed of via the lysosomal degradation pathway<sup>13</sup>. Mutations that increase the hydrophobicity of the CHR domain (such as A117V associated with the GSS syndrome, or artificially made K110I, H111I and 3AV<sup>12,14</sup>) cause neurodegeneration when expressed in transgenic mice. From these observations, it has been proposed that, in some TSE diseases, C<sup>mp</sup>PrP might be the neurotoxic species. During infection, the conversion of PrP<sup>C</sup> to PrP<sup>Sc</sup> may deplete the pool of available PrP<sup>C</sup> therefore stressing its biosynthesis and leading to a higher level of C<sup>mp</sup>PrP, thus causing neurodegeneration<sup>15</sup>.

The aforementioned observations are, among others, indications that membrane-CHR domain interactions are multifaceted and modulate the involvement of the prion protein in the disease. Several studies using circular dichroism and NMR<sup>15,16,17,18</sup> have taken a close look at these interactions in structural terms, but an atomic-level (high-resolution) description is not yet available. From these reports, Hornemann <i>et al.</i><sup>18</sup> have studied the interactions between DPC and the mouse prion protein (mPrP-(90-231)) and disease-related mutants. The data showed little or no interaction between the wild-type and the detergent while the mutants showed weak interactions. This study suggests that prion-membrane interactions may be held up by the folded domain. In fact, the two mutations that greatly enhanced hydrophobicity (KH->II and 3AV), and showed a higher affinity for DPC, precipitated at relatively low DPC concentrations thus preventing a complete characterization of the interactions at play.

Glover and co-workers<sup>17</sup> studied a peptide which includes residues 110-136 corresponding to the CHR domain and the first secondary structure element of the folded domain (a four residue β-strand in the human protein). Their results suggested that the peptide may adopt an alpha helical conformation spanning the bilayer when dissolved in lipid biceles (made with a 3:1 mixture of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and 1,2-dicaproyl-sn-glycero-3-phosphocholine (DHPC)). In this paper, we describe the determination of the three-dimensional structure of the prion peptide (110-136) in dodecylphosphocholine (DPC) micelles at high-resolution using NMR techniques in solution. Peptide uniformly labeled with stable isotopes (15N and 13C) was produced in <i>Escherichia coli</i> as a fusion partner with glutathione-S-transferase (GST) to allow easy isolation and purification. Peptide localization with respect to the micelle was determined using paramagnetic relaxation enhancement experiments in a similar fashion as described by Zangger<sup>19</sup>. Peptide-micelle surface interactions were probed by titration of PrP(110-136) solutions with DPC.

**Experimental Procedures**

<i>Protein expression and purification- </i>The plasmid pET19b-GST-TEV-PrP(110-136) was used for expression of the CHR of the prion protein. Four oligonucleotides (Invitrogen) (FW 1-5' GATCCGAGAACCCTTATTTT CAGGGTAAC ACATGCGAG TGCCCGCGCA GCAGG, FW 2-5'-AGCACTGGTA GGGGGT GAGCAGAACCCTTATTTT CAGGGTAAC ACATGCGAG TGCCCGCGCA GCAGG)
The DNA sequence was codon optimized for expression in *E. coli*. The synthetic DNA was inserted into the pET19b-GST expression vector at the BamHI/XhoI restriction site. This vector is a modified version of the pET19b vector (Novagen) where a glutathione S-transferase fusion partner has been incorporated into the multiple cloning site. Mutants K110E and Δ110 were obtained by site-directed mutagenesis (Stratagene). Expression of the 15N-PrP(110-136) and 13C,15N-PrP(110-136) peptides was carried out by incubating *E. coli* BL21(DE3) (Stratagene) cells harbouring the pET19b-GST-TEV-PrP(110-136) plasmid in minimal media (M9) at 37°C using 15N-enriched ammonium chloride and 13C-glucose as sole sources of nitrogen and carbon, respectively. Protein expression was induced by the addition of isopropyl thio-D-galactopyranoside (IPTG) at an OD600 of 0.8 and the temperature was lowered to 30°C. Cells were harvested 3 h post-induction by centrifugation and frozen at -80°C. Purification of the fusion protein was carried out by resuspending the cell pellets corresponding to a 1 L culture in 50 mL of lysis buffer (10 mM Tris-HCl, 100 mM sodium phosphate, 50 mM beta-mercaptoethanol, 50 mM MgCl2, 1 tablet of Complete™ Protease Inhibitors (Roche), 20 mg/ml lysozyme and 50 μg/ml DNase at pH 8.0) on ice. The cells were disrupted by sonication on ice using a 400 W Branson sonifier (ThermoFisher) or by French Press. After lysis, cell debris were removed by centrifugation. Three cycles of cell disruption-centrifugation were carried out. Supernatants were pooled and added to a slurry of Ni-NTA resin (Qiagen) (15 mL resin) and gently stirred at room temperature for 60 minutes before loading into a column. The column was washed with three column volumes of Buffer B (10 mM Tris-HCl, 100 mM sodium phosphate pH 8.0) followed by three column volumes of Buffer B + 60 mM imidazole to remove non-specifically bound material. The protein was eluted off the column with Buffer B + 250 mM imidazole. Protein quantification was carried out by UV absorption at 280 nm using the calculated molecular mass of the fusion protein of 32 403 g/mol and the theoretical molar extinction coefficient of 45 480 M⁻¹ cm⁻¹ calculated by the ProtParam utility. Typical yield was ~7 mg fusion protein per litre of culture. Prior to cleavage of the fusion partner, buffer was exchanged by dialysis with TEV protease cleavage buffer (50 mM Tris-HCl pH 8.0, 0.5 mM EDTA and 1mM dithiothreitol (DTT)). Cleavage of the peptide from the fusion partner was carried out using 10 U of AcTEV (Invitrogen) per milligram of fusion protein during 3 hours at 30°C. The reaction was monitored by SDS-PAGE. As soon as cleavage of the fusion protein reached about 80% completion, HPLC purification was immediately carried out to maximize peptide recovery. Use of AcTEV protease results in an extra glycine residue at the N-terminal: gly-KHMAGAAAAGAVGGLGGYMLGSAMSR.

NMR spectroscopy- The 13C, 15N huPrP110-136 NMR sample was obtained by dissolving 1.5 mg of doubly labelled peptide in NMR buffer (10 mM sodium phosphate, 1 mM 3-(trimethylsilyl)-1-propanesulfonic acid sodium (DSS) and 5% deuterium oxide). Next, 14 mg of DPC was added and pH was adjusted with 1N NaOH to 7.6 in a final
volume of 0.5 mL. NMR measurements were performed at 37°C on an AVANCE III 600 MHz spectrometer equipped with a triple resonance cryogenic probehead (Bruker, Milton, ON). Resonance assignment of the backbone atoms was obtained from 3D-HNCA, 3D-HNCACB, 3D-CBCA(CO)NH, 3D-HNHA, 3D-HBHA(CO)N, 3D-HN(CA)CO and 3D-HNCO experiments. Side-chain resonances were assigned using 3D-HCCH-TOCSY, 3D-HCCH-COSY, 15N- and 13C-edited NOESY HSQC experiments recorded with mixing times of 50 and 150 ms. All spectra were referenced relative to DSS and they were processed using NMRPipe and analyzed with NMRview.

DPC titration and paramagnetic relaxation enhancement (PRE) measurements- In order to test if the peptide inserts itself into DPC micelles, 10 µL aliquots from a stock solution of 280 mg/ml of DPC in the NMR buffer were added to either a 1 mM or 2 mM 15N-labelled peptide solution until a final amount of 14 mg DPC was obtained. After each addition, a 2D-HSQC spectrum was collected comprised of 64 FIDs with 1024 complex points and 16 scans per FID for a total acquisition time of 20 minutes. PRE experiments were carried out by addition of gadolinium diethylenetriaminepentaacetic acid (Gd-DTPA) to 15N PrP(110-136) inserted into DPC micelles. Additions of the corresponding relaxation agent were carried out on NMR samples containing 1mM of the labelled peptide with 14 mg of DPC at a volume of 500 µL. Effects of dilution were accounted for in plots of normalized intensity versus concentration of the paramagnetic agent.

Structure calculation- Structure calculations were performed using CYANA 2.1. Most of the NOEs were assigned manually and calibrated within CYANA. Backbone torsion angles were derived from chemical shifts using TALOS+. In addition, calculations were also performed with 22 hydrogen bond constraints inferred from the sequential short and medium range NOE pattern (see Table 1). Structure validation was performed using the Protein Structure Validation Software suite version 1.4 (PSVS) from the Northeast Structural Genomics Consortium (NESG, http://www.nesg.org). No further refinement was necessary in order to obtain a well-resolved NMR ensemble of structures. The protein chemical shifts and NMR structure ensemble were deposited in the Biological Magnetic Resonance Data Bank (BMRB, http://www.bmrb.wisc.edu) and the Research Collaboratory for Structural Bioinformatics (RCSB, http://www.pdb.org) under the accession numbers 17558 and 2LBG, respectively.

RESULTS

Studies of peptides that bind to membrane often face challenges in peptide preparation and isolation. The strategy used to circumvent the poor solubility of these peptides consists of producing the target peptide fused to a highly soluble partner such as GST. The fusion protein is then isolated using affinity purification chromatography. In addition, this approach facilitates the incorporation of nuclei such as nitrogen-15 and carbon-13 which are required to apply multidimensional NMR techniques. Unlike most membrane-binding peptides or proteins, the PrP(110-136) peptide is soluble in water. One would expect that this property should greatly facilitate the isolation of the peptide after it is cleaved from the fusion partner. However, the propensity of PrP(110-136) to form insoluble matter (probably amyloid material) under certain conditions led to very low yields when the process was scaled up in an attempt to isolate the milligram amounts required for structure determination. The formation of insoluble material however could be avoided by removing solvents in a two-step procedure as described in the methods section.

The NMR spectrum of resuspended PrP(110-136) in water (pH ~5.0) shows all features of an unfolded peptide (Supplemental Figure S1A). At pH 7.5, most resonances on a 2D-'H,15N-HSQC have disappeared indicating the fast chemical exchanges of the amide hydrogen with the bulk water that typifies unfolded peptides (Supplemental Figure S1B). The addition of lipids or detergents modifies the spectroscopic signature of the peptide. Initially, we considered using DMPC/DHPC bicelles as described by Glover and co-workers in their NMR study of a synthetic CHR peptide labeled with 15N-glycines. Within certain ratios, mixtures of these lipids form discoidal particles that closely mimic membrane bilayers, while allowing data collection of high-resolution NMR spectra for structure determination. At first, we

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dissolved our uniformly labelled peptide in DMPC/DHPC bicelles as described by those authors, and we were able to collect NMR spectra with the same spectroscopic features reported for the labelled glycines (Supplemental Figure S2). Unfortunately, in our hands samples had a short half-life (in the order of hours), which precluded data collection of triple resonance experiments. When DPC was substituted for bicelles, a similar resonance pattern was observed for glycines suggesting that this detergent could provide a suitable membrane-like environment, with the extra advantage of highly stable samples (half-life > year).

The three-dimensional structure of the CHR peptide was determined in DPC micelles using the standard NMR approach. The assigned HSQC of the peptide in DPC (see Figure 1) shows that residues 110-112 are not observed while residues S135 and R136 each have two resonances attributed to them. Analysis of the backbone chemical shift with TALOS+25, and the pattern of NOE interactions between backbone residues (Supplemental Figure S3) indicate that the peptide adopts an alpha helical conformation. Figure 2A shows an ensemble of the twenty lowest energy conformers calculated from NOE-based distance constraints and chemical shift derived torsion angles. The CHR adopts a curved alpha helical conformation in the presence of DPC micelles. Examination of the structure reveals that glycines and short-chain amino acids are lining the concave side of the curved helix while larger hydrophobic side-chains of valine and leucine residues are located on the convex side (Figure 2B). Suspecting that this curved helix may result from the lack of hydrogen-bond constraints, we attempted, without success, to measure coupling constants through hydrogen bonds in order to supplement the calculations with these constraints. Nevertheless, rounds of structure calculations using hydrogen bond constraints produced a straight helix but led to the violation of over 10 well-resolved NOE-derived distance constraints.

The next step in our study was to determine whether the peptide was spanning the micelle or if it was interacting with the surface. Localization of the peptide was determined via titration of PrP(110-136) samples in DPC with complexed paramagnetic cations. In a parallel experiment, we titrated a sample of PrP(110-136) in buffer with a solution of DPC in order to detect the presence of surface interactions, or whether peptide insertion could occur in the presence of pre-formed micelles. This experiment was designed to help rule out the possibility that the conformational behaviour of the peptide (i.e. the structure obtained) was ‘forced’ as an artificial result of the procedure used for making the NMR sample, i.e. the addition of solid DPC directly into a solution of the peptide in buffer.

Titration with DPC suggests surface interactions: Addition of aliquots from a concentrated solution of DPC into a 1mM 15N-CHR peptide at pH 7.6 was carried out by making sure that the first addition yielded a concentration of DPC (14 mM) in the sample that is well above the critical micelle concentration (CMC is 1.1 mM) of DPC in pure water29,30. Measurement of the CMC of DPC using 31P-NMR showed that the chemical shift of the headgroup phosphorous reached the value associated with a micellar environment at 3-4 mM29. In the absence of DPC, most of the resonances in a 2D-HSQC spectrum of PrP(110-136) are not visible due to fast exchange of amide protons with the solvent (Supplemental Figure S1B). But after the addition of the first aliquot of DPC, two sets of resonances were observed at a 1:1 ratio where one set corresponded to the alpha-helical structure of the CHR peptide, and the second to an unknown conformation (Figure 3A). At this stage, before resonance assignment, we assumed that we were observing only two populations: one that is folded and another that is of unknown conformation. A priori, one may not rule out the presence of the free peptide. But analysis of both spectra in Figure 3 at very low contour levels did not reveal the presence of any of the sharp resonances observed for the free peptide at pH 7.6 (Supplemental Figure S1B). Therefore, if a low population of the free peptide did remain, it must have been below 1-2% considering that the signal-to-noise-ratio is in the order of 100:1 for these datasets. Further additions of DPC resulted in an increase of the intensities of the folded set of resonances at the expense of the resonances of the unknown species. After the last aliquot was added, only the set of resonances associated with the helical conformation remained. Doubling the peptide concentration resulted in a helical-to-unknown ratio of 1:4 after the first aliquot of DPC was added (Figure 3B). This last observation indicates that the peptide had a higher affinity for the
micelles than for the bulk water. However, before further discussion of this observation, we must first describe the localization of these two conformers within the micelle. This was established with PRE experiments.

**Titration with PRE agents** - Gadolinium and manganese are two paramagnetic cations that enhance relaxation in a distance dependent manner. In structure determination studies, these are complexed to ligands that are covalently linked to the protein at strategic locations. Therefore, the degree of relaxation enhancement can be correlated to the inter-nuclear distance (paramagnetic probe-proton) and used as a distance constraint in structure calculations. PRE effects have also been used in NMR to provide localization information for peptides and proteins associated with detergent micelles or membrane mimetic systems. In this type of application, the PRE agent is in a soluble form, either free or complexed with DTPA, and transforms the solvent into a relaxation-enhancement medium. In this context, nuclei that are solvent-exposed will experience the most relaxation enhancement, while those that are buried in the protein hydrophobic core or in the detergent micelle will be less affected because they are farther away from the solvent-dissolved paramagnetic probe. Here, we used the complexed forms of the probe, Gd-DTPA in order to prepare stock solutions at high concentrations while maintaining solubility at the pH of the sample. In the absence of the PRE agent, the amide resonances of the first three residues in the sequence (110-KHM-112) are never observed. This is consistent with the latter being fully exposed to the solvent and undergoing fast chemical exchange. During titration with PRE agents, only the 113-AGA-115 region shows easily observable effects in contour plots. The effect of increasing the concentration of Gd-DTPA on the amide resonance intensity is represented in Figure 4A. In this experiment, the measured resonance intensity at a given concentration is divided by the intensity of that resonance in the absence of paramagnetic agent. Titration with Gd-DTPA was repeated twice.

**DISCUSSION**

The titration experiment shows that residues 113-AGAA-116 experience the greatest relaxation enhancement, while residues 125-LGG-127 experience the smallest effect. The latter are thus farther away from the relaxation media (water + paramagnetic probe) indicating that 125-LGG-127 are located at the centre of the micelle. Since these residues are not in the middle of the peptide sequence, and they are not in the centre of the helix shown in Figure 2B, we concluded that the folded peptide is inserted in the DPC micelles in a non-symmetrical fashion. Indeed, residue S135, at the carboxy-terminus of the peptide, display a relaxation enhancement that is comparable to the effect experienced by residue A117. Both residues (S135 and A117) are equally distant (eight residues away) from the 125-LGG-127 segment that forms the centre of mass of the helix-micelle assembly. S135 and R136 are a polar and a charged residue, respectively, and are probably interacting with phosphocholine headgroups.

In order to illustrate the above observations, we built the model of the peptide-micelle assembly shown in Figure 5. Using a micelle containing 54 DPC molecules obtained from molecular dynamic simulation, the structure of PrP(110-136) was manually inserted in such a way that the 125-LGG-127 segment was positioned in the centre of mass of the micelle. Four DPC molecules that showed van der Waals clashes between their headgroup and the peptide were removed. This handmade model is a simple and reasonable approach to describe the localization of the peptide in the micelle. Zangger and coworkers found, using NMR, circular dichroism and small angle X-ray scattering, that in the presence and absence of various peptides, the diameter of the DPC micelle was not significantly altered upon peptide binding. In our model and consistent with the PRE data, A117 and S135 are both positioned near or at the water-lipid interface allowing interactions between the charged guanidinium group of the arginine side-chain and the phosphocholine headgroup. This localization of the peptide maximizes hydrophobic interaction between the larger side-chains of non-polar residues and the core of the micelle, and places the 113-AGAA-116 segment at the edge of the detergent headgroup region, close to the bulk water. The model in Figure 5 can be used as a depth gauge to provide localization information on the unknown population in the micelle. The PRE data measured for the resonances associated with this population of unknown
conformation (Figure 4B) show relaxation enhancements along the whole length of the peptide that are similar to the measurements for residues A117 and S135 in the helical structure. The latter residues are more protected than 113-AGAA-116, but closer to the surface of the micelle than residues 125-LGG-127. This indicates that the population of unknown conformation of the PrP110-136 peptide is located within the phosphocholine headgroup layer, allowing peptide-detergent interactions, and excludes the possibility that this species is exposed to the bulk water. In the absence of any detergent, only a few resonances are observed for the peptide at pH 7.6. The fact that most resonances of this unknown can be observed at that pH in the presence of DPC suggests that it is interacting with the detergent micelle.

The structure of PrP(110-136) in micelles provides insight into the conformation of CmPrP. The length of the helical portion of the structure in Figure 2 is sufficiently long (~32 Å) to span a cellular membrane bilayer, thus this structure represents a reasonable description of the transmembrane domain of CmPrP. In the human normal cellular form (PrP C), the first secondary structural element of the folded globular domain is a four residues β-sheet starting with Tyr-128 (Syrian Hamster numbering). In the transmembrane domain of CmPrP, residues 128-136 are inserted in the membrane. This would prevent the formation of the small two strands β-sheet. This forces the remaining residues of the protein to fold in a different conformation.

Peptide-headgroup interactions: The localisation of the helical peptide in DPC micelles displays a particular feature: the helix is asymmetrically positioned within the spherical micelle. This indicates that it is preferable to bury polar residues such as S132 and S135 thus having the non-polar peptide 112-MAGA-115 at the micelle-water interface. Therefore, the asymmetry suggests the presence of stabilizing electrostatic interactions, perhaps of the salt-bridge type, involving the guanidinium group of R136 and the phosphate moiety of the detergent headgroup. Salt-bridge interactions have been observed in membrane bound cell-penetrating peptides such as penetratin and HIV-TAT.

Inserted and surface-bound conformers: The DPC titration experiments revealed the presence of two conformers. The peptide is soluble in water and upon addition of detergent it readily binds to micelles giving two populations (Figure 3A and 3B). One explanation for this observation is that the peptide in solution first binds to the micelle surface, then inserts into it. From the PRE data we can localize the surface conformer at the same depth as Ser-135 and Arg-136, hence it is reasonable to suggest that this salt-bridge interaction stabilizes the guanidinium-phosphate ion pair of Arg-136 of the conformer at the surface. This potential salt-bridge may thus be the driving force initiating peptide binding to detergent micelles. But what can be said about hydrophobic interactions? Upon addition of the first aliquot of DPC, the detergent concentration is 14 mM. We know that in the presence of micelles, the concentration of DPC monomers is about equal to the CMC. A snapshot taken at the end of a molecular dynamics simulation on the formation of DPC micelles performed at 20 mM showed an average aggregation number of 60 and revealed the presence of many monomers. Thus, it is more likely that hydrophobic interactions are initially driving the assembly of DPC micelles via binding of detergent molecules to the peptide, and inducing its folding into a helical conformation. Once all DPC molecules have been consumed to form peptide-detergent assemblies, the remaining free peptide molecules bind to the surface (Figure 6). This scenario is supported by the fact that doubling the initial peptide concentration led to a higher surface-bound to inserted peptide ratio (Figure 3B). A number of authors have proposed that electrostatic interactions might explain their observations. For example, electrostatic adsorption of PrP at the lipid bilayer/water interface was suggested as the first step in the reaction of conversion of normal PrP to an amyloid form. Morillas and co-workers have suggested that binding to the membrane surface alters the conformation of the globular domain. They showed that full-length PrP binds to acidic lipid-containing liposomes (PC/PS) with the strongest binding occurring at acidic pH, and a pH-independent binding was observed for a fragment consisting of residues 23-145. A different study reported that conversion was most efficient at pH 6-7 in the presence of membrane but it suggested that PrPC needs to transiently cycle through an acidic compartment to acquire a conversion-competent state.
prior to conversion\textsuperscript{39}. This preparation step (a low pH requirement) may be a partial unfolding required to disrupt the small beta sheet in order to allow interaction of R136 with the membrane surface.

In summary, the water-soluble peptide PrP110-136 that includes the conserved hydrophobic region of the prion protein shows a high affinity to DPC micelles. It adopts an $\alpha$-helical conformation in which the centre of the helix does not coincide with the centre of mass of the micelle. The asymmetrical insertion of the CHR peptide in DPC micelles can be rationalized by the presence of a guanidinium-phosphate ion pair interaction between the carboxy-terminal arginine and phosphocholine headgroup. In addition, this possible electrostatic interaction may be contributing significantly to the interaction of the peptide at the micelle surface. This study may reveal an important behaviour of the prion protein at the membrane surface and may represent a snapshot of the conformation of the full PrP along the conversion pathway leading to the generation of PrP\textsupersc{Sc}. Finally, our observations may contribute to design studies needed to characterize the interactions at play during the conversion process.

REFERENCES


FOOTNOTES

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The abbreviations used are: CHR, conserved hydrophobic region of the human prion protein; DPC, dodecylphosphocholine; PrP, the prion protein; PrP<sup>C</sup>, cellular form of the prion protein; PrP<sup>Sc</sup>, misfolded or infectious form of PrP; CemPrP, a transmembrane form of the prion protein; GSS, Gerstmann-Sträusler-Scheinker syndrome; Gd-DTPA, gadolinium diethylenetriaminepentaacetic acid; PRE, paramagnetic relaxation enhancement.
**FIGURE LEGENDS**

Fig. 1. 2D $^{1}$H-$^{15}$N HSQC of uniformly $^{13}$C, $^{15}$N labeled PrP 110-136 @ 600 MHz, 1 mM peptide in 10 mM NaPi pH 7.6 with 75 mM DPC, recorded at 37°C. Resonances of the backbone amides are labelled according to the syrian hamster prion protein sequence.

Fig. 2. Solution structure of PrP 110-136 in DPC micelles. A: An ensemble of 20 lowest energy conformer calculated using NOE derived constraints and dihedral angles obtained from TALOS+. B: Ribbon representation of the first conformer from the ensemble.

Fig. 3. Two dimensional $^{1}$H-$^{15}$N HSQC spectra recorded after adding the first aliquot of DPC (14 mM) to a (A) 1mM and (B) a 2mM sample of PrP110-136. The black and red contours correspond to the folded peptide and the unknown species, respectively. The folded-to-unknown ratio is ~ 1:1 in (A) and ~1:4 in (B). The intensities of the folded resonances appear lower in (B) because the contour level was lowered for clarity.

Fig. 4. Plots of the normalized intensities of peptide resonances as a function of the paramagnetic relaxation agent (PRA) concentration recorded on sample containing 2mM of PrP110-136 and 2.5 mM of DPC. A: Gd-DTPA titration of selected resonances of the folded PrP B: PRA titration of all well-resolved resonances of the unknown conformer. A rapid decrease of signal intensity is indicative of an amide pair in close proximity to the PRA whereas a slow decrease is indicative of an amide pair that is well buried into the DPC micelle. Lines are colour coded to reflect the effect of the PRA: blue is less affected (most buried) red is most affected. The black lines describe the decay of the resonances (not assigned) to the unknown species. The colour scheme is applied to the residues of the peptide sequence. Data points are fitted to a single exponential with a Y-intercept set to 1.0.

Fig. 5. Modelling PrP110-136 in a micelle of DPC. The average NMR structure of the peptide was manually positioned in a micelle containing 54 DPC molecules obtained by molecular dynamics simulation (see text). The position of the peptide was based on the results of the PRE experiments. The blue residues were positioned at the centre of mass of the micelle. This resulted in alignment of the yellow residues with the phosphate group of the detergent headgroup.

Fig. 6. Cartoon illustrating the proposed folding of the CHR followed by the binding of the unknown conformer at the micelle surface (see text).
## Table

Table 1: Statistics of the CHR (PrP 110-136) in DPC micelles (20 Structures)

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<td>Dihedral angle restraints from TALOS</td>
<td>50</td>
</tr>
<tr>
<td>( \Phi )</td>
<td>25</td>
</tr>
<tr>
<td>( \Psi )</td>
<td>25</td>
</tr>
<tr>
<td>Distances violations (no.)</td>
<td></td>
</tr>
<tr>
<td>Ramachandran plot for residues 5-25( ^{ii} )</td>
<td></td>
</tr>
<tr>
<td>( \Phi/\Psi ) in most favored region</td>
<td>94.4%</td>
</tr>
<tr>
<td>( \Phi/\Psi ) in additionally allowed region</td>
<td>5.6%</td>
</tr>
<tr>
<td>( \Phi/\Psi ) in generously allowed region</td>
<td>0.0%</td>
</tr>
<tr>
<td>( \Phi/\Psi ) in disallowed region</td>
<td>0.0%</td>
</tr>
<tr>
<td>Atomic rmsd (Å)( ^{iii} )</td>
<td></td>
</tr>
<tr>
<td>Backbone atoms (N, C( \alpha ), C)</td>
<td>0.02 +/-</td>
</tr>
<tr>
<td>Heavy atoms</td>
<td>0.29 +/- 0.09</td>
</tr>
</tbody>
</table>

\(^{i}\) Violated in > 10 structures  
\(^{ii}\) Calculated with CYANA ramaplot macro, excluding Gly and Pro  
\(^{iii}\) Calculated with CYANA over residues 5-25.
Figure 1
Figure 4

Concentration of DTPA-Gd (mM)
Interactions between the conserved hydrophobic region of the prion protein and
dodecylphosphocholine micelles
Simon Sauve, Daniel Buijs, Genevieve Gingras and Yves Aubin

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