Interaction between Spin Labels and DPPC Vesicles

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Introduction

Recently we could show by 1H NMR studies, that the spin label (1,14) which is located at the apolar end of the CH₂ fatty acid chains affects the entire structure of the lipids of spin labeled DPPC vesicles at temperatures above the phase transition temperature (~37 °C) [1]. This effect is more pronounced, of course, at the side chain than at the choline head groups. The spin label (12,3), which is located near the head groups, should, then, influence the head groups stronger and the side chains less than the spin label (1,14). This modification of the configuration of the lipids should also be reflected by the ESR spin label spectra. For this reason, 1H NMR and ESR studies on DPPC vesicles labeled with the spin labels (1,14) or (12,3) have been conducted at different temperatures.

Results and Discussion

The effect of the concentration of the two spin labels (1,14) and (12,3) on the line width of the 1H NMR signals of the choline head groups and the fatty acid side chains is shown in Fig. 1. As expected, the line broadening of the (~N'-(CH₃)₃) choline signal is more pronounced at 34 °C if the vesicles were labeled with the spin label (12,3). Even at 52 °C, this spin label causes a line broadening of the head group signal. It is interesting to note that the rate in line width change is almost comparable to that observed with the side chain signal.

Material and Methods

The preparation of the DPPC (dipalmitylophosphatidylcholine) vesicles, their labeling with the spin labels (1,14) or (12,3), and details of the 1H NMR (nuclear magnetic resonance) as well as the ESR (electron spin resonance) measurements were described recently [1, 2]. Na-ascorbate (NaASC) was a gift from Hofmann-La Roche, Basle.

The NMR studies have been conducted at two different temperatures (34 °C and 52 °C) below and above the phase transition temperature (~37 °C) for determining the influence of membrane fluidity on the spin label-lipid interaction. In the case of the ESR experiments, the temperature has been varied between 2 °C and 47 °C for determining the order parameter.

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Because of the strong immobilisation of the fatty acid chains at 34 °C, they cannot be observed at this temperature. At 52 °C, the spin label (12,3) affects the side chains less than the spin label (1,14), as expected.

From the results obtained one might conclude, that the spin label (1,14) also affects the head groups while the side chains are also influenced by the spin label (12,3).

For comparison of these results, it is very important to conduct all of these measurements at exactly the same time after vesicle preparation. In preliminary investigations we have observed that the permeation rate of ascorbate across DPPC membranes, using spin labels as an indicator, depends on the “age” of the DPPC vesicles (to be published). There is a considerable decrease in permeation rate with “age” of the DPPC vesicles reaching a plateau (that is, stabilization of the vesicles) after about 24 h. This effect might be explained by the fact that the vesicles are not in a thermodynamic equilibrium right after preparation.

For that reason, the influence of “age” of the vesicles on the line width was also investigated (s. Fig. 2). At 34 °C, the (-N(CH3)3) choline signal broadens considerably with time after preparation of the vesicles, especially if they are labeled with the spin labels. Here, too, a plateau is reached after about 24 h. Two results seem to be very interesting:

a) Addition of 50 mM NaASC to the vesicles right after their preparation results in a minimal line width change only, which is less than in the controls, indicating a stabilizing effect of NaASC on the membrane.

b) Addition of 50 mM NaASC to the spin labeled or to the control vesicles after about 30 h after their preparation results in a considerable reduction in line width. The line broadening produced by the spin label seems to be compensated by the addition of NaASC, an effect which cannot be explained yet.

At 52 °C, there is only a small increase in line broadening of the head group and side chain signals if the vesicles were labeled with the spin labels (12,3) and (1,14), resp., (results not shown).

The influence of the spin label (1,14) on the head groups should also be reflected by the ESR spectra. As can be seen in Fig. 3, only the low-field signal of the spectrum of DPPC vesicles labeled with the spin label (12,3) seems to be temperature-dependent, at least at the higher temperatures. At temperatures <20 °C, the high-field signal is broadened considerably. The dashed line can be used for determining the two components $T_\perp$ and $T_\parallel$ of the hyperfine coupling tensor and, thus, for calculating the order parameter $S$. The spin label (1,14) shows an opposite behaviour. At higher temperatures, only the high-field signal is modified, while between 2 °C and 5.6 °C a drastic change occurs in the low-field region.
Fig. 3. The influence of temperature on the ESR spectra of the spin label (12,3) located near the choline head groups of DPPC vesicles. $s \equiv$ sensitivity factor. Since the spectrum obtained at 2 °C is very similar to that obtained with spin label (12,3) at 24 °C (s. Fig. 4, two uppermost spectra), one might conclude that, at that temperature, the spin label (1,14) influences considerably the head groups. This might indicate the existence of a “phase” transition in regard to the head groups.


Fig. 4. The influence of temperature on the ESR spectra of the spin label (1,14) located at the apolar end of the fatty acid side chains of DPPC vesicles. For comparison, the ESR spectrum of DPPC vesicles containing the spin label (12,3) obtained at 24 °C is also shown. $s \equiv$ sensitivity factor.

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