Investigations on the Pretransition of Model Membrane Systems Using an Anthracenophane Fluorophor

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A variety of fluorescent dyes, among them 9,10-dimethylanthracene, are useful tools for the investigation of phase transitions in lipid membranes. A phane derivative of this chromophor, consisting of two anthracene molecules, which are bridged by two diaminopolyether rings (1), was found to be a very sensitive marker of the pretransition of zwitterionic phospholipids. The quantum yield of this fluorophor strongly depends on temperature, particularly in the region of the pretransition. The observed changes in the fluorescence intensity are due to changes in the distribution of the dye at the phase boundary, reflecting structural alterations of its membrane environment.

Preparations of small unilamellar vesicles obtained by sonication of dimyristoylphosphatidylcholine suspensions were incubated with micromolar amounts of the anthracenophane dye at a temperature above the phase transition temperature T_m of 297 K. A significant enhancement of the fluorescence by a factor of about 4 was observed only in the temperature range below the main transition. The vesicle preparations were slowly cooled with a rate of 0.2 K/min.

From the cooling curves two processes in the region of the pretransition could be resolved. In the temperature-jump experiments only very small perturbations (0.04 K) were applied. The high increase of the fluorescence of the dye in this region allows the investigation of the kinetics of this metastable system under quasi-equilibrium conditions. Between 291 K and 283 K two relaxation processes of τ_1 = 1-10 msec and τ_2 = 10-100 msec were obtained, which both show maxima of their times at the pretransition temperature (287 K); they may be attributed to the process of cluster formation in the phospholipid lattice.

Increasing the concentration of Ca^{++} results in a small decrease of τ_1, whereas τ_2 is constant in the range of 2-50 mM $CaCl_2$. The absence of larger changes in the relaxation times points out that the nucleation process in the pretransition range is influenced only to a small extent by the immobilization of the polar head groups on Ca^{++}-binding.

Considering the high cooperativity of membrane phase transitions, the rearrangement of the whole lipid matrix should take place in the range of minutes, as Lentz et al. (2) showed for multilayer suspensions. With the anthracenophane dye this process is now accessible also for vesicles. In preliminary experiments relaxation times of about 1 minute have been measured.

(1) Synthesized by P. Koo Tze Mew, F. Vögtle, Inst. Org. Chem. der Universität Bonn

(2) B.R. Lentz, E. Freire, R.L. Biltonen, Biochem. (1977) 16, 2674