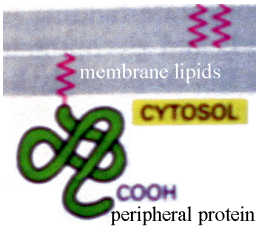
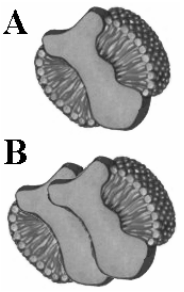
The conclusion is not justified. Though 0.1 M NaOH denatures "everything", if the peripheral protein interacts with membrane lipids through a covalent lipid chain (shown right, *MBOC4* pp.594), NaOH will not destroy it. You should use an extreme detergent to do a further test: if the protein is released by the detergent from the pellet, it is integral not peripheral.



The density measured by the equilibrium density gradient centrifugation only indicates that the pellet is a mixture of lipids, protein/CHO.
[$?_{protein}$=1.3~1.35; $?_{CHO}$=1.8; $?_{lipid}$=0.9~1.0]



DGK25 is a **homodimer**.
The molecular mass of SDS micelle is: (24500/6.02E-23)*1.657E-24=24.5 kD.
The molecular mass of ß-octylglucoside (ßOG) micelle is: (8000/6.02E-23)*1.657E-24=8 kD.
The molecular mass of the monomer of DGK25 is 25kD (by SDS-PAGE).

ßOG is a mild detergent, which can not break the interaction between two monomers, and can form a mix micelle in B form with a molecular mass of 25kD+25kD+8kD=58kD. SDS can partly break the interaction between monomers, and form mix micelles both in A or B form, with molecular mass of 25kD+24.5kD=49.5kD or 25kD+25kD+24.5kD=74.5kD.

Problems are:

1. **DGK25 is not enough**. Based on the data we have, it is easy to figure out that in every 100 liposomes, only one liposome has a DGK25 on it. [2.5ug/25kD : 320mg/(40*800kD) = 1 : 100] Though the substrate DAG is enough, the enzyme is so little that you can only measure a small amount of phosphorylated DAG.
2. **Ratio of PC/PE is not appropriate**. In the natural eukaryote membranes, PC/PE is around 2 (*Handout* 1-10). Here, PC/PE=8.5. The conformation of PC, which is not wedge like PE but cylinder, is not favorable in the formation of liposomes. The membrane fluidity decreases with the increase of the length of lipids' chains and the saturation. More PC will results in less membrane fluidity (compared with PE which has the same R_1 & R_2). The inappropriate ratio of PC/PE makes the enzyme only phosphorylate DAG very near it, which is measured at very early time points.
3. **No control in the assay**. The aim of the assay is to investigate whether the PE stimulates the DGK25. So you should set a control to distinguish the stimulation of PE.

Adjustments of the assay:

Sample 1: 1.25ug DGK (solubilized in 5%ß OG) + 1.6mg total lipid (65PC:30PE:5DAG)
Sample 2: 1.25ug DGK (solubilized in 5%ß OG) + 80 ug DAG [control]

Dialyze the samples respectively. Follow the same protocol to form the liposomes. Measure the phosphorylated DAG.

I will purchase substrate III.

Substrate I will lose radiation during the reaction. When formatting the isopentenyl pyrophosphate, $*CO_2$ releases. Substrate II labels on a-H, which is very active and may exchange with H_2O resulting in a radioactive water, a high background.

Two lipid parameters are: **the length of acyl chains**; **the number and the positions of the double bonds** in acyl chains.

Usually, 2 more carbons in the acyl chain will increase the transition temperature (T.T.) of the lipid by more than 10℃. For example: the T.T. of 14:0 PC is 24℃, the T.T. of 16:0 PC is 41℃, and the T.T. of 18:0 PC is 55℃. The longer of the acyl chain, the easier of lipid to form "ordered gel" phase, which has a high transition temperature than "liquid crystal" phase.

The import of double bonds into acyl chains will dramatically decrease the transition temperature. Double bonds will induce gauche conformation of the lipid, such as "kink", which will destroy the ordered structure of the lipid complex to decrease the transition temperature. And the position of double bonds will determine the extent of "kink" to influence the T.T. For example: the T.T. of 18:1?9 PC is much lower than the T.T. of 18:1?1 PC or the T.T. of 18:17?9 PC.