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Review

Molecular characterization of the detergent-insoluble cholesterol-rich membrane microdomain (raft) of the central nervous system

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Abstract

Many fundamental neurological issues such as neuronal polarity, the formation and remodeling of synapses, synaptic transmission, and the pathogenesis of the neuronal cell death are closely related to the membrane dynamics. The elucidation of functional roles of a detergent-insoluble cholesterol-rich domain (raft) could therefore provide good clues to the molecular understanding of these important phenomena, for the participation of the raft in the fundamental cell functions, such as signal transduction and selective transport of lipids and proteins, has been elucidated in nonneural cells. Interestingly, the brain is rich in raft and the brain-derived raft differs in its lipid and protein components from other tissue-derived rafts. Since many excellent reviews are written on the membrane lipid dynamics of this microdomain, signal transduction, and neuronal glycolipids, we review on the characterization of the raft proteins recovered in the detergent-insoluble low-density fraction from rat brain. Special focus is addressed on the biochemical characterization of a neuronal enriched protein, NAP-22, for the lipid organizing activity of this protein has become increasingly clear.

Keywords: Cholesterol; CNS; Synapse; GPI-anchor; Inner leaflet; NAP-22

1. Raft: structure and function

The clustering of cholesterol and (glyco)sphingolipids within the cell membrane causes the formation of distinct domains or lipid rafts [1–4]. GPI-anchored proteins and acylated proteins associate with the outer and the inner leaflet, respectively [5]. Owing to the highly saturated long-chain fatty acid and sphingolipid base moiety, sphingolipids in purified form would be in the gel phase at physiological temperatures. However, cholesterol preferentially associates with these sphingolipid domains and promotes conversion to a liquid ordered state [6]. The saturated acyl chains characteristic of GPI-anchored proteins associate with this liquid ordered state [5,7–11].

In contrast to the fairly well-characterized outer leaflet lipids, little information is available concerning the cytoplasmic leaflet lipids [4,12,13]. Since many acylated proteins localize on the inner leaflet of the raft, the elucidation of the raft structure in this sphingolipid-poor leaflet is of great importance [14–18]. Also important is the molecular characterization of the communication between outer and inner leaflet. The co-redistribution of the doubly acylated Src family protein kinases, after the cross-linking of cell surface GPI-anchored proteins or gangliosides using antibodies or toxins, suggests the presence of such communication [19,20]. The elucidation of such communication will be essential to understand the molecular roles of raft in signal transduction.

Another important role of the raft is the transport and retention of lipids and proteins within the cell. Site specific sorting of membrane components is essential to establish and maintain the cell polarity observed in epithelial cells and neurons [21,22]. It is from the studies on the specific sorting of membrane domains in epithelial cells that the concept of "raft" was embodied [23–25]. It is well recognized that cellular cholesterol levels are precisely controlled through biosynthesis, influx into and efflux from cells via lipoproteins. Since cholesterol is one of the major lipids in the raft, much attention has been paid to the roles of the raft in the intracellular trafficking and compartmentation of cholesterol [26–30].

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2. Brain, a cholesterol- and sphingolipid-enriched tissue

The largest pool and concentration of cholesterol in the body exists in the brain [31–34]. Being a constituent of cell membrane, cholesterol is important for the function of this organ, and inborn defects in cholesterol metabolism and transport are associated with serious neurological and mental dysfunctions [35–44]. Alterations in cholesterol metabolism occur with age [45–47] and have been implicated in the pathogenesis of Alzheimer's disease, because the production of Aβ is regulated by membrane cholesterol and the accumulation of the peptide occurs on the ganglioside GM_1 located on raft [48,49]. Studies on cholesterol metabolism discovered the functional roles of apolipoprotein E, and the elucidation of the allele type of apolipoprotein E as a risk factor for the Alzheimer's disease further illuminated its receptors as signaling molecules [50,51]. In addition, recent studies showed the importance of cholesterol-derived neurosteroids, cholesterol modification of proteins, and supply of cholesterol in early development, synaptogenesis, and synaptic functions [52–60].

There exist some pioneering works focused on the lipid composition, asymmetrical distribution, and its changes in the synaptic plasma membrane during brain development [27,61–64]. Interestingly, an asymmetrical distribution of cholesterol in the synaptic membrane (~ 85% in the inner leaflet) reduces after chronic ethanol treatment or aging [27]. Furthermore, knockout mice deficient in the low-density lipoprotein receptor, apolipoprotein E, or both proteins, also showed the increase of cholesterol in the outer leaflet [64].

Brain is also a rich source of gangliosides and the content and chemical characteristics of brain gangliosides are under genetic control, differing in various brain regions and during development. Much attention has been paid on the various functions of the gangliosides in various neuronal events, including differentiation and survival, signal transduction, synaptic transmission, and neuronal plasticity [65–71]. The study focused on the functional roles of cholesterol, and sphingolipid-enriched domain (raft) is therefore of central importance for the molecular understanding of the central nervous system.

3. Identification of a NAP-22 localized fraction as the brain-derived raft

The growth cone is a neuronal specialized sensory and motor apparatus localized at the tip of the neuronal process [72,73]. During the characterization of membrane components of the growth cone, we noticed a very acidic calmodulin-binding protein present in a Triton-insoluble membrane fraction. Since molecular cloning showed that the molecular mass of this protein is 22 kDa, the protein was termed NAP-22 (neuronal acidic protein of 22-kDa) [74]. Further studies showed the effect of calmodulin on the phosphorylation of NAP-22 with protein kinase C [75,76]. Although the homology between NAP-22 and CAP-23 (cortical cytoskeleton-associated protein) was not so evident at this time, these proteins were found identical after the recloning of CAP-23 [77,78]. The same protein is also called BASP1 [79]. From the studies using knockout and knockin mice, Frey et al. [80] showed the participation of CAP-23/NAP-22 on the membrane dynamics through the regulation of the actin dynamics. In contrast, the biochemical analysis was not enough to explain its cellular function. The protein was assumed to be a hydrophilic protein because this protein had no hydrophobic sequence. Biochemical fractionation studies of the brain tissue showed that the protein was present in the Triton-soluble and Triton-insoluble membrane fraction, not in the soluble fraction. Further characterization showed the N-terminal myristoylation of this protein [81]. Myristoylation, however, explains only the membrane association. The molecular mechanism of the Triton-insolubility was still to be elucidated. Interestingly, the Triton solubility of NAP-22 decreased gradually during the maturation of the brain. Since the localization of NAP-22 in the synaptic membranes, such as presynaptic membrane and synaptic vesicles, was very interesting [82,83], the fractionation of the Triton-insoluble structure was then attempted.

After a sucrose density gradient centrifugation, the NAP-22-enriched Triton-insoluble membrane domain was recovered in a low-density region (~ 0.6 M sucrose) as a white

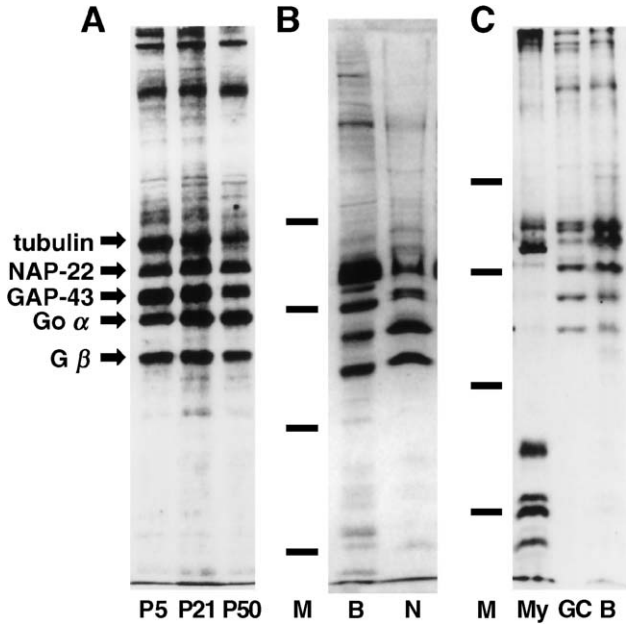

Fig. 1. SDS-PAGE patterns of the Triton-insoluble low-density fraction (raft) of rat brain. (A) Fractions isolated from P5, P21, and P50 forebrains were analyzed using a 10% acrylamide gel. (B) Rafts from the forebrain of 2-week-old rat (P14) (B) and from cultured cortex neurons (N, 8 days in culture) were compared using an 11% gel. (C) The raft fractions from myelin fraction (My), growth cone fraction (GC), and forebrain (B) were electrophoresed in a 12.5% gel. M, marker proteins (68, 43, 29, and 21 k, from top to bottom).

layer separated clearly from other more high-density fractions. Partial amino acid sequencing of some major proteins in this fraction showed the localization of GAP-43 and the trimeric G-protein Go in addition to NAP-22 [84]. Further 2D PAGE and Western blot analysis showed the enrichment of other G-proteins (Gs, Gi), Src family protein kinases (Src, Fyn, Lyn), and some GPI-anchored proteins [84]. Since Triton-insolubility, low-density, and the enrichment of GPI-anchored proteins and doubly acylated proteins were the characteristics of the raft, the NAP-22 enriched fraction was hence judged to be the brain-derived raft [25,85]. Fig. 1A shows the SDS-PAGE pattern of the raft components derived from three different developmental stage brains. A couple of major proteins exist in all three preparations. Since these proteins are also present in the raft fractions obtained from cultured primary neurons and the growth cone fraction but not myelin fraction (Fig. 1B and C), the relative amount of the myelin proteins in the raft fraction is fairly low. As described above, these major proteins were identified as NAP-22, GAP-43, α and β subunit of trimeric G-protein Go, and tubulin. Among these proteins, NAP-22 localized predominantly in the raft [84,86].

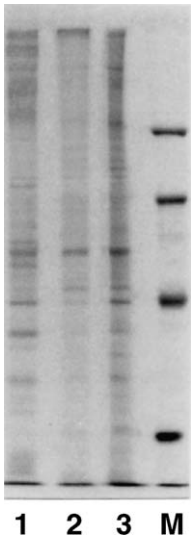

Fig. 2. SDS-PAGE analysis of the low-density fractions obtained from the synaptic plasma membrane fraction from 6-week-old rat forebrain treated with Triton X-100 [1], pH 11 buffer [2], and sonication [3] before a density gradient centrifugation. M, marker proteins (97, 68, 43, and 29 k, from top to bottom).

4. Isolation of the brain-derived raft

The raft domain is recovered in a low-density fraction after the treatment of the membrane with the nonionic detergent such as Triton X-100 and sucrose density gradient centrifugation [14,23,24]. The enrichment of cholesterol and sphingolipids is ascribed to be responsible for the detergent insolubility. As little expression of caveolin is observed in brain tissue, the caveola domain is hard to detect in the fraction obtained [14,15,17,87]. Since the detergent extraction leads to raft aggregation, it is difficult to separate individual rafts. Alternatively, nondetergent methods were applied to avoid detergent-induced membrane mixing. One of such methods employed sonication and the other used an alkaline treatment to disrupt membranes before the density gradient centrifugation [88–90]. The low-density fractions prepared from these methods were also considered to be the raft. The sonication method, however, was judged not to be applicable to the brain because the brain tissue contains much amount of low-density membrane fractions such as the myelin membrane and synaptic vesicles. Fig. 2 shows the SDS-PAGE patterns of the low-density fractions prepared from the synaptic plasma membrane using these three methods. A comparison of the protein components of these fractions clearly shows that these fractions are not identical. Here we focus on the protein components recovered in the detergent-insoluble raft because this method is able to handle a fairly large amount and also easy to reproduce. Very little protein recovery (~ 0.1% of total protein) in the raft fraction has been reported using nonneural cells and tissues [13,14,88]. In contrast, a fairy large amount of protein was recovered from the brain (more than 1%) even after repeated washes using Triton X-100. The brain is hence a raft-enriched tissue.

5. Extracellular proteins

The localization of GPI-anchored proteins to the raft is well recognized. Prion protein, probably the most famous GPI-anchored protein, also localizes in the raft [91,92]. Lipid modulations were shown to effect the formation of the Scrapie type prion protein [93]. Recent studies showed that prion is a synapse-localized protein associated with the dystrophin complex [94,95]. These results could provide a good clue to elucidate the neuronal function of the protein. Concerning the GPI-anchored cell adhesion molecules, Olive et al. [96] showed the localization of F3 in the raft and its interaction with L1 in cerebellum. F3 is also known to interact with paranodin (casper) in the course of myelinated axon formation [97]. Thy-1 is a small GPI-anchored protein abundant in brain and is known to participate in axonal outgrowth, synaptic regulation, and synaptic transmission [98–100]. A search for activity-regulated genes resulted in the identification of a small GPI-anchored protein, CPG-15 (activity-regulated candidate plasticity gene). CPG-15 promotes dendritic growth and axon arbor elaboration in an activity-dependent manner [101,102].

In order to identify the GPI-anchored proteins in the raft from 2-week-old rat brain, amino acid sequences of the major proteins were analyzed after the solubilization with PI-PLC treatment and deglycosylation. The presence of N-CAM-120, F3, T-cadherin, and Thy-1 was identified with this analysis and/or Western blot [103]. T-cadherin is a truncated form of cadherin, and a recent study suggested

that LDL is a physiologically relevant ligand for T-cadherin [104,105]. In addition to these proteins, a novel protein (Kilon), which belongs to a subfamily (IgLON family) of the immunoglobulin superfamily, was detected as one of the GPI-anchored protein [103]. Further immunohistochemical studies using specific antibodies to Kilon or OBCAM, another IgLON family protein, showed the localization of these proteins in the post synaptic density region [106]. The region-specific expression of these proteins in developmentally regulated manners suggests their important roles in the formation and maintenance of the CNS [107–111]. Different localization of various GPI-anchored proteins on the neuronal surface was also shown by Madore et al. [112]. Following this work, segregation of gangliosides GM1 and GD3 on the cell membrane is reported [113]. These results clearly show that there exist a variety of raft in the protein and lipid components.

Since GPI-anchored proteins have no transmembrane domains, the elucidation of the mechanism by which these proteins transmit the extracellular signal is important to understand the signaling pathways of cells. For example, GPI-anchored protein TAG-1 and ganglioside GD3 caused the activation of cellular tyrosine kinases through an unknown pathway [114]. In case of the glia-derived neurotrophic factor (GDNF) family ligand, the signal is transmitted through the recruitment of the signaling complex (ligand/GPI-anchored receptor/transmembrane protein, RET) to raft [115–119]. Future studies focused on the molecular interaction of GPI-anchored proteins are clearly needed to elucidate the background of the cell-to-cell interactions in the nervous system.

6. Transmembrane proteins

Curiously, there is no transmembrane protein as a major component in the brain-derived raft (Fig. 1). The identification through the Western blot analysis has hence been the major method to detect these proteins. One of the raft marker proteins, flotillin, originally found in the caveola of 3T3-L1 mouse fibroblast differentiated to adipocyte, was shown to be in the neuronal raft [120,121]. Since the localization of some ion channels in raft and the regulation of their function with lipids are just beginning to notice, further studies will be very promising to elucidate the functional role of raft in the neuronal signal transmission [122,123]. Another transmembrane raft protein was identified through the studies on the regulation of Src kinase family proteins in brain. This protein, called Cbp, regulates the Src-family protein kinases that are also localized in neuronal raft [124].

Although the enrichment of some receptor-type tyrosine kinases, the insulin receptor and TrkB (brain-derived neurotrophic factor receptor), in the brain-derived low-density fraction prepared by the sonication method was reported, further study showed the solubilization of these proteins with Triton X-100 [125,126]. Aβ is a 40–43-amino-acid peptide derived by the proteolytic cleavage of the integral membrane amyloid precursor protein (APP) [127]. Partial localization of APP in raft, Aβ binding to ganglioside GM_1, and the modulatory effect of cholesterol on the specific cleavage of APP indicated the importance of raft in the pathogenesis of Alzheimer's disease [48,49,128–136].

7. Proteins associated to the inner leaflet of raft

7.1. Molecular characterization of NAP-22

NAP-22 has properties very similar to GAP-43 (growth associated protein of 43 kDa, also called neuromodulin) and MARCKS (myristoylated alanine-rich C-kinase substrate) [137,138]. They are relatively small acidic proteins with abnormal behavior in SDS-PAGE and bind calmodulin, although the amino acid sequences of the calmodulin binding sites are different from each other. These proteins are resistant to boiling treatment or acid precipitation. Membrane association is due to the myristoylation (MARCKS and NAP-22) or palmitoylation (GAP-43), and the phosphorylation by protein kinase C inhibits the calmodulin binding [137–145].

Considering the low dissociation constant between NAP-22 and calmodulin (1.2 nM), the high cellular content of these proteins (60 μM calmodulin, 20 ~ 40 μM NAP-22), and the localization of NAP-22 in synaptic regions, calmodulin could participate in the neuronal membrane dynamics through the interaction with NAP-22 at the synaptic region [74,137,138]. Since alcohol extraction was effective to solubilize NAP-22 and GAP-43, these proteins were assumed to bind to the membrane through interactions with membrane lipids. The lipid components of brain raft were then studied.

7.2. Lipid components of raft and cholesterol-dependent lipid binding of NAP-22

An analysis of lipid components recovered in the Triton-raft from the 2-week-old rat brain showed the enrichment of cholesterol and sphingomyelin (SM) as observed in other rafts, although the enrichment of SM was not so evident compared to the case of cultured cells. Interestingly, the recovery of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) in the raft was clearly observed [85]. Since the enrichment of PC, PE, and cholesterol was also reported in the GPI-anchored protein-rich fraction (which corresponds to the raft) of chicken brain prepared with Nonidet P-40, there may be some factor(s) which assemble these lipids in the brain-derived raft [146].

An attempt to extract cholesterol using methyl-β-cyclodextrin (MCD) showed a dose-dependent and specific solubilization of NAP-22 (Fig. 3). It is calculated that the extraction of 460 cholesterol molecules causes the solubili-

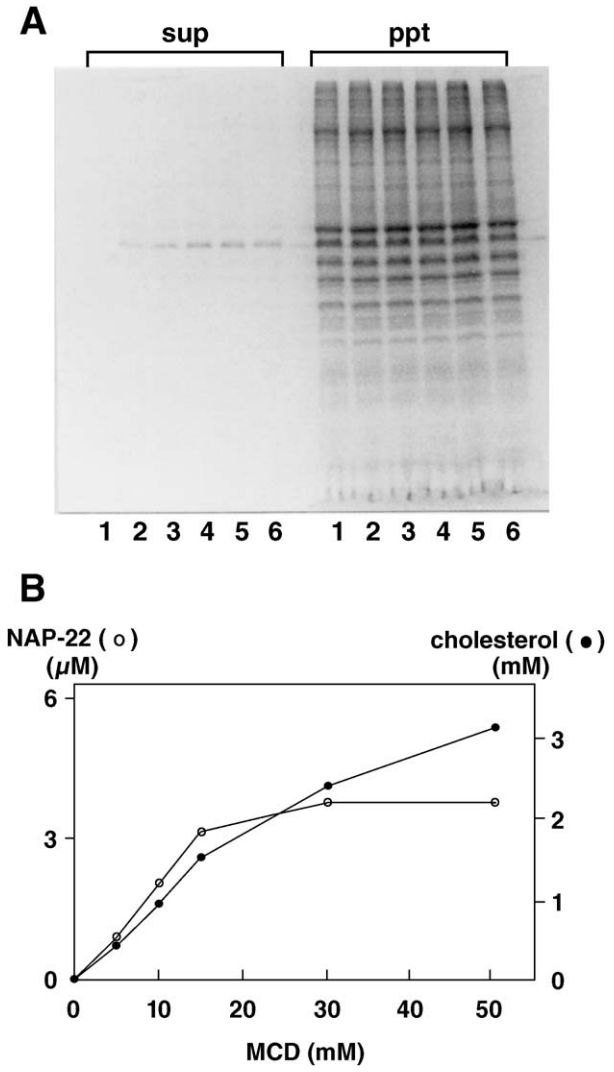

Fig. 3. Stoichiometric solubilization of cholesterol and NAP-22 with MCD. (A) After incubation on ice for 60 min with MCD (1, 0 mM; 2, 5 mM; 3, 10 mM; 4, 15 mM; 5, 30 mM; 6, 50 mM), the samples were centrifuged at 100,000 × *g* for 30 min to separate supernatant (sup) and pellet (ppt). Pellets were suspended in the original volume to represent the solubilizing effect after the analysis of the same volume in SDS-PAGE. (B) Solubilization of NAP-22 and cholesterol with MCD. The amounts of NAP-22 and cholesterol in the supernatants were plotted for used MCD concentrations.

zation of one NAP-22 molecule. Since PC was the main glycerolipid in the raft, the effect of cholesterol on the liposome binding of NAP-22 was studied using PC/cholesterol liposomes. Increasing amounts of NAP-22 to a constant amount of PC/cholesterol liposome proved a dose-dependent binding, and a Scatchard plot analysis showed a binding ratio of about 760 cholesterol molecules/1 molecule of NAP-22. Assuming an even distribution of cholesterol in the inner and outer leaflet of the liposome, this means one NAP-22 molecule covers a region composed of 380 cholesterol molecules in the liposome. This figure corresponds well with that obtained in the extraction experiment [85]. Interestingly, changing the headgroup from zwitterionic phosphatidylcholine to anionic phosphatidylserine virtually eliminated the binding of NAP-22 to liposomes even in the presence of cholesterol. This result was further confirmed by Epand et al. using multilamellar vesicles composed of 1-stearoyl-2-phosphatidylcholine, dioleoylphosphatidylcholine, and dipalmitoylphosphatidylcholine. They also showed that NAP-22 promotes the formation of cholesterol microdomain in liposomes composed of cholesterol and PC [147]. The lipid-organizing ability of NAP-22 to induce cholesterol microdomain in the membrane is thus different from other cholesterol binding proteins such as caveolin and START (StAR-related lipid transfer) domains [148,149].

Since NAP-22 shows a Ca^{2+}-dependent calmodulin binding, the effect of calmodulin on this binding was interesting. Calmodulin not only inhibited the binding but also caused the dissociation of NAP-22 from the liposomes. Further studies showed that NAP-22 also binds PC/PE liposome to some extent. Since PE has much higher T_m than PC because of its small headgroup, this result suggests the importance of PE in the raft formation in the inner leaflet. The three major lipid species in the brain-derived raft were therefore shown to interact with NAP-22.

In order to elucidate the molecular background of neurodegeneration, the mechanism of LDL, LDL receptor-related proteins (LRPs), apolipoprotein E, and Aβ in the maintenance of the transbilayer distribution of cholesterol and their effects on the signaling mechanism are under intense investigation [150–158]. Considering the asymmetric distribution of cholesterol in the synaptic plasma membrane, further studies on the role of NAP-22 on the distribution and organization of cholesterol within the neuronal membrane will be important to understand the lipid–protein interactions in signal transduction.

7.3. Localization and cellular function of NAP-22 in brain and neurons

Since NAP-22 shows a specific localization to the raft, NAP-22 provides a good marker to identify raft domains in neurons and in brain sections. The Western blot analysis showed a predominant expression of NAP-22 in brain but not in other tissues [74]. NAP-22 immunoreactivity was detected through the whole brain, and dense staining was observed in the forebrain including cerebral cortex, hippocampal formation, olfactory bulbs, basal ganglia, and thalamus. Immunoreactivity was distributed densely at the neurophil, whereas nerve cells and nerve fibers had little or no reaction. NAP-22 immunoreactivity was observed to be associated mainly with pre- and postsynaptic membranes and synaptic vesicles [82]. Investigation on the changes in the localization of NAP-22 during the development of the neuronal polarity in vitro and in vivo, using cultured hippocampal neurons and developing cerebellum neurons, showed a gradual localization of the protein to the synaptic region [159]. The time course of the accumulation was much slower than that of GAP-43 in hippocampal neurons.

In the cerebellar sections, the synaptic accumulation of NAP-22 was not evident in 1-week-old neurons, although the accumulation of VAMP-2, a synaptic vesicle protein, was already observed at this stage. These results suggest that NAP-22 plays an important role in the maturation and/or the maintenance of synapses by controlling cholesterol-dependent membrane dynamics.

Synergistic participation of GAP-43 and CAP-23 (NAP-22) on the neurite plasticity is observed in adult double transgenic mice [80,160]. Expression experiments of these proteins in COS7 and PC12B cells showed that GAP-43, CAP-23, and MARCKS modulate PI(4,5)P_2 at the plasma membrane raft and regulate cell cortex actin dynamics [161]. Strong binding of NAP-22 to liposomes containing PIP or PIP_2 is also observed [162]. These results suggest that NAP-22 organizes specific lipid species such as cholesterol, PE, PIP, and PIP_2 at the raft domain.

8. Calcium-dependent raft-localizing proteins

Calcium ions play multiple functions in the nervous system. One possible participation of Ca^{2+} ions is to reconstruct the raft through the interaction of calmodulin with NAP-22 as described above. To investigate the other possible participation, calcium-dependent raft binding proteins were screened. After the incubation of an EGTA-extracted cytosolic fraction with the raft in the presence of Ca^{2+} ions, the raft fraction was recovered and washed several times with a solution containing Ca^{2+} ions. Ca^{2+}-dependent binding proteins were then eluted with a solution containing EGTA. SDS-PAGE analysis revealed the specific association of several proteins. Amino acid sequencing and Western blot identified these proteins as annexin VI, protein kinase C α, and neurocalcin α. Although the Ca^{2+}-dependent membrane association of these proteins has been well known, this study identified their binding domain as the raft microdomain within the membrane [163]. Since little is known on the targets of these proteins, further investigation will provide much more information on the Ca^{2+}-dependent signaling mechanism. In addition, considering the specific lipid composition of the raft described above and the selective association of these proteins, identification of the membrane-binding region within these proteins will provide good probes to the membrane microdomains.

9. Localization of cytoskeletal proteins and their regulators

As described above, there exist three different membrane fractionation methods before the density gradient centrifugation to separate raft domains. The localization of Rho family proteins in the raft fraction was reported using the sonication method in fibroblastic cells [164]. To examine the heterogeneity of the components in these raft fractions, localization of these proteins in the sonication-raft and the Triton-raft obtained from the sonication-raft was compared using the synaptic plasma membrane fraction. Although the enrichment of Rac1, RhoA, and Cdc42 in the sonication-raft was confirmed, further extraction with Triton showed the localization of Rac1, but not RhoA nor Cdc42, in the Triton-raft. Interestingly, some part of Rac1 in the Triton-raft was solubilized after incubation with MCD. The localization pattern of Rac1 in neurons observed with an immunocytochemical technique, however, was quite different from that of NAP-22 [165]. This result further supports the idea that there exist various rafts even in one neuron [112,113].

The localization of Rac1, gelsolin, and an actin regulatory phospholipid (PIP_2) in the raft further suggests the participation of raft in the neuronal morphological change [80,160,161,165,166]. A recent finding that raft is the localizing place of a new type of phosphatidylinositol 4-kinase (PI4KII) indicates that raft is not only the storehouse of the signaling lipid molecules but also the factory of these important molecules [167,168]. Interestingly, tubulin, the microtubule building block, was also present as the major protein in the brain-derived raft [84]. The participation of lipid modification of tubulin on the localization was shown by Palestini et al. [169]. The localization is also ascribed to the presence of a palmitoylated neuronal tubulin binding protein, SCG10, in the raft [170]. Since the localization of tubulin was not so evident in the rafts from other tissues or cells, the elucidation of the localization mechanism and the role of tubulin in the raft could be a good clue to understand the function of neuronal raft.

10. Rafts and membrane cycling

Since the localization of NAP-22 on the synaptic vesicle (SV) was shown by an immunohistochemical study, the purification of the SV was performed to evaluate the amount of NAP-22 on SV. NAP-22 and synaptophysin, an SV marker, showed a resembled pattern during SV purification. An immunoprecipitation of this SV fraction with anti-synaptotagmin antibody showed the precipitation of synaptophysin and NAP-22 together with synaptotagmin. N-CAM, a synaptic plasma membrane protein, however, was not precipitated. These results further confirmed the presence of NAP-22 and hence the presence of the raft in SV. A densitometric analysis showed that NAP-22 comprised 1.3% of the total protein in SV; this means about 1.8 molecules of NAP-22 in one SV. Since two molecules of synapsin I are estimated to be in one SV, the molar amount of NAP-22 is comparable to that of synapsin I, a well-known synaptic vesicle protein [171]. The localization of vesicular H^+-ATPase in the rafts of the chromaffin granule membrane and SV and the finding that some SNARE proteins and synaptophysin have affinity to cholesterol suggest the participation of the raft in the neuronal membrane cycling [172–176].

11. Other raft proteins

In addition to the major protein components listed above, a variety of important proteins exist in the neuronal raft. For example, the raft-localization of some of the postsynaptic density proteins was shown recently [177,178]. At present, the identification of the raft-localized transmembrane proteins in neurons is poor compared to the outer leaflet- and inner leaflet-localized proteins. Some GPI-anchored proteins are known to recruit transmembrane proteins to the raft after the binding of their ligands [119,179]. The exclusion of raft proteins during the signal transduction is also known [180]. Further studies on the lipid-binding domains in the raft-localized transmembrane proteins will be useful to identify the raft-localized proteins from the protein database in silico. Introduction of novel lipid probes will also contribute to the further characterization of the lipid–protein interactions in various rafts [181–183].

12. Summary and perspectives

The characterization of growth cone membrane proteins resulted in the identification of an acidic protein, NAP-22, as a major neuronal raft component. Trimeric G-protein Go, GAP-43 (neuromodulin), tubulin, several GPI-anchored proteins, and various signal transducing proteins were also detected in the raft from CNS. The enrichment of cholesterol, PC, and PE was detected compared with the lipid components of raft fractions from cultured cells or other tissues. Further characterization of NAP-22 showed that this protein interacts with cholesterol within the membrane and induces a cholesterol-rich domain in the membrane. The enrichment of these lipids in the brain-derived raft is, hence, partly ascribable to the localization of NAP-22. Considering the high expression, cholesterol assembly, calmodulin binding, and C-kinase phosphorylation, NAP-22 seems to have a pivotal role in the regulation of the membrane dynamics in neurons. Through the characterization and localization studies of the brain-derived raft components, the variety of the raft not only in protein composition but also in lipid composition has become increasingly clear. In addition, there occur mixing and segregation of the raft components during the neuronal function. Since many neuronal functions such as secretion of neurotransmitters, internalization of various receptors, formation and remodeling of synapses are based on the membrane dynamics, the elucidation of the roles of the raft in these events is of great importance to understand the molecular mechanism of the central nervous system.

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