Entry of cell-penetrating peptide transportan 10 into a sinlge vesicle of lipid membranes and its induced pore formation

（課程博士・様式７）（Doctoral qualification by course work, Form 7）

学 位 論 文 要 旨

Abstract of Doctoral Thesis

専　攻：　Course : Bioscience

氏　名：　Name : Md. Zahidul Islam

論文題目：

Title of Thesis : Entry of cell-penetrating peptide transportan 10 into a single vesicle of lipid membranes and its induced pore formation.

論文要旨：

Abstract :

Plasma membranes of eukaryotic cells prevent the entry of biomacromolecules inside the cells. Cell-penetrating peptides (CPPs) have an ability to translocate across the plasma membranes to enter into cells. Transportan 10 (TP10), a synthetic CPP, has a cell-penetrating activity and thus can be used for the intracellular delivery of biological cargo such as proteins and oligonucleotides. However, the mechanisms of translocation and the delivery of large cargo molecules are still controversial. Many researchers investigated the interactions of TP10 with lipid membranes using a suspension of large unilamellar vesicles (LUVs). However due to the disadvantage of the LUV suspension method, elementary processes of the interaction of TP10 with lipid membranes remain unclear. In this thesis, the entry of TP10 into a single giant unilamellar vesicle (GUV) and the TP10-induced pore formation were investigated using the single GUV method.

[Chap.2]: To investigate the entry of TP10 into a GUV and the TP10-induced pore formation, a new method was developed to detect the entry of TP10 into a GUV using confocal microscopy. Using this method, interactions of carboxyfluorescein (CF)-labeled TP10 (CF-TP10) with single GUVs containing a water-soluble fluorescent dye, Alexa Fluor 647 hydrazide (AF647) and smaller vesicles were investigated. As lipid membranes, 20% dioleoylphosphatidylglycerol (DOPG) /80% dioleoylphosphatidylcholine (DOPC) was used. After starting the interaction of CF-TP10 with a GUV, the fluorescence intensity of the GUV membrane increased with time to a saturated value, then the fluorescence intensity due to the membranes of the smaller vesicles inside the GUV increased prior to leakage of AF647. This result indicates that CF-TP10 entered the GUV from the outside by translocating across the lipid membrane before CF-TP10-induced pore formation. The fraction of entry of CF-TP10 inside the GUVs before pore formation increased with an increase in CF-TP10 concentration and became

1.0 at and above 1.0 μM. The rate constants of CF-TP10-induced pore formation in lipid membranes were determined, which were larger than these TP10. Large molecules such as Texas Red dextran 40000, and vesicles with a diameter of 1-2 μm, permeated through the TP10-induced pores or local rupture in the lipid membrane. The interaction of CF-TP10 with single DOPC-GUVs were also investigated and essentially identical results as for 20%DOPG/80%DOPC-GUVs were obtained. These results provide the first direct experimental evidence that TP10 can deliver large cargo through lipid membrane.

[Chap.3] Plasma membrane of eukaryotic cells contain high concentrations of cholesterol. It is well known that there are many effects of cholesterol (chol) on physical properties of membrane. Thereby it is important to elucidate the effect of cholesterol on entry of TP10. The effect of cholesterol on the entry of TP10 into single GUVs of DOPG/DOPC/chol mixture and the TP10-induced pore formation in the membranes were investigated. CF-TP10 entered single GUVs of the membranes containing high concentrations of cholesterol before leakage of AF647, although a little higher concentrations of CF-TP10 were required for the entry compared with membranes without cholesterol. This result indicates that TP10 can translocate across lipid membrane regions in plasma membranes of eukaryote.

[Chap.4] The effects of stretching of lipid membranes on the entry of TP10 into single 20%DOPG/80%DOPC-GUVs and the TP10-induced pore formation were investigated. The binding of TP10 to a GUV membrane induced stretching of the membrane and during an increase in stretching pore formation occurred stochastically in the membrane. The fractional area change of a GUV membrane played an important role in the TP10-induced pore formation. Tension due to the external force decreased TP10-concentration required for the pore formation, and also increased the fraction of entry of CF-TP10 into a GUV before pore formation, and hence the rate of entry of CF-TP10 into a single GUV was increased.

The elementary processes of the entry of TP10 into a single vesicle and the TP10-induced pore formation in the lipid membrane were revealed. Based on the experimental results, it is concluded that the TP10 can enter GUVs of lipid membrane before TP10-induced pore formation. Therefore, TP10 can be used as a vector to deliver large cargo through lipid membranes without the need for special transport mechanisms such as those found in cells.