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Research Article

BIOPHYSICAL INVESTIGATION OF MOLECULAR INTERACTIONS BETWEEN ALLIIN AND ANIONIC DMPG MODEL MEMBRANE: AN FTIR STUDY

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Abstract *Garlic, which contains bioactive compound alliin, is a medicinal herb that has been traditionally utilized for its therapeutic properties against a range of illnesses. Our aim is to investigate the interactions between alliin and anionic dimyristoyl phosphatidylglycerol (DMPG) multilamellar vesicles (MLVs) at various temperatures and alliin concentrations (1, 3, 6 and 9 mol%) using Fourier transform infrared (FTIR) spectroscopy. The PerkinElmer Frontier spectrometer was used to collect spectra within the region of 4000-1000 cm^{-1}. The specimens were subjected to scanning within a temperature range of 0 to 40 $^{\circ}C$ using the Specac temperature control device. The analyses were conducted utilizing the Spectrum v10.3.7 program. By introducing both low and high concentrations of alliin to DMPG MLVs, the wavenumber values of the CH_2 antisymmetric stretching band decreased, while the bandwidth values increased, both in the gel and liquid crystal phases. During the gel phase, the presence of alliin resulted in a downward shift of the C=O stretching bands' wavenumber values. Opposite evidence occurred in the liquid crystal phase. The wavenumber values of the PO_2^- antisymmetric stretching band exhibited a shift towards lower values both in the gel and liquid crystal phases. In the present study, we investigated the biophysical effects of alliin on DMPG model membranes using parameters such as lipid order, dynamics and hydrogen bonding ability. The addition of alliin altered the physical characteristics of the DMPG MLVs by ordering the system, enhancing its dynamics, and promoting hydrogen bond interactions between the phosphate group of DMPG and alliin or water molecules, both in the gel and liquid crystalline phases. Moreover, alliin enhanced the strength of hydrogen bonding in proximity to carbonyl groups in the gel phase.*

Keywords: *Alliin, DMPG, MLV, FTIR, Model membrane*

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1. Introduction

The use of model membranes that mimic the complex and dynamic structure of biological membranes to investigate the pharmacological action mechanisms of drugs is frequently seen in the literature [1-3]. Garlic has been used for centuries in all cultures as a therapeutic agent on bacterial infections, cardiovascular diseases, diabetes, and even cancer thanks to its antibacterial, antioxidant, antidiabetic, and anticancer properties [4-8]. Alliin, which we used in our study, is one of the bioactive organosulfur compounds that promote the clinical use of garlic. When garlic is damaged for various reasons, alliin turns into allicin, another bioactive component of garlic [9].

It is possible to investigate the impact of drugs on membrane localization, membrane phase transition, order, and dynamics in the light of the unique data that can be obtained by examining the spectral bands belonging to the functional groups in certain biomolecules using FTIR spectroscopy. In this manner, the structural and functional properties of garlic as well as its clinical effects have been

investigated in the literature by Raman and FTIR spectroscopies [10-12]. However, there are only a few studies examining the interaction of alliin with biological membranes, which are the target systems of drugs, or with model membranes that are structurally and functionally remarkably similar to biological membranes. Allicin has been preferred in both clinical, physiological, and pharmacological studies [13-15]. For example, a reference noted the antioxidant effect of alliin by stabilizing the membrane in isoproterenol-induced myocardial infarction in male mice [16]. In a study examining the antiproliferative effect of alliin, it was reported that alliin inhibited the proliferation of gastric adenocarcinoma cells and caused a slight decrease in mitochondrial membrane potential [17]. Alliin has been reported to significantly reduce nuclear membrane damage in oogenesis cells in X-ray irradiated white mice [18]. Furthermore, one study showed that alliin and other sulphur compounds reduced the fluidity of tumour cell and platelet model membranes prepared with cholesterol and phospholipids, as well as candida cell model membranes prepared with ergosterol and phospholipids [19].

In our previous study [20], where we investigated the physical interactions between alliin and DMPC model membrane as a function of alliin concentration and temperature using FTIR spectroscopy, we revealed the regulatory effect of alliin on many parameters such as lipid order, dynamics, and hydrogen bonding capacity. To check whether these parameters change depending on the head group charge of the model membranes, in this study we examined the changes in the above-mentioned parameters caused by different alliin concentrations in the negatively charged DMPG model membrane, depending on temperature, using FTIR spectroscopy.

2. Materials and Methods

2.1. Chemicals

Alliin and DMPG were acquired from Sigma (St. Louis, MO, USA) and utilized without additional purification.

2.2. Sample preparation

The specimens for the FTIR investigation were prepared following the procedure identified by Severcan et al. [21]. To form liposomes, firstly 5 mg of DMPG solution was dissolved in chloroform and dry lipid films were obtained. It was then treated with nitrogen gas to remove excess chloroform from the solution. The additional solution was dried using a Christ LT-105 spin vacuum apparatus for 2 hours. The thin films were hydrated by adding 25 µl of a 10 mM phosphate buffer solution with a pH of 7.4. The formation of multilamellar liposomes was achieved by subjecting the mixture to vortex for a duration of 30 minutes at a temperature that was 20 ˚C higher than the main transition temperature [T_m] of DMPG, which is 43 ˚C. To create liposomes containing alliin, a certain quantity of alliin from the stock solution was first poured into the sample tube. To conduct FTIR measurements, a volume of 20 µl of liposomes was positioned between CaF_2 windows, resulting in a sample thickness of 12 µm.

2.3. FTIR studies and spectral analysis

The spectra were obtained using a PerkinElmer Frontier FTIR spectrophotometer. The fingerprint region is analyzed within the range of 4000-400 cm^{-1} using an FTIR spectrometer. Nonetheless, the utilization of the CaF_2 window restricts the wavenumber range that can be investigated. For this reason, a temperature ranges of 0-40 ˚C was used to scan the mid-infrared reagion of 4000-1000 cm^{-1}. The temperature was monitored using a Specac digital temperature controller. The samples were subjected to incubation at each temperature for a duration of 5 minutes prior to scanning the spectrum. The interferograms were combined by taking the average of 50 scans, with a resolution of 2 cm^{-1}. The water absorption bands in the range of 3050-2800 and 1700-1500 cm^{-1} strongly overlap with the corresponding

bands from lipid functional groups [1-3,20,21]. To enhance the clarity of the bands, the water bands were eliminated from the spectra using PerkinElmer Spectrum v10.3.7 software. The spectra were normalized in 3000-2800 cm^{-1} regions using the same software to visually represent and compare changes. FTIR experiments were repeated 3 times and the same results were observed.

3. Results

The current research used FTIR spectroscopy to examine the temperature dependence of DMPG MLVs with and without alliin concentrations ranging from 1 to 9 mol%. The examination of FTIR spectra focused on the investigation of CH_2 antisymmetric stretching, C=O stretching, and PO_2^- antisymmetric double stretching bands. The experiments were replicated a total of three and consistent patterns were detected on each occasion.

Normalized average FTIR spectrum of the DMPG liposomes with and without 9 mol% alliin in the gel (15 °C) and liquid crystal (35 °C) phases were shown in Figures 1(a) and (b), respectively. Visual representations of average spectra were used to demonstrate and compare the alterations in frequency, intensity, and bandwidth of particular bands of focus in the lipid with and without alliin.

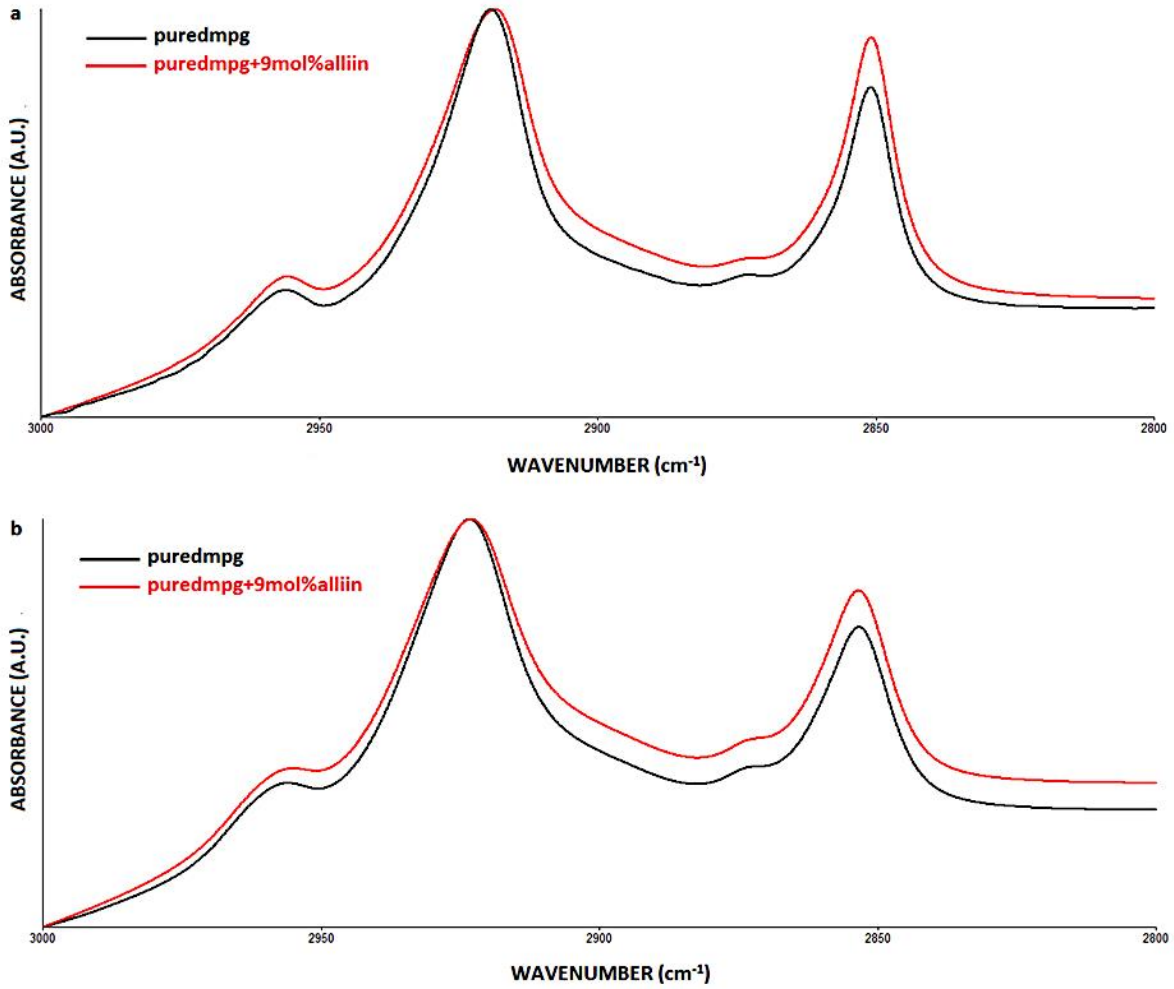

Figure 1. FTIR spectra of DMPG model membranes in the C-H stretching region at **(a)** 15 °C and **(b)** 35 °C with and without 9 mol% alliin.

Figure 2 illustrated the relationship between temperature and the frequency of the CH_2 antisymmetric stretching band of DMPG MLVs including data for both the absence and presence of

various doses of alliin. We conducted a frequency analysis of the CH_2 antisymmetric stretching band to acquire comprehensive insights into the phase transition behaviour and order-disorder condition of the system [2,3]. The main phase transition temperature (T_m) of the pure DMPG model membrane was determined at 23 °C, as in the DMPC model membrane, and the pre-phase transition temperature was determined at 15 °C (Figure 2). The gel phase is the state in which the lipid acyl chains are in a regular and tightly packed structure at temperatures below the main phase transition temperature whereas the liquid crystal phase is the state in which the lipid acyl chains are in a disordered and completely melted structure [1,2]. Our observation revealed that with an increase in alliin concentration, the frequency of the CH_2 antisymmetric stretching band decreased, leading to an enhancement in the trans configuration and order of the system in both the gel and liquid crystal phases.

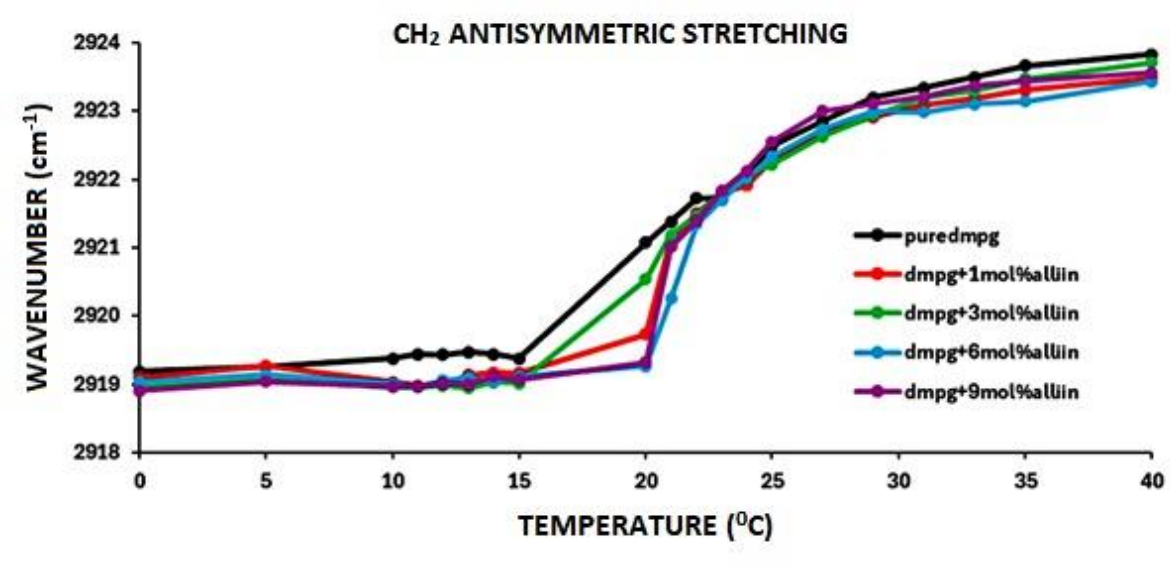

Figure 2. The frequency changes of CH_2 antisymmetric stretching modes of DMPG model membranes as a function of temperature, both in the presence and absence of varying doses of alliin.

Figure 3 displayed the variations in the bandwidth of CH_2 antisymmetric stretching in DMPG model membranes resulting from the introduction of alliin at varying concentrations, depending on the temperature. The bandwidth parameter provides information regarding the fluidity of the membrane, namely the movement of lipid acyl chains. The relationship between bandwidth and fluidity in a system is such that as bandwidth increases, system fluidity also increases. Conversely, as bandwidth values decrease, membrane fluidity decreases [2,3]. Adding alliin concentrations of 3, 6, and 9 mol% to DMPG MLVs demonstrated a rise in membrane fluidity in both the gel and liquid crystal phases. The inclusion of 1 mol% alliin concentration resulted in a notable reduction in membrane fluidity in both the gel and liquid crystal phases, thereby stabilizing the system (Figure 3).

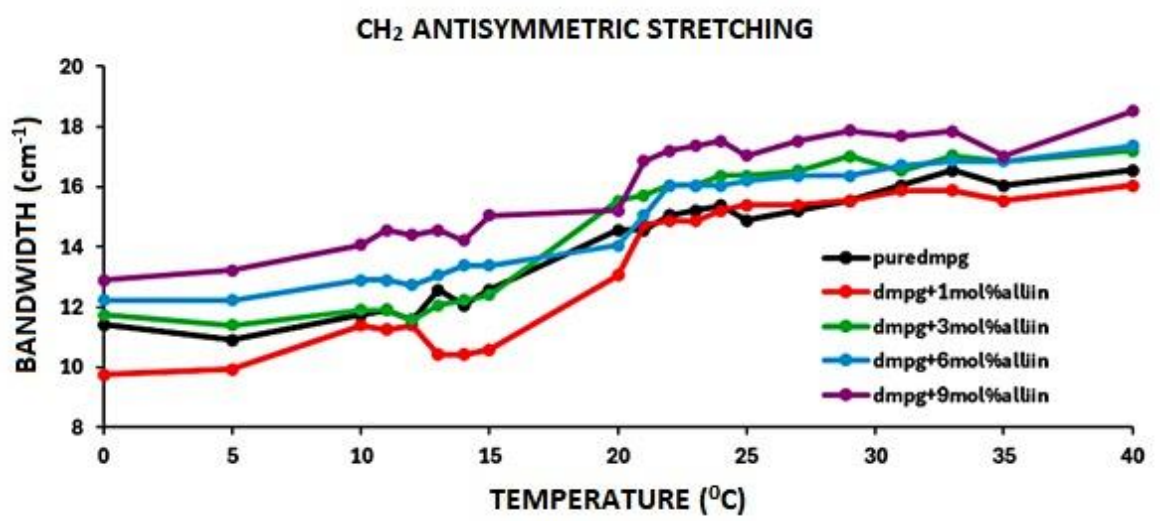

Figure 3. Temperature-dependent changes of the bandwidth of CH_2 antisymmetric stretching modes of DMPG model membranes in the presence and absence of varying concentrations of alliin.

Figure 4 demonstrated the variation in the frequency of the C=O stretching band as a function of temperature. Our study yielded contrasting outcomes about the alteration in the frequency of the C=O stretching band between the gel and liquid crystalline phases. While the presence of alliin in the gel phase resulted in a decrease in frequency, it had the opposite effect in the liquid crystal phase, causing an increase in frequency (Figure 4). This suggested that during the gel phase, alliin formed a robust hydrogen bond with the glycerol backbone near the head group of DMPG phospholipids in the interfacial area. In the liquid crystal phase, it revealed the presence of unbound carbonyl groups at the system [22].

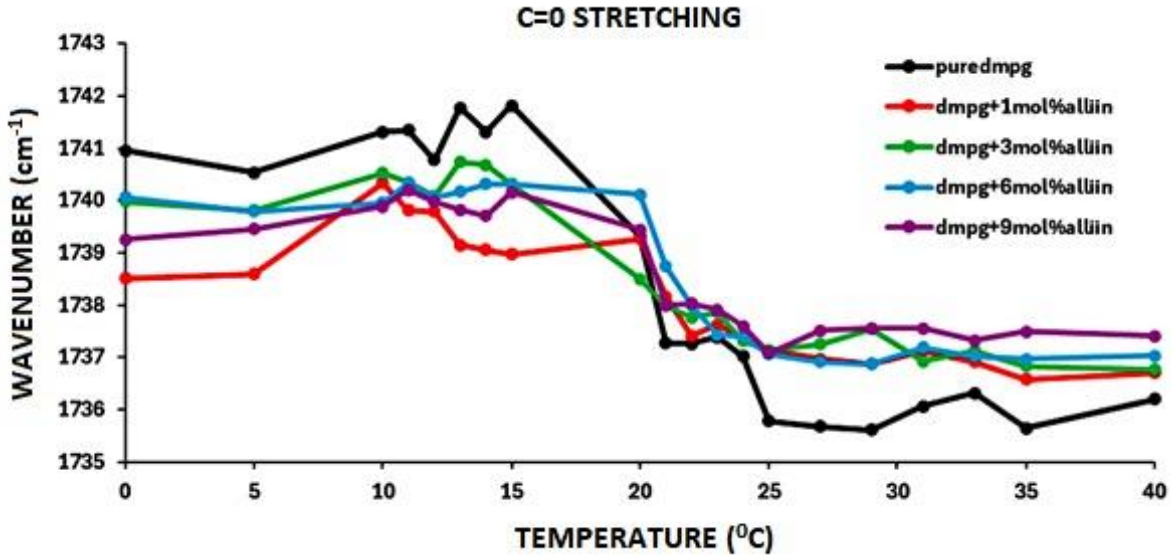

Figure 4. Changes of the frequency of C=O stretching modes of DMPG model membranes as a function of temperature, both in the presence and absence of low and high doses of alliin.

Figure 5 illustrated the change in frequency of the PO_2^- antisymmetric stretching band for DMPG MLVs both with and without varied amounts of alliin as a function of temperature. As depicted

in the diagram (Figure 5), the frequency decreased when alliin was added at both low and high concentrations. The impact was particularly significant in the gel phase, as it leaded to the reinforcement of the hydrogen bonding between the phosphate group of liposomes and the adjacent alliin or water molecules [1].

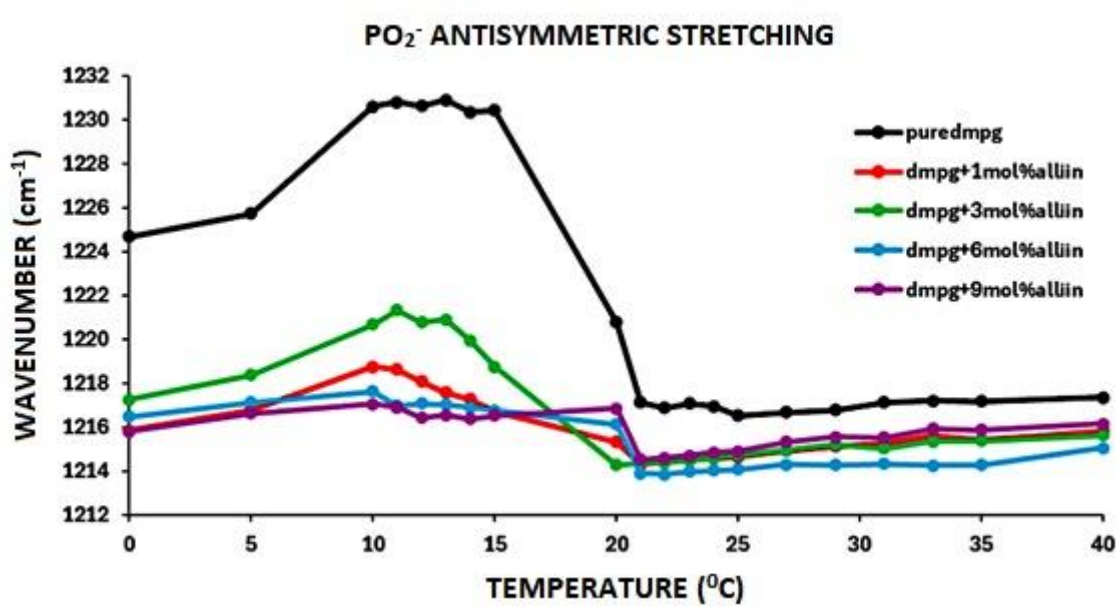

Figure 5. Changes of the frequency of PO_2^- stretching modes of DMPG model membranes as a function of temperature, both in the presence and absence of low and high doses of alliin.

4. Discussion

FTIR spectroscopy is based on the principle of measuring the vibration of chemical bonds by applying electromagnetic radiation at infrared wavelengths. The changes in the vibrations of chemical bonds in the infrared region and the absorption properties provide the formation of spectral peaks [23]. In the literature, the use of infrared spectroscopy to elucidate the therapeutic effect mechanism of drugs is increasing day by day [24]. If it is desired to investigate the changes caused by many bioactive compounds such as vitamins, minerals, hormones, etc. in model membranes, it is sufficient to examine the bands corresponding to different functional groups in FTIR spectra [1-3,20-22]. For example, the shift in the frequency value of the CH_2 antisymmetric stretching mode provides detailed information about the structure of the membrane, and the bandwidth provides detailed information about the mobility of lipid acyl chains [23].

In our study, analysis of CH_2 antisymmetric stretching vibrations showed that alliin reduced the acyl chain flexibility of DMPG liposomes in both gel and liquid crystalline phases, indicating an increase in the ordering of phospholipids. The chain order of membrane lipids has a significant effect on permeability. Increasing the order of lipid bilayers reduces agent diffusion [25]. Xiang and Anderson [26] studied the permeability of monocarboxylic acids in gel and liquid crystal phases of dipalmitoyl phosphatidylcholine (DPPC) model membranes with nuclear magnetic resonance (NMR) spectroscopy depending on lipid order and showed that membrane permeability depends on the cross-sectional area of the agent. In a study measuring water permeability using model membranes with different head groups, chain lengths and unsaturation, Mathai et al. [27] showed that membrane permeability is affected

by area/lipid and membrane thickness. In contrast, Gruhlke and colleagues [28] found that allicin increased membrane permeability, whereas Miron and colleagues [29] observed no effect.

An additional factor that must be scrutinized in order to comprehend the impact of a drug on membrane structure and function is the thickness parameter. Increased lipid order was experimentally associated with greater membrane thickness in a study by Kučerka et al. [30], in which cholesterol-model membrane interactions were examined by neutron diffraction methods. In addition, Balakrishnan and Kenworthy [31] observed that peroxidation, one of the properties that changes the structure and proper functioning of lipids, also leads to a decrease in the thickness of the bilayer and an increase in membrane permeability. According to our results, the antioxidant effects of alliin, which are widely commented in the literature [32-34], can be attributed to its ability to defend against lipid peroxidation, which may lead to membrane thickening and a decrease in permeability.

The fluidity of model membranes can be modified by the lipid composition and consequently alter the functional properties of the membrane [35]. We reported that the administration of alliin at varying doses increased the dynamics of negatively charged DMPG model membranes. This result is consistent with the results of our study conducted in our laboratory in 2017. We found that alliin also increased the mobility of zwitterionic DMPC model membranes [20]. Unexpectedly, the fluidity of the negatively charged DMPG membrane was reduced when 1 mol% alliin was introduced. This phenomenon could be attributed to the hydrophobic interactions occurring between alliin molecules and the acyl chains of DMPG MLVs. Consistent with our findings, a study employing electron paramagnetic resonance spectroscopy discovered that garlic extract caused a rise in the pliancy of red blood cell membranes [36]. A study conducted in 2008 by Tsuchiya et al. [37] demonstrated that sulphur compounds present in garlic caused an increase in fluidity and thickness of DPPC and DPPC: cholesterol MLVs. Pinilla et al. [38] found that the presence of phosphatidylcholine, oleic acid, and garlic extract resulted in an elevation of membrane rigidity under heat exposure.

Another factor that decreases the flexibility of the DMPG model membrane could be linked to the development of new hydrogen bonds or the reinforcement of existing hydrogen bonds between the hydroxyl groups of alliin and the carbonyl groups of DMPG [39]. Examining the C=O and PO_2^- functional groups allowed us to observe the changes that alliin produces in the interface and polar head group region of DMPG MLVs. Based on our findings, we observed that different concentrations of alliin in the gel phase resulted in an increase in the number of H-bonded carbonyls. However, in the liquid crystal phase, the C=O groups remained in their free form. The decrease in wavenumber values of the PO_2^- antisymmetric stretching band, upon the addition of alliin, suggests an increase in H-bond interactions between the phosphate group of DMPG and the surrounding alliin or water molecules [20].

Model membranes consist of lipids with diverse head groups and alkane chains. DMPG, an essential constituent of biological membranes, has a negative charge at the phosphate group. Consequently, the characteristics of the DMPG bilayer may be influenced by the counterion present [40]. One of the intermolecular attractive forces is the ion-dipole interaction between a charged ion and polar molecules, which can be attributed to the molecular interaction between alliin and DMPG. Consistent with our expectations, our research confirms that the charge status of the phospholipid head group determines the action mechanism of alliin with model membranes. Repulsion of negative charges in the head group of DMPG MLVs may have caused the acyl chains to interact less frequently, which explains this situation [3]. In addition, our agent is thought to localize in the DMPG lipid membrane's more polar or near-polar areas [22].

Within the scope of this research, we aimed to determine the biophysical effects of alliin, a bioactive component of garlic, on negatively charged DMPG liposomes using FTIR spectroscopy and to offer an alternative to the therapeutic mechanism of action. We examined the interactions of alliin with DMPG MLVs through structural and functional parameters and compared them with our previous

study. We showed that it may be possible to alter the membrane response and sensitivity of alliin depending on the charge difference in the head group region of the phospholipids. Given the variation in lipid content across different parts in the human body, comprehending medication-lipid interaction can aid in assessing the potential impacts of the medicine on actual biological systems. This work aims to biophysically analyse the molecular alterations induced by medicines in model membranes, with the goal of providing insights about the impact of garlic on biological systems.

Ethical Statement

The authors declare that this study does not require ethics committee approval.

Conflict of Interest

The authors have no conflicts of interest to declare.

Author Contributions

Concept: İ.S., Literature Review: N.E.O., İ.S., Design: N.E.O., İ.S., Data acquisition: N.E.O., B.K.T., Analysis and interpretation: N.E.O., B.K.T., Writing manuscript: N.E.O., Critical revision of manuscript: N.E.O., İ.S.

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