Medical care, Drug development

Analysis of endocytosis using synthetic membrane protein

Institute for Protein Research
Professor Hironobu Hojo
Researchmap https://researchmap.jp/read0014254

Abstract

Membrane proteins, exist on the cell membrane, exchange information and substances inside and outside the cell. Many of them are expected to be drug discovery targets, and their rapid functional elucidation is awaited. However, membrane proteins are extremely hydrophobic and difficult to be prepared even with recombinant DNA methods. In addition, they often have post-translational modifications such as sugar chains, resulting in heterogeneous structures, making it difficult to obtain highly pure samples required for functional analysis.

We have been engaged in the chemical synthesis of proteins. Chemical synthesis can provide proteins with various post-translational modifications at any sites, and their functions can be analyzed accurately. In this study, in order to elucidate the mechanism of caveolae endocytosis, in which substances are taken up into cells from cavities on the cell membrane called caveolae, we established a method for chemical synthesis of caveolin, a membrane protein that constitutes caveolae. As a result, it was proved that synthetic caveolin has a three-dimensional structure similar to that of natural products, showing the success of the synthesis.

Background & Results

When a protein is chemically synthesized, the entire polypeptide is divided into several peptide segments, and each of them is subjected to machine-assisted synthesis by the solid-phase method. After purification, they are condensed into full-length polypeptide chain. A problem in the synthesis of membrane proteins is that the transmembrane region is highly hydrophobic, so the peptide in this region has extremely low solubility, which hinders purification and condensation with other peptide segments.

In the synthesis of caveolin, for the highly hydrophobic membrane-inserted region, a solubilizing tag is added during the solid-phase synthesis, purification and the assembly of the entire polypeptide chain and then, these solubilizing tags are cleaved to obtain the desired caveolin. In addition, caveolin has three palmitoyl groups at the C-terminal region, which makes it much more hydrophobic. Therefore, we decided to introduce palmitic acid after the peptide chain assembly to avoid an increase in hydrophobicity during synthesis.

Finally, folding was performed by embedding it in liposomes and bicelle membranes. The synthetic caveolin showed circular dichroism spectrum similar to that of natural caveolin, indicating the success of the synthesis. In future, we can analyze the function of caveolin how it induces endocytosis, and the functions of multiple phosphorylation at the N-terminus.

Significance of the research and Future perspective

We established a method for the chemical synthesis of a membrane protein, caveolin, by incorporating the solubilizing tag. At the end of the synthesis the tag was easily removed and the caveolin was successfully embeded in the membrane. Since this method can be applied to the synthesis of other membrane proteins, we believe that it will greatly contribute to the elucidation of the functions of membrane proteins.

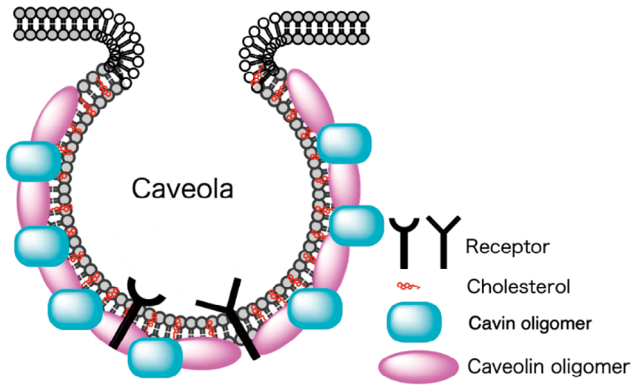

Figure 1. Structure of caveola. Oligomeric caveolin is important for caveola formation.

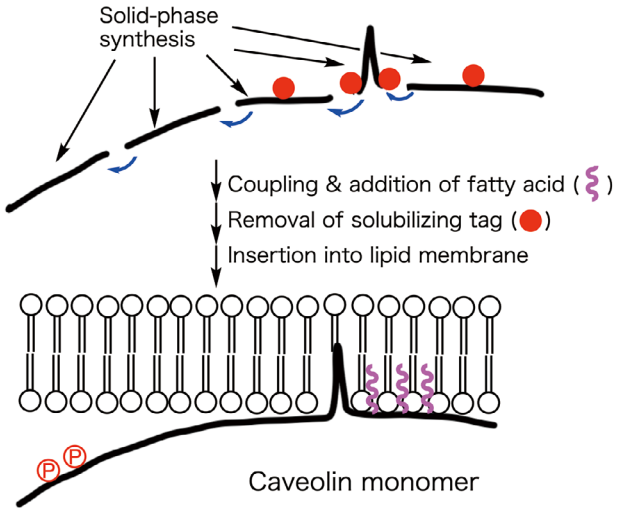

Figure 2. Chemical synthesis of caveolin. Ⓟ denotes the sites of phosphorylation.

Patent
Treatise
Hojo, Hironobu; Takei, Toshiki; Asahina, Yuya et al. Total Synthesis and Structural Characterization of Caveolin-1 Angew. Chem. Int. Ed. 2021, 60, p.13900–13905, doi: 10.1002/anie.202100826
Asahina, Yuya; Komiya, Shinobu; Ohagi, Ami et al. Chemical Synthesis of O-Glycosylated Human Interleukin-2 by the Reverse Polarity Protection Strategy Angew. Chem. Int. Ed. 2015, 54, p.8226–8230, doi: 10.1002/anie.201501847
URL

Keyword caveolin, membrane protein, chemical synthesis, solubilizing tag, post-translational modification