Small-angle neutron scattering from large unilamellar vesicles: An improved method for membrane thickness determination

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Small-angle neutron scattering (SANS) measurements were performed on large unilamellar vesicles (LUVs) in order to investigate solute effects on membrane properties. Although SANS is a well established technique for the measurement of membrane thickness in unilamellar vesicles, earlier measurements have depended on approximate treatments of the scattering function and have suffered from effects of multilamellarity or difficulty in sample preparation. More recent studies of temperature induced thickness changes in DPPC LUVs which have included explicit treatment of the full scattering function were complicated by disparities between the predicted and measured scattering curves. Here, we reexamine theoretical descriptions of SANS from LUVs. Motivated by our observations, we then introduce a new method for interpretation of SANS data, which we compare to established techniques and apply to our measurements.

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I. INTRODUCTION

Pure lipid membranes have been the subject of numerous studies because of their intrinsically interesting physical properties, their role as models of more complex biological membranes, and potential use in biotechnology. Large unilamellar vesicles (LUVs) are of particular interest in this regard because they share some of the basic properties of their biological counterparts, such as a semipermeable interface separating the outer medium from the vesicle interior and the ability to accomodate membrane proteins which can mediate transport of solutes across the membrane.

Properties of lipid vesicles (such as size, size distribution, shape, and membrane thickness) can be characterized by light [1], x-ray [2], and small-angle neutron scattering (SANS) [3]. In the case of large unilamellar vesicles (LUVs) prepared by extrusion, static (SLS) and dynamic light scattering (DLS) are the most appropriate techniques for studying vesicle size, size distribution, and shape. However, an absolute determination of membrane thickness is difficult to achieve by light scattering measurements [1]. Conversely, SANS measurements are relatively insensitive to the size of LUVs [3], but provide very accurate measurements of bilayer thickness for both multilamellar vesicles (MLVs) and unilamellar vesicles [4,5].

The determination of membrane thickness from SANS measurements of MLVs, LUVs, and small unilamellar vesicles prepared by sonication (SUVs) is a well established technique; there have been measurements to determine the size and location of proteins in LUV membranes [6–8], physical changes in membranes as a function of temperature and lipid composition [4,9–11], thickness changes due to the presence of detergents [12,13], and thickness changes due to lipid polymerization [14]. Most of these measurements have relied on the small angle approximation of the scattering function [6] and have either been complicated by vesicle multilamellarity [4] or have required elaborate sample preparation in order to obtain unilamellar vesicles [7].

Recent SANS measurements of membrane thickness on DPPC LUVs were performed in order to address open questions concerning the properties of the P'_β ripple phase in unilamellar vesicles [11]. In that study, the membrane thickness was determined in two different ways, by fitting SANS data to the exact form of the scattering function (convoluted with the instrumental resolution function), and by correlating the position of a minimum of the second derivative of a shoulder feature of the scattering data with a similar feature appearing in numerically simulated scattering curves. The first method is computationally intensive and suffers from large uncertainties in thickness determination. The second method appears to be effective, however, lacks a direct physical interpretation.

In this paper, we will reexamine the full form of the scattering function and demonstrate the links between it and various levels of approximation, leading to the small angle approximation for SANS from LUVs. We will compare the effectiveness of each scattering approximation in determining membrane thickness and comment on the physical basis for and limitations of each approximation. Finally, we will then use our preferred method for thickness measurements to determine the effects of ionic and nonionic solutes on the thickness of LUVs of dioleoylphosphatidylglycerol (DOPG) under conditions of maximum contrast, where we use protonated lipid in D_2O.

II. MATERIALS AND METHODS

The sodium salt of 1,2-dioleoyl-sn-glycero-3-[phosphorac-(1-glycerol)] (DOPG) was purchased from Avanti Polar Lipids, Inc. (Birmingham, AL) and used without further purification. Lipids were transported solubilized in methylene chloride ($MeCl_2$) and hermetically sealed in borosilicate glass ampoules under nitrogen. Ampoules were stored at $-40\,^{\circ}C$ immediately upon arrival. All other chemicals were reagent grade.

Large unilamellar vesicles were prepared by extrusion using the method of Nayar *et al.* [15]. Lipid was transferred from the ampoules to a 100 ml round bottomed flask and the $MeCl_2$ removed using a two step process, first by rotary

evaporation followed by vacuum pumping. The lipid film was then dispersed in MOPS buffer (20 m*M* MOPS in D_2O adjusted to a pH of 7.4) at a concentration of 50 mg/ml with the appropriate solute (NaCl or sucrose) when required. Under these conditions, we expect maximum contrast between the membrane hydrocarbon region and the medium. Osmolalities of buffer solutions were measured using a vapor pressure osmometer (Wescor, Logan, UT). The dispersion was freeze-thawed ten times by alternating immersion in liquid nitrogen and warm water baths. Frozen-thawed dispersions were then extruded with a hand held extrusion device [16] in two stages, first 5 times through a 200 nm pore size polycarbonate filter (Nucleopore Corp., Pleasanton, CA), followed by extrusion 15 times through a 100 nm pore size polycarbonate filter. Large unilamellar vesicles prepared in this way have a narrow size distribution and are known to be almost entirely unilamellar [16]. Vesicles prepared for SANS and DLS measurements were used within hours of extrusion.

Dynamic light scattering (DLS) measurements, as previously described [17] were performed using a diode-pumped, frequency-doubled, Nd:YAG laser (Model 532 DPSS, Coherent Laser Group, Santa Clara, CA) and a Brookhaven digital autocorrelator and software (BI-9000AT and 9KDLSW control program, Brookhaven Instruments Corporation, Holtsville, NY). Measurements were taken at a scattering angle of 90°. Vesicles used for DLS measurements were diluted to a final concentration of 0.05 mg/ml. Number distributions of vesicle radii were determined using a nonnegative least squares fitting routine with Rayleigh-Gans-Debye form factor corrections [18].

Small-angle neutron scattering measurements were performed using the Center for High Resolution Neutron Scattering (CHRNS) NG-3 30 m SANS spectrometer at the National Institute of Standards and Technology [19] using neutrons with a wave length of $\lambda=5$ Å ($\Delta\lambda=0.15$ Å). Two configurations of the instrument were used, sample to detector distances of 2.5 and 7.5 m. This corresponds to a total q range of $0.0088\ \text{Å}^{-1}<q<0.27\ \text{Å}^{-1}$, where q is the scattering vector defined as $q=4\pi\sin(\theta)/\lambda$. Small-angle scattering data was corrected for instrumental background and detector efficiency and then converted into absolute differential scattering cross sections per unit volume using standard methods [19]. Incoherent background scattering was determined from the asymptotic slopes of plots of $I(q)q^4$ vs q^4 [20].

III. THE SCATTERING THEORY

A. General features

The form factor for scattering from unilamellar vesicles is referred to as the Rayleigh-Gans-Debye (RGD) formula, which applies to light, x-ray and small-angle neutron scattering (SANS). In the case of SANS, the applicability of the RGD formula rests primarily on the assumption that the scattering length density of the membrane is uniform. In the case of protonated lipid in D_2O the contrast or scattering from the membrane is due primarily to the difference between the scattering length density of the hydrocarbon region of the bilayer and the medium. In the q range used here, we expect to be insensitive to any variation of scattering length density within the hydrocarbon region of the bilayer. Consequently, we expect to be able to approximate the bilayer as a uniform slab, with a thickness which corresponds to the thickness of the hydrocarbon region. The close agreement between our scattering data and fits (shown in Fig. 1) strongly indicates that the vesicle systems we are studying here do behave like shells with uniform scattering length density.

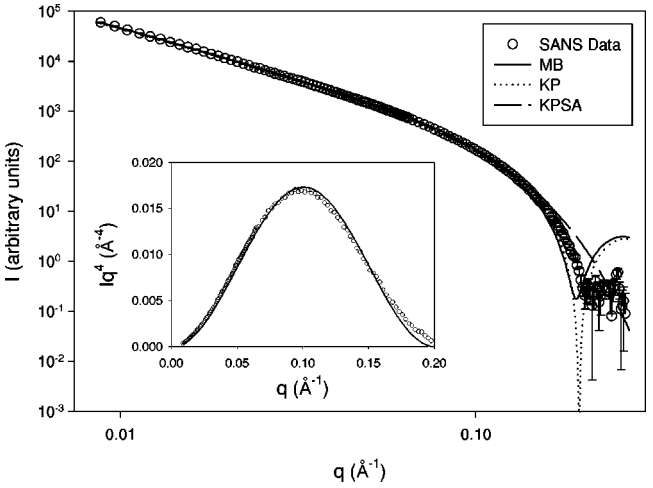

FIG. 1. Small angle neutron scattering from DOPG LUVs. The open circles correspond to experimental SANS data. The solid, dotted, and dashed lines correspond to fits using the MB, KP, and KPSA approximations. The inset to Fig. 1 shows an Iq^4 vs q plot of the data and corresponding modified KP fit (solid line).

The RGD formula for SANS can be derived by taking the Fourier transform of the scattering length density distribution for uniform shells and is given by

$$P(q)=\rho^2\left[\left(R+\frac{t}{2}\right)^3\frac{j_1\left[q\left(R+\frac{t}{2}\right)\right]}{q\left(R+\frac{t}{2}\right)}-\left(R-\frac{t}{2}\right)^3\frac{j_1\left[q\left(R-\frac{t}{2}\right)\right]}{q\left(R-\frac{t}{2}\right)}\right]^2, \tag{3.1}$$

where

$$j_1(x)=\frac{\sin(x)}{x^2}-\frac{\cos(x)}{x} \tag{3.2}$$

is the first order spherical Bessel function, q is the scattering vector, R is the average vesicle radius, t is the membrane thickness, and ρ is the scattering length density of the membrane. The RGD form factor is characterized in the SANS q range by a rapidly decreasing function modulated by two q dependent oscillations: a fast oscillation which depends on the radius of the vesicles and a slower oscillation which depends on the membrane thickness [11].

Experimental SANS measurements (shown in Fig. 1) do not show the fast oscillations predicted by RGD. The fast oscillations are absent in the experimental data because they are averaged away by polydispersity in vesicle size. In principle, instrumental resolution should also contribute to smearing of the scattering function, but the effects of poly-

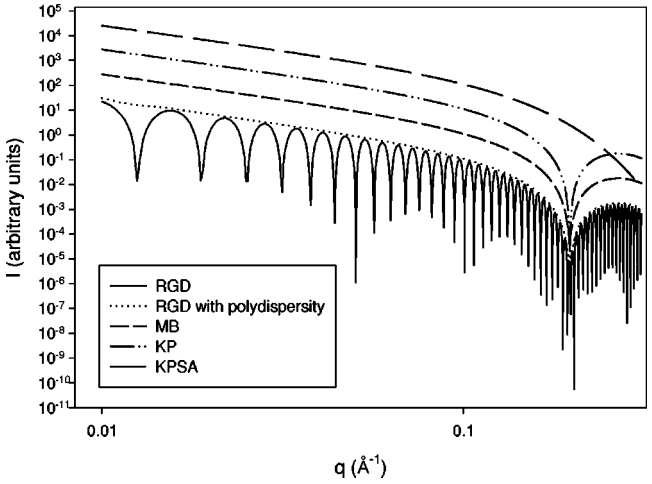

FIG. 2. Calculated scattering curves from LUVs. The solid, dotted, dashed, dash dotted, and long dashed curves correspond to the RGD, RGD convoluted with polydispersity, MB, KP, and KPSA approximations. All curves are plotted with $R=500$ Å, $\Delta=30$ Å, and $t=32$ Å. The scattering curves have been shifted in order to facilitate viewing.

dispersity are so significant that the instrumental resolution has no additional smearing effects, except at high q, near the first minimum of the scattering function. Although polydispersity effects almost completely average out the R dependence of the scattering function, the dependence on t is only slightly affected (as shown in Fig. 2). The local maximum predicted by RGD at high q (shown in Fig. 2) has been washed out by the incoherent background (not shown) in the experimental data.

In order to effectively use the RGD form factor to fit SANS data, it is necessary to include the effects of polydispersity by convoluting RGD with the vesicle size distribution. An approximate form of the RGD form factor, which includes effects of vesicle polydispersity, was introduced by Moody in 1975 [3] and later modified by Bouwstra *et al.* [2], and is given by

$$P(q)=\frac{\rho^2}{q^4}\left[(R^2+\Delta^2)\sin^2\left(\frac{1}{2}qt\right)+\frac{q^2t^4}{16}j_1^2\left(\frac{1}{2}qt\right)\right], \tag{3.3}$$

where Δ is the average polydispersity. The q dependent behavior of the Moody-Bouwstra (MB) approximation depends primarily on the membrane thickness t. The interference between the first and second terms in the MB approximation has the effect of smearing the slow oscillations due to the membrane thickness t. This smearing is an effect of the curvature of the membrane, a measure of the deviation from the ideal flat sheet behavior and depends on the value of R relative to t. For large values of R, there is effectively no smearing of the form factor (i.e., the membrane can be considered to be essentially flat). For intermediate values of R, sharp features of the form factor, such as the thickness dependent minima, become smeared. When the membrane radius approaches the value of the membrane thickness (the case of high curvature), the flat sheet approximation breaks down and oscillations in the MB approximation are no longer independent of R and Δ. For the system of interest here (large unilamellar vesicles), typical parameters are $R=500$ Å, $t=40$ Å, and $\Delta=30$ Å. In this range, the effect of membrane curvature is a slight smearing of the scattering function. At high q, near the first minimum in the scattering function, the MB approximation does not sufficiently match the smearing effect of the instrumental resolution. It is possible to match the effects of instrumental smearing in the MB approximation by decreasing the value of the radius and polydispersity, however, we have not investigated this in detail.

The leading term in the MB approximation corresponds to the form factor for randomly oriented thin sheets

$$P(q)=\frac{1}{q^2}\left[\int\rho(z)e^{-iqz}dz\right]^2=\rho^2\left[\frac{2}{q^2}\sin\left(\frac{1}{2}qt\right)\right]^2 \tag{3.4}$$

commonly referred to as the Kratky-Porod (KP) approximation [6], which is the Fourier transform of the scattering length density profile through the membrane (with a q^{-2} correction to include the effect of isotropic orientations). Although the KP approximation does not explicitly include effects of vesicle polydispersity or instrumental smearing, it is sufficient to describe scattering from LUVs over most of the q range we have examined, because it closely follows the behavior of both the smeared RGD form factor and the MB approximation, except near the first minimum of the scattering function, where the KP has a much sharper minimum than that predicted by either the polydispersity convoluted RGD or MB.

B. Asymptotic behavior of the scattering function

The small angle approximation to the KP form factor (KPSA) is often written as

$$P(q)=\frac{\rho^2}{q^2}e^{-q^2D^2}, \tag{3.5}$$

where D is the scattering length density weighted thickness, the one-dimensional analog of the radius of gyration (for membranes with uniform scattering length density $D^2=t^2/12$). This approximation is valid for $t<q^{-1}<R$. The upper bound for q comes from the small angle requirement that $qt<1$ (when qt approaches 1, the scattering function approaches its first minimum, corresponding to a q value of $2\pi/t$). The lower bound on q ensures that the vesicle radius does not significantly effect the scattering function. This effect can be seen in the second term of the MB approximation, which dominates the oscillations of the scattering function when q^{-1} is greater than R or of order R.

C. Extracting membrane thickness from scattering data

One of the aims of this paper is to compare several methods for membrane thickness determination. Before we proceed, it is worth while to examine how closely the MB, KP, and KPSA approximations follow the RGD approximation when effects of vesicle polydispersity are taken into account. In Fig. 2 the RGD function is plotted for vesicles with $R=500$ Å, $\Delta=30$ Å, and $t=32$ Å, with and without the effects of polydispersity. Also shown are the MB, KP, and KPSA

TABLE I. Parameters obtained from simulated SANS data using the RGD approximation convoluted with polydispersity. All units are in Å.

Method	t	R	Δ
Input for simulated RGD	32	500	30
MB	32.34 ± 0.88	500[a]	30[a]
MB	32.33 ± 1.8	371 ± 183	n/a[b]
KP	31.98 ± 0.84	n/a	n/a
KPSA	33.01 ± 0.89	n/a	n/a
Modified KP	$31.9942 \pm 4.3e-4$	n/a	n/a
Modified MB	$32.0018 \pm 3.9e-4$	500[a]	30[a]

[a]Values input as fixed parameters.

[b]R and Δ fit as single parameter.

approximations plotted using the same parameters. MB and KP appear to follow RGD over the entire q range, while the KPSA approximation follows the RGD approximation fairly closely at low angle, but deviates quite strongly as q^{-1} approaches the thickness of the membrane. MB differs from the polydispersity convoluted RGD over most of this range ($0.0175\ \text{Å}^{-1} < q < 0.3\ \text{Å}^{-1}$) by less than 0.3%. KP differs by about 0.6% in the same range, with the exception of the region near the first minimum of the scattering function ($0.175\ \text{Å}^{-1} < q < 0.2\ \text{Å}^{-1}$), where KP deviates by about 2%. At the position of the first minimum there is a significant difference between KP and MB, as discussed earlier.

An examination of both the MB and KP approximations shows that the scattering function is essentially the square of a sine function multiplied by q^{-4}, whose local maxima occur for qt equal to odd integer multiples of π. We propose an alternative method to determining membrane thickness, by finding the first local maximum in the scattering function by fitting Iq^4, which has a large maximum at $qt = \pi$ for both the MB and KP approximations.

In order to test the reliability of MB, KP, KPSA and the modified MB and KP approximations, we attempted to fit a theoretical curve generated using RGD convoluted with polydispersity. For the MB approximation, we fit the data in two ways: with R and Δ as fixed input parameters, or with $R^2 + \Delta^2$ as a fit parameter. The results summarized in Table I show the relative success of each method in extracting the correct thickness. The errors shown in Table I reflect the relative quality of the fits to the generated data. In several cases, the error in the fit is smaller than the difference between the estimated thickness and actual thickness. In those cases, a better estimate of the error is the difference between the actual and predicted thickness. It appears that the best two methods in determining the membrane thickness are the modified KP and modified MB fits, which give estimates of the membrane thickness to within 0.006 and 0.002 Å, repectively. These modified fits are significantly more effective because the dominant feature of the Iq^4 plots is the maximum at $qt = \pi$ rather than the q^{-4} falloff of the unmodified data.

IV. RESULTS AND DISCUSSION

A. Assessment of scattering approximations

Before performing neutron scattering measurements, we characterized large unilamellar vesicles of DOPG using DLS. Unilamellar DOPG vesicles prepared by extrusion through 100 nm diameter pore filters have a monomodal size distribution with an average radius of approximately 50 nm and a polydispersity of about 0.05. In the context of light scattering, a polydispersity of 0.05 in vesicle size is quite low and such vesicles are often referred to as monodisperse. However, the effect of such polydispersity on SANS measurements (shown in Fig. 3) is significant, and in fact has more of an effect than the instrumental resolution, $\Delta\lambda/\lambda$ (also shown in Fig. 3), except for higher values of q.

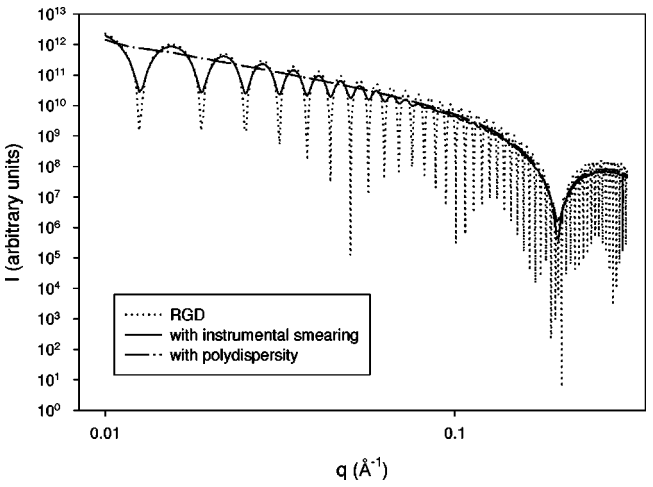

FIG. 3. Predicted effects of instrumental smearing (dash-dotted line) and vesicle polydispersity (dotted line) on the full RGD function (solid line). At low q, oscillations of the RGD function are partially smeared by instrumental effects and completely averaged away by vesicle polydispersity. At high q, the instrumental smearing is slightly more pronounced than that from vesicle polydispersity.

We have measured the small angle neutron scattering from DOPG LUVs in the absence and presence of increasing concentrations of ionic (NaCl) and non-ionic (sucrose) solutes. Before measuring possible changes in thickness due to solute effects, we tested the accuracy of several different scattering approximations in determining membrane thickness. As our test case, we chose to examine the scattering from DOPG LUVs in the absence of excess solute. As discussed earlier, the measured membrane thickness should correspond to the thickness of the hydrocarbon layer of the membrane [5]. For protonated lipid in D_2O, the scattering is due primarily to the contrast between the medium and the hydrogen in the hydrocarbon region of the bilayer. We expect the measured thickness of DOPG to be similar to that of the hydrocarbon layer of dioleoylphosphatidylcholine (DOPC) [21], $t = 32 \pm 0.4$ Å, because DOPG has the same acyl chain chemical structure as DOPC and a similar scattering profile in the acyl chain region of the bilayer [22].

The MB scattering function depends on the vesicle radius, as well as the membrane thickness. In this case, we fit data in two ways, either by using the average vesicle radius (and polydispersity) determined by dynamic light scattering, or by including the vesicle radius as a fit parameter. Fits to the scattering function which included both R and t as fit parameters gave a reasonable value for t, but with a large uncertainty in both t and R (shown in Table I). By including the average radius R (and polydispersity) as constants (as deter-

TABLE II. Parameters obtained from SANS data for RGD, MB, and KP fits. All units are in Å.

Method	t	R	Δ
MB	32.94 ± 0.71	484 ± 2 [a]	27 ± 2 [a]
MB	32.63 ± 6.4	$10757 \pm 1.5e7$	n/a [b]
KP	32.21 ± 2.4	n/a	n/a
KPSA	34.65 ± 0.08	n/a	n/a
Modified KP	31.29 ± 0.05	n/a	n/a
Modified MB	31.28 ± 0.05	484 ± 2 [a]	27 ± 2 [a]

[a]Values determined by DLS.
[b]Polydispersity of 0.05 assumed.

mined by DLS), we are able to reduce the error significantly in the MB fit. We also fit the data using the KP approximation, which is similar to the MB approximation, but does not include the effects of the vesicle size or polydispersity. The KP approximation yields essentially the same value for the membrane thickness as the MB approximation, but with a much larger uncertainty, because it fails to fit the data in the region corresponding to the first minimum in the scattering function. The MB fit is more accurate because it includes the smoothing effects of the vesicle radius on the scattering function.

The data was also fit using the small angle form of the KP approximation (KPSA). The KPSA appears to fit the scattering fairly well, but leads to an overestimate of the membrane thickness. We believe that this over estimate is a consequence of the deviation of the KPSA approximation from the KP at high q. In principle, it should be possible to improve the small angle estimate of the membrane thickness by progressively reducing the upper bound on the q range used in the fit, however, this results in concomitant increases in the uncertainty of the fit. It is noteworthy that, when DLS measurements of R and Δ are included in the fits, MB, KP, and KPSA all closely follow the scattering data over almost the full q range (as shown in Fig. 1).

Finally, we attempted to fit only the sinusoidal part of the scattering function, using a modified version of the KP approximation, in which the data and scattering function have been multiplied by q^4 (shown in the inset of Fig. 1). We also tested a similarly modified form of the MB approximation, which included the average vesicle radius R and polydispersity Δ as measured by DLS. These fits produced the smallest error and essentially the same values for the membrane hydrocarbon layer thickness 31.29 ± 0.05 Å, and 31.28 ± 0.05 Å, respectively, which is very close to that determined for DOPC, 32.0 ± 0.4 Å.

For each fitting method we attempted, the error in each fit (shown in Table II) is roughly the same magnitude or larger than that which we predicted from fits to the theoretical data. The larger errors in the fits to the experimental data are due to instrumental noise and may also be exaggerated by instrumental smearing of the data at high q. The errors in the modified KP and MB fits (shown in Table II) are much larger than those predicted theoretically. However, the modified KP and MB fits are still the most succesful in determining the membrane thickness with errors in each case of 0.05 Å, which still indicates a high degree of accuracy in each fit.

TABLE III. Thickness measurements of DOPG membranes in NaCl and sucrose.

Solute	Concentration, in mM	Osmolality, in mOsm	Thickness, in Å
None	0	37	31.29 ± 0.06
NaCl	125	268	31.65 ± 0.08
NaCl	250	472	31.63 ± 0.08
NaCl	375	692	31.69 ± 0.12
Sucrose	250	313	30.70 ± 0.06
Sucrose	500	667	30.16 ± 0.06

B. Solute effects on membrane thickness

Small-angle neutron scattering measurements were performed on a series of DOPG LUVs prepared in buffers with increasing isotonic concentrations of either NaCl or sucrose. These measurements were initially motivated by results from light scattering measurements, in which it was found that isotonic increases in NaCl concentration resulted in increased scattering from DOPG vesicles [1]. It was concluded that this change in scattering was due to physical changes in the bilayer corresponding to changes in membrane thickness or refractive index. Because DOPG is an anionic lipid, there was some question as to whether the effect of NaCl was primarily ionic or osmotic. By measuring changes in the thickness of the hydrocarbon region of DOPG membranes as a function of isotonic solute concentration for NaCl and sucrose, we hoped to investigate the magnitude and nature of the effects of both ionic and non-ionic solutes. In determining the membrane thickness as a function of solute composition and concentration, we chose to analyze the data using the modified KP approximation. We chose the modified KP approximation over the modified MB because it does not require information about vesicle radius or polydispersity.

We found that in going from an absence to presence of isotonic NaCl, there was an increase in the thickness of the DOPG membrane. However, increasing isotonic NaCl concentrations above the minimum concentration used had no significant effect on the membrane thickness (summarized in Table III). In the case of increasing isotonic NaCl concentration, we believe that in going from 0 NaCl to 125 mM NaCl, the surface charge on the PG head groups is partly or completely neutralized, either from counter ion binding or charge screening [23,24]. In the absence of NaCl, when the vesicles are fully charged, it is likely that the PG headgroups will be more widely spaced than in the partly, or fully screened case [25]. A reduction in the average head group spacing in going from the charged to uncharged state might result in a decrease in the hydration of the membrane [26]. A decrease in hydration would result in an increase in the effective thickness of the hydrocarbon layer [21], due to a reduction in the amount of water penetrating into the membrane.

Surprisingly, we found a significant decrease in membrane thickness as a function of increasing sucrose concentration (shown in Table III). When the isotonic concentration of sucrose is increased, we believe there is a concomitant increase in the hydration and surface area of the membrane. In the case of DOPC multilamellar vesicles, sucrose in the concentrations we have used is known to increase the hydra-

tion of the lamellar phase [27]. We suggest that the mechanism for increased hydration is association and hydrogen bonding of sucrose molecules with the PG headgroups [28]. If sucrose molecules were associated with the PG headgroups, they could act as spacers between headgroups, increasing the surface area, possibly allowing the passage of more water into the membrane.

V. CONCLUSIONS

In this paper we have developed an effective method for the determination of membrane thickness using SANS which provides a higher degree of precision than conventional methods and which does not require the additional information that other methods do. The modified KP fit does not require any information about vesicle size or polydispersity, in contrast to more conventional analysis methods, such as fits using RGD convoluted with polydispersity or MB. Futhermore, our comparison of various fitting methods using simulated data suggests that the modified KP fit is accurate to 0.1 Å, or better, which is more accurate that the 0.5 Å resolution of other fitting methods. As we have discussed, the main reason for the higher accuracy of the modified KP fit is that the main feature of the Iq^4 plot is the maximum of the function at $qt=\pi$, whereas the conventional fits are most sensitive to the overall q^{-4} behavior of the scattering function.

We then applied the modified KP method to the measurement of the effects of solute composition and isotonic concentration on membrane thickness. In the case of sucrose, we found that increasing the sucrose concentration caused a steady decrease in the membrane thickness. We believe the effects of sucrose are related to the hydration of the membrane. We found that in the presence of NaCl, the DOPG bilayer was approximately 0.4 Å thicker than in the absence of NaCl, but that increases in NaCl concentration above the minimum used had no effect. We attributed this behavior primarily to the effect of charge screening or counter ion binding. However, more detailed measurements are necessary in order to separate possible osmotic effects from purely ionic effects. Such measurements could include studies of NaCl effects at lower concentrations where the membrane surface charge is not fully screened, or measurements of the effects of other salts, such as LiCl and CsCl, which are similarly charged but have very different osmotic properties.

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