*J. Biosci.*, Vol. 15, Number 3, September 1990, pp. 145–148. © Printed in India.

# Role of nicotinic acid as modulator of liposomal microviscosity

M. CHATTERJEE*, R. BASU and P. NANDY†
Department of Physics, Jadavpur University, Calcutta 700 032, India
*Present address: Guha Institute of Biochemistry, 55/5 Purna Das Road, Calcutta 700 029, India

**Abstract.** Using the fluorescent probe 1,6-diphenyl-1,3,5 hexatriene, we have investigated the effect of nicotinic acid, a derivative of the toxic alkaloid nicotine, on the fluidity profile and activation energy of diffusion in the liposomal system of several lipids. We have also studied how the fluidizing property of nicotinic acid affects the intermediate fluid condition induced by cholesterol in these liposomal systems.

**Keywords.** Nicotinic acid; phospholipid; fluidity; phase transition.

## Introduction

Nicotinic acid (NA), a drug clinically used as vasodilatory agent, can lower serum cholesterol level when administered to man in large doses (Miller and Hamilton, 1964). From the observations in *in vivo* systems, it was concluded that the dilating effect is not correlated to the carboxyl group and the ring configuration probably is necessary in order to slow down the cholesterol biosynthesis. However, the exact mechanism of this drug is not clearly known as yet (Yovos *et al.,* 1982).

In order to investigate how this drug affects the membrane systems, we have recently studied its effect on the fluidity profile and activation energy of diffusion of several liposomal systems mimicking the actual biological membrane. These are liposomal systems of (i) dipalmitoyl phosphatidyl choline (DPPC) (Bhattacharyya *et al*., 1988), (ii) egg lecithin (Bhattacharyya *et al*., 1988), (iii) total lipid isolates of erythrocyte ghost membrane (Bhattacharyya and Nandy, 1986), (iv) sphingomyelin (Sph) (Bhattacharyya and Nandy, 1989) and (v) mixed lipid of Sph and DPPC (1:4 molar ratio) (Bhattacharyya and Nandy, 1989). The latter was chosen as it has lipid composition similar to that of rat liver plasma membrane (Bhattacharyya and Nandy, 1989).

For this study we have used the fluorescent probe 1, 6-diphenyl-l,3,5-hexatriene (DPH) whose polarisation when embedded in the membrane is an index of fluidity of its environment and thus any change in fluidity induced by an external agent can be detected (Shinitzky and Barenholtz, 1978). Our study indicates that this drug modifies the membrane fluidity, probably by mechanical interaction between the drug and membrane constituent molecules as there is no evidence of strong complex formation from spectral studies. We have also shown that the drug can disrupt the intermediate fluid condition induced by cholesterol in the cholesterol incorporated liposomal system.

†To whom all the correspondence should be addressed.
Abbreviations used: NA, Nicotinic acid; DPPC, dipalmitoyl phosphatidyl choline; Sph, sphingomyelin; DPH, 1,6-diphenyl-l,3,5-hexatriene.

## Materials and methods

Spectral grade solvents and AR grade chemicals were used. DPH, Sph and DPPC (Sigma, USA) were used. Liposomes were prepared by sonication of an aqueous dispersion of measured amounts of lipids (Bhowmik *et al.,* 1986). Cholesterol and drugs were incorporated into the liposomes (Bhattacharyya *et al*., 1988). Fluorescence measurements were performed with a Perkin-Elmer MPF 44B spectrofluorometer using a thermostated cell. Free drug was removed by gel filtration.

## Results and discussion

DPH, when incorporated in lipid bilayers, becomes an integral part of the system regardless of its phase or composition and from the measurement of polarization of this probe in the liposome, an approximate evaluation of viscosity ($\bar{\eta}$ ±15%) of the probe environment can be made (Shinitzky and Barenholtz, 1978).

From the viscosity temperature relation (Shinitzky and Barenholtz, 1978) the activation energy $E$ can be calculated. When In $\bar{\eta}$ is plotted with respect to $T^{-1}$ , a straight line is expected if the probe environment maintains the same structure, A deviation from linearity indicates a change in phase and/or structure (Shinitzky and Barenholtz, 1978).

**Table 1.** Effect of the drug nicotinic acid on the fluidity profile of some liposomal systems*.

| Liposomal systems | Lipid: cholesterol: NA | Viscosity (in poise) | | Max activation energy (ev) | Value of const. $b(K)^{-2}$ |
|---|---|---|---|---|---|
| | | 20°C | 40°C | | |
| DPPC** | 1:0:0 | 14·73 | 3·32 | 1·63 | 0·01 |
| | 1:1:0 | 6·63 | 5·58 | — | — |
| | 1:1:1 | 9·77 | 5·57 | — | — |
| | 1:0:1 | 5·36 | 2·31 | 1·54 | 0·02 |
| EPC** | 1:0:0 | 1·55 | 0·70 | 0·95 | 0·045 |
| | 1:1:0 | 4·39 | 2·71 | — | — |
| | 1:1:1 | 3·59 | 1·68 | — | — |
| | 1:0:1 | 1·00 | 0·37 | 0·69 | 0·02 |
| Sphingomyelin | 1:0:0 | 12·50 | 2·58 | 1·17 | 0·02 |
| | 1:1:0 | 8·80 | 6·20 | — | — |
| | 1:1:1 | 14·15 | 6·68 | 0·47 | — |
| | 1:0:1 | 8·37 | 1·91 | 1·14 | 0·02 |
| Mixed lipid of Sph and DPPC (1:4 molar ratio)† | 3:0:0 | 9·97 | 2·7 | 2·32 | 0·05 |
| | 3:1:0 | 7·76 | 3·50 | 0·72 | — |
| | 3:1:1 | 9·02 | 4·37 | 1·50 | — |
| | 3:0:3 | 8·50 | 1·82 | 2·16 | 0·06 |
| Total lipid isolates of erythrocyte ghost membrane†† | 1:0:0 | 9·02 | 2·41 | 1·02 | 0·03 |
| | 1:0:1 | 7·53 | 2·13 | 0·88 | 0·01 |

* Values are averages of 6–8 measurements with an average deviation of only 5%.
**Bhattacharyya *et al.* (1988); †Bhattacharyya and Nandy (1989); ††Bhattacharyya and Nandy (1986).

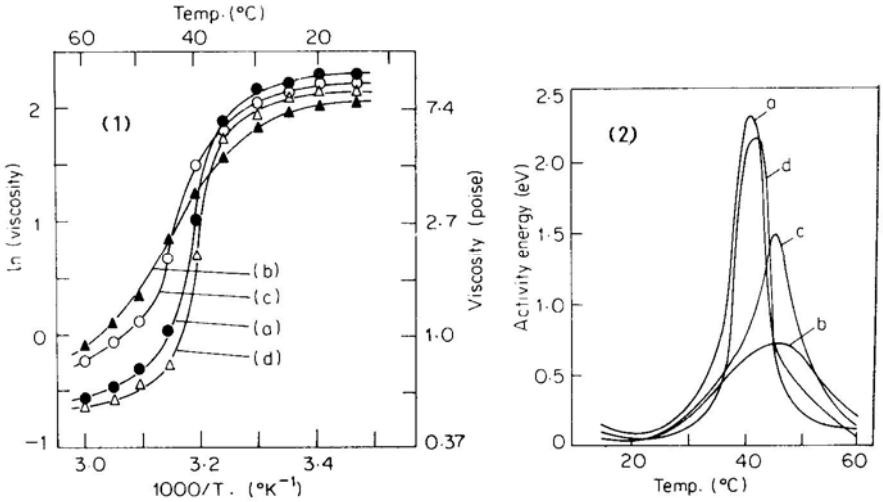
The fluidity profiles of these liposomal systems have a common feature though the absolute value of viscosity varies from system to system (table 1). Out of all these systems, let us consider as a representative one, the liposome of mixed lipids of DPPC and Sph in the molar ratio 4:1 (Bhattacharyya *et al.,* 1989). In figure 1 (curve a) we have shown how the liposomal microviscosity varies with temperature. Addition of cholesterol induces an intermediate fluid condition (figure 1, curve b). When NA is added to the cholesterol incorporated system, the original fluidity profile is brought back to some extent (figure 1, curve c). Addition of N A alone to the liposome merely fluidizes the system (figure 1, curve d).

In figure 2, we have plotted the calculated values of activation energy as a function of temperature and the empirical relation is

$$E = E_{\varnothing} \exp(-b(\Delta T)^2),$$

where $\Delta T = T - T_c$, $T_c$ being the critical temperature for each system and $b$ is a constant for the system. Values of $E_{\varnothing}$ and $b$ for the different liposomal systems are given in table 1.

Comparison of the results of mixed lipid liposomes with those in cases of individual lipid components shows that NA affects the fluidity of model membrane liposomes prepared from DPPC or Sph or mixture of both. The fatty acyl chains and the head group of lipid molecules probably interact mechanically with NA molecules as there is no spectral evidence of complex formation. *E,* the activation energy which is the energy barrier that must be overcome before the elementary flow process can occur, also reduces in the presence of NA. The observed vasodilatory effect of NA *in vivo* may be a natural consequence of the fluidizing effect recorded *in vitro.*




**Figures 1 and 2. (1)** Microviscosity *vs.* temperature curves and (2) activation energy *vs.* temperature for DPH-probed mixed lipid liposome. Concentration of DPPC, $10^{-4}$ M. Molar ratio of Sph and DPPC is 1;4. (a) liposome only, (b) cholesterol added to (a) in molar ratio 1:1, (c) N A added to (b) molar ratio of lipid, cholesterol and NA is 1:1:1, (d) NA added to (a) in molar ratio 1:1 (Bhattacharyya and Nandy, 1989)

## Acknowledgement

Financial assistance from the Council of Scientific and Industrial Research, New Delhi, is thankfully acknowledged.

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