Analysis of membrane protein-specific lipids using gold nanoparticle-based method

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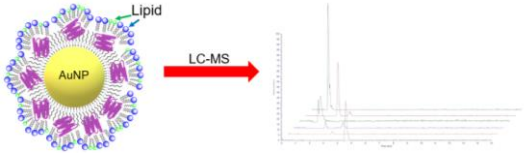
The interaction between membrane proteins (MPs) and surrounding lipids is important to regulate biological properties and stabilize the structure of MPs. In order to understand the MPs-lipid interaction, the analytical method was recently developed based on the surface plasmon resonance (SPR). [1] Briefly, the gold surface of SPR sensor chips was coated by self-assembled monolayer (SAM) containing mercaptohexanol and mercaptohexanoic acid which significantly enhance MP immobilization due to partly hydrophobic environment. After that, MP-lipid interaction was detected by introducing lipid into the MP-immobilized sensor chips. Applying this method to seven-transmembrane bacteriorhodopsin (bR), we found that a halobacterial lipid S-TGA-1 has the highest affinity to bR. [2] However, lipid isolation from the lipid mixtures extracted from biological membranes is necessary prior to the SPR analysis. Therefore, to overcome the limitation, a new method to identify MP-specific lipids without lipid purification was developed by using SAM-coated gold nanoparticles (AuNPs) based on the idea of SAM-based SPR method.

In this study, AuNPs were coated by SAM, followed by immobilization of bR through the EDC/NHS activation. The effects of SAM chain length, AuNPs size, and the ratio of mercaptoalcohol/mercaptocarboxylic acid were examined for the highest bR immobilization. Then, the lipid mixture extracted from purple membrane was incubated with the bR-immobilized AuNPs. The MP-bound lipid species were quantified by LC-MS and the affinity of each lipid to bR was roughly estimated by introducing the binding index which is the comparison of the MS peak intensity ratio between MP-bound lipids and the extracted lipid mixture. The bR-immobilized AuNPs successfully detected S-TGA-1 as bR-specific lipid which confirmed the result from the SPR-based method. More interestingly, the AuNPs-based method also identified S-TeGA-1 as the lipid with high affinity to bR, providing a new information about the bR-lipid interaction.

In conclusion, the AuNPs-based method, which enables simultaneous lipid screening, does not only reproduce the result for the bR-specific lipid, but also identify a new specific lipid otherwise undetected. This method can be further applied to various MPs to improve understanding on MP-lipid interactions, and thus provide a breakthrough for the study of lipids.



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