

INTERACTION OF MELATONIN WITH ZWITTERIONIC MODEL LIPID MEMBRANES

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Abstract

Melatonin is a key hormone secreted by the pineal gland in the brain. It interacts strongly with cell membranes and plays a protective role in several diseases such as Alzheimer's disease, cardiovascular diseases, and cancer. Hence, studies on the interaction of melatonin with lipid bilayer membranes have gained importance. In this regard, the present study aims to analyze the influence of different concentrations (0 to 15 mol%) of melatonin on the thermal and structural properties of zwitterionic stearyl-2-oleoyl-sn-glycero-3-phosphocholine (SOPC) lipid bilayer model systems by using Fourier transform infrared (FTIR) spectroscopy and differential scanning calorimetry (DSC). The DSC results have shown that melatonin interacts with the membrane in a concentration-dependent fashion, induces patch formation, and alters the phase behaviour by reducing the main phase transition temperature. FTIR data has shown that increasing the melatonin concentration caused a reduction in the wavenumber of C=O and PO₂⁻ antisymmetric stretching vibrations corresponding to the ester carbonyl and phosphate groups of SOPC lipids. This indicates the possibility of strong hydrogen bonding of melatonin with the SOPC lipids and nearby water molecules in the environment, suggesting its preferential location near lipid heads at the interfacial region. These results help to understand

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the organization and interactions of melatonin with membranes and provide insights to develop safe melatonin-based formulations for pharmaceutical applications.

Key words: melatonin, lipid membrane, biomedical applications

Introduction. Melatonin (N-acetyl-5-methoxytryptamine) is a hormone secreted mainly by a small endocrine gland, called the pineal gland in the brain, in most vertebrates [1]. It is also called “sleep hormone” as it plays a key role in synchronizing our day-to-day sleep-wake cycle and is essential for controlling various physiological processes in our body such as mood regulation, appetite, anxiety, ageing, and immunity [2]. Reports have shown that small molecules, such as melatonin and cholesterol can incorporate and interact strongly with the lipid molecules in cell membranes, leading to alterations in the structure and biophysical properties of membranes [3,4]. Therefore, it is important to study the molecular interactions of various concentrations of melatonin with cell membranes, as they have significant implications for health and various diseases like cardiovascular diseases, diabetes, Alzheimer’s disease, and cancer.

Since real cell membranes are complex and composed of different types of biomolecules such as proteins, lipids, and carbohydrates, it is difficult to study the mechanism of interaction of melatonin with pure membrane lipids. Hence, synthetic phospholipids that can self-assemble into lipid bilayer structures called “liposomes” are widely used as simple model systems to study the effects of various additives on membrane properties [5]. Therefore, we have used a zwitterionic phospholipid called 1-stearoyl-2-oleoyl-sn-glycero-3-phosphocholine (SOPC) to prepare model liposomal membranes to analyze the influence of melatonin. SOPC lipids are widely used for biophysical studies and are well-characterized [2–5]. The chemical structure of melatonin and SOPC lipids is shown in Fig. 1a and 1b, respectively.

In our previous study, we reported the effect of high melatonin concentrations (up to 50 mol%) on lipid membranes. Similarly, SEVERCAN et al. [6] and DIES et al. [7] have used high melatonin concentrations (up to 30 mol%) on lipid membranes. But these concentrations are much higher than the actual concentration of melatonin present normally in blood circulation. Hence, to get bio-

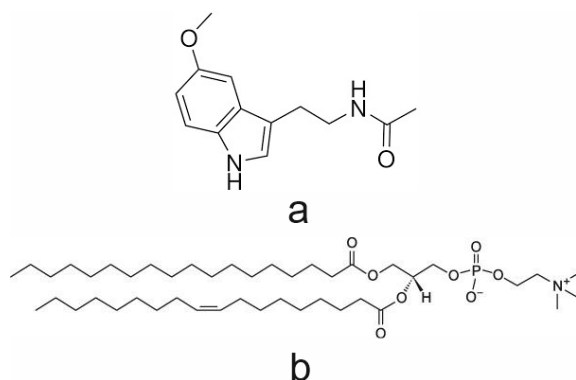


Fig. 1. Chemical structure of a) melatonin, and b) SOPC lipid

logically relevant information, it is essential to use less melatonin concentration, which is clinically appropriate or equivalent to the blood melatonin level. The present study aims to investigate the effect of melatonin interaction on the thermal and structural properties of SOPC lipid membranes incorporated with different melatonin concentrations (0 to 15 mol%) by using two sensitive techniques, namely Fourier transform infrared (FTIR) spectroscopy and differential scanning calorimetry (DSC). The obtained results will provide insight to understand the concentration-dependent effect of melatonin to induce phase transition of lipids, formation of lipid patches, and hydration state of the head group region of lipids, which, to the best of our knowledge, have not been previously reported for SOPC lipids.

Materials and methods. Materials. SOPC (C18:0/C18:1) lipid was purchased from Avanti Polar Lipids Inc. (Alabaster, Alabama, USA). Melatonin, ethanol, and chloroform (purity 99%) were purchased from Sigma-Aldrich Chemie GmbH.

Sample preparation and experimental conditions. The SOPC lipid was dissolved in chloroform and melatonin was dissolved in ethanol. To prepare SOPC multilamellar vesicles (MLVs) with different melatonin concentrations, appropriate quantities of both solvents were mixed in a calculated molar proportion to achieve final melatonin concentrations of 5, 10, and 15 mol% in SOPC MLVs. Then, the sample mixtures were evaporated under vacuum for 4–5 h, until the entire solvent was removed. The dry lipid film was hydrated with double-distilled water and kept in a warm ($\sim 35^\circ\text{C}$) ultrasonic bath, above their gel-to-fluid phase transition temperature for at least 12 h to achieve homogeneity of the lipid-water mixture. Pure SOPC MLVs were also prepared following the same procedure without adding melatonin for control experiments.

Differential scanning calorimetry. The phase transitions of SOPC MLVs were measured using a Discovery 250 differential scanning calorimeter (TA Instruments, USA). The samples were loaded into a calorimetric cell, and each sample was heated/cooled repeatedly in the temperature range from -5 to 30°C , with heating and cooling rates of $5^\circ\text{C}/\text{min}$. The obtained DSC scan was used to measure the phase-transition temperature (T_m) and the calorimetric enthalpy change (ΔH). Subsequent scans were used to assess the reversibility of the phase transition. The obtained values were analyzed using TRIOS data analysis software.

FTIR spectroscopy. About 20 μl of samples were spread on a transmission cell with silicon windows and the FTIR spectra were recorded using a Bruker Invenio R spectrometer (Bruker Optik GmbH, Germany) equipped with a deuterated tri-glycine sulphate detector and ATR attachment (diamond crystal). With a temperature control unit, 26°C temperature was maintained during the data acquisition. The FTIR spectra were measured in the frequency region $4000\text{--}600\text{ cm}^{-1}$, accumulating 128 scans at a resolution of 2 cm^{-1} . All experiments were repeated three times and a similar trend was observed after each repeat.

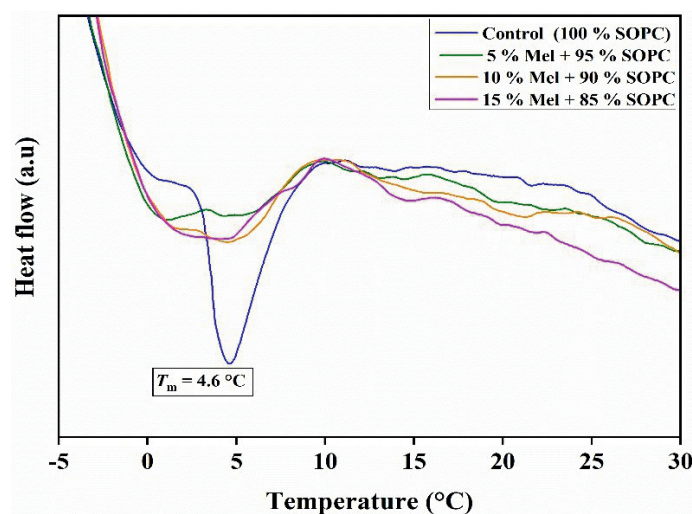


Fig. 2. DSC thermogram of SOPC MLVs containing 5, 10, and 15 mol% of melatonin

Results and discussion. Phase behaviour: phase transition temperature and lipid order. DSC is a sensitive thermoanalytical technique and it is used in this study to measure the concentration-dependent effect of melatonin in altering the phase behaviour of SOPC lipids. DSC thermograms of SOPC MLVs with different melatonin concentrations (0 to 15 mol%) are shown in Fig. 2.

All lipids have a characteristic melting temperature (T_m), at which they undergo a transition from the highly ordered gel phase ($L\beta$) to the less ordered liquid crystalline phase ($L\alpha$) [8]. The obtained DSC curve for pure SOPC MLVs in the temperature range of -5 to 30 °C indicates a first-order phase transition with a sharp symmetric peak around 4.6 °C, which corresponds to their well-known characteristic gel to a liquid crystalline phase transition temperature. This value coincides well with the phase transition temperature of SOPC lipids reported in our previous works [9,10]. Figure 2 shows that the inclusion of melatonin has a strong effect on the phase behaviour of SOPC lipids. It is interesting to note that even at the lowest melatonin concentration (5 mol%), a significant reduction in the intensity of the main phase transition peak, and broadening of the main peak occurs when compared to the pure SOPC MLVs taken as a control. As the melatonin concentration was further increased to 10 and 15 mol%, the characteristic main phase transition curve is smeared out and the heat flow curve was hardly detectable. This indicates the possibility of phase separation induced by melatonin in SOPC membranes, which coincides with the theory of phase transitions in the presence of admixtures, such as cholesterol and nanoparticles [11]. In such cases, there are more possibilities for the variation in lipid dynamics among the melatonin-rich and melatonin-poor phases in the membrane.

The obtained results are in good agreement with the literature data provided by various experimental techniques for other types of lipid membranes [6,7,9]. For

instance, Dies et al. [7] demonstrated that incorporation of melatonin (0–30 mol%) altered the lipid order in the 1,2-dimyristoyl-sn-glycero-3-phosphatidylcholine (DMPC) phospholipid membranes by inducing lipid patches or domains in a concentration-dependent fashion, by using 2-dimensional X-ray diffraction studies and Fourier analysis of the lipid and melatonin-enriched diffraction peaks. Their results have shown that the melatonin molecules at a lower concentration (2.5 mol%) align parallel to the lipid tails, just below the hydrophilic head group region of lipid molecules, and formed melatonin-rich domains in the membranes. Melatonin is a small amphiphilic molecule ~ 15 Å in size, and this orientation is more favourable for reducing the disruption of the lipid matrix. On the contrary, at high concentrations (30 mol%), melatonin molecules are uniformly distributed throughout the membrane and align parallel to the lipid membrane. This result clarifies that the melatonin molecules organize themselves in a different pattern in the lipid bilayer in a concentration-dependent manner, reduce the T_m values and cause a fluidifying effect.

FTIR spectroscopy. FTIR spectroscopy is a powerful technique to study the lipid conformational order in both model lipid and biological membranes [12]. The FTIR spectra precisely help to identify the different bonds and functional groups present within the molecule and provide a molecular fingerprint. Literature reports [13,14] have shown that several spectroscopic features help to monitor lipid phase transitions, such as (i) the shift of C–H symmetric and antisymmetric stretching frequency; (ii) the shift of PO_2^- antisymmetric stretching; (iii) the scissoring, rocking, and wagging band progressions of methylene moieties of lipid chains; (iv) bandwidth changes of the lipid CH_2 stretching, scissoring and rocking vibrational modes.

In this work, FTIR data was analyzed to explore the melatonin-induced variations in the structure of SOPC MLVs by monitoring the wavenumber shifts of various vibrational modes that correspond to conformational changes in acyl chains, the head group as well as at the interfacial region. Accordingly, the C–H stretching vibrations of the major functional groups of lipids in the spectral range corresponding to $1800\text{--}1000\text{ cm}^{-1}$, the C=O stretching mode corresponding to the ester carbonyl group of the fatty acyl chains, and the PO^- antisymmetric modes which provides information about the hydrogen bonding between the phosphate groups in SOPC lipid molecules and the surrounding water molecules were considered. Figure 3 shows the FTIR spectra of SOPC MLVs containing 5, 10, and 15 mol% of melatonin.

As seen from Fig. 3, increasing the melatonin concentration induced slight variations in the peak positions and the bandwidths of the FTIR spectral bands, in comparison with the control values. For instance, a sharp symmetric peak at 1633 cm^{-1} corresponding to the C–H stretching bands of control (pure SOPC MLVs) was changed to 1637 cm^{-1} at 15 mol% melatonin concentration. Figure 4a shows the changes in the frequency of the C=O stretching frequency mode in-

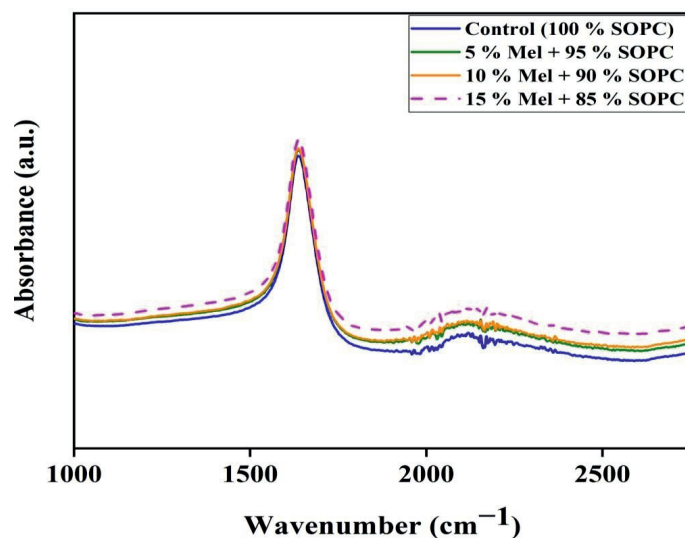


Fig. 3. FTIR spectra of SOPC MLVs with increasing melatonin concentration (0 to 15 mol%)

duced by incorporating various concentrations (0 to 15 mol%) of melatonin in SOPC MLVs. The C=O stretching frequency observed at 1739 cm^{-1} for pure SOPC MLVs was reduced to 1735 cm^{-1} by increasing the melatonin concentration up to 15 mol% in SOPC membranes. Reduction in the wavenumber in this frequency region indicates either the strengthening of existing hydrogen bonds or the formation of new hydrogen bonds between the components [15]. A similar result was reported by SAHIN et al. [16] who demonstrated that the inclusion of melatonin up to 15 mol% in 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) MLVs reduced the frequency of C=O stretching from 1734 cm^{-1} to 1730 cm^{-1} . The small variation in the wavenumbers between our results and the referred work could be due to the use of different lipids.

Another interesting band to probe the changes near the head region of SOPC lipid is the antisymmetric PO_2^- stretching vibration at 1223 cm^{-1} . Figure 4b shows the variation in the wavenumber of the antisymmetric PO_2^- stretching of SOPC MLVs with different (0 to 15 mol%) melatonin concentrations. Increasing the melatonin concentration in SOPC MLVs caused a slight reduction in this band frequency, which also signifies the hydrogen bonding between the phosphate group of SOPC lipids with melatonin and water molecules in the environment [17]. The strong hydrogen bonding induced by melatonin at the C=O and PO_2^- moieties of SOPC lipids with the nearby water molecules suggests that melatonin preferentially locates near the lipid head groups at the interfacial region. On the whole, FTIR data indicates the interactions of melatonin with the functional groups of lipid molecules and their organization near the lipid head groups at the interfacial region.

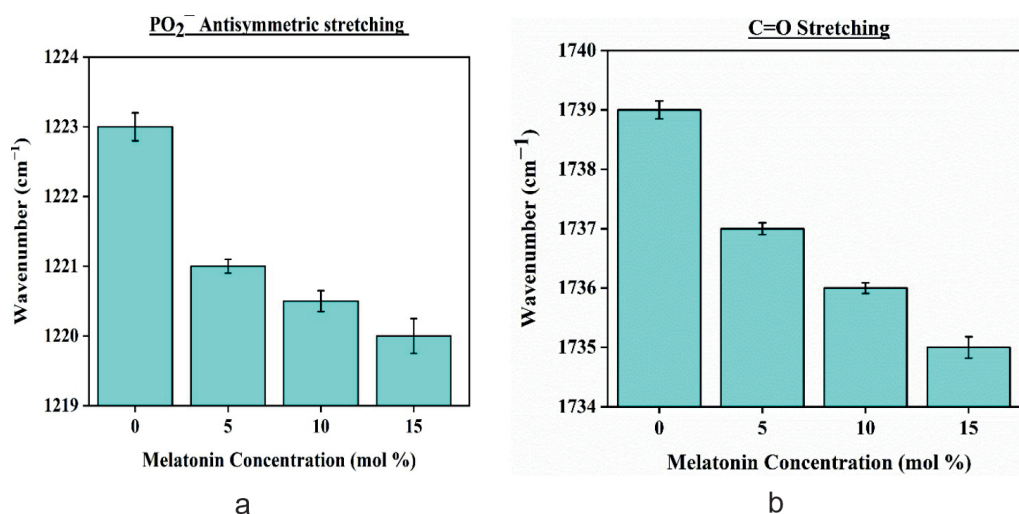


Fig. 4. Variations in wavenumber of a) C=O stretching mode, and b) PO_2^- antisymmetric stretching mode induced by the incorporation of 0–15 mol% melatonin in SOPC MLVs

Conclusions. The interaction of melatonin in a concentration-dependent fashion on the zwitterionic SOPC MLVs was investigated in the present study. DSC thermograms have shown that increasing the melatonin concentration up to 15 mol% in SOPC membranes induced notable changes in lipid order with the possibility of domain or patch formation. By combining DSC and FTIR data, we conclude that melatonin strongly interacts with the membrane lipids and its incorporation in the lipid matrix induces changes in the phase behaviour and lipid dynamics. These data will provide guidance to future studies on melatonin-induced lipid conformations in zwitterionic membranes. Understanding the organization of melatonin in cell membranes and their concentration-dependent effects on lipid dynamics will help to develop novel melatonin-based drugs with improved therapeutic efficacy.

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