LIGHT-SCATTERING AND CHROMATOGRAPHIC EVIDENCE OF ANTI-DEPRESSANT-PROMOTED LIPID VESICLE FUSION

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1. Introduction

Desipramine(5-(3-methylaminopropyl)-10,11-dihydro-5H-dibenz(b,f)azepine) (DMI), an anti-depressant-active molecule, has been shown to induce certain modifications in the dynamic and functional characteristics of phospholipid microvesicles (1).

At present magnetic resonance [1,2] and calorimetric data [3] exist which seem to indicate some gross alterations in the hydrocarbon molecular motion produced by this series of drugs.

In order to examine the possibility of desipramine-promoted vesicle fusion, we have measured some geometric characteristics of vesicles, because the high surface curvature of vesicles, compared with their unsonicated bilayer counterpart, causes the former to have considerably less thermodynamic stability.

Thus, in the presence of a possible surface active agent, such as DMI seems to be, the rate of fusion can become faster, and a careful examination of this possibility is necessary for the complete understanding of this type of situation.

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2. Theoretical and experimental methods

2.1. *Analytical gel filtration*

Preweighed samples of 1,2-dipalmitoyl or dimiristoyl lecithin were sonicated in a Branson sonifier for 2 min., being subsequently chromatographed in a Sepharose column or centrifuged at 1500 rev/min in a Superspeed RC-2 centrifuge. Column characteristics and operation are referred to in fig.1.

2.2. *Light-scattering*

Experiments were performed using a modified Perkin-Elmer 204 spectrofluorometer. Depolarization degrees were measured by means of Polaroid hn-38 polarizers.

Recrystallized benzene was used as reference for total intensity measurements.

Degrees of depolarization defined as:

$$\rho\sigma = \frac{i(\sigma,\pi)}{i(\sigma,\sigma)} \qquad \rho\pi' = \frac{i(\pi,\pi)}{i(\pi,\sigma)} \qquad \rho u = \frac{i(u,\pi)}{i(u,\sigma)}$$

where i (ϵ,η) refers to the state of polarization of the incident and scattered beams [4], having the values of π,σ,t,u, given the electric field vector parallel and perpendicular to the plane of observation, total intensity, and unpolarized radiation respectively, were

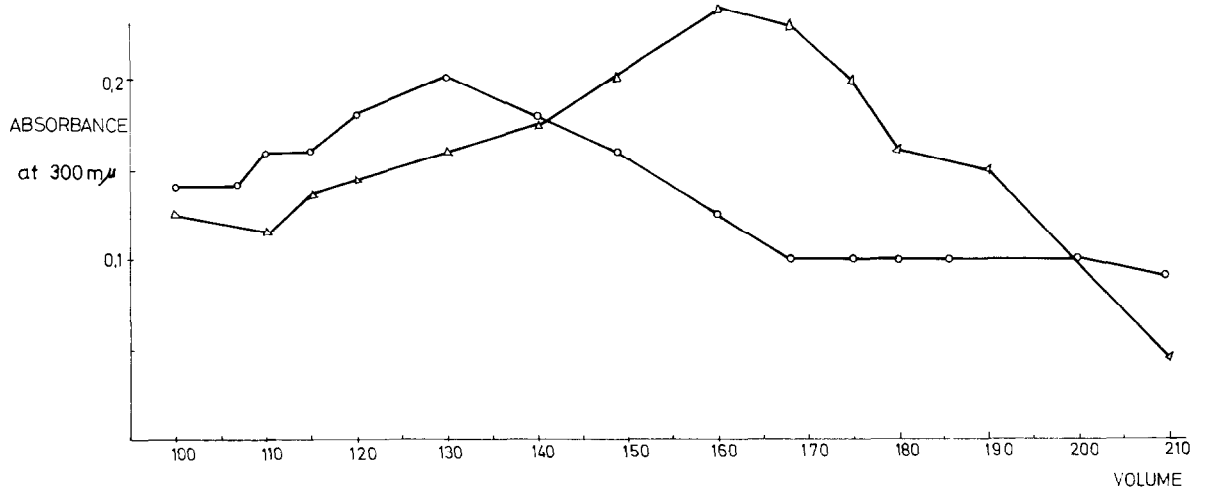


Fig.1. Elution patterns from the analytical Sepharose-4B (2.5 × 70 cm) column. Previously sonicated vesicles were first passed through the column collecting only the fraction corresponding to the 150Å diameter vesicle pool, and concentrated by means of a Bio-Rad Hollow Fibers device. Samples with a lipid concentration of 0.3 per cent were used. (Δ–Δ–Δ–) Lecithin. (o–o–o–o) Lecithin with 10% of added DMI.

used following the methods recently published by Heller et al. [4,5].

The contribution of particle optical anisotropy to the scattering was computed by application of the theory given by Pecora et al. [6]. Particle static polarization was calculated as usual (for instance Tinker [7]), using the numerical values given by Ohki [8], for the molecule-fixed polarizability tensor.

Numerical computations were performed in a Univac 1108 computer.

3. Results and discussion

Fig.1 shows two superimposed chromatograms demonstrating an increment in the averaged particle mol. wt from 2×10^6 to 5×10^6, which of course indicates some type of vesicle fusion.

The Stokes' radii a calculated from Ackers' [9] equation

$$a = a_0 + b_0 \, \mathrm{erfc}^{-1} \quad \epsilon$$

where a and b are calibration constants, were found to increase from 156 ± 10 A to 220 ± 32 A.

Total light-scattering intensity increases due to increasing amounts of the drug, are shown in fig.2, however interpretation of the data in terms of geometric constants is not feasible due to the strong optical anisotropy of the vesicles.

As a first approximation, measured degrees of depolarization and corresponding values of vesicle radius and non-sphericity are given in table 1.

Geometric features were obtained by the use of previously generated computer graphs, however corrections due to the anisotropy are included in the search for self-consistency of the data.

These contributions to the scattered intensity have the form:

$$C_{\mathrm{aniso}}(r,a) = \left[\frac{9\,(\mathrm{INT}(X)-\mathrm{INT}(XL))\,12(\mathrm{SIN}(XL)-\mathrm{SIN}(X)) + 3X\,(\mathrm{COS}(X)-L\mathrm{COS}(XL))}{X^3\,(1-L^3)}\right]^2$$

where r and a are the outer and inner particle radii and

$$X = q \cdot r \qquad L = a/r$$

$$q = \frac{4\pi}{\lambda}\,\mathrm{SIN}\frac{\Theta s}{2} \qquad \text{being } \mathrm{INT}(X) = \int_0^X \frac{\mathrm{SIN}(Z)}{Z}\,dz$$

are used as relative intensity increments, however discrepancies between theoretical and measured particle

Table 1
Apparent changes in vesicle characteristics induced by DMI. Data obtained by extrapolation to infinite dilution and 0.1/1 drug/lipid molar ratio

Magnitude used	Axial ratio	Layer thickness	Outer radius
$i(\sigma, \sigma)$	1	136.Å	211.Å
$\rho\mu$	1.001	–	235.Å
$\rho\sigma$	1.001	–	250.Å
$\rho\pi'$	1.0001	–	265.Å

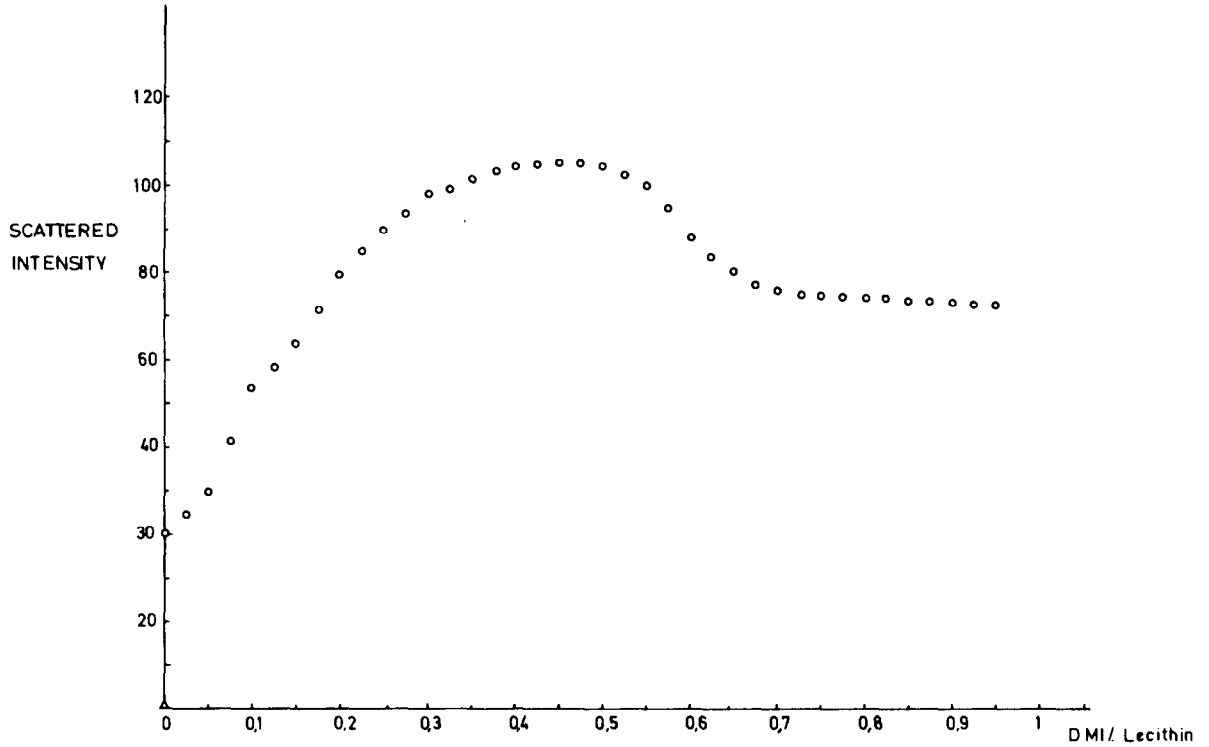
radii exist due to the nature of the theory used, because of polydispersity and distribution problems.

From the results presented here it is clear that considerable aggregation of lipid vesicles occurs. Fusion occurs because small vesicles possess high surface free energy due to the high surface curvature of the particle, and would undergo rearrangements in their molecular packing to attain a state of greater stability.

Uncorrected depolarization measurements were found to predict prolate spheroids with an axial ratio of 2.68, having only small variations upon addition of the drug.

Thus an adsorbed molecule, such as DMI seems to behave like can serve as a catalyst of the process, which can be initiated by clusters of this molecular species located at the surface of the vesicles.

However, this effect seems to have a strong and complex dependence on the drug/lipid molar ratio, having quite a different behaviour in the region of high ratios. On the other hand, temperature dependence of the process is not easy to explain as a previous paper indicates [2], and future work is necessary to obtain a clear picture of the physical situation; so, at high drug/lipid ratios and near or above the transition temperature, a clearly lytic effect of the drug on the vesicles can be demonstrated (J.B., unpublished), and the interpretation of this type of behaviour in terms of molecular parameters becomes difficult without the use of some quantitative model of the lipid conformation and phase transition [10,11].



Fig,2. Total intensity light scattering versus DMI/Lipid molar ratio. (△) Benzene, used as reference.

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