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# LIGHT SCATTERING TEMPERATURE JUMP RELAXATIONS IN MIXED SOLVENT SUSPENSIONS OF PHOSPHATIDYLCHOLINE VESICLES

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SUMMARY

Lipid vesicles were prepared from hen egg yolk phosphatidylcholine according to Huang's method. A relaxation of approx. 0.4 msec was observed in the intensity of white light scattered from an aqueous suspension of these vesicles following a rapid Joule heating temperature jump. This relaxation was observed to lengthen to about 1.5 msec as non-electrolyte (glycol, glycerol, 1,3-propanediol, or 1,4-butanediol) was added to the medium. The relaxation was attributed to passage of small molecules through the lipid membrane. The solvent viscosity was found to have a more pronounced effect upon the measured relaxation than did the size of the added non-electrolyte molecules.

## INTRODUCTION

Liquid crystals of hen egg yolk lecithin (phosphatidylcholine) in salt solutions have shown ion and water diffusion properties similar to biological membranes[1–3]. The shape of the liquid crystals in an aqueous salt solution was assumed[2,3] to be a bimolecular closed surface leaflet. As is true of biological membranes, the leaflets qualitatively exhibited a much higher permeability for anions and water molecules than for cations[1]. The rates of the temperature dependent swelling of egg yolk lecithin dispersions have been shown by previous investigators to be slower with glycerol than for glycol[4–6]. However, the individual size and shape of these liquid crystals was shown by electron microscopy to vary considerably.[2,3] HUANG[7] recently prepared hen egg yolk phosphatidylcholine vesicles that were very homogeneous with respect to size. We have prepared phosphatidylcholine vesicles according to HUANG'S[7] method and studied the kinetic effect of viscous non-electrolytes on the solution permeability of these artificial membranes. We have made our kinetic measurements by EIGEN'S[8] Joule heating temperature jump relaxation method which permits observation of reactions in liquids having half reaction times shorter than the usual shortest time of about 1 msec accessible to stopped-flow instruments.

SHA'AFI *et al.*[9] used a stopped-flow apparatus to measure the rate of human erythrocyte shrinkage in hyperosmolar solutions. They followed the changing volume

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of red cells as a function of time by recording the intensity of white light scattered at 90° to the incident beam. They estimated[10] the half time for the diffusion of water through the erythrocyte membranes to be 7 msec. More recently we have reported a light scattering temperature jump kinetic study of isotonic aqueous suspensions of human erythrocytes[11]. We tentatively attributed an approximately 0.8 msec relaxation to the passage of water molecules through the erythrocyte membranes. The work reported below represents a continuation of this type of kinetic study but in a membrane system with better known properties.

## METHODS

Phosphatidylcholine vesicles were prepared by HUANG's[7] method including Sepharose 4B chromatography. The phosphatidylcholine was extracted[12] from hen egg yolks and its purity was confirmed by thin-layer chromatography[13]. Ultrasonic irradiation of the aqueous phosphatidylcholine (0.01 M Tris–HCl buffer, 0.1 M NaCl, pH 8.5) yielded a milky opalescent dispersion of spherical vesicles that would not precipitate upon ultracentrifugation at 105 000 × $g$ for one hour. HUANG[7] states that the prepared vesicles are approximately 300 Å in diameter with a 70 Å thick wall holding a 160 Å diameter sphere of water inside the vesicle. An electron micrograph of our prepared vesicles (negatively stained with potassium phosphotungstate) also showed spheres homogeneous with respect to size with diameters of approx. 500 Å in the case of one preparation and approx. 3000 Å in another preparation.

All solvents (ethylene glycol, glycerol, 1,3-propanediol, 1,4-butanediol) used in the kinetic experiments were reagent grade and were used without further purification. Water used had been distilled and demineralized.

Light absorption detection is usually employed in temperature jump experiments, and the shortest measureable chemical relaxation times are of the order of 5.0 μsec. We discuss below the results of Joule heating temperature jump rate studies of phosphatidylcholine vesicle suspensions in which relaxations of approx. 0.4 msec have been detected in the intensity of white light scattered at 90° to the incident beam. Our light-scattering, Joule heating temperature jump apparatus has been described previously[11]. We have used the same apparatus to measure the rates of surfactant micelle dissociations[14,15].

Discharging a 0.1 microfarad 30 kV capacitor through the sample cell raises the temperature of approx. 1 ml of our sample suspension by approx. 8° in approx. 5 μsec, as verified by light absorbance measurements with methyl orange[16]. The initial sample suspension temperature was usually 17° and most of our reported relaxation times are for 25 ± 1°. Blank solutions of aqueous 0.11 M NaCl–Tris–HCl buffer were run previous to each experiment in order to verify the absence of detectable relaxations in the electrolyte. Straight base lines were also obtained in the absence of the vesicles when glycol or glycerol aliquots were added to the electrolyte solutions. The precision of the experimental relaxation times is approximately ± 15 %.

## RESULTS

A gold sol (particle size approximately 20–300 Å)[17] in aqueous 0.11 M NaCl–Tris–HCl was temperature jumped and no exponential relaxation curve was detected

between 0.1 and 10 msec. In a similar manner we tested an approx. 1 % suspension of solid 900 Å diameter, density 1.05 polystyrene latex spheres (Dow Chemical Co.). Again, no relaxation was noted. Thus "blank" runs (electrolyte, gold sol, and polystyrene spheres) suggest that the relaxations we subsequently observed in phosphatidylcholine suspensions are related to the permeability of the phosphatidylcholine vesicles.

Upon the addition of 3 ml of a 1.4 % suspension of phosphatidylcholine vesicles to 22 ml of a 0.11 M NaCl–Tris–HCl buffer solution (pH 8.5), relaxation curves of the order of 0.4 msec were observed. The relaxation time (defined as the time required for the amplitude of the exponential curve to decrease to 1/e of its original amplitude) in aqueous suspensions of phosphatidylcholine was found to be independent of phosphatidylcholine concentration (1.4–0.05 %), of vesicle size (500 and 3000 Å diameters) and of ionic strength (0.22–0.055 M). The data in Table I show that the relaxation time is, however, slightly dependent upon temperature.

TABLE I

LIGHT SCATTERING JOULE HEATING TEMPERATURE JUMP RELAXATIONS IN AQUEOUS SUSPENSIONS OF PHOSPHATIDYLCHOLINE

All data obtained at pH 8.5, 0.1 M NaCl, 0.01 M Tris–HCl buffer, and with light intensity measured at a 90° scattering angle.

| *Solvent* | *Temperature* | $\tau$ (*msec*) | $\eta$ (*cP*) |
|---|---|---|---|
| Water | 40° | 0.42 | 0.6560* |
| | 30° | 0.42 | 0.8007 |
| | 25° | 0.42 | 0.8937 |
| | 20° | 0.47 | 1.0050 |
| | 15° | 0.50 | 1.1404 |
| | 10° | 0.56 | 1.3077 |
| 0.0989 mole fraction glycerol | 38° | 0.65 | 1.60** |
| | 25° | 1.5 | 2.31 |
| | 15° | 1.8 | 3.08 |

* Coefficient of viscosity in centipoise of pure water at this temperature.
** Coefficient of viscosity of a water–glycerol mixture at each temperature as determined in this laboratory.

The effect of adding aliquots of glycol and glycerol to a 0.2 % suspension of phosphatidylcholine vesicles on the relaxation time is shown in Table II. We note that the relaxation time is lengthened by addition of either glycol or glycerol. Other experiments with 1,3-propanediol and 1,4-butanediol indicated that these non-electrolytes also have an effect on the solvent, intermediate between the effects of glycol and glycerol. Permeability retardation increases in the order: glycol < 1,3-propanediol < 1,4-butanediol < glycerol.

In order to conclude whether the effect of the non-electrolyte was a viscosity phenomenon, particle size characteristic, or a combination of the two, an experiment was run with a large viscous protein polymer added to the phosphatidylcholine vesicle suspension. A 1 % solution of 705 D-B Polymer (Stein, Hall and Co.) yielded a solution of viscosity 700–900 cP which is comparable to the viscosity of pure

TABLE II

LIGHT SCATTERING TEMPERATURE JUMP RELAXATIONS IN WATER–NON-ELECTROLYTE MIXED SOLVENT SUSPENSIONS OF PHOSPHATIDYLCHOLINE VESICLES

All data obtained at pH 8.5, 0.1 M NaCl, 0.01 M Tris–HCl buffer, 25° and light intensity measured at 90° to the incident white light beam.

| *Non-electrolyte* | *mole fraction* | $\tau$ (*msec*) | $\eta$ (*cP*)* |
|---|---|---|---|
| Glycol | 0.0 | 0.38 | 0.895 |
| | 0.025 | 0.50 | 1.07 |
| | 0.075 | 0.66 | 1.45 |
| | 0.125 | 0.80 | 2.00 |
| | 0.173 | 0.95 | 2.45 |
| | 0.246 | 1.10 | 3.35 |
| Glycerol | 0.0 | 0.41 | 0.895 |
| | 0.0049 | 0.43 | 0.96 |
| | 0.0195 | 0.60 | 1.15 |
| | 0.0554 | 1.10 | 1.39 |
| | 0.0898 | 1.50 | 2.40 |
| | 0.1128 | 1.51 | 3.40 |
| | 0.198 | 1.57 | 7.0 |
| 1,3-Propanediol | 0.0 | 0.42 | 0.895 |
| | 0.0553 | 0.65 | 1.54 |
| | 0.0830 | 0.90 | 1.77 |
| | 0.1382 | 1.00 | 2.56 |
| | 0.2074 | 1.10 | 3.80 |
| | 0.3457 | 1.10 | 4.93 |
| 1,4-Butanediol | 0.0 | 0.41 | 0.895 |
| | 0.0564 | 0.65 | 1.53 |
| | 0.1128 | 0.85 | 2.38 |
| | 0.1692 | 1.1 | 3.34 |

* Glycol viscosities taken from F. ACCASCINA, S. PETRUCCI, AND S. SCHIARO, *Scienza e Technica*, 3 (1959) 242; glycerol solution viscosities from D. F. MINER AND J. B. SEASTONE, *Handbook of Engineering Materials*, Vol. 3, Wiley, New York, 1955, p. 462. Viscosities of 1,3-propanediol and 1,4-butanediol solutions were determined in this laboratory.

glycerol (954 at 25°). Three successive 5 ml aliquots of this polymer solution were added to 25 ml of an aqueous phosphatidylcholine suspension with no noticeable effect on the temperature jump relaxation time.

Vesicles which were suspended in a deuterium oxide buffer solution immediately yielded relaxation times that were longer than those for similar suspensions in water. A relaxation time of approximately 0.6 msec at 25° was measured in 87% $^2H_2O$ compared to the value of 0.4 msec in water.

The fact that $^2H_2O$ penetrates biological membranes (erythrocytes) slower than water has been studied earlier[11,18], and similar decreases in the permeability time were detected.

## DISCUSSION

The objective of this work was to identify the process giving rise to an approx. 0.4 msec relaxation in the intensity of light scattered by lipid vesicles subjected

to a sudden temperature jump. The blank runs with electrolyte, a gold sol, and with solid polystyrene spheres indicate that the temperature dependence of the solvent refractive index does not account for the observed relaxation.

Since the Stein–Hall protein network does not affect the relaxation time, evidently only small molecules contribute to the relaxation process. Such a lack of correlation between solvent viscosity and the mobility of small molecules and ions when viscosity is increased by adding a macromolecule has been observed previously[19].

If we assume that the relaxation is due to the motion of small molecules or ions through the membrane, we can then begin to consider a model of the system. First let us assume that we have a one-step process which can be represented as

$$A_o \underset{k_r}{\overset{k_f}{\rightleftarrows}} A_i \tag{1}$$

where $A_o$ is an unknown species outside the vesicle, $A_i$ is the same species inside, $k_f$ the rate constant for the inward permeation, $k_r$ the rate constant for the outward permeation.

Since the relaxation is observed in aqueous media, we know that the species A must be either water or a component of the buffer such as chloride ion. The relaxation time for such a process is given by the equation

$$\tau^{-1} = k_f + k_r \tag{2}$$

This model is clearly inadequate to account for our data since it predicts a concentration-independent value of $\tau$.

Let us modify Eqn. 2 by assuming that $k_f$ is a function of viscosity as in the case of ionic association reactions. The equilibrium represented by Eqn. 1 is characterized by the equilibrium constant

$$K = \frac{[A_i]}{[A_0]} = \frac{k_f}{k_r} \tag{3}$$

Since the position of equilibrium is not a function of viscosity, we can conclude that if $k_f$ depends on viscosity so must $k_r$ and furthermore, they must have the same functional dependence. We will assume the functional dependence predicted by the Smoluchowski equation for diffusion controlled reactions (see, for example, ref. 20) and write

$$\tau^{-1} = \frac{k_f^*}{\eta} + \frac{k_r^*}{\eta} = \eta^{-1}(k_f^* + k_r^*) \tag{4}$$

where $k_f^*$ and $k_r^*$ are viscosity-independent. Thus a plot of $\tau^{-1}$ *vs.* $\eta^{-1}$ should be linear and pass through the origin. The ethylene glycol data of Table II yield precisely such a plot.

That solvent viscosity plays an important role in the process responsible for our observed relaxation time is evident from several of our other experiments, notably the fact when the viscosity of a glycerol–water solution is made equal to that of a glycol–water solution by raising the temperature of the former, the $\tau$ measured in the former is equal to that in the latter (*cf.* Tables I and II). Thus, over a fairly extended range of viscosities, $\tau$ is strongly influenced by $\eta$ and the choice of a means of varying the viscosity (temperature, $^2H_2O$, organic non-electrolyte) is not critical.

Since we see in Table II that $\tau$ reaches a limiting value at high viscosities with every non-electrolyte except glycol, Eqn. 4 cannot be a complete description of the relaxation process. Similar behavior was noted in ultrasonic absorption studies of $MnSO_4$ and of $ZnSO_4$ dissolved in water–glycol mixtures[21]. In the case of these inorganic salt solutions only the more rapid of two relaxations was observed and this relaxation was approximated[21,22] by the equation

$$\tau^{-1} = \frac{k_{12}^*}{\eta} + \frac{k_{21}^*}{\eta} + k_{23}^* + k_{32}^* \qquad (5)$$

where the specific rates $k_{ij}^*$ are all viscosity independent and $k_{12}^*, k_{21}^* > k_{23}^*, k_{32}^*$. Thus at low values of $\eta$ the first two terms on the right dominate and $\tau^{-1}$ depends upon $\eta^{-1}$. As $\eta$ increases $k_{23}^*$ and $k_{32}^*$ eventually dominate and $\tau^{-1}$ is viscosity independent. Assuming that an equation like Eqn. 5 applies, the linearity of the plot of $\tau^{-1}$ *vs.* $\eta^{-1}$ for the water–glycol system may reflect the fact that high enough values of glycol viscosity to reveal this leveling effect were not achieved.

It is interesting to speculate regarding the mechanism of a multistep membrane permeation process consistent with Eqn. 5. Let us suppose that following a rapid 10° temperature jump additional water enters the vesicle at a very high rate ($\tau \ll 10^{-3}$ sec). The entrance of more water will decrease the thermodynamic activity of all solutes inside the vesicle. This dilution produces a gradient of chemical potential across the membrane resulting in a flux of solutes through the membrane on a longer time scale.

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