Identification of a Common Sphingolipid-binding Domain in Alzheimer, Prion, and HIV-1 Proteins*

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The V3 loop of the human immunodeficiency virus (HIV)-1 surface envelope glycoprotein gp120 is a sphingolipid-binding domain mediating the attachment of HIV-1 to plasma membrane microdomains (rafts). Sphingolipid-induced conformational changes in gp120 are required for HIV-1 fusion. Galactosylceramide and sphingomyelin have been detected in highly purified preparations of prion rods, suggesting that the prion protein (PrP) may interact with selected sphingolipids. Moreover, a major conformational transition of the Alzheimer β-amyloid peptide has been observed upon interaction with sphingolipid-containing membranes. Structure similarity searches with the combinatorial extension method revealed the presence of a V3-like domain in the human prion protein PrP and in the Alzheimer β-amyloid peptide. In each case, synthetic peptides derived from the predicted V3-like domain were found to interact with monomolecular films of galactosylceramide and sphingomyelin at the air-water interface. The V3-like domain of PrP is a disulfide-linked loop (Cys179–Cys214) that includes the E200K mutation site associated with familial Creutzfeldt-Jakob disease. This mutation abrogated sphingomyelin recognition. The identification of a common sphingolipid-binding motif in gp120, PrP, and β-amyloid peptide underscores the role of lipid rafts in the pathogenesis of HIV-1, Alzheimer, and prion diseases and may provide new therapeutic strategies.

One of the hallmarks of prion diseases is the cerebral accumulation of an abnormal form of prion protein (PrP), the so-called PrPsc, which is derived from the normal cell surface glycoprotein, PrPc (1). The conformational change associated with the PrPc → PrPsc transition (chiefly an α-helix → β-sheet transformation) occurs in membrane microdomains enriched in sphingolipids and cholesterol (i.e. lipid rafts) (2, 3). Correspondingly, infectious prion rods were found to contain the two sphingolipids galactosylceramide (GalCer) and sphingomyelin, suggesting that both lipids may interact with normal and/or pathogenic prion proteins (4). Interestingly, the HIV-1 surface envelope glycoprotein gp120 also interacts with GalCer (5, 6), as well as with a few other sphingolipids found in membrane rafts, i.e. the ceramide trihexoside Gb3, the monosialoganglioside GM3, and sphingomyelin (6–10). Raft glycosphingolipids mediate lateral assemblies of the HIV-1 fusion complex and stimulate the conformational changes in HIV-1 envelope glycoproteins required for initiating the fusion process (9, 11–13). Moreover, a major conformational transition of the Alzheimer β-amyloid peptide is observed upon binding of the 1–40 β-amyloid peptide to ganglioside GM1-containing membranes (14). Taken together, these data suggest that conformational changes in prion, Alzheimer, and HIV-1 proteins may occur in lipid rafts under the control of specific sphingolipids.

The binding of HIV-1 to GalCer is mediated by the third variable (V3) loop of gp120, as demonstrated by various immunological, biochemical and biophysical approaches (15–17). Since GalCer is bound by both HIV-1 gp120 and prion proteins, we looked for a potential V3-like glycolipid-binding domain in human PrP. As a matter of fact, the search for molecules interacting with PrPsc is a major issue in the transmissible encephalopathies field. The presence of a similar motif in the Alzheimer β-amyloid peptide was also investigated. Structure similarity searches were carried out using the combinatorial extension (CE) method (18). A putative GalCer-binding motif was identified in the human PrP protein on the basis of its structural homology with the V3 loop. A similar motif was also found in Alzheimer β-amyloid peptide. The interaction of sphingolipids with the V3-like domain of human PrP and Alzheimer β-amyloid peptide was analyzed using the Langmuir film balance technology.

EXPERIMENTAL PROCEDURES

Materials—The chemicals used in this study, including GalCer, sphingomyelin, and the β-amyloid synthetic peptide (fragment 1–40) of the higher purity available were purchased from Sigma. The synthetic peptides P1 (KQHTVTTTGGFTETFVDKMMER) and P2 (KQHT-VTTTTGGFTETFVDKMMER) respectively derived from the human PrP protein and the E200K mutant were purchased from Euro Gene service (Evry, France). The peptides were purified by high performance liquid chromatography (purity >95%) and characterized by electrospray mass spectrometry (experimental M, of 2812.4 and 2810.8 for peptides P1 and P2, with a theoretical M, of 2811.4 and 2810.4, respectively).

Structure Analysis—Structure similarity searches were performed using the two chains calculation routine of the CE program (Ref. 18 and expasy.ch/spdbv/). PDB identification numbers were 1CE4 (HIV-1 gp120 V3 loop peptide), 1QLX (human PrP), and 1BJB (human Alzheimer peptide).

Surface Pressure Measurements—The surface pressure was measured with a fully automated microtensiometer (μTROUGH SX, Kibron Inc.). The apparatus allowed the recording of pressure-area compression isotherms and the kinetics of interaction of a ligand with the monomolecular film using a set of specially designed Teflon troughs. All experiments were carried out in a controlled atmosphere at 20°C ± 1°C. Monomolecular films of the indicated lipids were spread on pure water subphases (volume of 800 μl) from hexane:chloroform:ethanol...
This loop includes the pressure increases produced were recorded until reaching the equilibriums. The accuracy of the system under our experiment was injected in the subphase with a 10-min was allowed for solvent evaporation. To measure the interaction of the peptide with lipid monolayers, various concentrations of the ligand—e.g., Phe198 (dark), Phe20 of the gp120 V3 loop in green, and Tyr21 of gp120 loop in yellow; right panel, Tyr10 of Alzheimer peptide in yellow, and Phe20 of gp120 V3 loop in green.

RESULTS AND DISCUSSION

A V3-like Domain in PrP and Alzheimer β-Amyloid Peptide—Structure similarity searches revealed the presence of a HIV-1 gp120 V3-like motif in the human prion protein PrP and in the Alzheimer β-amyloid peptide (Fig. 1a). The V3-like domain of PrP consists of a helix-turn-helix motif formed by 33 of the 36 amino acid residues of a disulfide-linked loop (Cys179–Cys214). This loop includes the a2 and a3 helix of PrPnew (Fig. 1a). In the V3 loop of HIV-1 gp120, the motif is a hairpin structure with only one α-helix corresponding to a3 in PrP. This is also the case for the Alzheimer β-amyloid peptide. The V3-like motif of PrP and Alzheimer proteins has the same size as the V3 loop so that they can be easily superimposed (Fig. 1b).

The proteins are identified with their PDB entry numbers: 1CE4, HIV-1 gp120 V3 loop; 1QLX, human PrP; 1BJB, human Alzheimer peptide. The accuracy of the system under our experiment was injected in the subphase with a 10-min was allowed for solvent evaporation. To measure the interaction of the peptide with lipid monolayers, various concentrations of the ligand—e.g., Phe198 (dark), Phe20 of the gp120 V3 loop in green, and Tyr21 of gp120 loop in yellow; right panel, Tyr10 of Alzheimer peptide in yellow, and Phe20 of gp120 V3 loop in green.

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Thus, our study confirms the capacity of the CE program to detect specific motifs in unrelated proteins sharing little sequence homology (18, 23).
rafts (4). The identification of a V3-like domain in human PrP prompted us to study the interaction of this domain with GalCer and sphingomyelin. A peptide (P1) derived from the putative glycolipid-binding motif of human PrP (KQHTVTTTTK-GENFTETVDKMMER) was synthesized, and its interaction with sphingolipids analyzed using the Langmuir film balance technology. In these experiments, the peptide P1 was added in the aqueous subphase underneath a monomolecular film of lipid, and the resulting interaction was measured as an increase in the surface pressure of the film (6). This technique is one of the most sensitive for studying lipid-ligands interactions (24). The synthetic peptide P1 was found to interact specifically with GalCer, and the interaction was definitely dose-dependent (Fig. 2). The maximal surface pressure increase ($\Delta \pi_{\text{max}} = 8$ mN/m) was obtained with a peptide concentration of 100 nM. Similar data were previously obtained in our laboratory with a synthetic peptide derived from the conserved motif of the V3 loop crown (i.e. GPGRAF) (6).

To assess the specificity of GalCer-peptide P1 interaction, monomolecular films of GalCer were prepared at various initial pressures ($\pi_i$) and the maximal surface pressure increase ($\Delta \pi_{\text{max}}$) induced by the peptide on these films was determined after equilibrium had been reached. Below a $\pi_i$ of 30 mN/m, which corresponds to a fluid disordered (Lc) phase, the $\Delta \pi_{\text{max}}$ induced by the peptide was between 5 and 10 mN/m (Fig. 3). At a $\pi_i$ of 30 mN/m, the value of $\Delta \pi_{\text{max}}$ reached 16 mN/m. Then, for values of $\pi_i$ above 30 mN/m, which do correspond to raft-like liquid-ordered (Lo) phase domains, $\Delta \pi_{\text{max}}$ gradually...
decreased as $\sigma_i$ increased. The influence of the initial surface pressure on the compressibility of the sphingolipid monolayer demonstrates the high specificity of the interaction as previously established for several other lipids and ligands (6, 24). The critical pressure of insertion (i.e. the theoretical value of $\sigma$ extrapolated for $\Delta \sigma_{\text{max}} = 0$ mN/m) was 45 mN/m. Interestingly, the mean lipid density of cellular membranes corresponds to a surface pressure of at least 30 mN/m (25). Thus, these data suggest that the interaction of peptide P1 with GalCer requires a densely packed organization of the glycosphingolipid, which is likely to occur within a lipid raft (26).

Effect of Mutation E200K in Human PrP for Sphingolipid Recognition—The V3-like region of human PrP contains a mutation site (E200K) corresponding to the most common familial form of the Creutzfeldt-Jakob disease (1). Residue 200 is located at the N-terminal end of the second $\alpha$-helix of the sphingolipid-binding motif (i.e. a3 of human PrP). Another synthetic peptide similar to P1 but bearing the E200K substitution was therefore synthesized (P2), and its interaction with sphingolipids was also analyzed using the lipid monolayer assay. As for peptide P1, the interaction of peptide P2 with a monomolecular film GalCer ($\sigma_i = 10$ mN/m) was dose-dependent (Fig. 2). When the interaction was analyzed at various values of $\sigma_i$, a triphasic pattern of interaction was evidenced (Fig. 3b). When $\sigma_i$ was between 10 and 15 mN/m, the maximal surface pressure increase induced by the peptide gradually decreased as $\sigma_i$ increased. Between 15 and 25 mN/m, the peptide did not affect the surface pressure of the GalCer monolayer. Finally, for values of $\sigma_i$ greater than 25 mN/m, a specific interaction occurred, with a critical pressure of insertion of 42 mN/m. These data indicate that both peptides P1 and P2 could interact with GalCer, with a marked preference for densely packed films that mimic the organization of sphingolipids found in membrane rafts. The minor differences observed at low surface pressures ($\sigma_i < 25$ mN/m) may not have important implications, since these values of the surface pressure do not reflect the organization of lipids commonly found in biological membranes (25).

From a structural point of view, these data are consistent with the high level of structural homology between the wild-type and the E200K mutant of human PrP (27). The main effects of the E200K mutation are (i) major changes in the distribution of charges on the protein surface and (ii) the loss of a salt-bridge interaction between the side chains of Glu$^{200}$ and Lys$^{204}$. In any case, the E200K mutation has little (if any) effect on the orientation and accessibility of aromatic residues that are involved in binding to GalCer.

However, the redistribution of surface charges induced by the E200K mutation may dramatically affect the interaction of PrP with charged lipids such as sphingomyelin. As shown in Fig. 4b, the mutated peptide (P2) could interact with sphingomyelin only when the film was prepared at a very low $\sigma_i$ (<15 mN/m). In particular, no interaction occurred at the physiological pressure of 30 mN/m. In contrast, the wild-type peptide (P1) interacted with sphingomyelin films prepared at low and at high $\sigma_i$ (critical pressures of insertion of 25 and 40 mN/m, respectively) (Fig. 4a). These data show that the E200K mutation specifically affected the recognition of sphingomyelin. The replacement of an acidic residue (Glu) by a basic one (Lys) in the sphingolipid binding site is likely to affect the binding of PrP to this positively charged lipid. Impaired recognition of sphingomyelin in the raft environment may destabilize membrane-PrP$^\text{c}$ interactions and thus facilitate the conformational change associated with the PrP$^\text{c} \rightarrow$ PrP$^\text{sc}$ transition. Indeed, the depletion of sphingomyelin in neural cells treated with either a ceramide synthase inhibitor or sphingomyelinase resulted in a marked stimulation of the PrP$^\text{c} \rightarrow$ PrP$^\text{sc}$ conversion (28). Most importantly, the V3-like domain of PrP identified in the present study is involved in the dimerization of PrP, an early event that constitutes an important step on the pathway of the PrP$^\text{c} \rightarrow$ PrP$^\text{sc}$ conversion (29). Moreover, amino acid residues located in the vicinity of the putative raft-binding domain of PrP are thought to bind an auxiliary molecule essential to prion propagation (30). Taken together, these data support the view that the V3-like domain of PrP is involved in the PrP$^\text{c} \rightarrow$ PrP$^\text{sc}$ conversion and that raft sphingolipids may play an active role in this process. This hypothesis is consistent with recent data showing that the conformation of prion proteins is highly sensitive to the membrane environment (31).

We propose that sphingolipids such as GalCer and sphingomyelin stabilize the non-pathological conformation of PrP$^\text{c}$ in the lipid raft through specific interactions with the V3-like domain of PrP$^\text{c}$.

The $\beta$-Amyloid Peptide Interacts with GalCer and Sphingomyelin—Since a similar domain was characterized in Alzheimer $\beta$-amyloid peptide, we studied the interaction of this peptide with GalCer and sphingomyelin using the monomolecular film binding assay (Fig. 5). In both cases, $\Delta \sigma_{\text{max}}$ gradually decreased as $\sigma_i$ increased (critical pressures of insertion of 45 and 56 mN/m for GalCer and sphingomyelin, respectively). The ability of the $\beta$-amyloid peptide (fragment 1–40) to recognize both GalCer and sphingomyelin is likely due to the presence of both sugar-binding residues (Tyr$^{10}$, His$^{13}$, His$^{14}$, Phe$^{20}$, and acid residues (Asp$^2$, Glu$^{11}$) within the V3-like motif.

Conclusion—The finding of a common sphingolipid-binding motif in Alzheimer, prion, and HIV-1 proteins underscores the role of membrane rafts in the pathogenesis of the corresponding diseases. Further studies are warranted to assess whether raft lipids act as auxiliary molecules implicated in the conformational change of PrP (2, 3), as recently established for HIV-1 gp120 (13) and for the Alzheimer $\beta$-amyloid peptide (14). In any case, the main outcome of the present study is the finding of a structural homology between unrelated proteins known to induce major morphological and functional alterations of the central nervous system. Synthetic soluble analogs of GalCer bind to the V3 loop of gp120 and inhibit HIV-1 fusion (32). It would be of interest to evaluate the activity of such glycolipid analogs on the PrP$^\text{c} \rightarrow$ PrP$^\text{sc}$ conversion as well as on the formation of amyloid fibrils.

REFERENCES


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