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THE INFLUENCE OF Ca^{2+} ON THE LATERAL LIPID DISTRIBUTION AND PHASE TRANSITION IN PHOSPHATIDYLETHANOLAMINE/PHOSPHATIDYLSERINE VESICLES

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The lateral lipid distribution within dipalmitoylphosphatidylethanolamine (DPPE)/dipalmitoylphosphatidylserine (DPPS) vesicle membranes was investigated under the influence of Ca^{2+} using a lipid cross-linking method. To characterize the phase transition in DPPE/DPPS vesicles and to correlate the different phase states of the membrane lipids with the obtained lipid distribution ESR measurements using a fatty acid spin label were carried out. It is shown that Ca^{2+} has a significant influence on the lateral lipid distribution within the fluid phase of the membrane lipids; instead of a slight alternating lipid arrangement in absence of Ca^{2+} due to the electrostatic interaction between the DPPS headgroups after addition of Ca^{2+} a lateral cluster structure is characteristic of the fluid phase.

It has been shown that the lipid matrix of biological membrane influences the activity of membrane enzymes and, in particular, that a fluid lipid environment is necessary for the normal function of many membrane proteins [1–4]. Furthermore, the membrane lipids seem to be essential to membrane fusion [5–7], membrane transport [8,9] and membrane receptor clustering. On the other hand, it has become evident that the different lipids of the lipid matrix are not randomly distributed within the membrane [10–13]. Therefore, the lateral lipid distribution and its variation possibly induced by Ca^{2+} can be regarded as an important factor for the functions of biological membranes mentioned above.

Recently we have reported a lipid cross-linking method which allows to determine the nonideality of lateral mixing of the aminophospholipids dipalmitoylphosphatidylethanolamine (DPPE) and dipalmitoylphosphatidylserine (DPPS) within vesicle membranes [14]. The basic assumptions for evaluating this method were (i) the same reactivity of the lipid cross-linker 1,5-difluoro-2,4-dinitrobenzene (DFDNB) to the aminogroups of DPPE and DPPS and (ii) the concentrations of the various bis-derivatives of the crosslinking reaction are proportional to the numbers of different nearest-neighbour contacts of the lipids within the membrane. Taking into account these assumptions the nonideality parameter ν of the binary mixture [14,15], which is a qualitative measure for the lateral lipid distribution, could be calculated.

In this paper the influence of Ca^{2+} on the lateral lipid distribution is investigated by cross-linking for different temperatures as described in Ref. 14. The buffer (120 mM NaCl, 40 mM $NaHCO_3$, pH 8.5) used for the preparation of unilamellar DPPE/DPPS vesicles additionally contains 0.1 mM $CaCl_2$. On pure DPPE and pure DPPS vesicles it was proved that the reactivity of DPPE and DPPS to the cross-linker DFDNB does not change significantly after addition of 0.1 mM $CaCl_2$, so that assumption (i) can be sustained for

Abbreviation: DFDNB, 1,5-difluoro-2,4-dinitrobenzene.

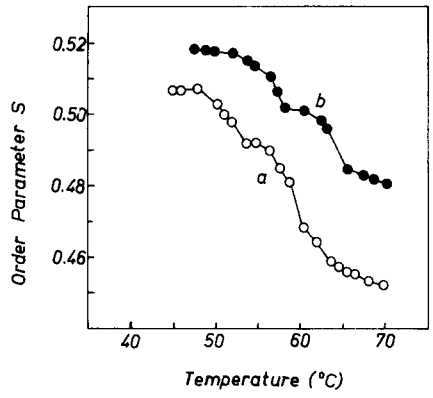

Fig. 1. Temperature dependence of the lipid order parameter S obtained by ESR measurements, using the fatty acid spin label I(10,3) (relation between ESR spin label and lipids: 1:100 (n/n)). The ESR spectra were recorded at a Varian E 3 spectrometer, using a microwave frequency of 9.15 GHz, a microwave power of 20 mW and a modulation amplitude of 1 G. The points indicated are the result of two independent series of measurements. Curve a (without Ca^{2+}) and curve b (0.1 mM $CaCl_2$) show a decrease of the lipid order parameter S with increasing temperature. Each curve is the result of the two phase transitions of DPPS (pure DPPS: $t_c = 55°C$ [27]) and DPPE (pure DPPE: $t_c = 63°C$ [27,28]), respectively. Without Ca^{2+} (curve a) the phase transition temperatures of the two aminophospholipids in mixture are shifted to lower temperatures compared with the pure components (DPPS in mixture: $t_c \cong 40°C$ and DPPE in mixture: $t_c \cong 58°C$). similar results for DPPE/DPPC mixtures have been determined by NMR experiments by Arnold and co-workers [29]. Addition of Ca^{2+} (curve b) causes an increase of the absolute values of the lipid order parameter S as well as a shift of the critical transition temperatures to higher values as in curve a (DPPS in mixture (0.1 mM $CaCl_2$ added): $t_c \cong 55°C$ and DPPE in mixture (0.1 mM $CaCl_2$ added): $t_c \cong 63°C$).

the evaluation of the cross-linking results.

The influence of Ca^{2+} on the phase transition in DPPE/DPPS vesicles was investigated by ESR measurements, using the fatty acid spin label I(10,3) (2-(3-carboxypropyl)-2-decyl-4,4-dimethyl-3-oxazolidinyloxyl). From the ESR spectra obtained the lipid order parameter S was calculated [14,16–18]. The decrease of the lipid order parameter with increasing temperature is presented in Fig. 1. For both preparations (curve a: without Ca^{2+} and curve b: 0.1 mM $CaCl_2$) two temperature regions in which the order parameter S alters drastically could be detected. These changes of the order parameter S correspond to the thermotropic phase transitions of the two different types of lipids as shown by others [19,20]. However, addition of Ca^{2+} leads to an increase of the lipid order parameter S and to a shift of the transition temperatures to higher values possibly due to electrostatic interaction between Ca^{2+} and the headgroups of the lipid molecules [21,22].

In Fig. 2. the temperature dependence of the nonideality parameter ν calculated from the measured concentrations of the cross-linking derivatives is shown. Curve a is the result of cross-linking of mixed DPPE/DPPS vesicles in Ca^{2+}-free buffer [14], while curve b indicates the data obtained, using a buffer with additional 0.1 mM $CaCl_2$. It can be seen (cf. Figs. 1 and 2) that the phase transitions of the lipids DPPE and DPPS in the mixture influence the lateral lipid distribution in both preparations. Comparing both curves of Fig. 2 the lateral lipid distribution in the gel phase

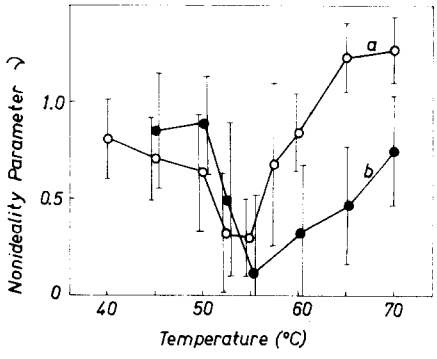

Fig. 2. Temperature dependence of the nonideality parameter ν as a qualitative measure of the lateral lipid distribution in a binary mixture of DPPE/DPPS (1:1, n/n) in vesicles. The nonideality parameter ν attains its minimum $\nu = 0$ for a total lateral separation of the two lipids. $\nu = 1$ means ideal mixing and ν becomes maximum $\nu = 2$ and $\nu = 4/3$ for a total alternating lipid arrangement in a square and hexagonal lattice, respectively. The nonideality parameter ν was determined from the obtained cross-linking data as described by Ref. 14. Vertical bar indicates S.E. of the mean of ten samples. Curve a (without Ca^{2+}) as well as curve b (0.1 mM $CaCl_2$) show the influence of the lipid phase transition detected by ESR (cf. Fig. 1) on the lateral lipid distribution. Significant differences between the two curves appear above the phase transition of DPPE within the fluid phase of the membrane lipids: without Ca^{2+} (curve a) the lipids are arranged in an alternating structure, possibly due to the electrostatic repulsion between the negatively charged headgroups of DPPS, and addition of Ca^{2+} (curve b) produces a cluster structure of the lipids also in the fluid phase. Statistical evaluations were carried out by means of Student's t-test. The ν-values of both preparations differ significantly (at 60°C, 65°C and 70°C) using the sensitive one-sided t-test with $P = 0.1$.

of the vesicle lipids (at a temperature below the critical phase transition temperature of DPPS) remains nearly unchanged by addition of Ca^{2+}. Within the temperature interval between the phase transitions of DPPS and DPPE (cf. Fig. 1) an increase of the lipid cluster size is caused by phase separation in both preparations. Addition of Ca^{2+} possibly enhances the lipid clustering within this region (the mean value of the nonideality parameter ν is altered from $\nu \cong 0.3$ without Ca^{2+} to $\nu \cong 0.1$, using 0.1 mM $CaCl_2$). Assuming these values of ν, a rough estimation of the mean cluster size [23] provides a Ca^{2+}-induced increase from approximately 100 to 600 lipids per cluster. Statistical significant differences between both preparations exist only in the fluid phase state of the vesicle lipids (above the phase transition temperature of DPPE). In this temperature region Ca^{2+} produces a transition from an alternating lipid arrangement ($\nu = 1.2$) to a lipid cluster structure ($\nu = 0.7$).

This result indicates that a prerequisite for Ca^{2+}-induced alteration of the lateral lipid distribution is the fluid phase state of the membrane lipids. If it is assumed that the lateral lipid distribution is an essential factor for regulating the membrane function it becomes evident that the fluid state of the membrane lipids is necessary for optimal membrane function [24–26].

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