Influence of Temperature on the Transport of Ascorbate across Artificial Membranes as Studied by the Spin Label Technique

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

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

Z. Naturforsch. **41c,** 348–350 (1986);
received November 8, 1985

Spin Label, DPPC Membranes, Ascorbate, Temperature

The effect of temperature on the permeation of vitamin C across membranes of DPPC vesicles labelled with the spin label 1,14 has been investigated. It could be shown that at temperatures above 40 °C the permeation rate is diminished considerably despite the fact that, at these temperatures, the spin label can rotate freely (above phase transition temperature) indicating that the membrane is more fluid. The results obtained agree well with previous findings (W. Lohmann and D. Holz, Biophys. Struct. Mech. **10,** 197 (1984)) according to which the furanoid ring formed by the side chain of this vitamin opens at these temperatures. The existence of a bicyclic side-chain structure seems to be a prerequisite for the permeation of vitamin C.

Introduction

Recently we could show by the reduction of the spin label 1,14 located at the apolar end of the CH_2 chain within DPPC vesicles, that Na-ascorbate can permeate membranes [1]. An assumption for this permeation is that vitamin C exists as an electroneutral radical with a cyclic side chain structure at physiological pH and temperature. At higher temperatures (> 40 °C), the radical disappears due possibly to the opening of the furanoid ring formed by the side chain [2]. Since the cyclic side chain structure might be a prerequisite for permeation, there should be no or, at least, a diminished reduction of the spin label 1,14 located within DPPC vesicles by vitamin C at temperatures above 40 °C despite the fact that this is above the phase transition temperature of this artificial membrane.

Material and Methods

The preparation of the DPPC (dipalmitoylphosphatidylcholine) vesicles and their labelling with the spin label 1,14 as well as the electron spin resonance (ESR) parameters used were described recently [1]. In contrast to previous investigations, the down-field line of the spin probe spectrum was used to monitor the kinetics of ascorbate transport. In this way, an interference with the signal of the ascorbyl radical can be avoided.

The ESR cavity temperature was adjusted by a variable Varian temperature unit. For the temperature measurements, the samples were placed into a hematocrit tube right after preparation. In each case, the final concentrations of the DPPC vesicles (17 mM) and of the spin label (0.39 mM) were kept constant throughout the experiments.

Ascorbate solution (pH 7.4) was prepared immediately before the ESR measurements by dissolving the appropriate amount of ascorbic acid (ASC, Hoffmann-La Roche, Basle) in NaOH. The reaction started when 10 µl of Na-ascorbate (NaASC) solution were added to a 100 µl vesicle suspension, resulting in a final concentration of NaASC of 50 mM. Both have been kept separately at the temperature to be investigated before mixing.

Results and Discussion

The effect of temperature on the spin label 1,14 spectrum of a label located within DPPC vesicles is shown in Fig. 1. As can be seen, the spectrum resembles a free spin label spectrum (can rotate freely) above the phase transition temperature of DPPC membranes (≈ 37 °C), while at low temperatures the label is rather immobilized. Concomittantly, an increase in peak height is observed with increasing temperature reaching a plateau at about 45 °C. It should be pointed out that there is almost no change with temperature in the peak height, if the label is investigated only.

If NaASC is added to a labelled DPPC vesicle suspension, the reduction rate in peak height is diminished at higher temperatures, as can be seen in Fig. 2. This is surprising since the membrane is more vulnerable and fluid, at least, at temperatures above the phase transition. Since addition of NaASC (50 mM) to a spin label 1,14 solution (0.39 mM) only, *e.g.* at 47 °C, reduces quickly the spin label, it must be concluded, that NaASC is unable to permeate the membrane perhaps due to an opening of the furanoid ring formed by the side chain.

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

Reprint requests to Prof. Lohmann.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/86/0300–0348 $ 01.30/0

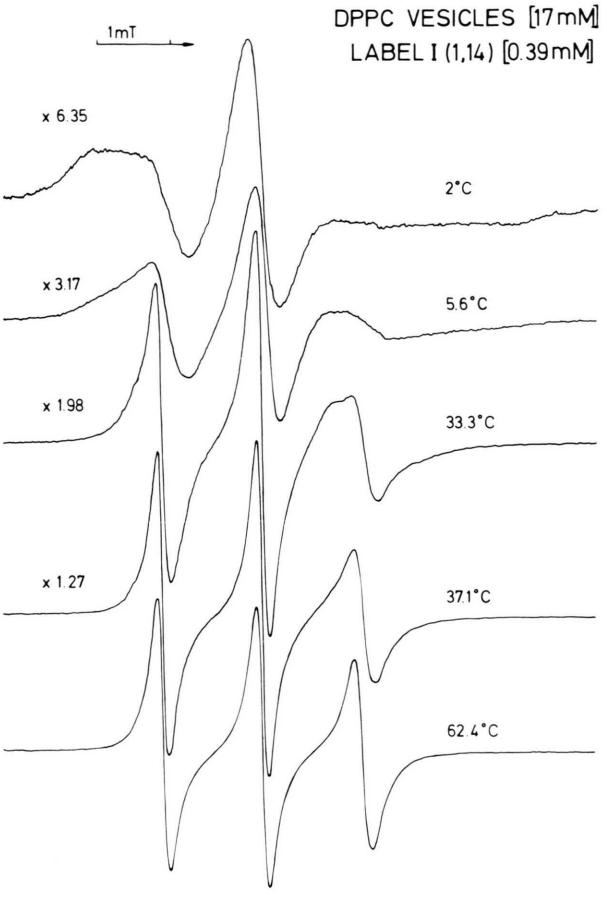

Fig. 1. The effect of temperature on the spin label 1,14 located within DPPC vesicles. (On the left hand side: sensitivity factor.)

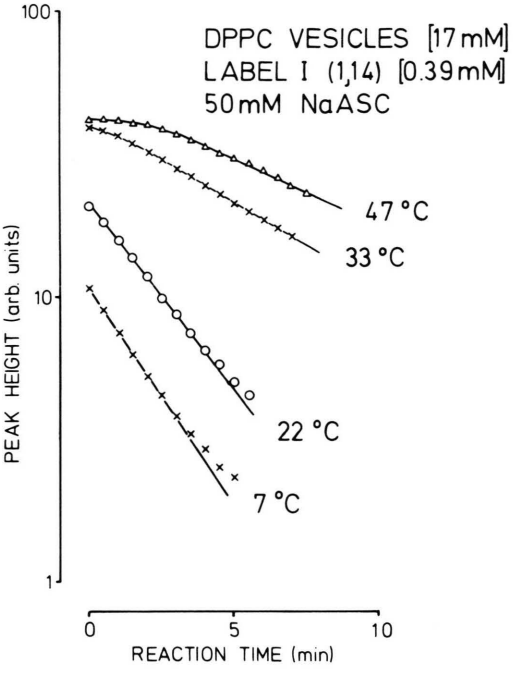

Fig. 2. The effect of temperature on the Na-ascorbate induced reduction rate of spin label 1,14 located within DPPC vesicles. S.D. $\leq 5\%$.

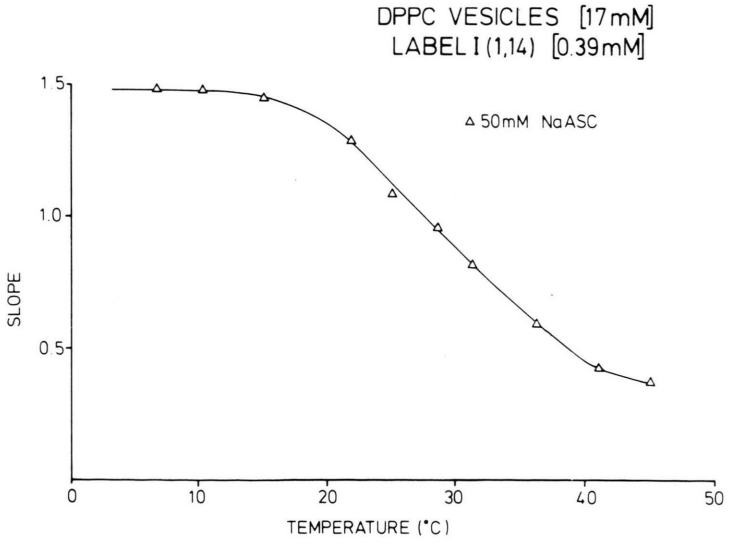

Fig. 3. The influence of temperature on the slope ($\triangleq$ reduction rate) of the exponential part of the curves shown in Fig. 2. S.D. $\leq 5\%$.

Furthermore, it should be pointed out that at higher temperatures several reactions might occur, as can be seen by the shoulder of the inactivation curves (s. Fig. 2). After this initial response, the slopes exhibit an exponential response. The influence of temperature on this latter part is shown in Fig. 3. The change in slope is almost a sigmoidal response with a $T_m \approx 30$ °C. Above 47 °C, the change in slope is minimal up to the highest temperature measured (62 °C).

The results obtained suggest that vitamin C cannot permeate membranes even above their phase transition temperature which might be due to structural changes in the vitamin configuration. Modifications of the membranes caused by the spin label cannot be ruled out.

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 and J. Winzenburg, Z. Naturforsch. **38c**, 923–925 (1983).

[2] W. Lohmann and D. Holz, Biophys. Struct. Mech. **10**, 197–204 (1984).