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DYNAMIC LIGHT SCATTERING STUDY OF CALCIUM-INDUCED FUSION IN PHOSPHOLIPID VESICLES*

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Summary

Acidic sonicated phospholipid vesicles can undergo dramatic morphological changes due to fusion in the presence of divalent metal ions. For example, small spherical phosphatidylserine vesicles can form scroll-like cylinders which precipitate in the presence of Ca^{2+} above a threshold concentration. Subsequent addition of EDTA will yield large, unilamellar vesicles. These events have previously been established through the combined use of differential scanning calorimetry and freeze-fracture electron microscopy. We have applied the technique of dynamic light scattering to follow these fusion events rapidly, accurately, and non-perturbatively as they occur in solution at calcium concentrations slightly below threshold for precipitation.

Sonicated phospholipid vesicles have been studied extensively during the past decade as models of biological membranes. Of particular interest is vesicle fusion and the relationship of this process to similar cellular events. Of significance in this context is the fact that acidic sonicated phospholipid vesicles can undergo dramatic morphological changes due to vesicle fusion in the presence of divalent ions.

Fig. 1 gives a schematic summary of observations by Verkleij et al. [1] on 1,2-didodecanoyl-*sn*-phosphatidyl-1′-*sn*-glycerol and by Papahadjopoulos et al. [2] on phosphatidylserine. In each case small, spherical, sonicated vesicles (30—50 nm diameter) were shown to change into large, scroll-like cylinders (700—1000 nm length), named cochleate cylinders by Papahadjopoulos, in the presence of Ca^{2+} above a threshold concentration. Freeze-fracture electron

*Supplementary data to this article, giving details of information supporting the data in Fig. 3, are deposited with, and can be obtained from: Elsevier Scientific Publishing Company, BBA Data Deposition, P.O. Box 1527, Amsterdam, The Netherlands. Reference should be made to No. BBA/DD/064/71316/470 (1977) 503.

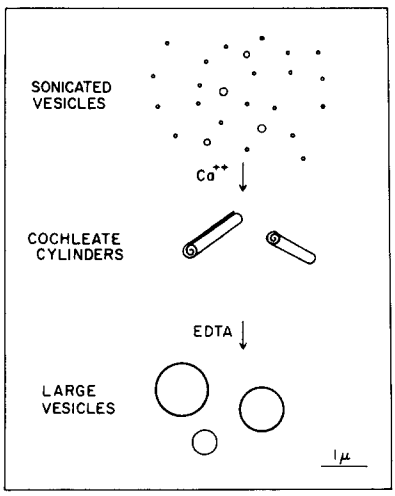

Fig. 1. Schematic representation of morphological changes induced in sonicated acidic phospholipid vesicles by calcium and EDTA.

microscopy gave unambiguous evidence of cochleate cylinder formation as a precipitate from solution at the calcium concentrations used in those studies. Differential scanning calorimetry has established that the cylinders were formed by fusion resulting from vesicle-vesicle collisions and not by phospholipid exchange due to monomer diffusion between vesicles [3]. Addition of the chelating agent EDTA following completion of cylinder formation resulted in the formation of large, spherical, unilamellar vesicles (300—1000 nm diameter) as represented schematically in Fig. 1. Freeze-fracture electron microscopy gave clear evidence of this second dramatic morphological change.

We have used the technique of dynamic light scattering to follow these morphological changes as they occur in solution [4]. This technique measures the hydrodynamic diameter of the diffusing vesicles through the time behavior of the fluctuations in scattered light intensity [5—7]. Previous techniques for studying vesicle fusion kinetics suffer from being perturbative such as ESR labels [8] and fluorescent labels [9], or applicable to only limited vesicle size range such as NMR [10], or ambiguous to interpret such as NMR [11] and turbidity [12], or incapable of measuring vesicle size changes such as biochemical assay techniques [13]. The only disadvantage of the dynamic light scattering technique is the need to deal with monodisperse populations of vesicles [14] The extra effort involved in first separating vesicles by size is not a true disadvantage since radius of curvature is one of the interesting variables in the study of vesicle fusion. The advantages of dynamic light scattering are that it is non-perturbative, fast, and accurate, giving a measure of vesicle hydrodynamic diameter as this dimension changes in solution.

The well-characterized process of calcium-induced fusion of phosphatidylserine vesicles was chosen for this study in order to evaluate dynamic light scattering as a technique for studying fusion kinetics in solution. Turbidity measurements were taken simultaneously to evaluate the reliability of turbidity as a measure of these changes. There are two differences between our study of sonicated phosphatidylserine vesicles and that of Papahadjopoulos et al. [2].

First, we purposely kept the calcium concentration slightly below threshold in order to avoid precipitation. This made it possible to follow cochleate formation in solution. Second, we passed our vesicle preparation through a Sepharose 4-B column before adding calcium in order to have an initially monodisperse population of vesicles. This was necessary to make interpretation of the dynamic light scattering data straightforward.

Highly purified phosphatidylserine from beef brain was supplied to us by Dr. D. Papahadjopoulos of the Department of Experimental Pathology, Roswell Park Memorial Institute, Buffalo, N.Y. [15]. Vesicles were prepared in a buffer solution containing 0.1 M NaCl, 2 mM *N*-tris-(hydroxymethyl)methyl-2-amino ethane-sulfonic acid (TES), 2 mM L-histidine, and 0.1 mM EDTA adjusted to pH 7.4 with NaOH. In a typical experiment tritiated dipalmitoyl phosphatidylcholine ([^{3}H]dipalmitoyl phosphatidylcholine) was added at less than 1 mol % to phosphatidylserine to label phospholipid concentration in the fractions to be collected from the Sepharose 4-B column. A sample containing 20 μmol phosphatidylserine in 2 ml buffer was shaken mechanically on a vortex mixer for 10 min and sonicated for 1 h at 24°C in a bath-type sonicator. The sample was kept under nitrogen atmosphere at all times. The sample was next centrifuged at 48 000 x g for 15 min and then passed through a 50 cm column of Sepharose 4-B and collected in 1-ml fractions which were divided into 0.1-ml subfractions for radioactivity count and 0.9 ml samples under nitrogen for study with dynamic light scattering and/or turbidity techniques.

Radioactivity was measured on a Packard Model 3380 TriCarb scintillation spectrometer after adding 10 ml ASC to each 0.1 ml subfraction.

Dynamic light scattering measurements were made using light from a Spectra Physics 5 mW 120 He-Ne laser or from a Spectra Physics 2 mW 133 He-Ne laser radiating at 633 nm focused on each 0.9 ml sample contained in a test tube under nitrogen atmosphere. The scattered light was detected by an ITT FW 130 photomultiplier after passing through two pinholes 25 cm apart. The pinholes and photomultiplier sat on an optical bench which could be rotated about an axis at the sample location so that the scattering angle could be varied. The scattering angle was usually set at 60°. The photocurrent $I(t)$ was amplified and its time-correlation function $R_I(\tau) = \langle I(t)I(t+\tau)\rangle$ computed with a Honeywell SAI-42A correlation and probability analyzer for 100 values of delay time τ separated by a selectable constant value. The data was stored on paper tapes with a Friden high-speed paper-tape punch. Data analysis was performed using a time-share terminal connected to the university CDC Cyber computer. The measured intensity correlation function $R_I(\tau)$ was fitted by the method of least squares to the expression

$$R_I(\tau) = a[1 + b \exp(-2\tau/\tau_c)].$$

Higher order corrections were tried and found unnecessary in fitting the data reported here. To extract the hydrodynamic diameter of the vesicles from τ_c, we calibrated the instrument over the size range of interest at the appropriate scattering angle by measuring the correlation times for various monodisperse polystyrene spheres of known radii obtained from Dow Diagnostics suspended in the buffer solution under the same conditions. This set-up is sensitive enough to measure 30 nm diameter vesicles in minutes at a phosphatidylserine

concentration of 0.5 mM using the 5 mW laser. The largest vesicles (200 nm diameter) can be easily measured at one-hundredth this phosphatidylserine concentration.

Turbidity measurements were made by detecting the transmitted light with a Spectra Physics Model 385 photodiode and Model 404 power meter whose output was recorded on a Houston 5233-5 Omniscribe strip-chart recorder.

The distribution of radioactivity in the collected fractions is shown in Fig. 2a. When these same fractions were measured for vesicle size using dynamic light scattering they were found to range in diameter from 30 nm to 300 nm in the expected way as shown in Fig. 2b.

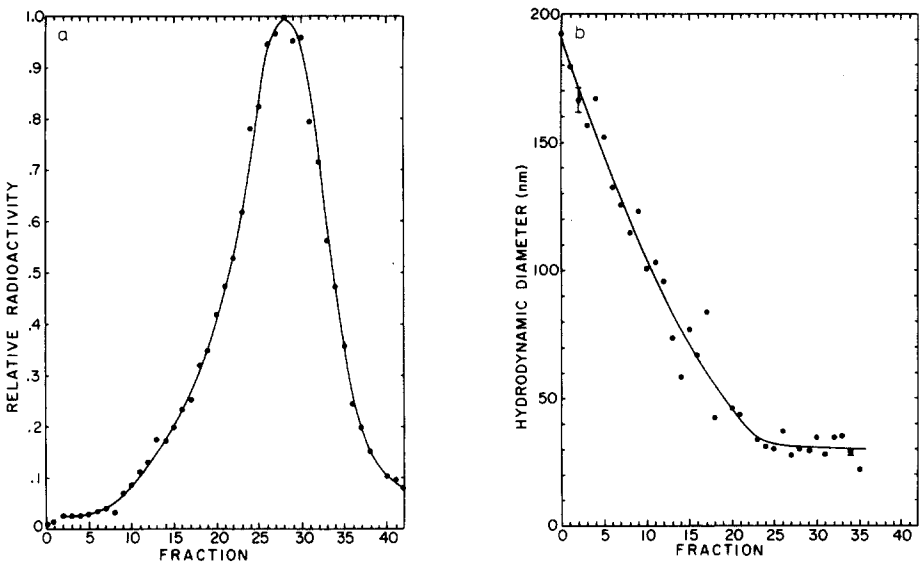

Fig. 2. (a) Relative phospholipid concentration as measured by radioactivity of [^{3}H]dipalmitoyl phosphatidylcholine label vs. 1 ml fraction number collected from Sepharose 4-B column. (b) Size of phospholipid vesicles by dynamic light scattering vs. same 1 ml fraction number.

Scroll formation was observed by adding Ca^{2+} to a concentration of 0.9 mM to one of these fractions and following the resulting change in vesicle size with both dynamic light scattering and turbidity. Typical results at 24°C are shown in Fig. 3. The hydrodynamic diameter for fraction 14 as measured by dynamic light scattering increased from 50 nm to 2300 nm while the transmitted intensity decreased to 10% of its previous value. Both dynamic light scattering and turbidity yield comparable half times for scroll formation under these conditions. This size change and the time for completion of cochleate formation are consistent with the data of Papahadjopoulos et al. [2], where scroll formation was observed using freeze-fracture electron microscopy after incubation for 1 h at 37°C following calcium addition.

Following completion of scroll formation the chelating agent EDTA was added and vesicle size was measured using dynamic light scattering. The data was taken well after equilibration approximately 15 min after adding EDTA. It was found that the hydrodynamic diameter changed from 2300 nm to

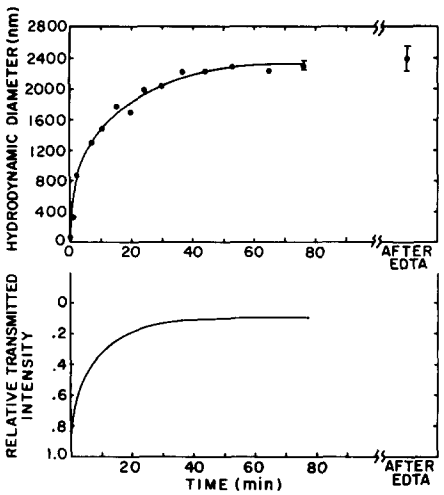

Fig. 3. Comparison of kinetics of cochleate cylinder formation as measured by dynamic light scattering (top) and turbidity (bottom). Data were collected simultaneously from the same sample after 0.9 mM Ca^{2+} was added to fraction 14 collected from the Sepharose 4-B column. The final point is the equilibrium value after adding EDTA.

2400 nm and did not return to its original value at 50 nm. This is consistent with the formation of large spherical unilamellar vesicles as observed by Papahadjopoulos et al. [2]. Simultaneous measurement of the transmitted intensity indicated the limitations of turbidity as a technique for following these dramatic morphological changes in solution. Unlike the dynamic light scattering data, the turbidity returned to 65% of its initial value. This misleading behavior of turbidity may be explained by the change from a multilayer structure of the light scatterers to a unilamellar structure [16].

Similar behavior was shown in other sets of measurements. Once again dynamic light scattering and turbidity data gave consistent results for the kinetics of scroll formation following calcium addition. However, the hydrodynamic diameter as measured by dynamic light scattering remained large upon addition of EDTA indicating formation of large, unilamellar vesicles. Turbidity decreased returning more closely to its initial value. Details of these kinetics are being published separately.

We have shown that dynamic light scattering and the simpler technique of turbidity measurement give consistent data for the kinetics of cochleate formation following calcium addition. However, dynamic light scattering, but not turbidity measurement, gives an accurate picture of what happens in solution when EDTA is added following completion of cochleate formation. We conclude that the technique of dynamic light scattering is appropriate to the study of vesicle fusion as it occurs in solution. The technique is especially powerful when used in conjunction with freeze-fracture electron microscopy. Electron microscopy serves as a necessary check on the morphology and population homogeneity of the light scatterers whose diffusive behavior can then be accurately, rapidly and non-perturbatively measured in solution by the technique of dynamic light scattering. Turbidity, though simpler than dynamic light scattering, gives more ambiguous and less quantitative results and should be used with caution.

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