New and Notable

Lipid Chirality Revisited: A Change in Lipid Configuration Transforms Membrane-Bound Protein Domains

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Since its discovery by Louis Pasteur in 1848 (1), chirality has been—and still is to this day—one major topic for chemists and biochemists alike. Many chirality-related phenomena, from alteration of polarized light to enantioselectivity of enzymes for their substrate, form an integral part of today's university courses in chemical and biological sciences. Similar to the enzymes in charge of their synthesis, phospholipids, glycolipids, and sterols found in biological membranes are chiral. However, even though the effect of chirality on the organization of lipid membranes has been studied (see for example, Weis and McConnell (2)), little is known about the impact of lipid configuration on biological function. In this issue of the *Biophysical Journal*, Schütte et al. (7) resort to cutting-edge chemical synthesis to generate the unnatural enantiomer of Globotriaosylceramide 3 (Gb3, a glycolipid of established biological importance), and investigate the consequences of changing Gb3 configuration on membrane binding and deformation by Shiga toxin, for which Gb3 is the biological receptor.

The Shiga toxin of the bacteria *Shigella dysenteriae* is a protein that binds Gb3 on the plasma membrane via its B subunit (STxB), and forms membrane-bound protein domains. Importantly, by doing so Shiga toxin induces its own internalization in cells by causing inward curvatures of membranes. This phenomenon is reproduced in vitro by adding STxB to giant unilamellar vesicles (GUVs) containing a small fraction of Gb3, in conditions favoring low membrane tension (3). Like in cells, Gb3 used in in vitro assays is the Gb3-*R* enantiomer.

By replacing Gb3-*R* with its unnatural enantiomer Gb3-*S*, Schütte et al. (7) observe that STxB-induced tubulation of GUVs is significantly enhanced, based on experiments with a total of 1253 GUVs. Although the affinity of STxB was the same for Gb3-*S* as for its natural counterpart, reflectometric interference spectroscopy suggested that the domain height and protein distribution in Gb3-*S* membranes significantly differed from that in Gb3-*R* membranes. Those observations were confirmed by atomic force microscopy (AFM) imaging of STxB domains formed in mica-supported planar bilayers composed of DOPC, DPPC, and Gb3. Taken together, those observation show that, in the words of the authors,

> …Gb3-*R* favorably induces large and tightly packed protein clusters, while a lower protein density is found on Gb3-*S* doped membranes.

This could be the molecular basis for the increase in tubulation frequency observed in presence of the unnatural Gb3-*S* enantiomer. The authors also propose

> …that invaginations are favored if the Gb3 molecules bound to STxB are 1) unsaturated leading to a large area per molecule requirement and 2) STxB is capable of forming protein clusters, which results in asymmetric reduction in membrane area, which is proposed to be prerequisite for membrane invaginations…

Clearly, many questions remain to be answered regarding how exactly interaction of STxB with the Gb3-*S* enantiomer increases the frequency of membrane deformation events. Nevertheless, this means that the authors' approach to studying membrane remodeling by STxB may in the future provide a more detailed picture of the molecular mechanisms of this process.

Overall, the study of Schütte et al. (7) is thorough and very robust, and goes from the nanoscopic level all the way to a physiologically relevant, in vitro functional assay. Importantly, the authors unambiguously demonstrate how a change of binding properties of a protein to a lipid bilayer affects the organization of entire protein patches, with very clear consequences on one of the main functions of Shiga toxin, which is to produce invaginations in lipid bilayers. This is one out of just a very few reports of how chirality affects binding of proteins to their membrane receptors (4).

Based on the above, one can surmise that nanometer-scale alterations in the structure of the proteins with their membrane receptor will produce similar effects on protein-protein interactions in, or at the surface of a lipid bilayer, leading to mesoscale rearrangements of membrane protein patches, with potential consequences on biological function. In such context, being able to study protein-protein interactions in biomembranes at physiological surface density and with biomolecular spatial and temporal resolution becomes crucial, as proven by the use of AFM in this study. Because the recent developments of AFM shed new light on the formation of protein domains in prokaryotic and eukaryotic biomembranes (5,6), AFM will be one method of choice to deepen our understanding of the relationship among the stereochemistry protein-lipid interaction, the structure of membrane-bound protein domains, and their biological function.

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