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Hypothesis

Curvature factor and membrane solubilization, with particular reference to membrane rafts

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Abstract

The composition of membrane rafts (cholesterol/sphingolipid-rich domains) cannot be fully deduced from the analysis of a detergent-resistant membrane fraction after solubilization in Triton X-100 at 4°C. It is hypothesized that the membrane curvature-dependent lateral distribution of membrane components affects their solubilization. The stomatocytogenic, Triton X-100, cannot effectively solubilize membrane components, especially with regard to the outward membrane curvature.

Keywords: detergent-resistant membrane; membrane curvature; raft; solubilization; Triton X-100

1. Introduction

The raft hypothesis, initially proposed to explain the enrichment of glycosphingolipids at the apical surface in polarized epithelia cells, suggests that self-association of sphingolipid and cholesterol drives the lateral segregation of lipids and proteins, resulting in the formation of specific functional DRM (detergent-resistant membrane) microdomains (Simons and Ikonen, 1997). The 'membrane rafts' are defined as small (10–200 nm), heterogeneous, dynamic, sterol- and sphingolipid-enriched domains that compartmentalize cellular processes, which can also be stabilized to form larger platforms (Pike, 2006). DRM fraction analysis after solubilization of membranes with TX-100 (Triton X-100) at 4°C and isolation using ultracentrifugation on density gradients (Brown and Rose, 1992; Arni et al., 1998; Brown and London, 1998) is widely used to determine the composition of rafts (Simons and van Meer, 1988; Simons and Ikonen, 1997). The DRM fraction is structurally a mixture of vesicles and membrane sheets (Giocondi et al., 2000; Magee and Parmryd, 2003) that is typically enriched in sphingolipids, including G_{M1} (ganglioside G_{M1}), cholesterol and specific proteins. There are numerous examples in which changes in the abundance or post-transcriptional status of signalling molecules in DRM fractions correlated with functional changes, thereby rendering DRM analysis useful in identifying subtle alterations in membrane organization. However, in view of the current concept of membrane rafts in which they are assumed to be small, short-lived, heterogeneous lipid inhomogeneities in the membrane that may eventually be stabilized by specific proteins, the relationship between membrane rafts and DRM has to be critically evaluated (Simons and Vaz, 2004; Hancock, 2006; Pike, 2006). On the one hand, it has to be taken into consideration that artefacts may arise during solubilization by detergent; on the other, it has to be taken into account that various types of membrane domains with respect to size and composition (that presumably coexist in the membrane) become merged in DRM preparation.

Several studies have indicated that DRM composition does not perfectly reflect raft composition (Madore et al., 1999; Heerklotz, 2002; Pike, 2004; Lichtenberg et al., 2005; Gaus et al., 2005; Brown, 2006; Ayuyan and Cohen, 2008). DRM fraction analysis is an indirect method, and therefore a more direct examination of native membrane raft composition has been demanded. Transmission electron microscopic and fluorescence microscopic observations have indicated that membrane proteins and cholesterol in DRM fractions are not necessarily located in G_{M1}-enriched domains (Fra et al., 1994; Wilson et al., 2004; Wüstner, 2007). Similarly, no lateral accumulation of the DRM-associated outer leaflet GPI (glycosylphosphatidylinositol)-anchored CD59 or integral inner leaflet proteins, stomatin, and cytoplasmic synexin and sorcin to CTB (cholera toxin subunit B) plus anti-CTB-induced G_{M1} patches, was observed by fluorescence microscopy in the human erythrocyte membrane (Salzer et al., 2002; Murphy et al., 2004; Mrówczyńska and Hägerstrand, 2008; Mrówczyńska et al., 2010). However, these proteins possess a similar membrane curvature-dependent distribution to G_{M1} by accumulating, but poorly co-localizing, with G_{M1} in ionophore A23187 plus calcium-induced spiculae. To explain these findings, i.e. how the distribution of certain membrane proteins in G_{M1} patched and curved membrane relates to DRM fraction composition, we suggest a novel mechanism effective in TX-100-induced solubilization. In this model (Figure 1), outward curved membrane areas remain unsolubilized (DRM) due to the weak ability of TX-100 to accumulate in these regions, because of its preferential inner leaflet distribution and conical shape.

2. Hypothesis

Amphiphile (detergent) molecular properties can in specific ways affect bilayer bending in erythrocytes, resulting in either stomatocytic (invaginated) or spiculated echinocytic (exvaginated) cell shapes (and further in endo- or exo-vesiculation respectively;

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Abbreviations: CTB, cholera toxin subunit B; DRM, detergent-resistant membrane; G_{M1}, ganglioside G_{M1}; GPI, glycosylphosphatidylinositol; TX-100, Triton X-100.

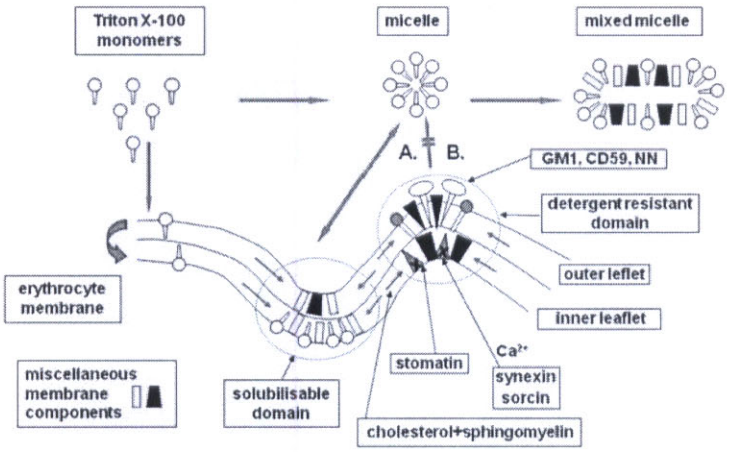

Figure 1 Schematic cartoon showing a hypothetical mechanism effective in TX-100-induced solubilization

The principal effective molecular shapes of TX-100 and some selected membrane components are shown. TX-100 may not solubilize outward curved membrane areas, and has reduced efficiency in flat membrane. The course of events suggested is: monomeric TX-100 intercalates into nearly flat membrane areas of erythrocytes (Salzer and Prohaska, 2001) or ghosts (Palek et al., 1974; Murphy et al., 2004); accumulates in the inner membrane leaflet, thereby striving to bend the membrane inwards; and redistributes laterally, locally creating (due to variation of the lateral composition of the membrane) small membrane invaginations and hence also indirectly creating outward bend (or otherwise segregated) membrane areas at the lips of small invaginations or in vesicle-like domains (buds), where outward-curvature-preferring membrane components are accumulated and protected from TX-100 solubilization. Similarly, other membrane components are accumulated in flat membrane areas, where the concentration of TX-100 monomers is low.

Deuticke, 1968; Fujii et al., 1979; Kuypers et al., 1984; Isomaa et al., 1987; Hägerstrand and Isomaa, 1989, 1992), and thereby in curvature-dependent lateral segregation of membrane components (Hägerstrand and Isomaa, 1994; Hägerstrand et al., 2006; Mrówczyńska and Hägerstrand, 2008). Physico-chemical properties and the balance of the hydrophobic and hydrophilic molecular parts of amphiphiles determine the type of membrane perturbation. We propose a mechanism where membrane-curvature-dependent segregation of membrane components during solubilization contributes to the specific fingerprint of TX-100-induced DRM (Figure 1). This mechanism is related to the stomatocytogenic (inward membrane bending) effect of TX-100. Preferential interaction of TX-100 with inner leaflet membrane components results in its accumulation in the inner membrane leaflet and hence a relative expansion of this leaflet compared to the outer (Deuticke, 1968; Deuticke et al., 1990; Hägerstrand and Isomaa, 1992; Schwarz et al., 1999; Hägerstrand et al., 2001). According to the bilayer-couple model, this specific imbalance in the area of the membrane leaflets causes stomatocytic shape transformation of the erythrocytes (Sheetz and Singer, 1974, 1976; Iglic, 1997; Lim et al., 2002). We found a negative correlation between the primary membrane curvature induced by TX-100 in erythrocytes and that preferred by DRM-associated components stained for fluorescence microscopy (Mrówczyńska and Hägerstrand, 2008; Mrówczyńska et al., 2010). Namely, G_{M1}, CD59, stomatin, synexin and sorcin accumulated in outward curved membrane spiculae on echinocytic shape transformation of erythrocytes induced by elevated intracellular calcium. However, the proteins only poorly co-localized with G_{M1} in the same membrane protrusions, indicating that the segregation of these components (to the protrusions) is not interdependent. This implies that these components are not specifically associated in the same type of raft of the intact membrane. Thus, during solubilization, TX-100 induces and accumulates in membrane invaginations. TX-100 thereby indirectly creates outward bent membrane areas at the borders of these invaginations where outward-bilayer-curvature-preferring (echinophilic) components are accumulated and protected from TX-100 solubilization (Figure 1). Furthermore, intermediate zero curvature areas should contain only low TX-100 concentrations and hence solubility is reduced. A low temperature (4°C) used during solubilization may work to slow down membrane intercalation of monomeric TX-100, resulting in a more complete lateral segregation of membrane components prior to final solubilization; a balance between TX-100 intercalation/solubilization and membrane component redistribution in TX-100-induced curved membrane can be reached. Accumulation of TX-100 in invaginations (solubilizable domains) contributes, due to unfavourable interaction of TX-100 with cholesterol (Tsamaloukas et al., 2006a, 2006b), to segregation of cholesterol (and sphingomyelin) into detergent-resistant domains.

In agreement with our idea that echinophilicity promotes DRM partition, the echinophobic (Discher et al., 1994; Mrówczyńska and Hägerstrand, 2008) transmembrane glycoprotein CD47 was reported to not associate with the erythrocyte DRM fraction (Murphy et al., 2004). Furthermore, the increased DRM partition of G_{M1} on CTB binding (Hagmann and Fishman, 1982; Panasiewicz et al., 2003) can be ascribed to more pronounced echinophilicity. Also in line with our model, the ability of TX-100 to preferably solubilize inner leaflet lipids (Pike et al., 2005; Delaunay et al., 2008; Chen et al., 2009) and merge microdomains into larger DRM vesicles (Giocondi et al., 2000; Koumanov et al., 2005) has been suggested. A mechanism where membrane components laterally redistribute due to changes in membrane curvature during solubilization may be particularly effective in erythrocytes that have a strong tendency to undergo shape transformation (Deuticke, 1968; Fujii et al., 1979; Kuypers et al., 1984; Isomaa et al., 1987; Hägerstrand and Isomaa, 1989, 1992, 1994; Iglic, 1997; Lim et al., 2002). Other cell types may respond to detergent

intercalation with different membrane bending patterns than human erythrocytes. The cell type- and the model bilayer-specific response to detergent solubilization (Schuck et al., 2003; Arnulphi et al., 2007) may be a sign of this. However, factors other than molecular shape and curvature-dependent component segregation must also be taken into account to understand more fully the process of TX-100-specific membrane solubilization and DRM generation. Specifically in erythrocytes, the spectrin–actin skeletal network is not solubilized by TX-100 at 4°C and remains tightly associated with the DRM unless it is stripped under basic conditions after lysis (Salzer and Prohaska, 2001; Ciana et al., 2005). Cytoskeletal association of membrane components (both raft and non-raft) is certainly an important factor influencing the specific solubilization behaviour and the fraction of a given component that is found unsolubilized in DRM.

While the precise mechanism by which amphiphiles solubilize membranes is not fully known, in addition to the interaction of detergent with the membrane, the uptake of membrane components into detergent micelles is also considered to be important (Helenius and Simons, 1975; Kragh-Hansen et al., 1998; le Maire et al., 2000; Lichtenberg et al., 2005). TX-100 monomers have conical shape (Israelachvili, 1997) and preferentially form small spheroidal micelles (Lichtenberg et al., 1983). Thus the inability of TX-100 spheroidal micelles to readily incorporate large monotopic membrane proteins possessing high intrinsic positive curvature may be an additional reason for their tendency to end up in the DRM (Figure 1). More generally, it is evident that most of the typical DRM protein markers are monotopic membrane proteins: (i) the GPI-anchored proteins, which are linked to the extracellular membrane leaflet by a single lipid anchor, (ii) signalling components such as G-proteins, which are attached to the cytoplasmic membrane leaflet by the (reversible) addition of one or more lipid moieties and (iii) members of the stomatin, flotillin and caveolin protein families, which are bound to the cytoplasmic membrane leaflet both by fatty acid modifications and a monotopic insertion of a hydrophobic stretch into the membrane; the latter proteins, moreover, share the common characteristic of forming higher order hetero- or homo-oligomers and are implicated as functioning in some kind of endocytic process. The shape of all of these components is that of a cone with a very bulky hydrophilic part compared with a rather small lipid pole, thereby conceivably indicating that their uptake into TX-100 micelles is highly unfavourable (at least, at 4°C). Additionally, oligomerization may increase the intrinsic (average) positive curvature of membrane components (Hägerstrand et al., 2006).

Minor attempts have been made to relate DRM composition to detergent-specific properties such as intrinsic molecular shape, charge, transmembrane distribution or effect on membrane curvature. It can be foreseen that various detergents, especially those widely differing in their effect on bi- and mono-layer bending, will produce DRM fractions of different compositions. Data from several DRM experiments using erythrocytes (Domingues et al., 2009), other cell types (Mairhofer et al., 2002; Schuck et al., 2003; Rouvinski et al., 2003; Garner et al., 2008) and model bilayers (Arnulphi et al., 2007) already support this assumption. In line with our model, dependence of solubilization dynamics on DRM composition has been suggested previously (Heerklotz, 2002; Lichtenberg et al., 2005; Babiyshuk and Draeger, 2006; Ingelmo-Torres et al., 2009). Furthermore, the effect of bilayer detergent saturability and predisposition to form lipid–detergent mixed micelles has been indicated (Arnulphi et al., 2007). The possibility must be considered that membrane intercalated detergent molecules may form oligomers, or complexes with membrane components, that act as effective entities and may have increased intrinsic (average) curvature. Notably, since ligand binding may affect the molecular shape and clustering of studied membrane components and thereby their true curvature-dependent lateral distribution (Panasiewicz et al., 2003; Cantu et al., 2009; Tian and Baumgart, 2009), this issue should be further investigated.

In conclusion, we present a hypothesis that suggests a coupling between curvature-dependent distribution of laterally mobile membrane components and DRM composition after detergent solubilization (TX-100, 4°C). The relevance of our model for solubilization of different cell types and for membrane domain composition studies in general remains to be elucidated. It is our hope that the model encourages systematic studies aimed at revealing the relationship between detergent molecular properties and solubilization characteristics. Although DRM may not fully represent raft composition, well-controlled and -understood solubilization in DRM analyses will continue to play an important role in membrane studies.

Author contribution

All of the authors (i) substantially contributed to the conception and design of the hypothesis, (ii) drafted and revised it for intellectual content and (iii) approved the version to be published.

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