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Abstract

Cellular prion proteins, PrP(C), carrying the amino acid substitutions P102L, P105L, or A117V, which confer increased susceptibility to human transmissible spongiform encephalopathies, are known to form structures that include transmembrane polypeptide segments. Herein, we investigated the interactions between dodecylphosphocholine micelles and the polypeptide fragments 90-231 of the recombinant mouse PrP variants carrying the amino acid replacements P102L, P105L, A117V, A113V/A115V/A118V, K110I/H111I, M129V, P105L/M129V, and A117V/M129V. Wild-type mPrP-(90-231) and mPrP[M129V]-(91-231) showed only weak interactions with dodecylphosphocholine micelles in aqueous solution at pH 7.0, whereas discrete interaction sites within the polypeptide segment 102-127 were identified for all other aforementioned mPrP variants by NMR chemical shift mapping. These model studies thus provide evidence that amino acid substitutions within the polypeptide segment 102-127 affect the interactions of PrP(C) with membranous structures, which might in turn modulate the physiological function of the protein in health and disease.
Prion Protein-Detergent Micelle Interactions Studied by NMR in Solution*

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Cellular prion proteins, PrPC, carrying the amino acid substitutions P102L, P105L, or A117V, which confer increased susceptibility to human transmissible spongiform encephalopathies, are known to form structures that include transmembrane polypeptide segments. Herein, we investigated the interactions between dodecylphosphocholine micelles and the polypeptide fragments 90–231 of the recombinant mouse PrP variants carrying the amino acid replacements P102L, P105L, A117V, A113V/A115V/A118V, K110I/H111I, M129V, P105L/M129V, and A117V/M129V. Wild-type mPrP-(90–231) and mPrP [M129V]-(91–231) showed only weak interactions with dodecylphosphocholine micelles in aqueous solution at pH 7.0, whereas discrete interaction sites within the polypeptide segment 102–127 were identified for all other aforementioned mPrP variants by NMR chemical shift mapping. These model studies thus provide evidence that amino acid substitutions within the polypeptide segment 102–127 affect the interactions of PrPC with membranous structures, which might in turn modulate the physiological function of the protein in health and disease.

Transmissible spongiform encephalopathies (TSEs), 2 such as Creutzfeldt-Jakob disease and the Gerstmann-Sträussler-Scheinker syndrome in humans, are accompanied by the appearance in the brain of an aggregated “scrapie” isoform of the host-encoded prion protein, PrPSc (1–3). The cellular form, PrPC, consists of an unstructured N-terminal “tail” of residues 23–125 and a globular domain of residues 126–231, and is attached by a C-terminal glycosylphosphatidylinositol (GPI) anchor to the outer plasma membrane. This structure ensures a role of membrane interactions in the physiological function of PrPC and probably also in the disease-related events leading to TSEs. For example, transgenic mice expressing a prion protein variant lacking the GPI membrane anchor did not develop the typical clinical signs of TSE after inoculation with infectious brain homogenate, although significant amounts of PrPSc accumulated in the brain (4). This finding led to the conclusion that membrane-association of PrPC is necessary for the development of a TSE. Independent evidence for the importance of membrane interactions for the onset of prion diseases was derived from cell-free conversion assays and cell culture experiments (5, 6).

Data have also been presented that indicate that in addition to the normal form with the C terminus linked to a GPI anchor and the C-terminal domain located on the cell surface, PrPC can adopt two different transmembrane topologies, CtmPrP and NtmPrP, which have the C-terminal polypeptide segment located in the lumen of the endoplasmic reticulum (CtmPrP) or in the cytoplasm (NtmPrP) (7–9). The population of the CtmPrP variant is <10% of the total wild-type prion protein present during cellular biosynthesis but is increased to 20–30% for the pathogenic mutations P102L, P105L, and A117V of human PrP and the designed variant mouse PrPs obtained with the amino acid exchanges A113V/A115V/A118V and K110I/H111I (10–13). The population of the CtmPrP variant is further increased when an additional mutation, L9R, was present in the N-terminal signal sequence (14), so that ~50% of the PrP was synthesized as the CtmPrP variant in granule neurons obtained from transgenic mice expressing a prion protein construct carrying the four amino acid replacements L9R, A113V, A115V, and A118V (15). Quite generally, an increase in the population of CtmPrP was also shown to be associated with severe neurodegeneration in transgenic mice, and it has been suggested that CtmPrP may be the proximate cause of neuronal death in certain prion disorders (10, 11, 15).

In vitro studies on interactions of full-length and N-terminally truncated forms of recombinant PrP showed that acidic membranes caused the N-terminal part of the protein to become more structured, whereas the C-terminal domain was destabilized (16–19). Furthermore, zwitterionic gel-phase dipalmitoylphosphatidylcholine or raft-like membranes were shown to induce increased α-helical structure in recombinant Syrian hamster PrP-(90–231) at pH 7.0 (18, 19). Membrane interactions of polypeptides representing sequence motifs found in the prion protein have also been studied (20–23).

In this report we describe investigations of PrP interactions with a membrane mimetic and focus on the mutations P102L, P105L, and A117V, which have been linked with familial Gerstmann-Sträussler-Scheinker syndrome in humans (2, 24, 25). Our interest in these variant proteins is related to open questions about the mechanisms by which pathogenic mutations predispose humans for prion diseases. We studied the interactions of a recombinant wild-type mouse prion protein frag-
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For example, the mutations P105L and A117V are only pathogenic to humans to prion diseases and on the disease phenotype. Reported to have a significant influence on the susceptibility of humans to prion diseases and on the disease phenotype.

For the titration experiments, 150 μm samples of 15N-labeled protein were prepared in 5 mM sodium phosphate at pH 7.0, which contained 10% D2O. The experiments were carried out by the addition of aliquots of a 129 mM stock solution of DPC in 5 mM sodium phosphate at pH 7.0, which contained 10% D2O. Two-dimensional 15N-H, 2D-NOESY spectra were recorded at each DPC concentration to measure the 15N chemical shifts. A mean dissociation constant for the micelle-protein complex, $K_{D}$, was obtained from the dependence of the $^{15}$N chemical shifts of selected prion protein residues on the DPC concentration for mPrP[105L]-[90–231], using Equation 1 (32).

\[
\Delta \delta^{15N} = \Delta \delta_{\text{max}} \frac{[M_i]}{K_{D}n + [M_i]} - \text{cmc}
\]  

(Eq. 1)

In Equation 1, $\Delta \delta^{15N}$ is the chemical shift difference between the backbone amide nitrogen resonances at a given DPC concentration and in the absence of DPC, $\Delta \delta_{\text{max}}$ is the chemical shift difference at the final DPC concentration, $[M_i]$ is the monomeric DPC concentration, cmc is the critical micelle concentration, and n is the aggregation number of DPC. The latter two values were used as specified by the manufacturer (Anatrace, Maumee, OH), i.e. cmc = 1.5 mM and n = 54.

**Calculation of Hydrophobic Moments and Free Energies of Transfer for Residues 90–130 from Water to Membrane**

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were also produced as uniformly $^{13}$C, $^{15}$N-labeled proteins (29, 30).

**NMR Spectroscopy**—Uniformly $^{15}$N- or $^{13}$C, $^{15}$N-labeled protein samples were prepared in 5 mM sodium phosphate buffer at pH 7.0 in 90% H2O/10% D2O. The protein concentration was 1.0–1.4 mM, unless otherwise indicated. Tentative initial backbone $^{1}H^{N}$, $^{15}$N, $^{13}$C$,^{1}H$, and $^{13}$CO resonance assignments for mPrP-[90–231] at pH 7.0 were generated by transfer of the published assignments of mPrP-[121–231] at pH 4.5, and of human PrP-[90–230] at pH 7.0 (29–31). The assignments were then confirmed using standard triple resonance NMR experiments with the uniformly $^{13}$C, $^{15}$N-labeled mPrP-[90–231] at pH 7.0. The $^{1}H^{N}$, $^{15}$N, $^{13}$C$,^{1}H$, and $^{13}$CO resonance assignments for all except two (see below) of the prion protein variants were guided by the thus obtained assignments for wild-type mPrP-[90–231], using HNCA, HNCO, and HN(CA)CO spectra measured with the variant proteins. For mPrP[A113V, A115V, A118V]-[90–231], additional CBCA(CO)NH and HNCACB spectra were used to confirm the assignments. For mPrP[105L,M129V]-[90–231] and mPrP[117V,M129V]-[90–231] the assignments were derived from comparison with the chemical shifts of the corresponding single-residue variants.

The $^{13}$CO assignments for uniformly $^{13}$C, $^{15}$N-labeled mPrP-[90–231], mPrP[105L]-[90–231], and mPrP[117V]-[90–231] in the presence of 12.9 mM DPC were obtained using HNCA experiments recorded at a protein concentration of 135 μm.

All NMR experiments were performed at 20 °C on a Bruker DRX500 spectrometer equipped with a triple tunable cryogenic probehead. The NMR data were processed with the programs XWINNMR and TOPSPIN 2.0, and the program CARA (www.nmr.ch) was used for the spectral analysis (31).

**Titration Experiments with the Detergent DPC**—For the titration experiments, 150 μm samples of $^{15}$N-labeled protein were prepared in 5 mM sodium phosphate at pH 7.0, which contained 10% D2O. The experiments were carried out by the addition of aliquots of a 129 mM stock solution of DPC in 5 mM sodium phosphate at pH 7.0, which contained 10% D2O. Two-dimensional $^{15}$N-H, 2D-NOESY spectra were recorded at each DPC concentration to measure the $^{15}$N chemical shifts. A mean dissociation constant for the micelle-protein complex, $K_{D}$, was obtained from the dependence of the $^{15}$N chemical shifts of selected prion protein residues on the DPC concentration for mPrP[105L]-[90–231], using Equation 1 (32).

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In Equation 1, $\Delta \delta^{15N}$ is the chemical shift difference between the backbone amide nitrogen resonances at a given DPC concentration and in the absence of DPC, $\Delta \delta_{\text{max}}$ is the chemical shift difference at the final DPC concentration, $[M_i]$ is the monomeric DPC concentration, cmc is the critical micelle concentration, and n is the aggregation number of DPC. The latter two values were used as specified by the manufacturer (Anatrace, Maumee, OH), i.e. cmc = 1.5 mM and n = 54.

**Calculation of Hydrophobic Moments and Free Energies of Transfer for Residues 90–130 from Water to Membrane**

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Environment—Hydrophobic moments were calculated according to Eisenberg et al. (33) using the program EMBOSS version 5.0.0 with the function hmoment and a window size of 7 residues (34). Free energy changes for transferring amino acid residues between a lipid and a water environment were calculated according to White & Wimley using the function octanol from the program EMBOSS (35).

RESULTS

The prion proteins used for this study were cloned, expressed, and purified as described under “Experimental Procedures.” To establish a platform for studies of protein–detergent micelle interactions at a resolution of individual amino acid residues, we determined sequence-specific backbone assignments for all the proteins in 90% H2O/10% D2O containing 5 mM sodium phosphate buffer at pH 7.0 and T = 20 °C. The NMR assignments were based on standard triple resonance experiments (36), using a strategy described under “Experimental Procedures.” The backbone assignments are complete, with the following exceptions: in all the proteins the HN, 15N, 13Cα, and 13CO resonances of Gly93 and Gly94 are degenerate and could only be assigned as a group. In the tetrapeptide segment Ala115–Ala116, Ala117–Ala118, the resonances of Ala115 and Ala118 could be assigned from the sequential connectivities to Gly114 and Gly119 (Fig. 1C). The HN and 15N resonances of residue 117 are at chemical shifts typical of Val residues. The resonance assignments have been deposited in the BioMagResBank (accession numbers: mPrP-(90–231), 16071; mPrP[M129V]-(91–231), 16075; mPrP[P102L]-(91–231), 16076; mPrP[P105L]-(91–231), 16077; mPrP[A117V]-(90–231), 16078; mPrP[A113V,A115V,A118V]-(90–231), 16079; and mPrP[K110I,H111I]-(90–231), 16080).

Screening for a Membrane Mimetic—At the beginning of our study, we tested several detergents and phospholipid bicelles as possible membrane mimetics. The best results were obtained with DPC, whose phosphocholine head groups are expected to simulate a membrane interface, and which is the most widely used detergent for NMR studies of both single α-helical membrane proteins and integral membrane β-barrel proteins (38, 39). These studies include proteins and peptides that have been reported to be associated with the endoplasmic reticulum membrane such as the N-terminal membrane anchor segment of thromboxane A2 synthase (40), the N-terminal membrane anchor domain of prostaglandin I2 synthase (41), and cytoplasmic domain of the canine Sec61 gamma protein from the protein translocation pore of the endoplasmic reticulum membrane (42).

Prion Protein Interactions with the Detergent DPC—Interactions of the detergent DPC with mPrP-(90–231) and the prion protein variants shown in Fig. 1C were characterized by observing 15N chemical shift changes of the backbone resonances in the two-dimensional 15N,1H-HSQC spectra caused by addition of increasing amounts of DPC at pH 7.0. The resonance assignments for the free prion proteins were used as a starting point, and the positions of the backbone 15N,1H signals were then recorded as a function of the concentration of added DPC. Using this approach, the backbone HN and 15N resonances could be assigned up to a DPC concentration of 40 mM in all the proteins except mPrP[K110I,H111I]-(90–231) and mPrP[A113V,A115V,A118V]-(90–231). For the latter two proteins, assignments could only be obtained up to concentrations of 6.8 mM and 18.6 mM DPC, respectively, because at higher DPC concentrations there was line-broadening and reduced solubility that prevented reliable assignment of the resonances. In all proteins the HN,15N cross peak of His111 could not

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be followed after addition of DPC, due to resonance overlap. Fig. 2 shows superpositions of an expanded region of the two-dimensional $^{15}$N,$^{1}$H-HSQC spectra obtained for wild-type mPrP-(90–231), and for the two variants mPrP[P105L]-(91–231) and mPrP[A117V]-(90–231) in the absence and presence of 12.9 mM DPC. Whereas the wild-type protein shows only small chemical shift differences upon addition of DPC, individual $^{15}$N resonances in the two variants exhibit significant changes in their resonance positions and many of the affected resonances show significant line-broadening of the shifted cross-peaks. Because the $^{1}$H resonances were only slightly affected, we performed our analysis using the $^{15}$N chemical shift changes. However, analysis performed with combined $^{1}$H and $^{15}$N chemical shift changes do not change our conclusions (data not shown).

Identification of DPC Binding Sites in the Protein Sequence—Histograms displaying the amide $^{15}$N chemical shift changes after addition of 6.8 mM DPC for the individual residues in the proteins (Fig. 3) reveal that DPC interactions cause mainly upfield shifts of $^{15}$N resonances. In all the proteins, the largest $^{15}$N chemical shift changes between the free and DPC-bound states of the proteins were found for the residues 107–127, identifying this polypeptide segment as the DPC binding region. All other amino acid residues in the proteins were at most only slightly affected, except that the binding region is N-terminally extended to residue 102 for mPrP[P102L]-(91–231), and to residue 105 for mPrP[P105L]-(91–231) and mPrP[P105L,M129V]-(90–231) (Fig. 3, C–E). Judging from the size of the $^{15}$N chemical shift changes, the two designed variants mPrP[A113V,A115V,A118V]-(90–231) and mPrP[K110I,H111I]-(90–231) (Fig. 3, H and I) show the strongest interaction with DPC, followed by the other proteins in the order mPrP[P105L]-(91–231) > mPrP[A117V]-(90–231) > mPrP[P102L]-(91–231). Only weak interactions were observed for mPrP-(90–231) and mPrP[M129V]-(91–231).

Within the aforementioned binding regions, the largest chemical shift changes were observed for the residues Val$^{113}$, Val$^{118}$, and Gly$^{123}$ in mPrP[A113V,A115V,A118V]-(90–231), and for the residues Val$^{112}$, Ala$^{113}$, and Gly$^{123}$ in mPrP[K110I,H111I]-(90–231). The resonances that show the largest $^{15}$N chemical shift changes upon addition of DPC also exhibit the greatest degree of line-broadening.

Comparison of pairs of proteins carrying either methionine or valine at position 129, with otherwise identical amino acid sequences, showed that they have nearly identical $^{15}$N chemical shift changes upon addition of DPC (in Fig. 3, compare A and B, D and E, and F and G, respectively), indicating that the M129V polymorphism does not noticeably influence the interactions of the proteins with DPC.

Variation of the DPC Concentration Shows that PrP Interacts with Detergent Micelles—To further investigate the nature of the detergent interactions causing the $^{15}$N chemical shift changes in Fig. 3, two-dimensional $^{15}$N,$^{1}$H-correlation NMR spectra of mPrP[A113V,A115V,A118V]-(90–231) were measured during a titration with small DPC concentrations. Below the critical micelle concentration of 1.5 mM, no changes in the $^{15}$N chemical shifts were observed, indicating that the protein does not interact with DPC monomers, but is rather interacting with DPC micelles (Fig. 4).

To obtain an estimate of the stability of the PrP-detergent micelle complexes, we measured $^{15}$N chemical shift changes for selected well resolved resonances upon stepwise addition of DPC in the range above the cmc up to a concentration of 40 mM.
Fig. 5 shows for all the proteins studied that over this range of concentrations the upfield chemical shift changes increase monotonously with increasing DPC concentrations. The slope of the $\Delta\delta^{(15N)}$ versus the DPC concentration over the near-linear part of the curves in Fig. 5 was highest for $m$PrP$[A113V,A115V,A118V]-(90–231)$ and $m$PrP$[K110I,H111I]-(90–231)$, and for the other proteins it decreased in the order $m$PrP$[P105L]-(91–231)$, $m$PrP$[P105L,M129V]-(91–231)$, $m$PrP$[A117V]-(90–231)$, $m$PrP$[A117V,M129V]-(90–231)$, $m$PrP$[P102L]-(91–231)$, $m$PrP$[M129V]-(90–231)$, and $m$PrP$-(90–231)$ (Fig. 5). The addition of DPC micelles also caused line broadening and precipitation of variable fractions of the individual proteins. For $m$PrP$[A113V,A115V,A118V]-(90–231)$ and $m$PrP$[K110I,H111I]-(90–231)$, these side effects limited the data analysis to DPC concentrations below 18.6 and 6.8 mM, respectively (Fig. 5). Although the $^{15N}$ chemical shifts show a linear dependence on the detergent concentration over most of the DPC concentration range covered in Fig. 5, saturation at the higher DPC concentrations could be measured and fitted with Equation 1 (see “Experimental Procedures”) for $m$PrP$[P105L]-(91–231)$, which shows the strongest binding among the proteins for which data could be obtained up to 40 mM DPC concentration (Fig. 5). This approach yielded a dissociation constant, $K_d$, of $0.9 \pm 0.2$ mM, which can be considered to represent an upper limit of the affinity of DPC micelles for the $PrP$ species of Fig. 1C with the exception of the last two species listed. These may bind more tightly. However, the limited solubility of these two species at elevated DPC concentrations prevents a quantitative statement regarding their affinity for DPC micelles.

$^{13C}$ Chemical Shifts Show That DPC Induces Helical Structure in the Micelle-binding Region of $PrP$—The proteins $m$PrP$[P105L]-(91–231)$ and $m$PrP$[A117V]-(90–231)$ were selected for further studies, based on the fact that they show large chemical shift changes upon interaction with DPC and are associated with TSEs in humans, to quantify the population and determine the residue boundaries of helical conformation induced by the interaction with the micelles. The $^{13C}$ downfield chemical shifts induced by the addition of DPC in these two proteins (Fig. 6, B and C) indeed manifest increased $\alpha$-helical character of the polypeptide segments 102–124 and 110–122, respectively, which had independently been identified as the DPC binding regions (Fig. 3). Wild-type $m$PrP$-(90–231)$ was used as a control, and it showed no specific DPC effects on $^{13C}$ shifts (Fig. 6A).
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FIGURE 4. Dependence of protein $^{15}$N chemical shifts on the DPC concentration for selected residues in mPrP[A113V,A115V,A118V]-(90–231) that are identified in the upper left corner. $\Delta \delta(15N)$ is the difference between the $^{15}$N chemical shifts in the absence of DPC and in the presence of the DPC concentrations given on the horizontal axis. The dashed vertical line indicates the cmc of DPC.

DISCUSSION

The striking result of this in vitro study is that amino acid exchanges in PrP, which are otherwise known to affect the susceptibility of healthy mammalian organisms to TSEs, have readily measurable effects on PrP interactions with a membrane mimic. Thus, the amino acid substitutions P102L, P105L, and A117V, which are linked with familial Gerstmann-Sträussler-Scheinker syndrome in humans, specifically enhance the interaction with DPC micelles. Gradual change of the $^{15}$N chemical shifts with increasing detergent concentration (Fig. 5) indicates that there is fast exchange between free and micelle-associated protein, and that the population of bound protein is enhanced as the detergent concentration increases. The different extent of the $^{15}$N chemical shift changes for the different proteins provides a qualitative indication of the variable affinities for binding to DPC micelles, whereby the estimated upper limit for $K_D$ of 0.9 mM for the disease-related variants corresponds to only a modest affinity. Weak binding is independently supported by an analysis of the changes in the $^{13}$C$\alpha$ chemical shifts observed upon interaction of PrP with DPC micelles (Fig. 6). Based on the fact that a fully populated $\alpha$-helix has $\Delta \delta(13C)\alpha$-values of 2.0 to 4.0 ppm (43, 44), the $\Delta \delta(13C)\alpha$ values of $\leq 0.4$ ppm observed for mPrP[P105L]-(91–231) (Fig. 6B) suggest that $\sim 20–20\%$ of the polypeptide segment 102–127 adopt helical structure characteristic of the bound state. Similar results were obtained with variants of mPrP that had been specifically designed to increase the amount of the GliPrP population in in vitro and in vivo studies (10, 15). We consider the presently evidenced weak PrP-micelle interactions to be significant, because even a modest further increase in membrane affinity can lead to significant changes in the population of particular membrane-associated states of a GPI-anchored protein.

Molecular simulations of GPI-anchored membrane-bound human PrP-(90–230) have shown that the N-terminal fragment 90–125 is very close to the membrane (45). The local concentration of membrane binding sites for the N-terminal fragment of PrP can therefore be expected to be greatly increased when PrP is GPI anchored at the membrane, leading to significant populations of bound states despite weak affinity.

The $^{15}$N chemical shift changes within the polypeptide segment of residues 102–127 show similar patterns for all the proteins with sizeable DPC affinities, indicating that they adopt similar conformations upon interacting with DPC micelles (Fig. 5). Upfield $^{15}$N chemical shift changes have been reported for other proteins that take on a helical conformation upon interaction with micelles (46). The observation that addition of DPC causes upfield shifts of nearly all backbone $^{15}$N resonances indicates that the micelle-binding site of the studied PrP variants take on a more helical conformation in the micelle-bound state (Fig. 5). This conclusion is supported by the downfield shifts of the $^{13}$C$\alpha$ resonances for the residues 110–122 of both mPrP[P105L]-(91–231) and mPrP[A117V]-(90–231), actually indicating that the binding polypeptide segment adopts $\alpha$-helical conformation in the micelle-bound state (Figs. 3 and 6) (46, 47).

For mPrP[A113V,A115V,A118V]-(90–231) and mPrP[K110I, H111I]-(90–231), the affinity for DPC micelles is the highest among all the studied variants consistent with the large initial slope of the DPC concentration-dependent curves (Fig. 5, H and I). The higher affinity of mPrP[A113V,A115V,A118V]-(90–231) and mPrP[K110I,H111I]-(90–231) for DPC micelles presumably results in enhanced aggregation. However, these variants show the same pattern of upfield $^{15}$N shift changes indicating that they take on a similar conformation to the naturally occurring variants in the micelle-bound state.

It is worth noting that amino acid substitutions at the periphery of the micelle-binding site, such as P102L or P105L, affect the affinity of the entire binding region, which has a length of $\sim 20$ residues. This suggests that the PrP polypeptide segment 107–127 has a predisposition to bind to lipidic micelles, so that a mutation anywhere within the binding site can lead to an increased population of the micelle-bound state. This conclusion is supported by the fact that replacement of proline, which is an “$\alpha$-helix breaker,” by leucine is expected to increase the $\alpha$-helical propensity of the local polypeptide segment, and it indeed results in increased DPC micelle affinity (Figs. 3 and 5).

Calculations of the hydrophobic moments for residues 90–130 of mPrP show amphipathic helical character for the segments 98–102 and to a lesser extent for 109–113. The substitutions Pro$^{102}$ and Pro$^{105}$ tend to increase the amphipathic character of the two respective regions and the simultaneous substitutions K110I and H111I eliminate the second amphipathic stretch. The other substitutions have little effect. On the other hand the tendency of each residue of this segment to partition into a bilayer shows a good correlation with the order of binding affinity for DPC micelles which we observe experimentally. The average calculated $\Delta G$ values for membrane insertion of residues 102–127 are 0.0, 0.02, 0.16, 0.21, 0.23, 0.64, 0.67, 1.97, and 2.56 kcal/mol for mPrP-(90–231), mPrP[M129V]-(91–231), mPrP[P102L]-(91–231), mPrP[P105L]-(91–231), mPrP[P105,M129]-(91–231), mPrP[A117V]-(90–231), mPrP[A117V,M129V]-(90–231), mPrP[A113V,A115V,A118V]-(90–231), mPrP[K110I,H111I]-(90–231) relative to mPrP.

No significant differences of the protein-detergent micelle interactions are observed between proteins carrying either
valine or methionine at position 129. This finding might be rationalized by the fact that, in contrast to all other variable sequence locations in Fig. 1C, the residue 129 is located within the globular C-terminal domain (Fig. 1B). It might therefore be less readily accessible for contacts with detergent micelles. The negligible effect of the substitution M129V may alternatively be due to the minimal change in hydrophobicity caused by this substitution. The well documented impact of the M129V polymorphism on disease-related processes in humans (1, 2, 48, 49) can thus not be traced to an influence on the membrane affinity, indicating that it is due to other mechanisms.

Previous NMR studies of interactions between non-globular proteins and detergent micelles have shown that micelles tend to interact locally with hydrophobic clusters, which typically consist of tryptophan and/or histidine residues flanked by hydrophobic polypeptide segments (50–54). It has also been shown that micelle-binding hydrophobic clusters may change conformation upon binding to micelles (52). The micelle binding described in this study seems to be different from these previously reported hydrophobic cluster-micelle interactions, in that they do not require the presence of aromatic residues in the binding polypeptide segment. Actually, replacing His by Ile in mPrP[K110I, H111I]-(90–231) enhances the micelle-protein interaction.

In addition to PrP, the Aβ1-42 peptide related to Alzheimer disease, which is derived from cleavage of the amyloid precursor protein (APP) by γ- and β-secretases, has been shown to specifically interact with membranes (55, 56). Recent studies have shown that PrP seems to play a crucial role in inhibiting the β-secretase cleavage of APP, thereby regulating the production of the neurotoxic amyloid β-peptide (57). To be able to down-regulate the β-secretase, PrP must be integrated in lipid rafts, where it has been found to interact with the β-secretase via charged residues within the polypeptide segment 95–111 (Fig. 1B and C). Interestingly, PrP carrying the substitution A117V loses this protective function. The present study now shows that the substitut-
tion A117V enhances the affinity for ordered lipids of the polypeptide segment 110–122 (Fig. 3), which is located immediately C-terminal to the aforementioned /H9252-secretase interaction site. The thus implicated increased membrane affinity could result in PrP being less accessible for interactions with the /H9252-secretase.

Conformational rearrangements induced by interaction with lipid phases have been discussed to be operative in the insertion of proteins into membranes (35, 58). In vitro translation experiments with microsomes show that all variant prion proteins of Fig. 1C exhibit an increased population of CtmPrP when compared with mPrP, with the amount of CtmPrP increasing in the order wild-type PrP < PrP[A117V] < PrP[K110I/H111I] < PrP[A113V/A115V/A118V] (10, 12). A similar trend is observed when comparing the strength of the protein-micelle interaction affinities (Figs. 3 and 5). Consistent with the assumption of a predominant role of hydrophobic contacts in the protein-micelle interactions, variant proteins carrying the designed mutations A113V/A115V/A118V and K110I/H111I show higher micelle affinity than the variants carrying the A117V amino acid replacement (Figs. 3 and 5). There is thus an apparent correlation between the present data and the increased population of CtmPrP observed in the aforementioned in vitro translation experiments. In contrast, reports that methionine at position 129 significantly increases the population of the CtmPrP variant when compared with otherwise identical proteins containing a valine at this position (59) do not receive support from the present study (Figs. 3 and 5) (see also text above). The higher affinity of the polypeptide segment 102–127 for the endoplasmic reticulum membranes induced by the disease-related mutations might lead to an enhancement in the amount of the CtmPrP form and to increased transport of the potential neurotoxic CtmPrP form to the plasma membrane, where its different conformation might cause cell death and disease progression.

In conclusion, the present study indicates that the amino acid substitutions P102L, P105L, and A117V, which have been associated with the prion disease Gerstmann-Sträusser-Scheinker syndrome in humans (2, 24, 25), as well as the specifically designed substitutions K110I/H111I and A113V/A115V/A118V increase the affinity of the prion protein segment spanning the residues 102–127 for binding to ordered lipid structures. The implicated enhanced affinity of the mPrP variants carrying the amino acid substitutions P102L, P105L, and A117V for cellular membranes could either cause a loss of PrPC function or lead to a gain of function, either of which might ultimately impact the physiological roles of PrPC in health as well as in the development of TSEs.

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