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Partial molecular volumes of cholesterol and phosphatidylcholine in mixed bilayers

Original Paper

Gallová J.✉, Želinská K., Balgavý P.

Comenius University in Bratislava, Faculty of Pharmacy, Department of Physical Chemistry of Drugs, Bratislava, Slovak Republic

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Abstract Dispersion of multilamellar liposomes of dimyristoylphosphatidylcholine (DMPC) and cholesterol (CHOL) were studied by vibrational densitometer for the CHOL mole fractions $X = 0–0.54$ in the temperature range 18–50 °C, both below and above the main phase transition. DMPC-CHOL bilayers served as a simple model for lipidic part of biological membrane. Volumetric parameters are essential not only to evaluate the data obtained by scattering and diffraction methods on model membranes but can provide valuable information about molecular packing in bilayers and the phase behaviour of lipid-CHOL mixtures. In this paper, preliminary results regarding the changes in the specific volume of lipid bilayer with increasing temperature and CHOL content are presented. Different values of apparent molecular volume of CHOL for different CHOL mole fraction pointed out the non-ideal mixing of DMPC and CHOL.

Keywords – *cholesterol, dimyristoylphosphatidylcholine, specific volume, densitometry*

INTRODUCTION

In biological systems, CHOL is known to play a fundamental role as a modulator of physical properties and lateral organization of the membrane lipid bilayer. Thus, many studies of the interaction of CHOL with phospholipid bilayer model membranes have been performed, utilizing a wide range of physical techniques (for a review, see Marquardt et al., 2016). In our recent paper (Gallová et al., 2015), we determined the molecular volumes of CHOL and of a series of monounsaturated diacylphosphatidylcholines with the number of carbon atoms in the acyl chain 16, 18, 20, 22 and 24 in the temperature range 20–40 °C in mixed bilayer. This work is devoted to the preliminary study of volumetric parameters of DMPC and CHOL in mixed bilayers. The knowledge of these parameters is essential to evaluate the data obtained by scattering and diffraction methods on model membranes. Greenwood et al. (2006) studied the binary systems of CHOL with different phospholipids at limited temperature points. In this paper, changes in volumetric parameters of CHOL and DMPC in mixed bilayers induced by increasing temperature and CHOL content are studied.

MATERIAL AND METHODS

Chemicals: 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) was purchased from Avanti Polar Lipids (Alabaster, Alabama), CHOL from Sigma Aldrich (Germany) and chloroform, p.a. from Slavus (Bratislava, Slovakia).

Sample preparation: DMPC and CHOL were weighed in a dry state into a glass vial and then co-dissolved with a small amount of chloroform. The solution was mixed thoroughly. The solvent was evaporated to dryness under a stream of gaseous nitrogen, followed by evacuation in a vacuum chamber. The dry lipid film was hydrated by the weighed amounts of deionised water to obtain the final concentration of lipid ≈1–3 % (w/w). Multilamellar liposomes were formed during vortexing and brief sonication in a bath sonicator. Samples were heated to 30 °C and degassed before measurement. The density of prepared samples and also of deionised water was measured using vibrational densitometer DMA 4500M (Anton Paar, Austria) in the temperature range 16–50 °C with step 1 °C. Weighing errors were ± 0.0001 g; the precision of the density measurement was ± 0.00005 g/cm^3.

* *E-mail: gallova@fpharm.uniba.sk*

Data treatment: The specific volume of the sample, v_S, is a reciprocal value of the sample density measured by a densitometer. According to (Greenwood et al., 2006; Klacsová et al., 2010) we assumed that the specific volume of water in our samples is the same as in pure bulk water. Under this precondition, the apparent specific volume of DMPC, v_{PC}, was calculated from the specific volume of the control sample v_{S0} without cholesterol, according to the equation 1

$$v_{PC} = \frac{v_{S0} - (1 - w_{PC})v_W}{w_{PC}} \quad (1)$$

where, v_W is the specific volume of water and w_{PC} is the mass fraction of DMPC in the control sample defined as

$$w_{PC} = \frac{m_{PC}}{m_{PC} + m_W} \quad (2)$$

where, m_{PC} and m_W are masses of DMPC and water in the sample, respectively. The apparent specific volume of the nonaqueous part (DMPC + cholesterol) of the sample, v_{PC+CH}, was calculated in a similar way:

$$v_{PC+CH} = \frac{v_S - (1 - w_{PC+CH})v_W}{w_{PC+CH}} \quad (3)$$

where, w_{PC+CH} is the mass fraction of lipid part in the sample defined as

$$w_{PC+CH} = \frac{m_{PC} + m_{CH}}{m_{PC} + m_{CH} + m_W} \quad (4)$$

If we further suppose that the specific volume of DMPC in the sample does not depend on the CHOL content, the apparent specific volume of CHOL, v_{CH}, can be calculated using the equation:

$$v_{CH} = \frac{v_{PC+CH} - (1 - w_{CH})v_{PC}}{w_{CH}} \quad (5)$$

where, w_{CH} is the mass fraction of CHOL in the lipidic part of the sample defined as

$$w_{CH} = \frac{m_{CH}}{m_{PC} + m_{CH}} \quad (6)$$

where, m_{CH} is the mass of CHOL in the sample. The apparent specific volume of CHOL was converted to the apparent molecular volume V_{CH} using the following relation:

$$V_{CH} = \frac{v_{CH} M_{CH}}{N_A} \quad (7)$$

where, M_{CH} is the molar mass of CHOL and N_A is the Avogadro's number.

RESULTS AND DISCUSSION

The density of samples containing multilamellar liposomes of DMPC and CHOL was measured in the temperature range 16–50 °C. The apparent specific volume of the lipid (nonaqueous) part of samples was calculated for the control sample without CHOL, v_{PC}, and for samples containing CHOL, v_{PC+CH}, according to equations 1–4. Because the partial molecular volume of water located in bilayers is the same as the molecular volume in the bulk water (for references, see Uhríková et al., 2007), the expression 'apparent' is omitted and the fully hydrated lipid bilayers in multilamellar liposomes were treated as a separate phase. As expected, the specific volume v_{PC+CH} increases with increasing temperature for various mole fractions of CHOL (Fig. 1).

It is well known that the hydrated bilayers of DMPC are in a solid-ordered S_o phase at $t<t_p$ (temperature of pretransition), in a rippled $P_ß$ phase at $t_p<t<t_m$ and in a disordered fluid phase L_d above the main phase transition temperature t_m. The main phase transition is clearly visible from the temperature dependence of v_{PC} (CHOL-free DMPC bilayers). According to McMullen and et al. (1993), a small amount of CHOL is able to abolish the pretransition in DMPC bilayers. In this work, the pretransition was not included in the measured temperature

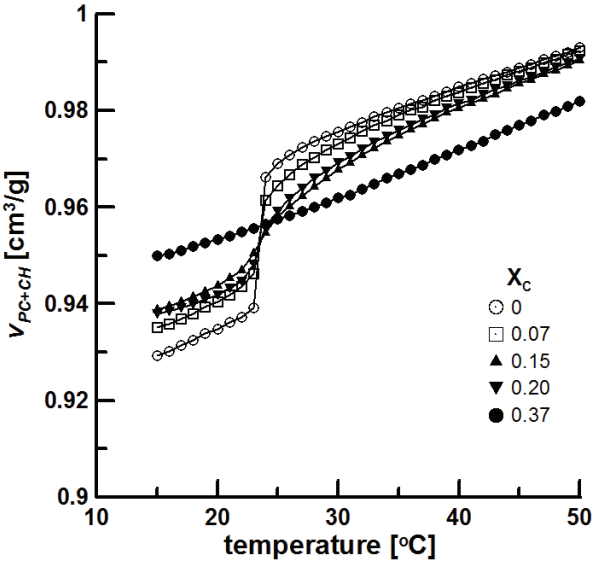

Figure 1. Temperature dependence of the specific volume of DMPC-CHOL bilayers with varying content of CHOL. Numbers denote mole fraction of CHOL

range and all the samples were heated above t_m just before the measurement to avoid a rippled $P_ß$ phase formation. The observed main phase transition temperature t_m = 23–24 °C in the control DMPC sample is in agreement with data from the literature (McMullen and et al., 1993). The values of DMPC specific volume are similar to the data of Nagle & Wilkinson (1978). The main phase transition at low CHOL mole fractions ($X < 0.1$) was narrow and only a small decrease of t_m (within the temperature range of 1 °C) was observed (Fig. 1).

The transition was broadened around $X = 0.1$ and was not detectable at $X \geq 0.3$ (Fig. 1). These observations are similar to those in McMullen et al. (1993). It is widely accepted that cholesterol can induce a liquid-ordered L_o state in lipid membranes containing high amount of cholesterol. L_o state was described by Vist & Davis (1990) as a phase with properties intermediate between S_o and L_d phases. The L_o phase is stable in a wide temperature range. We can therefore suppose that the samples with high CHOL content, where the main phase transition is not visible (Fig. 1), contain DMPC-CHOL bilayers in liquid-ordered L_o state.

The apparent specific volume of CHOL, v_{CH}, was calculated (eq. 5,6) and converted further to the apparent molecular volume of CHOL, V_{CH} (eq. 7). As seen from the Fig. 2, V_{CH} changes when the mole fraction X of CHOL increases, especially at low X. Our preliminary results confirm the fact that CHOL and DMPC do not mix ideally.

In conclusion, we have determined the temperature dependence of specific volume of DMPC bilayers with various amount of CHOL and the temperature dependence of apparent molecular volume of CHOL in the mixed DMPC-CHOL bilayers. We have confirmed a non-ideal mixing of CHOL and DMPC. The paper, where the partial molecular volumes of CHOL and DMPC will be calculated in dependence on temperature and CHOL content, is being in preparation.

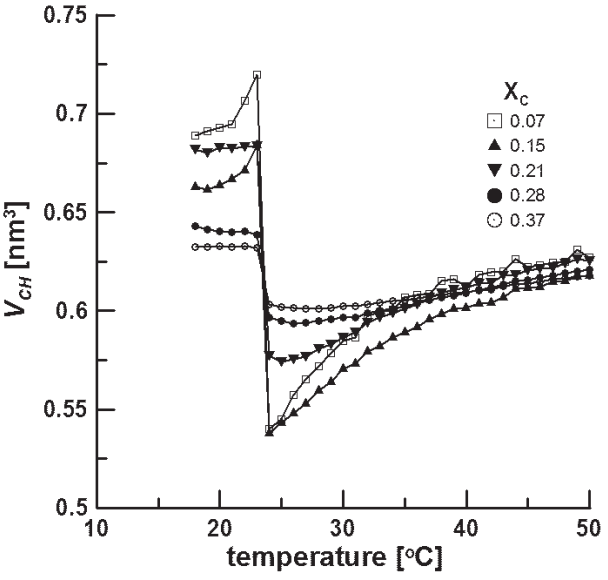

Figure 2. Temperature dependence of the apparent molecular volume of CHOL in DMPC-CHOL bilayers with varying contents of CHOL. Numbers denote mole fraction of CHOL

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