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The organization of melatonin in lipid membranes

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ABSTRACT

Melatonin is a hormone that has been shown to have protective effects in several diseases that are associated with cholesterol dysregulation, including cardiovascular disease, Alzheimer's disease, and certain types of cancers. We studied the interaction of melatonin with model membranes made of dimyristoylphosphatidylcholine (DMPC) at melatonin concentrations ranging from 0.5 mol% to 30 mol%. From 2-dimensional X-ray diffraction measurements, we find that melatonin induces a re-ordering of the lipid membrane that is strongly dependent on the melatonin concentration. At low melatonin concentrations, we observe the presence of melatonin-enriched patches in the membrane, which have a characteristic of smaller membrane spacing. The melatonin molecules were found to align parallel to the lipid tails in these patches. At high melatonin concentrations of 30 mol%, we observe a highly ordered melatonin structure that is uniform throughout the membrane, where the melatonin molecules align parallel to the bilayers and one melatonin molecule associates with 2 lipid molecules. Understanding the organization and interactions of melatonin in membranes, and how these are dependent on the concentration, may shed light into its anti-amyloidogenic, antioxidative and photoprotective properties and help develop a structural basis for these properties.

1. Introduction

The interactions of proteins and small molecules with lipid membranes play a large role in maintaining the integrity and functionality of the cell membrane, and significant changes in these interactions are involved in the pathology of many diseases [1]. Melatonin is a hormone that is produced in the central nervous system by the pineal gland for circadian cycle regulation. However it has also been shown to be produced by several peripheral tissues, which suggests that it may have other physiological roles [2]. Melatonin has been of recent interest in the study of membrane/small molecule interactions; it acts as an antioxidant, may be preventative against cardiovascular disease and may also inhibit the formation of toxic amyloid structures [3,4].

While cholesterol, which is speculated to be correlated to an increased risk for Alzheimer's disease, leads to a decrease in membrane fluidity, melatonin is a largely hydrophilic amino acid derivative hormone, which has been shown to reside in the head group region [5], increasing membrane fluidity and causing a corresponding increase in head group area and a decrease in bilayer thickness [5–7]. Increased fluidification of the membranes is speculated to inhibit peptide insertion, as the amyloid peptide has been found to preferably interact with gel phase membranes [8].

Although cholesterol and melatonin are highly integrated into the biochemical pathways of the cell, previous studies have identified that their mechanism of influence is at least in part structural; many of their effects are as a result of biophysical interactions that influence the protein and membrane structure [6,9,10]. Choi et al. have recently shown through Langmuir–Blodgett methods and molecular dynamics simulations that melatonin is able to offset the rigidifying effects of cholesterol in dipalmitoylphosphatidylcholine (DPPC) monolayers [11]. Additionally, Saija et al. have suggested that melatonin's fluidifying action may lead to a photoprotective effect, which could extend to internal cellular components as melatonin is able to permeate membranes [7]. Melatonin's ability to permeate even the hemato-encephalic (blood–brain) barrier means that it is accessible to almost all somatic cells, further extending its potential influence and emphasizing the importance of understanding its interactions with cell membranes [7].

Sahin et al. investigated the effects of melatonin on membrane properties in multilamellar vesicles, and showed that the effects were strongly dependent on the melatonin concentration [12]. Previous studies by Severcan et al. have shown that in dehydrated DMPC model membranes, the addition of even a very small molar percentage of melatonin resulted in a phase separation within the membrane [13]. The inductance of a different phase has also been predicted by molecular dynamics simulations of membranes containing large amounts of

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ethanol (also a small hydrophobic molecule which, similarly to melatonin, has been shown to increase membrane fluidity) [14].

Drolle et al. [6] used neutron diffraction and small angle scattering in combination with computer modelling to study the interaction between melatonin with bilayers made of DPPC and DOPC. The location of the melatonin molecules was determined at melatonin concentrations of ~10 mol% (in experiment and simulation) and ~30 mol% (in experiments). Melatonin was found to reside in the head group region of the bilayers and to lead to a decrease in bilayer thickness indicative of an increase of bilayer fluidity. Dies et al. [10] then presented experimental evidence that melatonin inhibits the insertion of amyloid-β_{25-35} peptides in anionic lipid membranes made of DMPC and DMPS at high melatonin concentrations of 30 mol%, which is considered to be an important step in protein oligomerization and toxic fibril formation. This observation supports the assumption of a potential protective role of melatonin in the formation of amyloid plaques in Alzheimer's disease.

In this study, we investigate the position and organization of melatonin at different concentrations in phospholipid membranes. This is achieved through the preparation of lipid membranes containing 1,2-dimyristoyl-sn-glycero-3-phosphatidylcholine (DMPC), a 14 chain saturated phospholipid with an overall zwitterionic nature, as shown in Fig. 1. Different amounts of melatonin ranging from 0.5% to 30% were included in the membranes. Using 2-dimensional X-ray diffraction, the in-plane and out-of-plane structure of the membranes was determined.

2. Results

Synthetic lipid membranes made of DMPC were prepared as highly oriented, multi-lamellar membrane stacks on silicon wafers. Lipids and melatonin at different concentrations were dissolved in a solvent and applied as thin films to the wafers. Five different membrane complexes were prepared for this study, as detailed in Section 5 and listed in Table 1. As depicted in Fig. 1b, the samples were oriented such that the $q_{||}$-axis probed lateral membrane structure and the perpendicular axis, q_z, probed out-of-plane structure of the multi-lamellar membrane complexes. The samples were kept in a temperature and humidity controlled chamber, a so-called humidity chamber, during the measurements. Data were collected at $T = 28$ °C and in a 100% H_2O atmosphere to ensure full hydration of the membranes to study structure in the fluid, physiologically relevant state of the membrane complexes.

Table 1
List of all the samples prepared for this study and their molecular composition.

DMPC (mol%)	Melatonin (mol%)	d_{z1} (Å)	d_{z2} (Å)	d_{z3} (Å)	Lateral structure
100	0	66.17	–	–	
99.5	0.5	64.24	59.30	–	
99	1	61.63	59.30	48.18	
97.5	2.5	64.24	59.30	–	
95	5	65.89	55.06	–	
					$a_1 = 12.4$ Å, $b_1 = 10.1$ Å, $\gamma_1 = 90°$
70	30	66.17			$a_2 = 5.8$ Å, $b_2 = 5.3$ AA, $\gamma_2 = 98.5°$

Two-dimensional X-ray data are shown in Fig. 2. The data cover a large area of reciprocal space to determine the in-plane and out-of-plane structure of the membranes simultaneously. These maps are also important to identify potential scattering features from, e.g., molecular tilts, which may occur outside of the q_z and $q_{||}$ directions. The diffracted intensity shows one well developed in-plane Bragg peak along the $q_{||}$-axis at $q_{||} \sim 1.5$ Å^{-1}, related to the packing of the lipid acyl chains (with the exception of the 30 mol% melatonin sample, which will be discussed further in later sections). The intensity has a distinct rod-like shape, typical for a 2-dimensional system.

The out-of-plane scattering along q_z shows pronounced and equally spaced Bragg intensities due to the multi-lamellar structure of the membranes, as reviewed for instance in Refs. [15,16].

2.1. The low melatonin concentration membranes

For a quantitative analysis of the diffracted intensity, the 2-dimensional data were cut along the out-of-plane and in-plane axes.

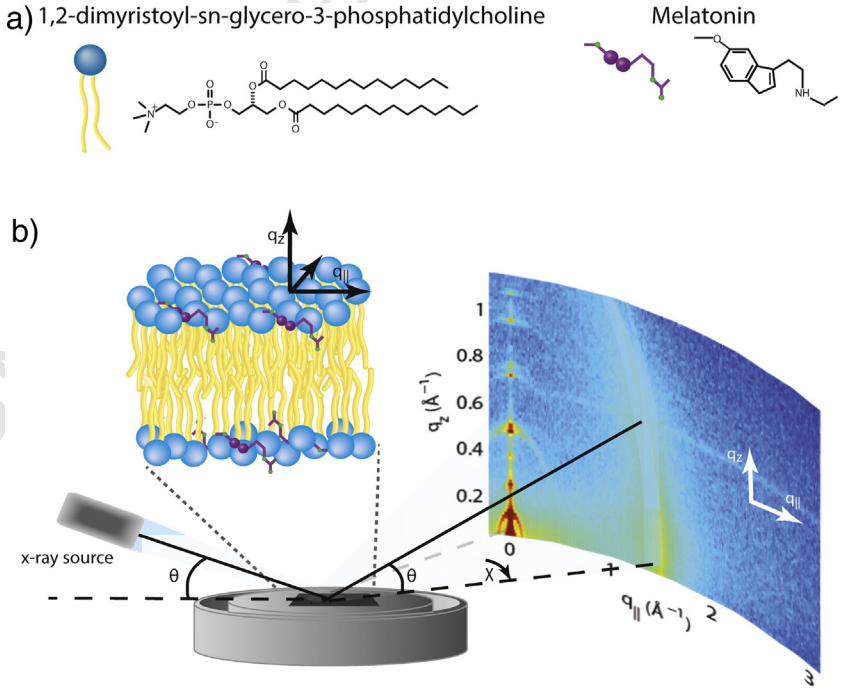

Fig. 1. (a) Schematic representations of DMPC and melatonin molecules. (b) Diagram of the experimental setup used for the X-ray diffraction measurements. Two-dimensional data sets were collected to study molecular structure perpendicular to the solid supported membranes (out-of-plane) and parallel to the membranes (in-plane). Abbreviations: DPPC — dipalmitoylphosphatidylcholine, DMPC — diymyristoylphosphatidylcholine, DMPS — dimyristoylphosphatidylserine, DB — Drug Bank, FWHM — full width half maximum, AFM — atomic force microcopy, FTIR — Fourier transform infrared spectroscopy, DCM, dichloromethane, TFE — trifluoroethanol, RH — relative humidity.

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