Determination of Internal H^+ concentration in Lecitin Vesicles Using an ESR Method

M. BRAGADIN, E. R. VIOLA

Facoltà di Chimica Industriale, Università di Venezia; Calle Larga S. Marta 2137, 30123 Venice, Italy

and M. G. BETTOLI

Università di Bologna, 40100 Bologna, Italy

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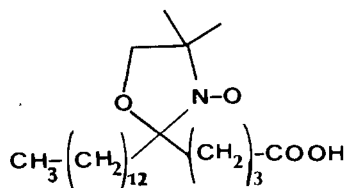
Differences in hydrogen ion concentration across biological membranes play an important intermediary role in a number of cellular processes, including oxidative and photosynthetic phosphorylation.

Many techniques and probes have been used [1–3] for the determination of internal H^+ concentration. In a previous paper [4] the authors observed that the ESR spectrum of a spin-labelled fatty acid dissolved in lecitin dispersions shows a marked pH dependence. In the present paper we make use of the pH dependence of the ESR signal of the same spin labelled fatty acid to measure the internal pH in sonicated lecitin vesicles and to verify the impermeability to protons of sonicated lecitin membranes.

Experimental

The spin labelled fatty acid utilized is the N-oxyl-4',4'-dimethyl oxazolidine derivative of 3-ketostearic acid:



supplied by Syva Inc., Paolo Alto, California. Pure synthetic lecitin was supplied from SIGMA.

Samples were prepared by suspending 50 mg of lecitin in 5 ml of a medium containing KCl 50 m*M*, Tris 20 m*M* (at various pHs) EDTA 0.1 m*M* and about 10^{-4} *M* of spin label. The suspension was then sonicated. ESR spectra were run at 22 °C on a Varian E-4 instrument using an aqueous sample cell.

To remove the ESR signal due to the spin label placed in the external bilayer, a slight excess of sodium dithionite was added to the solution. The ESR signal height was thus immediately reduced to one third of the initial one and no further significant lowering of the ESR signal height was observed. The order parameter of the spin label was determined by eqn. 1:

$$S = \frac{T_{\parallel} - T_{\perp}}{T_{zz} - 0.5(T_{xx} + T_{yy})} \cdot \frac{a}{a'} \tag{1}$$

where the ratio a/a' is a correction introduced by Hubbel and McConnel [5] for the polarity of the medium. The following values were used for T_{xx} = 31.91, T_{yy} = 5.83 and T_{zz} = 6.31. $T_{\perp}$ and $T_{\parallel}$ were determined from the ESR spectra [6].

Results and Discussion

Figure 1 shows the variation of the order parameter of the spin labelled stearic acid at various pHs. The suspension of lecitin containing the probe was sonicated at various pHs and the external signal quenched as indicated in the Experimental. The observed signal is due only to the contribution of the spin labelled fatty acid retained in the internal bilayer of the internal aqueous surface, since no changes in the ESR signal are observed if the external pH is changed. (On the contrary the external ESR signal

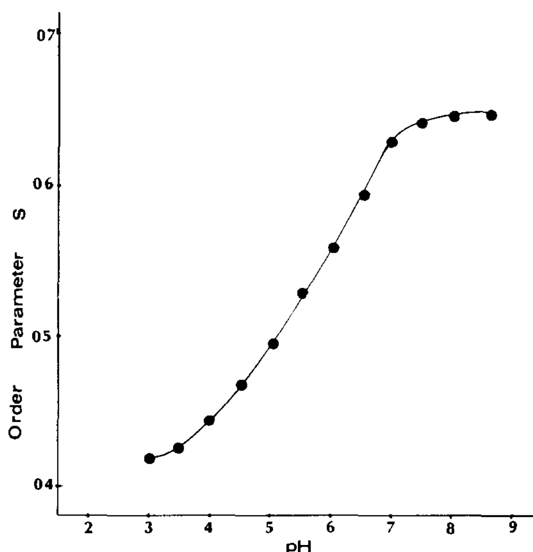


Fig. 1. The figure shows the variation of the order parameter of the spin labelled stearic acid at various pHs. In the sonicated suspension of lecitin containing the probe the external ESR signal has been quenched as indicated in the Experimental. The signal is thus only due to the contribution of the spin labelled fatty acid retained in the internal bilayer of the internal aqueous surface.

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due to the spin label added after sonication shows a marked pH dependence and addition of dithionite causes complete disappearance of the ESR signal). This behaviour is evidence that lecitin membranes are not permeable to protons. However, if the external pH variation is performed in the presence of both valinomycin and uncouplers (such as dinitrophenol), the internal pH assumes the external value at once, thus confirming the role of H^+ carriers of the uncouplers and of the K^+ carrier of valinomycin.

This method for internal pH measurement may have some advantages with respect to other probes or systems for analogous use. In fact removal of external probes does not need chromatography as in the case of pyranine [2] or dialysis to remove the external phosphate as in the case of methods based on NMR [1]. Moreover, our method may be used to study the permeability through the lecitin membrane of substances (such as for example ascorbic acid) able to pass through and to reduce rapidly the internal spin label signal. The relative stability of the internal ESR signal may be used to establish the flip–flop rate of some spin label fatty acids, as in the present case of the spin label derivative of 3-ketostearic acid.

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