^{1}H-NMR Studies on the Effect of Spin Label on Lipids

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^{1}H-NMR studies on DPPC vesicles labelled with the spin label (1,14) have been performed below (34 °C) and above (52 °C) the phase transition temperature. At both temperatures, the spin label, located at the apolar end of the CH_2 chain, affects the whole structure of the lipids. Structural and functional investigations of membranes using spin labels have to consider always the effect described.

Introduction

Recently we could show that the permeation rate of Na-ascorbate across membranes of DPPC vesicles decreases concomittantly with increasing concentrations of spin label (1,14) [1]. Since this spin label is located at the apolar end of the CH_2 chain, it was concluded that it might cause certain reinforcements between the lipids. These results are supported by the observation that even at temperatures > 40 °C, that is above the phase transition temperature, the ESR spectrum of a spin labelled membrane of DPPC vesicles is very similar to a free spin label spectrum and yet the permeation rate of NaASC is reduced considerably.

To elucidate the influence of the spin label (1,14) on the configuration of DPPC membranes, ^{1}H-NMR studies on DPPC vesicles labelled with the spin label (1,14) have been conducted at 34 °C and 52 °C resp.

Material and Methods

The preparation of the DPPC (dipalmitoylphosphatidylcholine) vesicles in D_2O and their labelling with the spin label (1,14) were described recently [2]. The ^{1}H-NMR (nuclear magnetic resonance) experiments were performed by means of a Varian XL-100-15 FT-spectrometer operating at 100 MHz with a 5 mm insert. Internal D_2O provided the field-frequency lock. Deuterated phosphate buffer (100 mM, pD 7.4) was used for maintaining constant pD conditions. Acetate (10 mM) was added to the vesicle solution as an internal standard. Acquisition times of 4 s have been used. The 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.

Results and Discussion

The ^{1}H-NMR spectrum of DPPC vesicles is shown in Fig. 1, uppermost spectrum. At the temperature of 34 °C only the $[-N^+(CH_3)_3]$ and $-CH_2-$ signals of the choline head groups can be detected. The $-CH_2-$ and $-CH_3$ resonances of the side chain are broadened considerably and, thus, cannot be observed. This effect indicates a strong immobilization of the fatty acid chains.

The presence of the spin label (1,14) results, at this temperature, in a considerable line broadening of the head groups. Such an effect could not be expected, since the label is located at the end of the apolar side

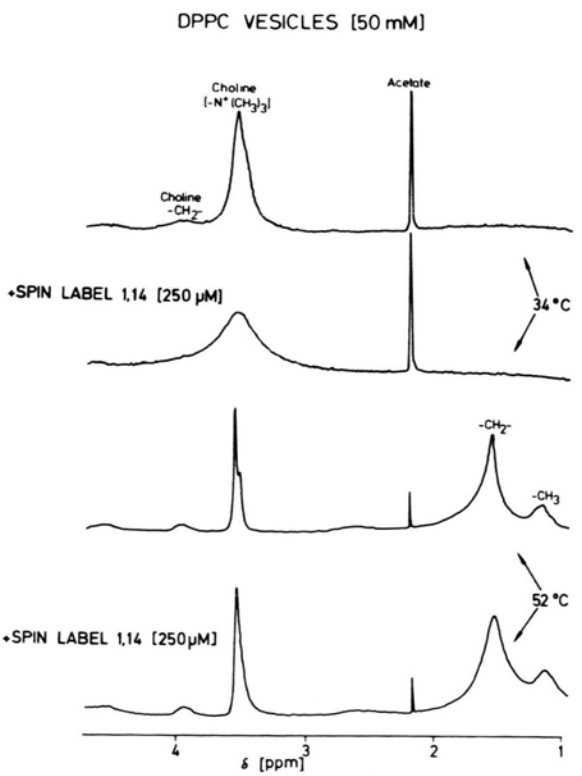

Fig. 1. The influence of the spin label (1,14) on the ^{1}H-NMR spectra of DPPC vesicles at 34 °C and 52 °C, resp.

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chain. The acetate peak doesn't exhibit any paramagnetic line broadening after addition of the spin label demonstrating the presence of the spin label within the membrane. Despite its position, the spin label exerts a long range influence on the choline head groups.

At 52 °C, the protons of the side chain can also be detected (s. Fig. 1, 3rd spectrum from top). As expected, the line width of the choline proton signals is more narrowed. The two slightly resolved $[-N^+(CH_3)_3]$ peaks belong to the two different head groups located at the inside and outside of the double layer.

It is interesting to note that even at 52 °C the presence of the spin label (1,14) causes a line broadening of all proton signals shown in Fig. 1, bottom spectrum. And this, as we have shown recently [3], despite the fact that the spin label can rotate almost freely indicating that the spin label-lipid interaction should be weak, at the most. Again, the acetate peak is not influenced indicating that even at this temperature the spin label is located within the membrane. From this it can be concluded, that the spin label might influence the side chain at 52 °C and, therefore, also at temperatures below the phase transition temperature. As can be seen, the spin label affects the whole structure of the lipids; at temperatures below the phase transition temperature it exerts its strongest influence, of course. The very small influence of the spin label on the signals of the choline head groups at 52 °C indicates that the effect observed at 34 °C is mainly due to the modifications of the configuration of the lipids and not due to the paramagnetism of the label.

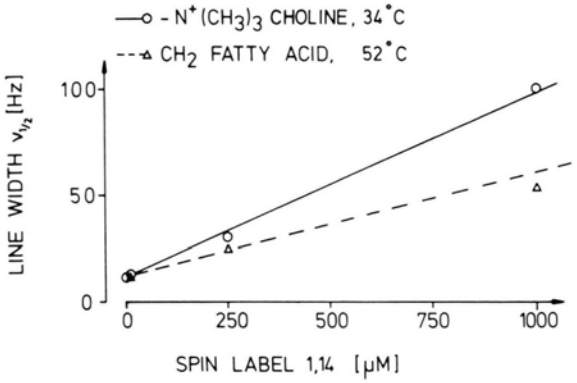

Fig. 2. The influence of different spin label (1,14) concentrations on the line width of the proton signals of the choline head groups and of the fatty acid side chain, resp.

The influence of spin label concentration on the change in line width of the choline head group- and the CH_2 side chain-signals as observed at 34 °C and 52 °C, resp., is shown in Fig. 2. As can be seen, the line width increases with increasing spin label concentration, at least up to a concentration of 1 mM, that is a DPPC: spin label ratio of 50:1. At higher spin label concentrations, dipol-dipol interactions as well as spin exchange interactions will modify the effect of the spin label on the lipids.

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