NMR STUDIES OF pH-INDUCED TRANSPORT OF CARBOXYLIC ACIDS ACROSS PHOSPHOLIPID VESICLE MEMBRANES[1]

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SUMMARY

Proton NMR spectroscopy was used to demonstrate that transmembrane pH gradients across single-bilayer vesicle membranes effect the transport and concentration of carboxylic acids. The results obtained indicate that this transport occurs via selective permeation of the membrane by the protonated (uncharged) form of the acid.

INTRODUCTION

The active transport across biological membranes of small hydrophilic molecules is important to the metabolic and regulatory functions of the cell. The possible mechanisms for accomplishing this transport are quite varied. One of the simplest, however, utilizes a pH gradient to directly raise the chemical potential of a permeable form of a titratable solute on one side of a membrane, resulting in the passive diffusion of the solute. It is widely accepted that the neutral form of a molecule, such as a carboxylic acid, is more lipid soluble and hence more permeable than its ionic counterparts. Thus, lowering the pH of a carboxylic acid solution, which is external to a closed vesicular membrane, should concentrate the acid in the interior volume of the vesicle. An analogous mechanism has been proposed for the concentra-

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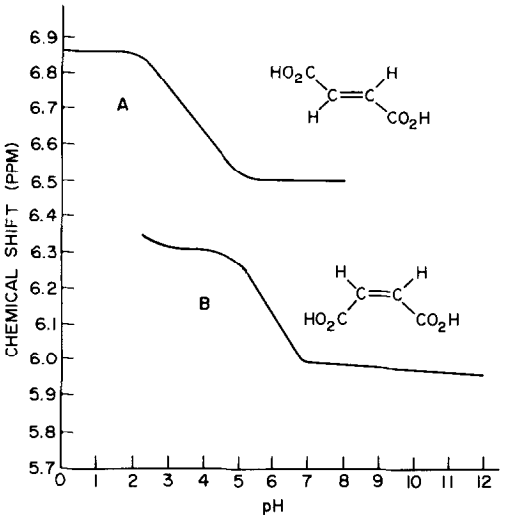

Figure 1. Chemical shift titration curve for the vinyl protons of fumaric (A) and maleic (B) acids. Curve A is taken from work reported by L. Pratt and B. B. Smith (3) and is for a 0.04 M aqueous solution containing t-butanol as an internal standard (δ = 1.25). The shifts for curve B are referenced to sodium 2,2-dimethyl-2-silapentane-5-sulfonate (δ = 0.0) and are for an 8.6 mM D_2O solution containing phosphatidylcholine-cholesterol vesicles.

tion of catecholamines within chromaffin vesicles (1) and has led to recent studies of the correlation of pH gradients with transport phenomena (2).

It is often not a simple task to quickly and accurately obtain pH and concentration information for solutions external and internal to vesicular structures less than 1000 Å in diameter. High field proton NMR offers a means of accomplishing this for many systems. We report here an illustration of this fact, using the pH-induced transport across phospholipid vesicles of fumaric and maleic acids. These acids exhibit vinyl proton resonances which are resolved from vesicle absorptions and which exhibit a strong chemical shift dependence on pH as shown in Figure 1.

MATERIALS AND METHODS

Single-bilayer 320 Å diameter vesicles containing maleate dianion were prepared by sonicating a 10% (w/v) egg yolk phosphatidylcholine-cholesterol (2:1 mole ratio) suspension in a 0.19 M maleic acid solution in D_2O at pH 7. The exterior maleate solution was replaced by a pH 7 equiosmolar D_2O solution

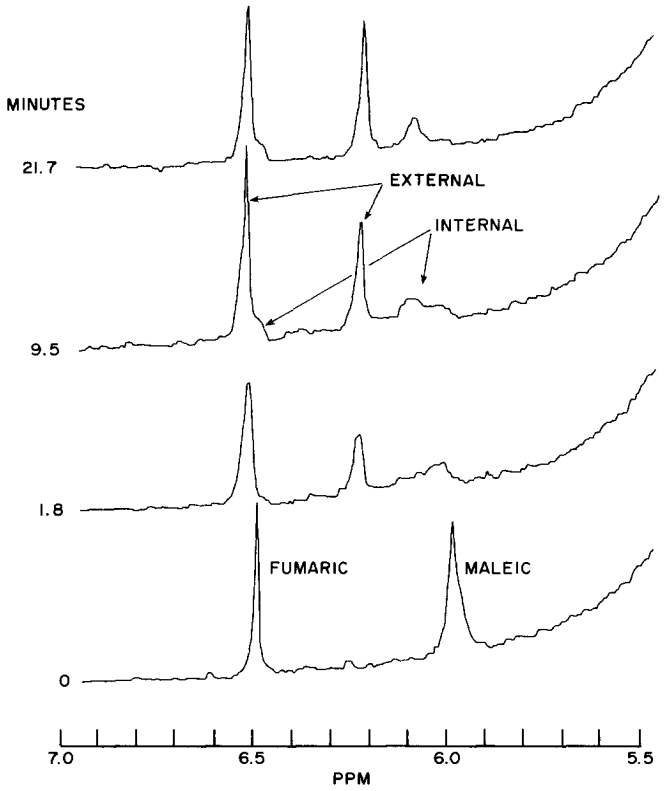

Figure 2. Vinyl proton NMR spectra recorded at various times after a transmembrane pH gradient was established by lowering the extravesicular pH to 5.5.

containing fumarate dianion (9.3 mM), NaCl (0.26 M), and sodium 2,2-dimethyl-2-silapentane-5-sulfonate (4.6 mM) which served as an internal NMR reference. This solution replacement was accomplished by passing the vesicle preparation through a Sephadex G-50 column, which had previously been equilibrated with the above fumarate solution.

A pH gradient was established across the vesicle membrane by lowering the exterior pH through the addition of 0.4 N DCl. Sequential FT NMR measurements were then made with a Bruker HX270 spectrometer to monitor the pH and carboxylic acid concentrations for both the exterior and interior regions of the vesicle preparation.

RESULTS

Figure 2 presents a series of spectra observed when the external pH was lowered to 5.5. Lowering the outside pH serves to drive the transport of external fumaric acid into the interior of the vesicles. This is demonstrated by the growth of an inside fumaric acid peak, which appears as a high field shoulder on the external acid resonance at 6.50 ppm. The gradual downfield

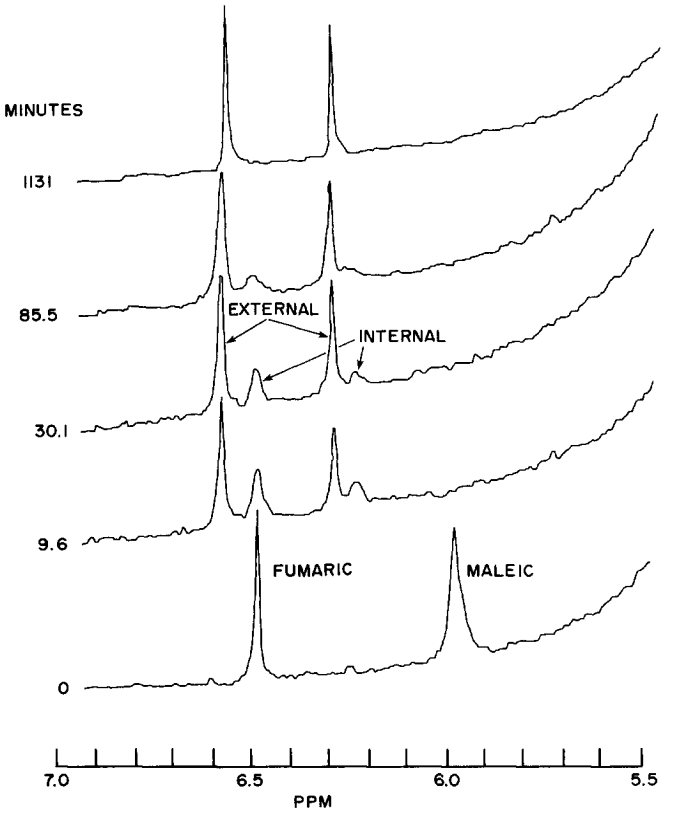

Figure 3. Vinyl proton NMR spectra recorded at various times after a transmembrane pH gradient was established by lowering the extravesicular pH to 4.7.

shift of the internal maleic acid peak from 6.0 to 6.1 ppm, in concert with the growth of the inside fumaric acid signal, indicates that fumaric acid is being transported with protons, resulting in the titration of maleate dianion. The broad appearance of the inside maleic acid signal at 1.8 and 9.5 minutes indicates that a range of internal pH values exists, which probably reflects the inhomogeneity of the vesicle preparation with respect to permeability and size.

Figures 3 and 4 reveal that adjusting the outside pH to a lower value (4.7 compared to 5.5) results in a rapid and greater accumulation of internal fumaric acid, followed by a slow simultaneous leakage of both acids. Note, in contrast to the case just considered, the inside fumaric acid peak in

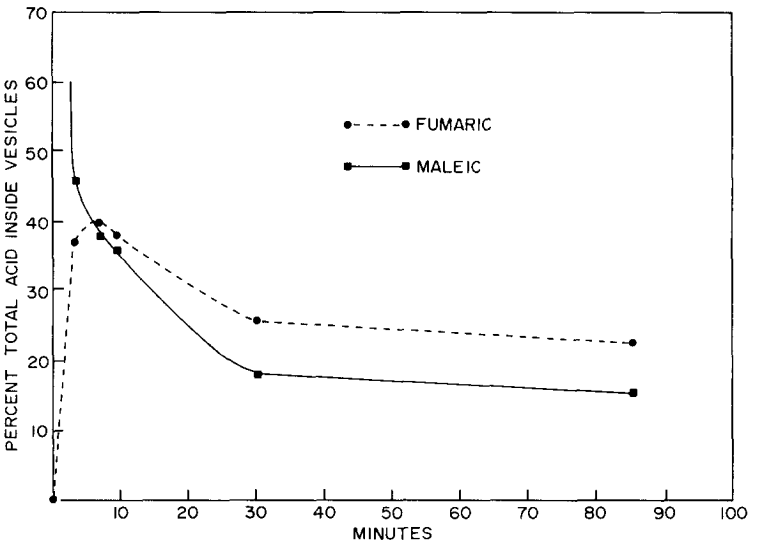

Figure 4. Time variation of internal acid concentrations for data presented in Figure 3.

Figure 3 is clearly resolved from the outside signal in accord with the increased chemical shift dependence on pH at pH values less than 5, as shown in Figure 1. Figure 4 demonstrates that the pH gradient in this case drives the transport of 40% of the total fumaric acid. This corresponds to internal and external concentrations of 74 mM and 5.9 mM, respectively, if one assumes the internal volume of the vesicle preparation to be 5% of the total volume. Thus, in this instance the pH gradient serves to concentrate fumaric acid by a factor of 13.

DISCUSSION

Fumaric and maleic acids exist as neutral molecules (H_2A), monoanions (HA^-), or dianions ($A^=$). On the basis of the passive diffusion model presented earlier, these acids would be predicted to undergo preferential transport in their more lipid-soluble fully protonated forms (H_2A). This would result in a tight coupling of proton and carboxylate transport. On this basis we predict that, following a pH perturbation, an equilibrium con-

dition, corresponding to zero net transport, should be reached where the internal and external H_2A activities are equal.

Since activities in these systems are difficult to predict, we will deal here only with concentrations. The H_2A concentration for dicarboxylic acids may be expressed as a function of total acid concentration (C_T), pH, and ionization constants (K_1 and K_2) as shown in Equation 1.

$$[H_2A] = \frac{C_T \times 10^{-2pH}}{10^{-2pH} + K_1 \times 10^{-pH} + K_1K_2} \qquad (1)$$

Calculation of H_2A concentration for fumaric acid using the observed pH values and concentrations, corresponding to maximal internal accumulation in Figure 4, yields 5×10^{-5} M and 4×10^{-5} M for inside and outside acid, respectively. These values are within experimental error of being equivalent and therefore offer direct evidence for the coupling of proton and carboxylate transport via the selective transport of the fully protonated acid form.

On the basis of this coupled transport scheme, the initial rate of fumaric acid accumulation, in the experiments we have described, should be a linear function of the initial concentration of the H_2A species. Since the concentration of H_2A is a strong function of pH, we would expect more rapid initial transport in the experiment described by Figures 3 and 4, where the outside pH was lowered to 4.7, compared to the case in Figure 2 where the pH was only lowered to 5.5. On the basis of Equation 1 the concentration of H_2A is 16 times greater at pH 4.7 than at pH 5.5. Initial transport rates observed in these experiments, although based on a small number of data points, are consistent with this factor and thus provide further support for the transport mechanism discussed above.

The nonselective leakage of both fumaric and maleic acids depicted in Figure 4 at longer times is probably the result of vesicle rupture in response to the osmotic stress provided by the early selective transport of protonated

fumaric acid. This contention is supported by our observation of enhanced leakage of normally impermeant $N(CH_3)_4^+$, when $N(CH_3)_4Cl$ is included in the vesicle preparation.

The NMR spectra in this study were acquired in less than 70 seconds in FT mode. The spectrometer used has the capability of accumulating sequential spectra in a computer automated manner, thereby greatly facilitating the study of rapid transport phenomena. The use of other NMR kinetic procedures and the use of vesicles of differing size can further extend the transport time scales which are amenable to study. The variety of solutes yielding resolvable and pH sensitive resonances is large and promises a variety of applications in the future.

ACKNOWLEDGMENT

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