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Determination of osmotic volumes and pH gradients of plant membrane and lipid vesicles using ESR spectroscopy

Terri L. Lomax [a,*] and Rolf J. Mehlhorn [b]

[a] *Carnegie Institution of Washington **, Department of Plant Biology, 290 Panama St., Stanford, CA, 94305 and* [b] *Department of Physiology / Anatomy and Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720 (U.S.A.)*

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Volumes and pH gradients were determined with spin probes in liposomes and zucchini membrane vesicles by quantitating the internal concentrations of probes in the presence of an impermeable line-broadening agent, manganese + EDTA. Volume shrinkage in response to increasing external concentrations of MnEDTA was consistent with perfect osmotic behavior of both vesicle populations. Buffer additions were used to impose pH gradients on the vesicles; liposome gradients measured with a spin-labeled weak acid were slightly smaller than the maximum theoretical imposed gradients, whereas above a threshold magnitude, measured gradients for the plant membranes were significantly smaller than imposed gradients. However, the residual pH gradient in the zucchini vesicles decreased at about the same rate as the liposome gradient. Moreover, this residual gradient was not completely collapsed in the presence of the proton ionophore, FCCP, indicating that the vesicles were impermeable to ions; indeed, ion permeabilities of both vesicle preparations appeared to be similar during the slow phase of the pH gradient collapse. Thus, zucchini membrane vesicles are tightly sealed and appear to have a mechanism for dissipating pH gradients rapidly when these gradients exceed some threshold value.

Introduction

Many transport phenomena involve proton gradients as driving forces for the movement of other solutes. In recent years, interest has increased in plant membrane transport phenomena (for reviews see, e.g., Refs. 1–4), including hormone transport [5]. To elucidate how proton gradients are coupled to other solute gradients it is useful to measure accurately the component of the proton-motive force. Generally, such measurements have been plagued by inaccuracies, primarily because the inference of intravesicular parameters hinges upon assumptions that are difficult to verify. Since cell, organelle and vesicle volumes may change considerably under different metabolic conditions, determinations of volumes under specific conditions are critical for accurate calculations of ΔpH and other bioenergetic parameters. The standard technique for determining the internal water volumes has been to use tritiated water and a labeled membrane-impermeable solute such as

* To whom all correspondence should be addressed.

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Abbreviations: ΔpH transmembrane proton gradient; FCCP, carbonyl cyanide trifluorophenylhydrazone; Tempone, 2,2,6,6-tetramethyl-4-oxopiperidine-*N*-oxyl; Tempacid, 2,2,6,6-tetramethyl-4-carboxylpiperidine-*N*-oxyl; $TMA_2MnEDTA$, tetramethylammonium MnEDTA.

[^{14}C]sorbitol or sucrose [6]. However, this method requires either the sedimentation or filtration of vesicles to separate them from the external probe. Those steps require both time and considerable amounts of material and may cause errors as a consequence of breakage of previously sealed membranes during the mechanical separation or misinterpretation of tracer measurements due to membrane adherence or association with unstirred layers or Debye layers.

A recently developed method using spin-probe measurements of bioenergetic parameters overcomes these difficulties because intravesicular probe concentrations are measured directly. Moreover, both volume and pH are measured with the same technique, so that the pH of a well-defined intravesicular compartment, i.e., the aqueous compartment that is sequestered from the added paramagnetic ions, is reliably measured. Nitroxide spin-probe spectra measured with electron spin resonance (ESR) spectroscopy can provide unique information not available from other methods: volume measurements are independent of cell shape, probe binding is quantitated and corrected for, and kinetic measurements are feasible.

The nitroxide spin-probe method is particularly well-suited for volume measurements because rapid measurements can be made with only a minimal amount of material (40 μl containing membranes at 1–5 mg protein/ml is generally sufficient) and without disturbing vesicle integrity. The method consists of quantitating the probe signal in a vesicle suspension by measuring the aqueous line heights of a membrane-permeable, yet highly water-soluble probe in the absence of a membrane-impermeable, paramagnetic line-broadening agent (quencher). A schematic diagram representing volume measurements by ESR is shown in Fig. 1. The probe, Tempone, was chosen because it is rapidly membrane-permeable and is inert towards electric or ionic gradients, and its narrow linewidths are efficiently quenched by minimal concentrations of paramagnetic agents, thus minimizing osmotic effects of the quencher [7]. Quantitating probe concentrations by direct measurements of spectral line heights avoids the need for calibration curves from other 'model' systems by using membrane impermeable paramagnetic broadening agents to eradicate signals

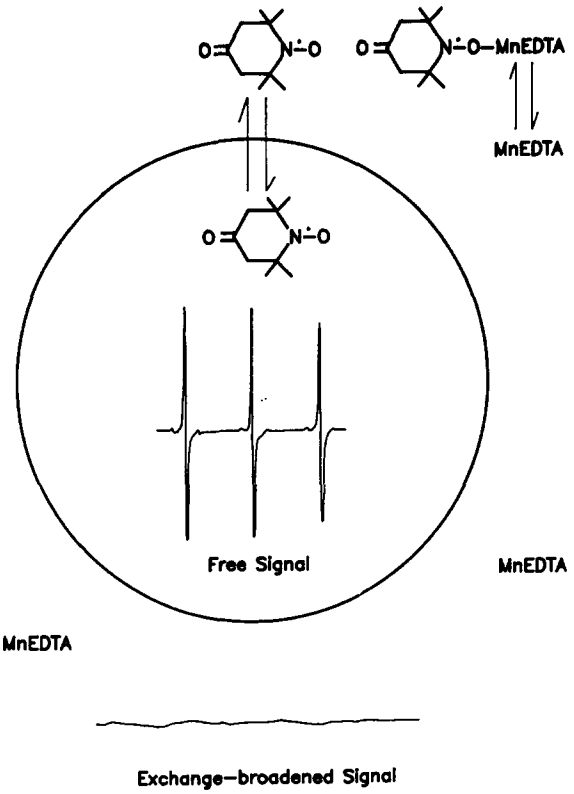

Fig. 1. Schematic diagram of volume measurements by ESR. The nitroxide spin probe, tempone, is freely permeable whereas the quenching agent, $TMA_2MnEDTA$, cannot permeate the vesicle. Signals represent the aqueous line heights of electron spin resonance measurements.

selectively from the extravesicular water (Fig. 1). When intracellular signal changes are observed, these are linearly related to probe concentrations.

In most of the techniques utilized for the evaluation of ΔpH across vesicle membranes, the internal activity of the probe is evaluated on the basis of the probe mass balance and of the volume of the inner compartment measured with independent techniques. The amine distribution method is the technique most widely used for measurement of pH differences across membranes [6]. It has, however, inherent limitations. For both radioactively labeled amines and fluorescent amine dyes (such as 9-aminoacridine), deviation from the ideal behavior of the ionic probe can arise from interaction of the probe with itself, with other ions or with membrane surfaces, and cause errors in the evaluation of ΔpH [8]. Also, as with the volume

measurements, the physical disruption of the vesicles caused by this method may influence the results.

Spin-labeled amines and carboxylic acids have proven useful for following proton movements across membranes. Unprotonated amines and protonated carboxylate spin probes are freely permeable to membranes, whereas the charged species are not [9]; hence, the relative concentration of the probes within the vesicle provides a direct measure of the pH gradient. ESR spectroscopy monitors probe partitioning between the aqueous and membrane phases and gives easily resolvable signals. Any interaction of the spin-labeled molecules with each other or the membrane will result in a characteristic distortion of the ESR spectrum that can be easily detected. Thus, no assumption of ideal behavior for the inner probe is necessary. Since the probes equilibrate extremely rapidly, the method can be used for kinetic studies. The high sensitivity of spin probes and availability of both weak acid and amine labels permits measurement of steady-state pH gradients of small magnitude ($\Delta pH < 0.1$).

The effectiveness of the spin-labeled nitroxide probe method for determining biologic volumes and pH gradients has been well-documented in both bacterial and animal systems. Mehlhorn and Probst [10], using simultaneous determination of internal volume and ΔpH, found that the addition of acid or alkaline buffer solution resulted in a rapid and irreversible decrease in the apparent internal volume of *Halobacterium halobium* vesicles. Melandri et al. [11] systematically compared the values of pH measured with a spin-labeled amine probe, tempamine, with those measured with the fluorescence of 9-aminoacridine in the chromatophores of *Rhodopseudomonas sphaeroides* and found good agreement provided that corrected osmotic volumes were used. With plants, however, the technique has been applied only to cyanobacteria, where Blumwald et al. [12] measured the osmotic volume of cells, or, to higher plant thylakoids where both volumes [13] and pH gradients have been determined [9].

Here, we extend the spin-label method to microsomal vesicles isolated from a higher plant, *Cucurbita pepo*. Zucchini was chosen on the basis of previous evidence that sealed, pH-tight vesicles can be isolated from that tissue [14,5] and current interest in the pH-driven uptake of the plant hormone, auxin, by those vesicles [15]. The results with the vesicles isolated from zucchini were compared with parallel measurements with vesicles prepared from soybean lipids. The intravesicular volume was determined by spin probe methods using Tempone. The nitroxide spin probe, Tempacid, was used to determine pH gradients across vesicle membranes.

Materials and Methods

Plant material. Seeds of zucchini squash (*C. pepo* L., cv. Dark Green, Ferry Morse Seed Co., Mt. View, CA) were planted in moist vermiculite and grown in plastic boxes at 26°C and 95% relative humidity for 5 days in total darkness.

Zucchini membrane vesicle preparation. Membrane particles were prepared from 2-cm sections of the hypocotyl cut 5 mm below the hook following the procedures described in Hertel et al. [5], modified as described below. The vesicles were made by homogenization in 2 vol. per g FW tissue of extraction medium (250 mM sucrose/50 mM Tris-citrate (pH 7.9)/3 mM Na_4EDTA/0.1 mM $MgSO_4$, with 1 mM dithiothreitol added immediately before use). The homogenates were then filtered through a narrow-mesh nylon cloth and centrifuged for 10 min at $6000 \times g$, and the resulting supernatants were recentrifuged for 30 min at $50\,000 \times g$. The pellets from the second centrifugation were resuspended in 2 vol. extraction medium per g FW tissue and recentrifuged at $50\,000 \times g$. The final microsomal pellets were resuspended in medium buffered at pH 5.5 (250 mM sucrose and Tris-citric acid), thereby creating a pH gradient across any sealed membranes, or with Tris-citric acid medium buffered at pH 7.9 followed by external additions of 0.5 M H_3PO_4 to impose a given pH gradient.

Liposome preparations. Lipid vesicles were prepared by sonication according to Miyamoto and Stoeckenius [16]. Briefly, this method consists of a prolonged sonication of soybean lipids under argon and at low temperature to ensure that exclusively unilamellar vesicles are formed and to avoid oxidative damage. The sonication was conducted

in 250 mM sucrose and 50 mM Tris-citrate at pH 7.9.

Spin probes and quencher. Tempone· was purchased from Aldrich Chemical Co., Milwaukee, WI. Tempacid was prepared from Tempone [17]. The paramagnetic quenching agent introduces a substantial osmotic effect; hence, a stock solution of minimum osmotic strength was prepared as follows. An aqueous solution of free acid-EDTA was stirred on an ice bath and one equivalent of MnO was added slowly, maintaining a temperature below 25°C throughout the mixing procedure. The resulting slurry was stirred at room temperature overnight and treated slowly with tetramethyl ammonium hydroxide until the pH of the stock solution reached 7.0. Approx. 2 equiv. of base were required for this pH adjustment. The final solution was 0.9 M.

ESR measurements. ESR spectroscopy of nitroxide-labeled probes was performed with a Varian E-109E spectrometer at a microwave power of 10 mW, modulation amplitude of 1.0 G (0.4 G for Tempone measurements), time constant of 0.128 s, and scan rate of 0.4 $G \cdot s^{-1}$. Vesicles at concentrations as given in the figure legends were mixed with 0.5–2.0 μl of a 1 M quencher solution, TMA_2MnEDTA. The sample, contained in 75 μl capillary tubes, had a total volume of 40–50 μl.

The absolute volume of the inner aqueous phase of zucchini membrane vesicles was evaluated using the nitroxide probe, Tempone (TK). Tempone, which is an uncharged molecule, does not show a pH-dependent distribution between internal and external space. The procedure consists of obtaining a Tempone spectrum in the presence of vesicles and a second spectrum with added quenching agent. The final aqueous signal is expressed as a fraction of the initial signal and converted to membrane concentration in terms of milligrams of protein.

Quantitating pH with spin probes consists of an intravesicular spin signal measurement as described above, but using spin-labeled amine and acid probes. In the absence of a pH gradient, the 'effective volume' should be equal to the volume determined with Tempone, after correcting for binding [10]. The nitroxide probe used here, a weak acid (Tempacid, TC), partitions preferentially into basic environments. In the presence of a pH gradient across the vesicle membranes, the effective volume will be different from the true volume as estimated by Tempone. Any difference in the measured apparent volumes is due to a pH gradient, which can be expressed as:

$$\Delta pH = \log\{vol_{TC}(1 - vol_{TK})/(1 - vol_{TC})vol_{TK}\}$$

This formulation holds as long as the total concentration of tempacid can be equated to the concentration of the charged species. This assumption is valid for these studies, since the pH never was less than 5.5. Calculations using the formulation for the protonated species of the probe at even the lowest pH gave a correction factor of at most 0.07 pH units and so were neglected.

The extravesicular pH of vesicle preparations was measured with a glass pH electrode (Sensorex S 900C). None of the subsequent additions altered the external pH.

Results and Discussion

Osmotic volume determinations

A typical experiment showing the Tempone ESR spectra of either liposomes or zucchini membrane vesicles in the presence of increasing amounts of the paramagnetic quenching agent, TMA_2-MnEDTA, is depicted in Fig. 2. Even in the presence of 200 mM quencher, spin probe signals did not change over several hours, indicating that the vesicles are highly impermeable to TMA_2Mn-EDTA and remained intact. The membrane vesicles did not reduce the nitroxide radical to its nonparamagnetic hydroxylamine derivative (data not shown), a major shortcoming of such volume measurements in some membrane preparations [11]. The spectra show indications of probe membrane binding only in the case of the liposomes, where the bound probe signal is easily separated from the signal peak for the unbound Tempone (Fig. 2). Thus, membrane interactions do not distort the measured response of the spin probe to the quencher.

As can be seen in Fig. 2, the quencher-inaccessible space was sensitive to the osmolarity of the external buffer. Increasing the concentrations of osmoticum in the external medium (using 0.02–0.20 M quencher) reduces the volume of the

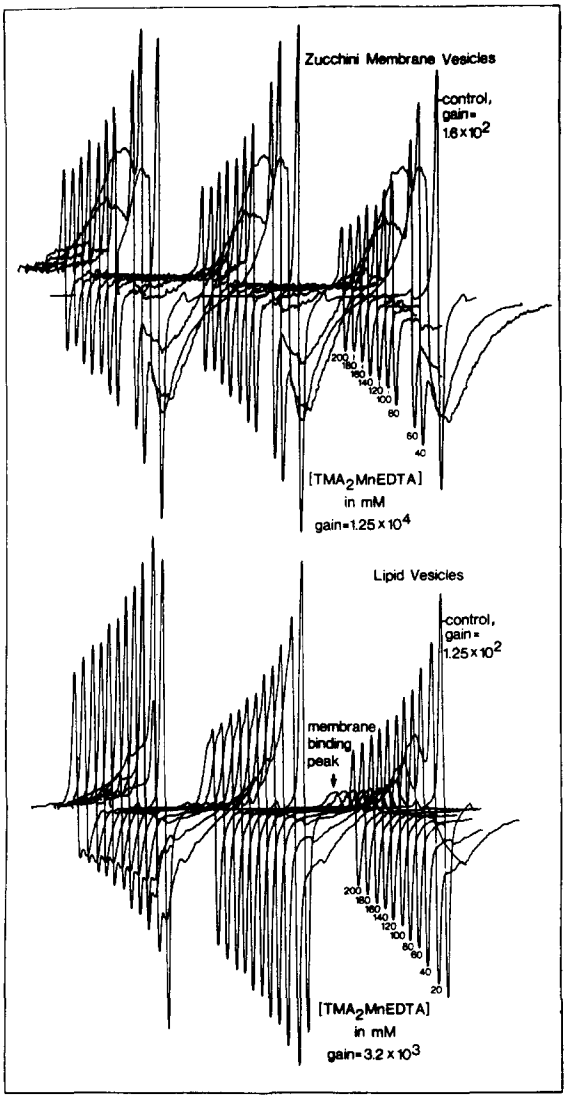

Fig. 2. ESR spectra of Tempone vesicle volume measurements in the presence of increasing amounts of $TMA_2MnEDTA$ (0.02–0.20 M) at 4°C. Zucchini membrane concentration, 4 g FW equiv./ml; liposome concentration, = 30 mg/ml; Tempone, 1 mM.

vesicles (Fig. 2). Conversely, decreasing the external osmoticum increases the volume of preshrunken vesicles (data not shown). Since vesicles are freely permeable to water, they exhibit a predictable osmotic response. For the internal water compartment, a plot of volume against 1/osmolarity should extrapolate to zero volume at infinite osmolarity [6]. The determination made here with both the zucchini hypocotyl membrane vesicles and with lipid vesicles exhibit a linear relationship between volume and the osmotic concentration (Fig. 3). While the volumes (signal line heights) of the liposomes were considerably larger than those of the zucchini membrane preparation, a linear regression of both curves is seen to extrapolate to zero volume at infinite osmolarity (Fig. 3). This subsequently allowed the adjustment of all volume determinations to give the volume found in the absence of quenching agent.

These experiments demonstrate that the vesicles prepared from zucchini hypocotyl tissue are capable of expanding and contracting in response to external osmotic levels and provides convincing evidence as to their sealed nature, in good agreement with the indications of earlier studies [5,14]. The ideal osmotic behavior of volumes observed with both the plant membrane vesicles and liposomes using these ESR methods strengthens the contention that the ESR method detects the full, osmotically active intravesicular volume. If there were a substantial fraction of water whose properties differed significantly from those of bulk water, e.g., 'bound water', near the membrane surface, and whose volume was insensitive to the osmotic strength of the surrounding medium, then the plot of ESR volumes as a function of inverse osmolarity would not intercept the origin. Such a possibil-

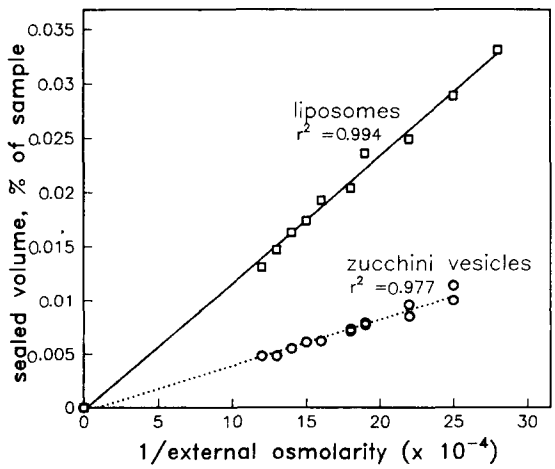

Fig. 3. Relationship of external osmotic concentration and Tempone signal height as measured by ESR. Calculated from the data given in Fig. 2,

ity appears to be excluded by the Tempone data.

In various experiments performed on six different days, the iso-osmolar volume of the sealed vesicle population in suspensions of zucchini membranes prepared as described in Materials and Methods was found to be about 0.7% ± 0.2% of the sample volume, after correction for the quencher concentration. This value corresponds to about 10 μl/mg protein. The volume size remained the same whether measurements were made at 4°C or at room temperature. For the liposomes, the average iso-osmolar volume under these buffer conditions was found to be about 5.2% ± 0.5% of the sample volume, or about 1.75 μl/mg asolectin.

pH gradient determinations: kinetics and sensitivity to ionophores

A time-course for the decay of the pH gradient across zucchini membrane vesicles prepared under iso-osmolar conditions with an internal medium pH of 7.9 and external medium pH of 5.5, is shown in Fig. 4. The zero timepoint represents the time of resuspension of the vesicles in the lower pH medium, thus creating a pH gradient across any sealed membranes. In the control sample (i.e., without ionophores present) the pH gradient decays slowly (approx. 0.002–0.003 pH units/min). After an initial rapid decay phase, which can be at least partially ascribed to electrogenic proton influx [18] and may also reflect a property of the membranes (see below), the decay in pH is approximately linear with respect to time and therefore represents a negative exponential decline in $[H^+]$, as might be expected, due to some 'leakiness' in the vesicles. In general, these data indicate that the zucchini membrane vesicles must be relatively tightly sealed to maintain such a slow decay.

Further evidence as to the sealed, pH-tight nature of the zucchini membrane vesicles was obtained by the addition of ionophores. The electroneutral H^+/K^+ exchanger, nigericin, rapidly and totally dissipates the pH gradient (Fig. 4). These experiments included 20 mM K^+ to allow nigericin to fully collapse the ΔpH. The electrogenic protonophore, FCCP, should reduce the pH gradient only to the point at which the protonmotive force is reduced to zero by the opposite but equal electrical potential formed. The ESR measurements depicted in Fig. 4 show clearly that FCCP

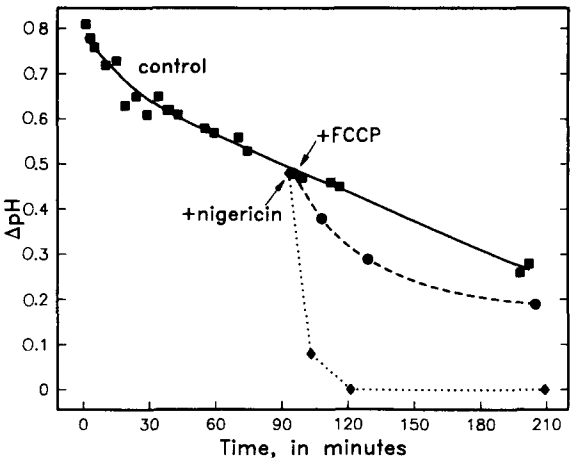

Fig. 4. Time-course of ΔpH in zucchini membrane vesicles in the presence and absence of ionophores. At zero time, vesicles which had been made with pH 7.9 buffer (50 mM Tris-HCl) internally were resuspended in pH 5.5 buffer (10 mM sodium citrate-HCl). Ionophore additions were made as indicated by arrows, the final concentration for each was $1 \cdot 10^{-5}$ M. Tempone, 1 mM; Tempacid, 200 μM; $TMA_2MnEDTA$, 0.2 M. The external pH remained at 5.71 throughout the measurements. Gain, $6.3 \cdot 10^4$.

does not totally dissipate the ΔpH. Instead, the FCCP sample reaches an equilibrium ΔpH to which the control sample eventually (after 2 h or more) declines. FCCP acts slowly, since it is not interfering with a proton pump; rather, it merely allows the diffusion of protons across the membrane. Similar responses to the ionophores, i.e., complete and rapid ΔpH dissipation with nigericin and only partial dissipation with FCCP, were observed with the soybean lipid vesicles (not shown).

The ESR measurements providing the data depicted in Fig. 4 were made at 4°C. When the ESR assay temperature was raised to 25°C, then further to 32°C, the rate of decline of the pH gradient increased markedly (data not shown). This result suggests that the vesicles become more permeable to ions at higher temperatures and is in agreement with the findings of Hertel et al. [5] that increased temperature decreased the pH-dependent accumulation of the plant hormone auxin by zucchini membrane vesicles.

Comparison of the response of zucchini membrane vesicles and synthetic liposomes to imposed pH gradients

The size of the pH gradient in the measurements shown in Fig. 4 was about 0.8 pH units directly after resuspension. This was considerably smaller than the expected value of about 2.4 pH units, and was consistently observed in other experiments of a similar type. In these experiments, the pH gradient is imposed by resuspending vesicles with a high internal pH in a low pH medium. Although the vesicles were resuspended in pH 5.5 medium, some breakage or leakage may have taken place during resuspension, resulting in a measured external pH of 5.73. In addition, breakage and resealing of vesicles during resuspension may trap some of the external, lower-pH media inside the membranes, resulting in a lower overall pH gradient.

In an effort to resolve why the measured pH gradient differed from expected values, we turned to an alternative method for imposing the pH gradient: resuspending the membranes in a buffer with a pH identical to that of the internal medium (7.9) followed by titration of the external medium with 0.5 M H_3PO_4. In this way pH gradients of a range of magnitudes could be imposed upon a single membrane preparation. For comparison, liposomes made from asolectin were treated in a similar manner under identical pH and ionic conditions. Fig. 5 shows that with the zucchini membrane and liposome preparations for ΔpH up to 1.3, there is a remarkably close correlation between the measured and imposed ΔpH. This provides and excellent demonstration of the accuracy of the ΔpH measurements made using Tempacid and ESR with these preparations.

The maximum gradients observed in the case of the plant membrane vesicles are substantially smaller than those seen with the liposomes (Fig. 5). Moreover the relationship between the imposed gradient and the observed maximum gradient appears to differ significantly for the two membrane preparations. The liposomes exhibit a smooth curvature above a ΔpH of about 1.5, continuing to increase the ΔpH with decreasing external pH, while the plant membranes show a difference in their response to imposed pH, suggesting a maximum sustainable gradient of about 1.4 pH units in these preparations.

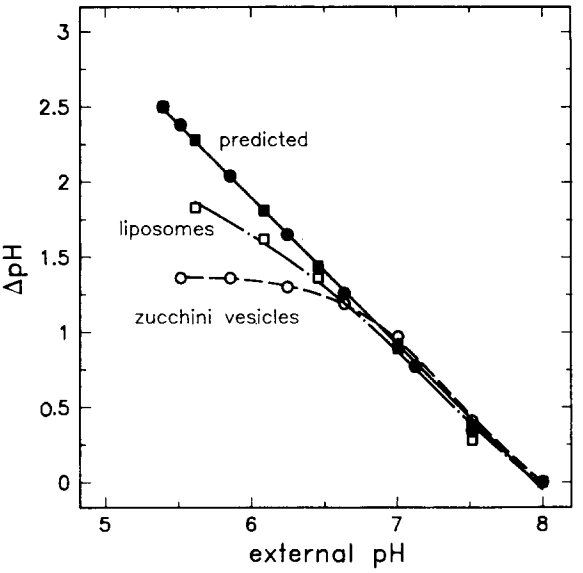

Fig. 5. The magnitude of the proton gradient across membrane vesicles in response to different externally imposed pH values. The vesicles were either isolated from zucchini hypocotyl tissue (4 g FW/ml) or prepared from asolectin (30 mg/ml). Both were prepared and resuspended in identical media (250 mM sucrose/50 mM Tris-citrate (pH 7.9)). Aliquots of 0.5 M H_3PO_4 were added to individual samples to achieve the desired external pH. Tempone, 1 mM; Tempacid, 20 μM; $TMA_2MnEDTA$, 0.2 M.

The liposome pH gradient measured at greater than 1.5 pH units was smaller than expected when 50 mM buffer was used for preparing the vesicles. However, in other experiments (not shown) at higher initial buffer concentrations, e.g., 500 mM phosphate, the gradient calculated from the spin probe distribution was exactly equal to the expected value, up to even 3 pH units. A possible explanation for anomalous pH gradients at low buffering strength is that dissolved CO_2 had introduced bicarbonate into the solution and that this bicarbonate moved across the membrane as the protonated acid species or (CO_2) in response to the imposed pH gradient, thus rendering the intravesicular compartment more acidic. At pH 7.9, the bicarbonate concentration in equilibrium with atmospheric CO_2 will be about 300 μM. In the presence of a pH gradient of about 2 units the intravesicular concentration of bicarbonate could reach 30 mM, which would be more than sufficient to reduce the magnitude of the pH gradient to the

extent observed in the liposomes.

On the other hand, the observation of an apparent plateau in the pH gradient that could be achieved in the plant membrane vesicles suggests that vesicle breakage or 'leakiness' alone does not explain the lower than expected ΔpH values, but rather that some control mechanism sensitive to the magnitude of the gradient must exist. A possible membrane event is the triggering of ion 'gates' when the pH gradient reaches a threshold value of about 1.3 units. Threshold phenomena are common in neuronal membranes, although the gating mechanisms usually appear to involve electrical potentials. Further work to characterize the phenomenon of limited maximum pH gradients in these plant membranes seems warranted but is beyond the scope of the present study. A previous study of pH gradients in liposomes found that pH gradients were associated with vesicles by virtue of anionic lipids within the membranes [19]. This suggests that a portion of the gradients seen in the present study may be the result of anionic lipid effects. However, the large buffering capacity of vesicles in the present study relative to the earlier work would argue against such an effect playing a predominant role in the data of Fig. 5. It is possible to utilize specialized spin probes with ESR to measure membrane potential and surface potential, respectively [20,21]. Such measurements with the zucchini membrane preparations described here would allow description of the complete protonmotive force across the membrane vesicles.

Decay kinetics for externally imposed pH gradients

The difference in the magnitude of the ΔpH observed at externally imposed gradients greater than about 1.2 pH units between zucchini microsomal and synthetic liposome preparations seen in Fig. 5 is not due to differences in the rate of decay of the pH gradient. When the decay kinetics for both were measured in identical experiments, it was found that the rate of decline for both the zucchini microsome and asolectin liposome preparations was nearly identical (Fig. 6). Over the 30 min measured, the decay rate was again linear and relatively slow, with kinetics similar to those seen earlier (Fig. 4). With an imposed gradient of 2.2 pH units, once again the zucchini membrane

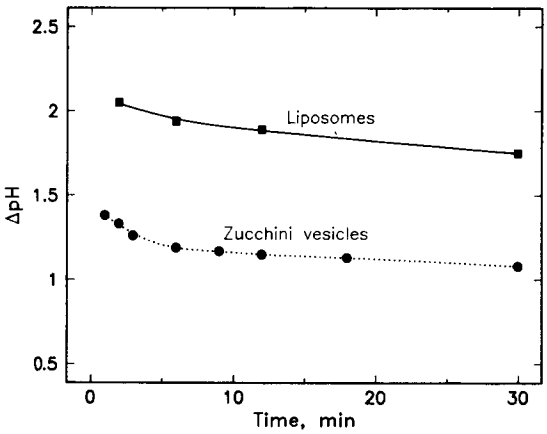

Fig. 6. Comparison of decay kinetics of ΔpH for soybean lipid and zucchini membrane vesicles. Conditions identical to Fig. 5.

vesicles had a measured maximum ΔpH of approximately 1.4 units while the maximum ΔpH across the soybean lipid vesicles was higher (approx. 2 pH units), agreeing well with the data in Fig. 5, where the pH gradient was also formed by external addition of H_3PO_4 and in contrast to Fig. 4, where the proton gradient was established by resuspension of the vesicles in the lower pH medium, reaching only about 0.8 pH units, presumably as a result of vesicle disruption during resuspension (see above).

Conclusions

It has been shown that the ESR-detectable distribution of a spin-labeled weak acid, Tempacid, can be used as an accurate and sensitive method to determine the intravesicular pH of *C. pepo* membrane vesicles and soybean lipid vesicles, and that precise, simultaneous quantitation of the intravesicular volume can be readily measured as the quencher impermeable space using the spin probe, Tempone. Kinetic studies were shown to be feasible for both ΔpH and volume determinations.

The zucchini membrane preparations were shown to contain osmotically responsive and pH-tight vesicles which could maintain a pH gradient of greater than 1 pH unit for several hours. The pH gradient was sensitive to both temperature and the ionophores, FCCP and nigericin. Similar results were obtained with liposomes, which could,

however, maintain considerably higher pH gradients with no indication of a threshold for maximum possible pH difference across the membrane as seen for the zucchini membrane preparations.

Reinhold and Kaplan [1] have pointed out that a major step forward in elucidating transport mechanisms can be expected when transport can be observed in isolated membrane vesicles, where ΔpH and electrical gradient can be both quantitated and independently varied, and where the compartmentation problem commonly encountered in higher plant cells is no longer a factor. The availability of the rapid, accurate methods for the determination of volume and ΔpH in higher plant vesicles described here will now expedite the study of ion, solute and hormone transport by such tissues. These techniques have already proven useful in a detailed quantitation of the uptake of the plant hormone, auxin, by these zucchini membrane preparations [15].

Acknowledgements

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