The Influence of Spin Label on the Transport of Ascorbate across Artificial Membranes

W. Lohmann, P. z. Tian*, and D. Holz

Institut für Biophysik der Universität, Leihgesterner Weg 217, D-6300 Gießen, Bundesrepublik Deutschland

Z. Naturforsch. **41c,** 351–354 (1986);
received November 8, 1985

Spin Label, DPPC Membranes, Ascorbate

Spin label 1,14 studies on DPPC membranes revealed an increased rigidity of the membrane with increasing spin label concentrations. This causes a retardation in permeation rate of Na-ascorbate. At temperatures > 40 °C, that is above the phase transition temperature, the ESR spectrum of a spin labelled DPPC membrane is very similar to a free spin label spectrum and yet the permeation rate of NaASC is reduced considerably. From this it can be concluded that the spin label has caused certain reinforcements between the lipids, an effect which cannot be reversed by temperatures of about 60 °C.

Structural and functional investigations of membranes using spin labels have to consider always the effect described.

Introduction

Recently we could show that at temperatures above 40 °C (that is, above the phase transition temperature) the permeation rate of Na-ascorbate across membranes of DPPC vesicles is reduced considerably as indicated by a diminished reduction rate of spin label 1,14 located at the apolar end of the CH_2 chain [1]. This effect might be due to either structural changes in the vitamin C configuration or in the membrane configuration resulting in the inability of vitamin C to penetrate the membrane. Since at these temperatures the membrane behaves like a fluid, the permeation of the vitamin should be facilitated rather than inhibited. If the inhibition observed is caused by modifications within the membrane, they should be caused by the spin label incorporated.

To elucidate the influence of the spin label 1,14 on the configuration of DPPC membranes and thus on the transport of ascorbate across these membranes, the effect of different spin label concentrations on this transport has been investigated.

Material and Methods

The preparation of the DPPC (dipalmitoyllecithine) vesicles and their labelling with the spin label 1,14 as well as the electron spin resonance (ESR) parameters used were described recently [2]. In each case, the DPPC vesicle concentration was kept constant (17 mM), while the spin label concentration varied between 0.06 mM and 2.5 mM.

Na-ascorbate (NaASC) solutions (pH 7.4) were prepared immediately before the ESR measurements by dissolving the appropriate amount of ascorbic acid (Hoffmann-La Roche, Basle, Switzerland) in NaOH. The reaction started when 10 µl of NaASC solution were added to a 100 µl labelled vesicle suspension. All measurements were done at room temperature. The down-field line of the spin probe spectrum was used for monitoring the effects.

Results and Discussion

The influence of 50 mM NaASC on the peak height of DPPC vesicles labelled with different concentrations of the spin label 1,14 is shown in Fig. 1. As has been observed previously [2], the reduction occurs biphasicly. An initial steep decrease ("fast reaction" rate) is followed by a slow decrease ("slow reaction" rate). An explanation cannot be given yet for this biphasic behavior.

It can be seen, that the inactivation rate, and thus the permeation of NaASC across the membrane, decreases with increasing label concentration using the "fast reaction" phase as an indicator (s. also Fig. 2). At constant label concentration, the slope of the inactivation rate curve changes linearly with the NaASC concentration which agrees well with previous findings.

Furthermore, it should be pointed out, that the peak height of the spin label 1,14 located in the DPPC membranes increases linearly with its concentration up to about 0.5 mM (s. Fig. 3). At higher concentrations, dipol-dipol interactions as well as spin exchange interactions result in a line broadening. The peak height, thus, is no more an indicator for spin concentration.

It is interesting to note that at still larger label concentrations (*e.g.* 2.5 mM), the lines are broadened even further, however, a free label spectrum is superimposed (s. Fig. 4). This indicates that the spin label causes an increased rigidity of the

---

* On leave of absence from the Department of Biophysics, Academia Sinica, Peking, China.

Reprint requests to Prof. Lohmann.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/86/0300–0351 $ 01.30/0

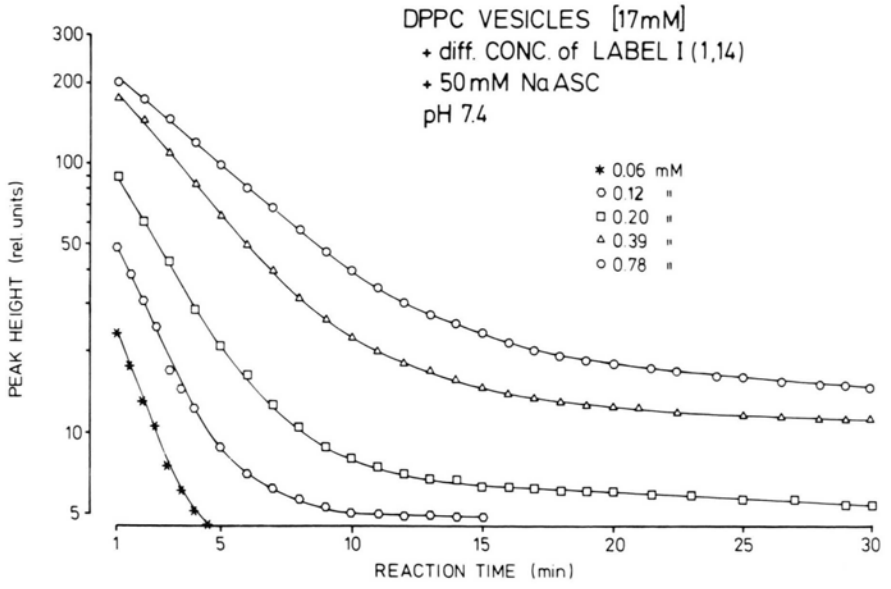

Fig. 1. The influence of Na-ascorbate on the reduction rate of different concentrations of spin label 1,14 located within DPPC vesicles. S.D. ≤ 5%.

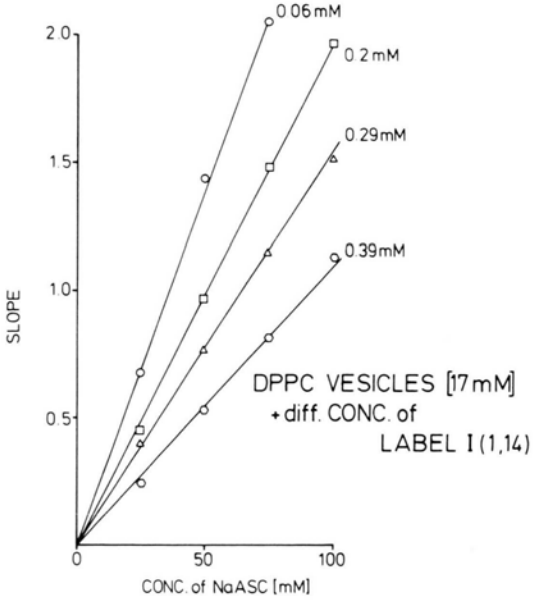

Fig. 2. Effect of Na-ascorbate concentration on the "fast reaction" rate of the inactivation of different concentrations of spin label 1,14 located within DPPC vesicles. S.D. ≤ 5%.

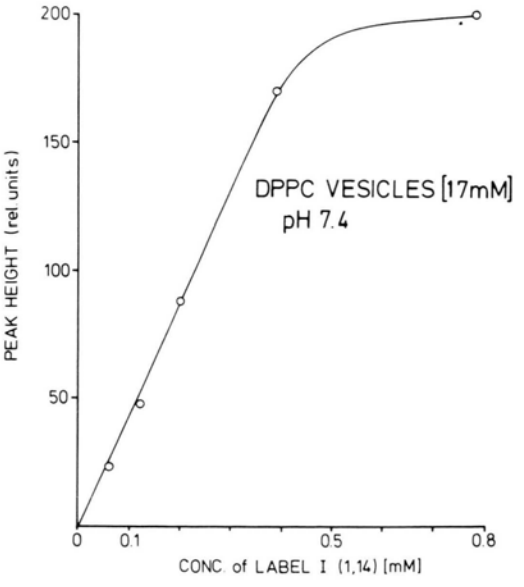

Fig. 3. The effect of label concentration on the peak height of the spin label 1,14 located within DPPC vesicles. S.D. ≤ 5%.

membrane which, at a certain molar ratio of DPPC: spin label, is so intense that any additional spin label cannot be built in any more. This fraction will appear as the free spin label spectrum.

Addition of NaASC to such a suspension will reduce this "free" portion first. The reduction of the other portion is diminished since the liquid crystal state retards the permeation of NaASC. Thus, after about 60 min interaction time the peak height (≙ spin concentration) is considerably larger than in those cases with smaller spin label concentrations registered even at 30 min interaction time (s. traces 3 to 5 in Fig. 5; the doublet in the center is the ascorbyl radical).

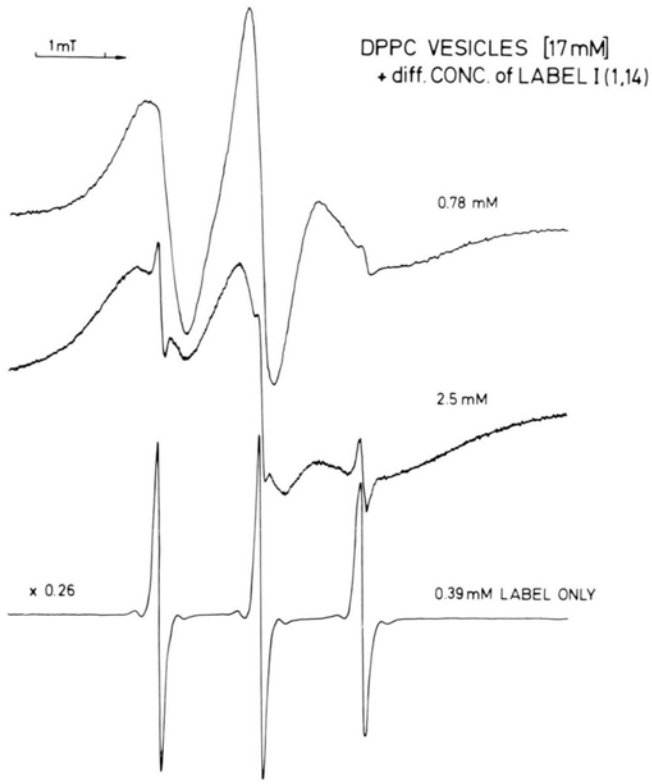

Fig. 4. The influence of label concentration on the spectrum of the spin label 1,14 located within DPPC vesicles. For comparison, a free spin label (0.39 mM) spectrum is shown with a different sensitivity factor.

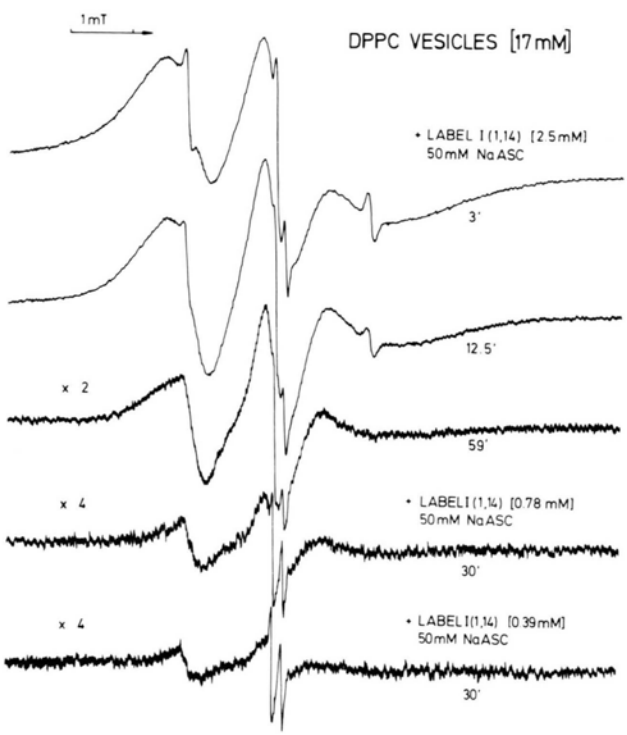

Fig. 5. The effect of Na-ascorbate and interaction time on the spin concentration of the label 1,14 located within DPPC vesicles. On the left hand side sensitivity factor.

From the results obtained it can be concluded that the spin label 1,14 causes an increased rigidity of DPPC membranes with increasing concentration resulting in a diminished permeation rate of NaASC. It seems very likely that other spin labels which can be attached to the hydrophobic side chain of lipids will affect the membrane in a similar way. This has to be considered if investigations concerning structure and function of membranes are conducted with spin labels. At certain concentration ratios of DPPC : spin label, relative flux measurements, *e.g.*, can be done, however, it has to be always considered that the membrane has been modified structurally by the incorporation of the spin label. It should be pointed out, that 50 mM NaASC can reduce very quickly a 2.5 mM spin label concentration.

Acknowledgements

The excellent technical assistance of Mrs. P. Mokkenhaupt and Miss P. Ferber is appreciated. This work was supported in part by grants from the Bundesministerium für Forschung und Technologie and from the Fonds der Chemischen Industrie.

[1] W. Lohmann, P. z. Tian, and D. Holz, Z. Naturforsch. **41c,** 348–350 (1986).

[2] W. Lohmann and J. Winzenburg, Z. Naturforsch. **38c,** 923–925 (1983).