Phase transitions of lipids in membranes

Method of DSC

In biological membranes the lipid layer, according to all available data, represents a liquid body with viscosity close to viscosity of sunflower oil. Strictly speaking fluidity of a membrane is limited to an internal hydrophobic phase, which consists of hydrocarbon chains of fatty acids. This phase, however, not always happens liquid. At cooling up to temperatures lower than 10^{o}C, the membranes freeze, i.e. the liquid phase hardens, getting properties of a two-dimensional crystal.

In membranes, composed of synthetic lipids, the phase transition from liquid to a solid state can occur at higher temperatures, depending on chemical structure of the phospholipid. In the table 1 the temperatures of phase transitions of some synthetic phosphatidylcholines (lecithins) are given.

Table 1. Temperatures of melting of some synthetic phospholipids

Fatty acids	The name of the fatty acid residue	Abbreviation	The melting temperature ,^{o}C
14:0	Myristoyl	DML	23
16:0	Palmitoyl	DPL	41
18:0	Stearoyl	DSL	58
18:1	Oleyl	DOL	–21 (*cis*-form)

For study of phase transitions at heating a method of differential scanning calorimetry is used (in abbreviated form - DSC). Not stopping on description of the device, we shall note only, that in the final account the curve of a heat capacity is being measured, i.e. the dependence of a heat capacity of lipids or membranes in a suspension from the temperature.

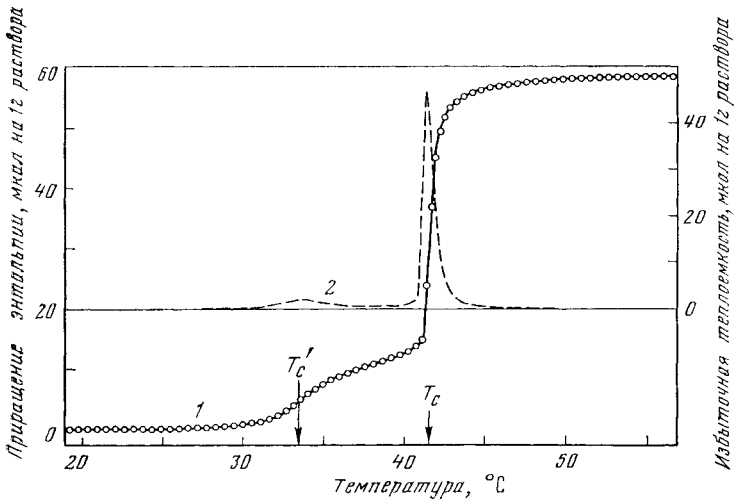

Figure 1. Phase transitions in a suspension of phospholipid vesicles (liposomes) as measured by a differential scanning microcalorimeter (DSC)

The heat capacity is plotted along the ordinate, the temperature along the abscissa.

The method refers to as *differential*, because the heat capacity of the suspended lipids is essentially less than that of the solvent, and for this reason the measurement of the *difference* between the sample and blank solvent is being measured. An example of such curve is given in Fig. 1, where the curve DSC for dipalmitoyl phosphatidylcholines (DPL) is given.

Parameters of DSC curves

In figure 2 parameters of a curve of a DSC at the left are in more detail designated. At the first stage we will be interested by(with) three of them:

1 - temperature of phase transition ("melting") Tc.

2 - temperature interval ("width") phase transition.

3 - total of heat Q, absorbed during the melting. It represents the area under the curve of DSC, i.e. function $C = f(T)$

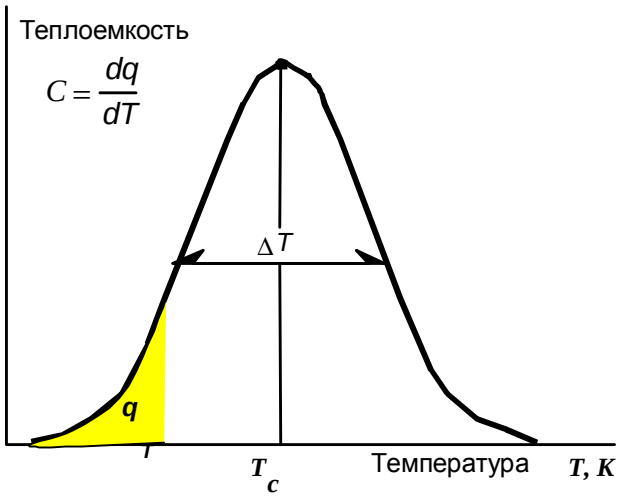

Figure 2. The characteristics of phase transitions in lipids on the data of differential scanning calorimetry.

C- heat_capacity. *T* –the temperature, T_c - temperature of phase transition (of melting). The shaded area corresponds to the amount of heat absorbed in the course of heating to the temperature *T*.

Melting curves

The melting curves shows the dependence of a fraction of the liquid phase in total of investigated substance, in our case - lipids of membranes in the suspension under study.

Let's designate the amount of lipids in the liquid phase through m_l, and that of lipids in the solid phase through m_s. Then the fraction of liquid lipids will be

$$\alpha = \frac{m_l}{m_l + m_s} \qquad (2)$$

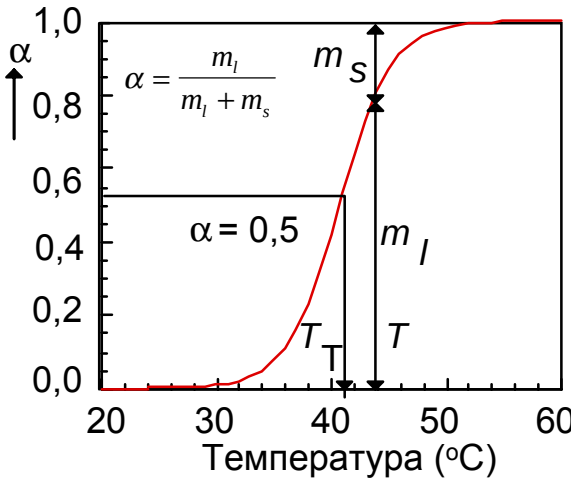

Figure 3. A curve of a melting of lipids in liposomes prepared from ÄÏË

α - a fraction of the liquid phase, *T* - temperature, T_c - temperature of a melting (= 0,5), m_l – the amount of the lipid in the liquid phase, m_s the amount of the lipid in the solid phase.

$$C = \frac{dq}{dT} = f(T)$$

In Fig. 3. the melting curve of dipalmitoyl phosphatidylcholine (or dipalmitoyl lecithin, DPL) is given.

Methods of measurement of the melting curves

For determine the fraction of liquid phase in bulk volume of investigated material (in our case – in a lipid layer of membranes), a variety of methods can be used.

The analysis of DSC curves

One method is based on the analysis of curves received by the method of differential scanning calorimetry. Let's address again to Figure 2.

Assume specific heat of melting to be equal Q_m, and the amount of lipids in the sample is m moles. Total of energy absorbed by the sample in the interval of temperatures of melting T_1-T_2 equals obvious of the area under the curve $C = f(T)$, i.e.

$$Q = Q_m \times m = \int_{T_1}^{T_2} C dT \qquad (3)$$

In the interval of temperatures from T_1 up to the current temperature T the amount of moles of the lipid m_l is melted as a result of absorption of the heat q:

$$q = q_m \times m = \int_{T_1}^{T} C dT \qquad (4)$$

(Shaded area in Fig. 2).

At the temperature T the molar fraction of lipids in a liquid phase is equal to

$$\alpha = \frac{m_l}{m} = \frac{m_l}{m_l + m_s} = \frac{q}{Q} \qquad (5)$$

Thus, by measuring the areas under the curve $C = f(T)$ at different temperatures, we can drow the melting curve: $a = f(T)$.

Usage of fluorescent probes

Many fluorescent substances have such a property that their spectra and (or) quantum yields of their fluorescence depend strongly on the environment¸ in particular, on the polarity, viscosity and other characteristics of the medium. One example is famous fluorescent dye ANS (1-anilino, 2-naphtalen-sulfonate). The structures of theses and some other ***fluorescent probes*** are shown in Fig. 4.

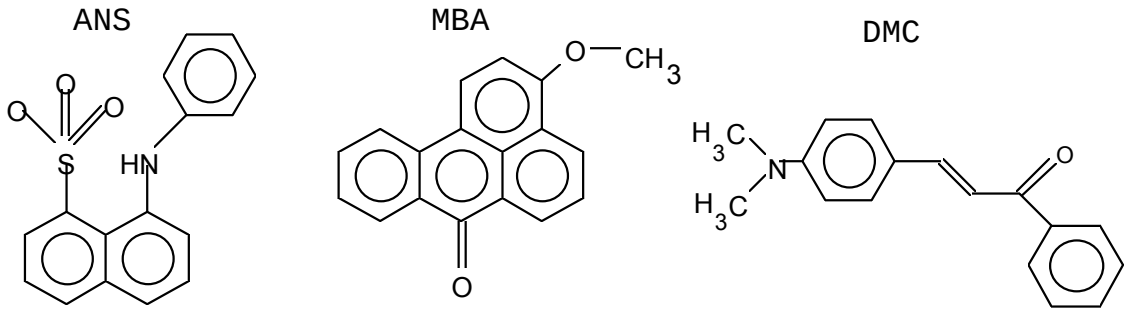

Figure 4. The structural formulas of some fluorescent probes used for measurement of structural reorganizations in a lipid layer of biological and phospholipid membranes

When being added to a suspension of membranes, ANS is distributed between water and lipid phases. Meanwhile, only ANS fraction, dissolved in the lipid phase, emits fluorescence under illumination by UV light. Therefore the intensity of fluorescence of the dye grows during the melting of lipids in membranes and drops at freezing.

Two other fluorescent probes, shown in Fig. 4, are also used to study the lipid phase transitions.

Several other methods could also be employed:

- spin probe method
- Rhaman spectroscopy
- light-scattering

Phase equilibrium

Within the temperature interval of phase transition, equilibrium is established, if only the melting occurs slowly enough:

$$\text{Liquid state} \rightleftarrows \text{solid state}$$

One may assume that all membrane consists of domains of liquid lipids and those of solid lipids. Then the reversible process of phase transition can be considered as a process of transformation of such domains to each other with speeds proportional to concentration of domains. In other words, the phase equilibrium can be considered as a reversible chemical reaction:

$$l \leftrightarrow s$$

with the constant of equilibrium

$$K = \frac{[l]}{[s]} = \frac{m_l}{m_s},$$

where [l] and [s] are the concentrations of lipids in liquid and solid phases, respectively, and m_l and m_s are the amounts of the lipid in liquid and solid phases. The change of free energy at melting ΔG is equal to the change of the enthalpy H minus the change of the thermal energy $T\Delta S$:

$$\Delta G = \Delta H - T\Delta S \qquad (6)$$

On the other hand,

$$\Delta G = RT \ln K = RT \ln \frac{m_l}{m_s} \qquad (7)$$

From here we find

$$\ln K = \frac{\Delta H}{R} \times \frac{1}{T} - \frac{\Delta S}{R} \qquad (8)$$

Hence, the dependence of $\ln K = \ln \frac{m_l}{m_s} = \ln \frac{q}{Q-q}$ upon inversed absolute temperature $\frac{1}{T}$ represents a direct line with the angular coefficient $\frac{\Delta H}{R}$ and the cut-off on the ordinate $\frac{\Delta S}{R}$. Several examples of such straight lines are given in Fig. 7. The explanation of the value n in this figure will be given below.

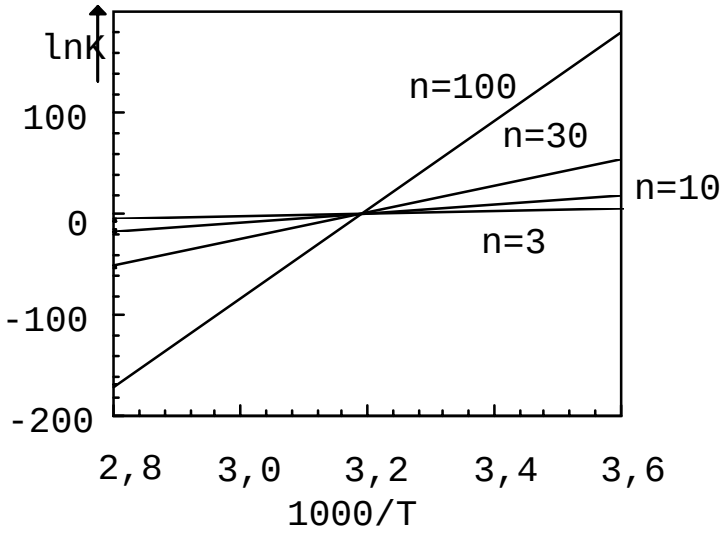

Fig. 7. The dependence of ln*K* from inversed absolute temperature.

The cut-off on the ordinate ($\Delta S/R$) allows to find the entropy of melting ΔS, whereas the angular coefficient ($\Delta H/R$) shows the enthalpy of melting ΔH; n is the size of the *cooperative unit* of melting (see next paragraph for explanation).

Thus, from the melting curve measured experimentally, one can find the thermodynamic parameters of the process ΔH and ΔS. For this purpose:

1. We find the ratio $K = \frac{m_l}{m_s}$ at different temperatures T, ^oC (see Fig. 3).
2. We plot ln$K$ against inversed absolute temperature ($1 / (T^oC + 273^o)$).
3. The angle of inclination of the line is $\Delta H/R$. From that one can find ΔH.
4. The cut-off on the ordinate is $\Delta S/R$. From that we can find ΔS.

Cooperativity of phase transitions

From a ***heat_capacity curve*** (such as given in Fig. 2) we find heat Q of melting of the sample and molar heat of a melting $Q_m = Q/m$, where m is the amount of moles of the lipid in the sample. From the ***melting curve*** (see Fig. 3), we find the enthalpy of melting ΔH. On the first sight Q_m and ΔH should be equal, as the system does not make mechanical work. Unexpectedly, ΔH exceeds Q_m by a factor ten or, sometimes, hundreds.

What is the matter?

Let's return to the basic equation 6 on page 4. Its application is based on the following statement (is quoted): "... The phase equilibrium can be considered examined as an reversible chemical reaction: $s \leftrightarrow l$ with the equilibrium constant $K = \frac{m_l}{m_s}$ ".

The question is, however, what "molecules" l are transformed in this reaction into the "molecules" s. It is obvious, that these are not individual molecules of the phospholipid, as a single molecule may not be in liquid or in solid state. It should be an assembly of the molecules, forming a domain, or a "cluster", which can be either in liquid or in solid state. Such a cluster is called the ***cooperative unit***. Each unit can change its phase state by the law "all or anything", absolutely independently on other clusters. In this sense a cooperative units represents a “super-molecule” that can pass from state l to a state s and back. The change of free energy ΔG, entalphy ΔH and entropy ΔS in the equation 6 on page 4 concerns to the Avogadro number of such "super-molecules". It is rather obvious, that if each cooperative unit is formed by n molecules of the phospholipid, then

$$\Delta H = nQ_m, \qquad (9)$$

where n is the size of cooperative unit, i.e. the number of phospholipid molecules in the cooperative unit.

Thus from the melting curve we find the heat of melting in account on n moles of the ***phospholipid***, and to receive these characteristics in account on ***one mole*** it is necessary to find at first the size of cooperative unit n. It can be made, only if the calorimetric curves allow to determine the melting heat Q, and we are able to calculate the molar melting heat Q_m. Having divided Q_m by ΔH, we receive n. Similarly, we can calculate ΔS, in account on one mole of the phospholipid. In the table 2 the thermodynamic parameters are shown of the melting of synthetic phospholipids (in account on one mole of the phospholipid) .

The table 2. Thermodynamic parameters of transitions of melting for 1,2-substituted-L-phophatidylcholines (on M.C. Phillips, 1972).

Phospholipid	T_c, °C	ΔH, kcal/mole	ΔS, kal/mole	
DOL	(18:1)	-21	7,6	30,3
DML	(14:0	23	6,64	22,4
DPL	(16:0)	41	8,66	27,6
DSL	(18:0)	58	10,67	32,4
DBL	(22:0)	75	14,88	42,8

Questions

1. Phase transitions of lipids in membranes. Differential scanning microcalorimetry. The basic parameters of DSC curves.
2. Determination of the degree of melting of a lipid layer by using DSC curves.
3. Theory of phase transitions. Temperature dependence of the equilibrium constant in the liquid - solid phase transitions.
4. Cooperative unit of a melting. Its experimental determination.
5. Dependence of parameters of phase transition on the sizes of cooperative unit of melting.
6. Methods of study of phase transitions in membranes.
7. Influence of a cholesterol on phase transitions. The facts and explanations.