Reconstitution of human ion channels in lipid bilayers formed in microfabricated apertures

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The phospholipid bilayer serves as a major barrier against ion movement, and specific membrane proteins permit ions to pass through it. Of these, ion channel proteins function as gated pores in cell membranes that permit passive ion flow following an electrochemical gradient. Functional reconstitution of ion channel proteins in artificial bilayer lipid membranes (BLMs) provides an excellent system for drug screenings [1]. However, two major problems associated with BLM systems reduce experimental efficiency and prevent them from being widely used, namely, membrane instability and a low incorporation efficiency of ion channels into BLMs. Previously, we succeeded in the formation of mechanically stable BLMs by preparing membranes in microapertures fabricated in silicon (Si) chips [2,3]. The key feature for BLM stabilization is probably the tapered shape of the pore edge, which allows reducing the stress on the bilayer at the pore edge. The remaining issue is the integration of ion channels into BLMs. In this presentation, we report on our recent approach to improve the integration efficiency by the use of centrifugal force to accelerate fusion between the BLMs and membrane vesicles containing ion channels [4]. Vesicles containing the human *ether-a-go-go*-related gene (hERG) channel, the human cardiac sodium channel ($Na_v1.5$) and the human $GABA_A$ receptor ($GABA_AR$) channel were formed, and the functional reconstitution of the channels into BLMs via vesicle fusion was investigated. Ion channel currents were recorded in 67% of the BLMs that were centrifuged with membrane vesicles under appropriate centrifugal conditions (14-55 x *g*). The present method for enhancing the probability of vesicle-fusion promises to dramatically increase the experimental efficiency of BLM reconstitution systems, leading to the realization of a BLM-based high-throughput platform for functional assays of various membrane proteins.

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References

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