Biological Membranes: Detection of Melting Processes using Differential Scanning Calorimetry

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In the previous article we learned that a lipid bilayer can exist in different phases. We introduced the terms solid ordered (gel) and liquid disordered (fluid). The first one is a highly ordered phase. The lipids are arranged on a lattice and their structure is ordered. If the membrane is in the other pahse the lipids have lost their internal ordering and they lack their lattice ordering as well. Therefore, a membrane which melts is subject to two different melting processes.

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In the literature various methods have been described in order to understand melting processes in lipid and biological membranes. In recent years techniques which are able to detect coexisting phases have obtained much attention. Approaches which have been successfully applied in the past are still useful tools. One of these is Differential Scanning Calorimetry (DSC). Studies on lipid membranes using DSC started in the late 1960s and early 1970s. Already then the attention was not only on artificial lipid membranes, but the focus was also put onto phase separation processes in biological membranes. Before we want to discuss some results, we need to understand how this method works. We will see that DSC is a simple, but very powerful approach.

Differential Scanning Calorimetry (DSC) allows to detect differences in heat capacity of a sample and a reference substance. It has a sample and a reference cell as it is also depicted in the respective figure (see fig. 1). A measurement starts from a given temperature and this one is changed with a constant scan rate to the final temperature value. This needs the addition or removal of heat to the two independent cells. Increasing temperature the heat capacity of a substance tells one how much energy is needed to increase the temperature of it by one degree. If the reference substance is a buffer solution and the sample solution are lipid membranes dissolved in the buffer solution one can detect differences in heat capacity between both the buffer solution and the lipid suspension. The calorimeter allows to monitor the power differences needed to change the temperature in both cells. Measuring only with buffer solutions in the two cells one would obtain a power difference of zero during a complete scan. If besides the buffer liposomes are present additional energy needed for heating the sample is due to phase separation processes in the lipid membranes. The power difference monitored can be related to the excess heat capacity.

An example result is shown in the graphic displayed. The heat capacity profile was obtained adding so called DMPC lipids to a buffer solution. This lipid belongs to the family of phospholipids which have a phosphor in their head group. It has two fatty acid chains with each of the chains containing fourteen carbon atoms. Given into the buffer solution it forms multilamellar lipid vesicles. These vesicles can be imagined to be similar to an onion. In this case the layers are built by lipid bilayers.

From the heat capacity profile it can be recognized that the transition happens over a small temperature regime. The half width of the transition is in the range of a few tenth of degree. This behavior means that the melting of one lipid influences the melting of other lipids in a strong way. One talks about a highly cooperative transition. There is the possibility to produce liposomes which are built from only one lipid bilayer by pressing multilamellar vesicles repeatingly through pores with a diameter of about 100nm or even less. Vesicles of this kind are termed large unilamellar vesicles. Determining the heat capacity profile of a suspension of these vesicles and comparing to the ones of multilamellar vesicles it is found that the heat capacity profile is broadened. This means that the transition is less cooperative.

The details of a phase separation process such as the melting temperature and the cooperativeness depend on different factors. We have already seen that the kind of vesicle plays a role. Further melt lipids with a smaller head group at higher temperatures. The chains are characterized by carbon and hydrogen bonds. If double bonds are present one talks about an unsaturated chain. A lipid with a double bond in one of its chains displays lower melting temperatures. The longer the chains of a lipid the higher the melting temperature. Outer parameters such as pH or ion concentration have an nfluence too.

Often studies about phase separation processes in lipid membranes were and are opposed with the comment that there are no such processes in biological membranes. This is, however, not true at all. Already after publishing the first heat capacity scans on artificial lipid membranes work on biological membranes was presented in the early 1970s.

The commonly accepted textbook picture assumes that a biological membrane is in a liquid disordered phase. Proteins are randomly distributed in the lipid membrane which only obtain a passive role. They are like water for ships. However, for decades researchers have been puzzled about the fact that the lipid synthesize of bacteria is adopted in dependence of their growth temperature. In other words the lipid composition depends on the temperature at which a bacteria grows.

This fact can be observed in heat capacity measurements too. Changes in lipid composition immediately mean that the melting behavior is changed.

In a master's thesis from the group of Prof. Thomas Heimburg (Membrane Biophysics Group, NBI, Denmark) D. Pollakowski could show that the lipid composition of membranes of the bacteria Echerichia Coli is adopted in a way that the phase transition ends at temperatures below the growth temperature. This is true for the conditions under which the heat capacity curves were determined. In this context one has to consider that changes in pH or ion concentration shift the melting profile to lower or higher temperatures (see the corresponding figure 3; the graph is published in Heimburg and Jackson, Biophys. J., in press). In the upper graph one does not only find an increase of the heat capacity at lower temperatures due to the melting of the lipid membrane. At higher temperatures further maxima appear. These are due to denaturation processes of proteins which are present in the membrane.

These findings suggest that the lipid membrane does not only play a passive role, but that the physical behavior of membranes is important. We do not want to go into detail in this article, but we want to tell so much that at the beginning researchers believed that for a proper protein function containing a certain fluidity of the membrane is necessary. This means that for a proper protein function the membrane needs to have a well defined viscosity. Nowadays, the view that the existence of domains plays a role has obtained much attention. A discussion of this shall follow in another article of this series. Before, however, we will learn more about domain formation processes in biological and artificial membranes and their detection by fluorescence microscopy and atomic force microscopy.

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