PARTIAL SPECIFIC VOLUMES OF LIPID AND WATER IN MIXTURES OF EGG LECITHIN AND WATER

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ABSTRACT We describe in this paper measurements of the partial specific volumes of lipid ($\bar{v}_L$) and water ($\bar{v}_w$) in mechanical mixtures of egg yolk lecithin and water over a range of hydrations (5–55 wt% water) that includes the region at which excess water appears. $\bar{v}_L$ and $\bar{v}_w$ are found to be very nearly 1 cm^3/g over the entire range. The water activities of the mixtures were also determined and found to be the same as for lipid deposited as oriented multilayers on solid substrates.

It is widely assumed that the partial specific volumes of both the lipid ($\bar{v}_L$) and water ($\bar{v}_w$) in bulk lamellar lipid phases are close to 1.0 cm^3/g regardless of water content. Neutron diffraction measurements, however, suggest that $\bar{v}_L$ and $\bar{v}_w$ may be considerably different from 1.0 cm^3/g for lipid multilayers at small water contents oriented on glass surfaces (King et al., 1985; White and King, 1985). This raised the possibility that $\bar{v}_L$ and $\bar{v}_w$ might be different from 1.0 cm^3/g in bulk lamellar lipid phases at low hydrations. We report here measurements of the hydration and mass density of egg lecithin liposomes over a range of hydrations (5–55 wt% water) that includes the region at which excess water appears (~35 wt%). $\bar{v}_L$ and $\bar{v}_w$ are found to be very nearly 1 cm^3/g over the entire range.

The assumption of ideal volumetric mixing is thus excellent for bulk mixtures. However, this finding cannot be reconciled with x-ray and neutron diffraction measurements obtained from lecithin oriented on glass or quartz substrates at low hydrations (Torbet and Wilkins, 1976; King et al., 1985). White and King (1985) have suggested that the polar headgroups of oriented lecithin bilayers occupy an anomalously large volume at low hydrations. As a result, the partial molecular volumes of lecithin and water were estimated to be 1,600 and 7 $Å^3$, respectively, rather than the 1,300 and 30 $Å^3$ observed in bulk mixtures.[1]

It occurred to us that the hydration behavior of oriented bilayers might be different from that of bulk-phase lipid/water mixtures. That is, the water content vs. activity curves might be different for lipid multilayers deposited on substrates than for bulk mixtures. We therefore measured the water activities of bulk egg lecithin/water mixtures and compared them with published values for multilayers deposited on Teflon. To rule out the possibility of a substrate dependence, we also measured the hydration of dioleoyllecithin on glass substrates using radiolabeled lipid and water. Regardless of orientation or substrate, the composition vs. water activity curves are identical within experimental errors for all systems.

METHODS AND MATERIALS

Materials

Egg yolk phosphatidylcholine (EYPC) and dioleoylphosphatidylcholine (DOPC) were purchased from Avanti Polar-Lipids, Inc., Birmingham, AL and ^{14}C-labeled DOPC from New England Nuclear, Boston, MA. The purity of the lipids was checked by thin layer chromatography.

Partial Specific Volumes

The major problem in measuring the density of lecithin/water mixtures at low hydrations is the retention of air bubbles in the viscous mixture resulting from mechanical mixing. We have overcome this problem as follows. Mixtures whose compositions were determined gravimetrically were homogenized with a glass rod in polyethylene microfuge tubes and spun at 15,000 *g* to remove air bubbles. The mixtures were drawn very slowly via a carefully controlled suction into calibrated capillary tubes (10 μl nominal volume) of volume V_c and mass M_c. Samples containing a significant number of air bubbles upon microscopic examination were discarded. The total mass of the system $M_t = M_{Lw} + M_c$ was then measured (M_{Lw} = mixture mass). The specific volume of the lipid/water mixture was calculated from $V_c/(M_t - M_c)$. The capillaries were calibrated with water and all masses were determined with an electronic microbalance. Triplicate determinations of specific volume were made at each composition. All measurements were made at 23°C.

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[1]The small partial molecular volume of water does not mean that the water is super dense. Rather, it means that the total volume of the system does not increase very much upon the addition of water because of a large amount of preexisting free volume in the headgroups. It is a situation equivalent to throwing buckshot into a box of ping-pong balls.

Lipid Mixture Water Activities

The water activities of the mechanically mixed lipid/water mixtures were determined by the isopiestic method (Lewis and Randall, 1961). Briefly, a given mixture was distributed among several microfuge tubes which were sealed in jars containing saturated salt solutions of known relative humidity. After ~24 h, the jars were opened, and the microfuge tubes sealed and weighed. The percentage change in weights of the tubes were plotted against relative humidity. These changes were generally linear in the region of zero weight change, and the activity of the water in the mixture could be easily determined by interpolation.

Water Uptake by Oriented DOPC Multilayers

The composition/water activity curves of dioleoyllecithin oriented on glass surfaces were determined using ^{14}C labeled lipid and ^{3}H labeled water. The samples were prepared as described by King et al. (1985) and sealed in a thermostated chamber containing saturated salt solutions that had been prepared with labeled water. The lipid was deposited on glass cover slips that could be covered with another cover slip before the chamber was opened to minimize loss of water to the atmosphere during transfer to the scintillation fluid. Control experiments in which the time between removal from the chamber and immersion in the scintillation fluid was varied revealed that it was impossible to transfer the samples without a few percent loss in water. Thus, the tracer experiments always tend to underestimate slightly the amount of water present. We found the most accurate method of determining the water content was to make lipid samples containing different amounts of lipid and determine number of waters/lipid from plots of ^{3}H counts against ^{14}C counts.

RESULTS AND DISCUSSION

The specific volumes of the EYPC/water mixtures are plotted against the wt% water in Fig. 1. The data were fitted to a straight line (solid line) constrained to originate at the partial specific volume of 100% water ($\bar{v}$ = 1.0024; 23°C). The line terminates at 0% water at the partial specific volume ($\bar{v}_L$) of the lipid, which is found to be 0.9826 cm^3/g.[2] The rms deviation of the points about the line is ±0.3%. The results of an unconstrained linear regression of the data are shown in Table I.

Even though the rms deviation of the points from the line is only ±0.3%, the points are not randomly distributed about it. The points seem to fall into three groups marked in Fig. 1 by arrows corresponding to a transition from two phases to one lamellar phase at 12% water, and to the appearance of excess water at ~35% as determined by x-ray diffraction (data not shown). The general phase behavior is similar to that reported by Small (1967).

Because of the apparent correlation between the phase behavior and distribution of the data points, we have also fit the data with two straight lines as shown by the dashed and broken lines in Fig. 1. The upper straight line (broken line), constrained to originate at the partial specific volume of pure water, was fit to the points above 12% water (±0.22% rms deviation). The lower straight line (dashed line), constrained to originate at 0.9847 cm^3/g (Elworthy, 1959), was fit to the points below 12% water (±0.08% rms deviation). The $\bar{v}_L$ in the two cases are, respectively, 0.9855 and 0.9847 cm^3/g. Below 12% water, $\bar{v}_w$ is ~0.95 cm^3/g. Unconstrained linear regressions of the data were also performed and the results are shown in Table I. Overall, it seems safe to say that $\bar{v}_L$ = 0.983 ± 0.003 over the entire hydration range. This compares favorably with values of lipid in excess water of 0.983 reported by Reiss-Husson (1967), 0.981 by Huang and Charlton (1971), and 0.987 by Tardieu et al. (1973).

TABLE I
RESULTS OF LINEAR REGRESSIONS OF SPECIFIC VOLUME AGAINST WT% WATER FOR MIXTURES OF EYPC AND WATER

Hydration range	Intercept	Slope	$\bar{v}_L$	$\bar{v}_w$
0.00–1.00	0.982 ± 0.001	+0.025 ± 0.001	0.982 ± 0.003	1.007 ± 0.003
0.00–0.12	0.984 ± 0.001	−0.032 ± 0.001	0.984 ± 0.001	0.952 ± 0.001
0.12–1.00	0.985 ± 0.001	+0.019 ± 0.001	0.985 ± 0.002	1.004 ± 0.002

$\bar{v}_L$ and $\bar{v}_w$ are the partial specific volumes of the lipid and water, respectively. The error estimates shown for the intercepts and slopes are standard deviations and for the partial specific volumes the standard error of the estimate. Hydration is given as weight fraction of the mixture (wt% ÷ 100). All other terms have units of cm^3/g.

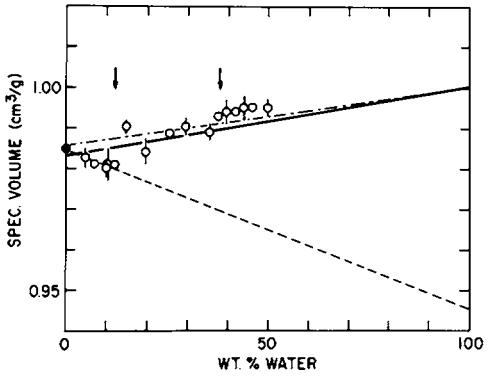

FIGURE 1 The specific volume of egg lecithin/water mixtures at various hydrations (23°C). The standard deviations of the measurements are indicated by the diameter of the data point or the error bar. The closed point (0.9847 cm^3/g) at 0% water is from Elworthy (1959). The solid line is a least squares fit (±0.3% rms deviation) of a straight line originating at the known specific volume of pure water at the temperature of our measurements. The partial specific volume of the lipid ($\bar{v}_L$) is given by the intercept of the line at 0% water and is 0.9826 cm^3/g. The arrows indicate phase boundaries (12% and 38% water) of the mixtures (see text). The data seem not to be randomly distributed about the line in a way suggesting minor changes in specific volume at the phase boundaries. The upper straight line (broken line) originating at the partial specific volume of pure water ($\bar{v}_w$) was fit to the points above 12% water (±0.22% rms deviation); the lower straight line (dashed line) originating at 0.9847 cm^3/g was fit to the points below 12% water (±0.08% rms deviation). The $\bar{v}_L$ in the two cases are, respectively, 0.9855 and 0.9847 cm^3/g. Below 12% water, $\bar{v}_w$ is ~0.95 cm^3/g.

[2]See Lewis and Randall (1961) for a detailed explanation of the determination of partial specific volumes from plots of specific volume against composition.

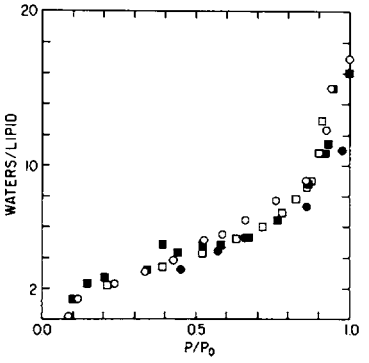

FIGURE 2 Bilayer water content as a function of water activity (P/P_0). P is the vapor pressure of the water in the mixture and P_0 the vapor pressure of pure water. In all cases the relative humidity (P/P_0) was controlled with saturated salt solutions in sealed chambers. Circles are measurements on dioleoylphosphatidylcholine (DOPC) and squares, egg lecithin (EYPC). The open circles are the gravimetric measurements of Jendrasiak and Hasty (1974) for DOPC deposited on Teflon surfaces and possibly oriented. The closed circles are measurements on DOPC oriented on glass slides made in our laboratory using ^3H_2O. The open squares are gravimetric measurements of Elworthy (1961) made on dried bulk EYPC in glass containers. The closed squares are isopiestic measurements of mechanically mixed bulk samples made in our laboratory.

Our measurements of the water activity of mechanically mixed bulk EYPC/water mixtures and of the uptake of water by DOPC multilayers on glass as a function of water activity are reported in Fig. 2. These results agree with the water uptake measurements of Jendrasiak and Hasty (1974) for lecithin multilayers on Teflon and of Elworthy (1961) for bulk lecithin in glass containers. We conclude that the variation in composition with water activity does not depend upon whether the lipid is in a bulk mixture or deposited on a surface nor does it depend upon the nature of the surface.

An interesting feature of Fig. 2 is that at 100% pH there are ~16–17 waters per lipid. However, x-ray diffraction measurements show that the lamellar Bragg spacing of lipid dispersed in water becomes constant (indicating the appearance of bulk water) at ~30 waters per lipid (Small, 1967; Reiss-Husson, 1967; Parsegian et al., 1979; McIntosh and Simon, 1986). Why are these two numbers different when the water activity is the same in both cases? There are two immediately obvious possibilities. First, the water in the vapor phase case might not be at true equilibrium because of nonuniform temperatures, stagnant vapor layers, etc. in the test chamber. However, the maximum water content seems to be quite reproducible among several laboratories using different equipment, so we do not think this is the only cause of the difference.

The second possibility is that the lipid in contact with water vapor alone might have a different physical state than lipid in contact with liquid water. That is, if one imagined lipid suspended in the vapor phase of a dispersion of liposomes in excess water, the chemical potentials of the suspended and dispersed lipids would be different. In such a system the water would be in equilibrium in all phases, whereas the lipid would not because it cannot exchange through the vapor phase. A possible reason for the difference in state is that lipid lamellae in excess water are likely to have more degrees of freedom than lipid in contact with vapor alone.

Whatever the reasons for the differing water contents of the systems at high water activities, the equivalence of the water contents in the regions of low water activity seems excellent and it is in these regions that the apparent discrepancy of the partial specific volumes of water between the oriented and unoriented systems exists. We have made a number of serious but unsuccessful attempts to measure directly the mass density of lipid multilayers oriented on glass surfaces. Until such measurements can be accomplished, the question of whether the apparent anomalous packing of lecithin on glass surfaces at low water contents is real or merely an artifact of inadequate diffraction analytical methods remains unanswered.

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