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Investigation on the interactions between pirarubicin and phospholipids

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ABSTRACT

Differential scanning calorimetry (DSC), Fourier transform infrared (FTIR) spectroscopy and quantum calculation based on molecular modeling were applied to investigate the interaction between pirarubicin (THP), an anthracycline antibiotic frequently used in chemotherapy, and zwitterionic distearoylphosphatidylcholine (DSPC) or anionic distearoylphosphatidylglycerol (DSPG). DSC and FTIR studies suggested that DSPG bilayers were less perturbed by THP than those of DSPC, and this might be due to the strong interactions between NH_3^+ of THP and the phosphate (PO_2^-) group in the polar head of DSPG, which limit the further access of THP into its bilayers. Quantum calculation results based on molecular modeling could further confirm the DSC and FTIR conclusions. Meanwhile, it could well translate the calorimetric and spectroscopic phenomena into the underlying physical knowledge. Interactions between THP and phospholipids can play a critical role in the liposomal drug delivery system, especially in the safety mechanism elucidation and rational formulation design.

1. Introduction

Anthracycline-based antibiotics such as doxorubicin, pirarubicin and daunorubicin have gained widespread applications in the cancer chemotherapy. Pirarubicin (THP, structure shown in Fig. 1) is a commonly used anthracycline against several solid tumors, acute leukemia and malignant lymphoma [1]. When THP crosses the cellular membrane by passive diffusion, it can intercalate into the base pairs of DNA, thus inhibiting the replication process of cells [2]. However, severe clinical side effects, such as cardiotoxicity and myelosuppression, need more concern, and the prevailing liposomal drug delivery system can provide an alternative solution.

Side effects of doxorubicin, whose structure is similar to THP, such as cardiotoxicity can be greatly reduced when encapsulated into the liposome. Meanwhile, it is interesting to find that interaction between doxorubicin and lipids can inhibit the formation of doxorubicin and cardiolipin complex (which might play a role in the cardiotoxicity) [3]. Therefore, interactions between anthracycline and phospholipids have profound influence on the safety mechanism elucidation of anthracycline liposomes. Furthermore, it can determine the partitioning, allocation, orientation and conformation of exogenous substance in the bilayers, thus playing an important role in the liposomal drug delivery system [4–6].

Up to now, only a few data are available to investigate the interaction of anthracycline with phospholipids from the perspectives of thermotropic phase behavior of phospholipids [7,8]. There are no systematic investigations between anthracycline and phospholipids from the overall techniques of differential scanning calorimetry (DSC) and Fourier transform infrared (FTIR) spectroscopy, which can well probe the interaction between drug and phospholipids [9,10]. Moreover, there are no access to the quantum calculation results about the interaction between anthracycline and phospholipids.

Various studies concerning the interaction of anthracycline with model or cellular membranes have already been performed. Interactions of anthracycline with monolayers of unsaturated zwitterionic palmitoyloleoylphosphatidylcholine (POPC) and anionic dipalmitoylphosphatidic acid (DPPA) (POPC–DPPA 80–20 mol%) had been examined by means of surface-enhanced resonance Raman scattering (SERRS) and surface pressure studies [11]. Fluorescence anisotropy, ATR–FTIR and 31P NMR techniques were combined to investigate the interactions between ciprofloxacin with dipalmitoylphosphatidylcholine and dipalmitoylphosphatidylglycerol [12]. Recently, an array of theoretical approaches based on the molecular mechanics, such as molecular dynamics (MD), Monte Carlo simulations and *ab initio* quantum chemical calculations were performed to study the interactions between drug and phospholipids from the perspective of molecular mechanism [13].

In this paper, THP, an anthracycline commonly used in chemotherapy, was selected as a drug model. To mimic the bulk lipids in eukaryotic and bacterial membranes in vivo compartment [14,15], zwitterionic distearoylphosphatidylcholine (DSPC) and anionic

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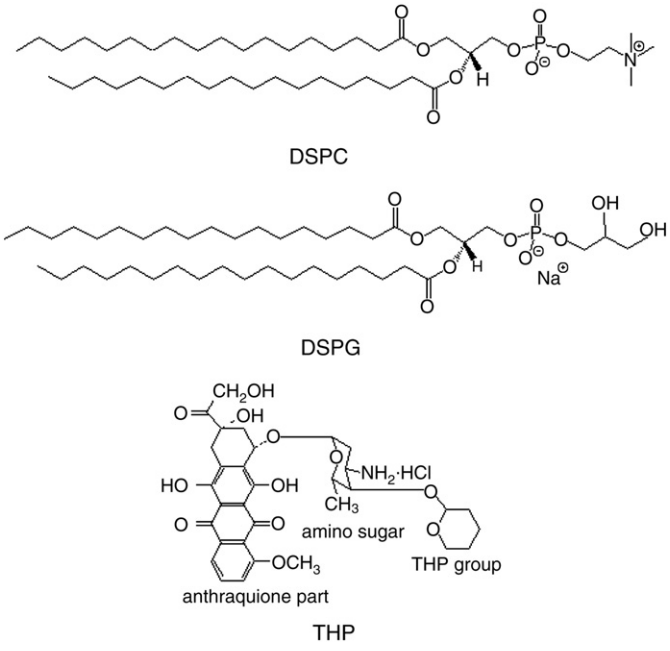

Fig. 1. Structural formulae of DSPC, DSPG and THP.

distearoylphosphatidylglycerol (DSPG) (structure shown in Fig. 1), which are frequently used in the liposomal drug delivery system, were used to form model membranes. Our efforts were mainly concentrated on the following three aspects: (i) To compare the thermotropic phase behaviors of DSPC and DSPG in the presence of a series of different amounts of THP using DSC technique. (ii) To observe the changes of acyl chain conformations and characteristic PO_2^- bands in the polar heads of DSPC and DSPG in the presence and absence of THP using FTIR spectroscopy, respectively. (iii) To investigate the interaction between NH_3^+ of THP and PO_2^- in the polar heads of lipids through quantum calculations based on molecular modeling.

2. Materials and methods

2.1. Materials

Distearoylphosphatidylcholine (DSPC, 99.5% purity), distearoylphosphatidylglycerol (DSPG, at least 98.5% purity) were purchased from the Lipoid GmbH (Ludwigshafen, Germany). Pirarubicin hydrochloride (THP, 99.5% purity) was obtained from Shenzhen Olympic Star Pharmaceutical Co., Ltd. (Guangdong, China). These materials were used without further purification. All organic solvents were of analytical grade. Double deionized water with a resistivity of about 18.2 MΩ·cm and D_2O (99.9% of deuterium, from Cambridge Isotopes) were used for the preparation of the lipid dispersions.

2.2. Methods

2.2.1. Sample preparation

The pure DSPC, DSPG, mixed THP/DSPC and THP/DSPG multilamellar vesicles were prepared by the traditional Bangham method [16]. According to the nature of the experiment (DSC and FTIR spectroscopy), the appropriate amounts of DSPC and DSPG were weighed in small glass tubes and then diluted in chloroform. And a series of adequate volume of THP stock solutions were added to obtain the mixtures of required molar ratios.

Then, the sample tubes were placed under vacuum at room temperature for about 2 days in order to eliminate any traces of solvent. Then, a film of fully dehydrated lipids was formed on the wall of the glass tubes. Finally, sample was obtained by adding the appropriate volume of deionized H_2O with a resistivity of about 18.2 MΩ·cm (1/6, w/w) for DSC measurements. For FTIR experiments, appropriate amount of H_2O and D_2O (1/6, w/w) were used, respectively. To well capture the frequency changes of $\nu_s CH_2$ of acyl chains of lipids, D_2O was used for the lipids dispersions preparation, and all samples were scanned three to four times until identical thermograms were acquired.

2.2.2. DSC measurements

The calorimetric data were obtained with a differential scanning calorimeter DSC821^e equipped with the high sensitivity sensor HSS7 (Mettler-Toledo Co., Ltd., Switzerland). The scan rate is 1 °C/min.

2.2.3. FTIR spectroscopic studies

FTIR spectra were carried out using a Nicolet 5700 Fourier transform infrared spectrometer equipped with a DTGS detector. The scan range is 4000–900 cm^{-1}, with a spectral resolution of 2 cm^{-1} and a zero filling factor of 2. The frequency precise is better than 0.1 cm^{-1}. Samples were coated onto the inner surfaces of a pair of CaF_2 windows, which were mounted on a Linkam heating–cooling stage for temperature control. The exact temperature of the sample in two CaF_2 windows was measured by a Pt100 standard resistor connected with a digital multimeter. A spacer with the thickness about 10 μm was used in the experiment. The equilibration time at each temperature is 5 min, and the heating rate of sample is 1 °C/min. For each spectrum, 16 scans were required and the spectra were recorded every 30 s.

2.2.4. Quantum calculation of the interaction energy between NH_3^+ of THP and the phosphate (PO_2^-) group in the polar heads of DSPC or DSPG using Gaussian 03W

Gaussian 03W had been successfully used in the molecular modeling between lipids and exogenous substances [17]. To well comprehend the DSC and FTIR results, Gaussian 03W was used in the molecular modeling between THP and the polar heads of phospholipids. In this experiment, AM1 algorithm, a frequently used semi-empirical method [18,19], had been exploited. Although its precision is limited, the rapid calculation makes it suitable to the large complex structure in the molecular modeling.

To simplify the quantum calculation process, we use EthylPC (which means a neutral head group with two ethyl chains) and EthylPG (which means a negative charged head group with two ethyl chains) instead of DSPC and DSPG, respectively. Meanwhile, water, as an important medium in our experiments, was not taken into considerations. As our main objective of quantum calculation in this paper is to shed light on the interaction between NH_3^+ of THP and PO_2^- in the heads of phospholipids, the interaction groups considered in the molecular modeling are only the NH_3^+ of THP and PO_2^- of the polar heads of phospholipids. Interaction energy (E_{int}) is a thermodynamic measure [17], which can reflect the stability difference among various complexes. The calculation of E_{int} is based on the following equation:

$$A + B \rightarrow AB \tag{1}$$

where A is THP, B is EthylPC or EthylPG and AB is their complex. E_{int} can be described by:

$$E_{int} = E_{AB} - E_A - E_B. \tag{2}$$

The E_{int} value of the interaction between THP and EthylPC or EthylPG is a sensitive index in describing the association degree between drug and the polar heads of phospholipids and it can also indicate the thermodynamic stability of complex.

All calculations were performed in Gaussian 03W program and AM1 algorithm was exploited. The geometrical structure of EthylPC, EthylPG and THP was optimized according to the minimum energy principle. Then, the optimized conformations of THP and EthylPC or

EthylPG with a 1:1 molar ratio were used to perform molecular modeling. And then a different set of complexes for EthylPC–THP or EthylPG–THP were obtained and yielded different E_{int} values. Finally, the most stable conformations were chosen based on the minimum energy principle.

3. Results and discussion

3.1. DSC studies

The pre and main transitions are often observed in PC or PG lipids. The pre-transition, which corresponds to the conversion of a lamellar gel phase to a rippled gel phase, is mainly related to the polar region of phospholipids; while the main transition reflects the change from a gel phase to a liquid crystal phase. It is also suggested that the main transition process is closely related to the acyl chains of phospholipids bilayers, which can probe the interaction between the acyl chains of phospholipids and exogenous substances. Moreover, the width of its DSC peak is an index of the cooperativity of this conversion: the narrower the peak, the higher the cooperativity [20,21].

3.1.1. The phase behaviors of DSPC/THP and DSPG/THP dispersions

The DSC heating scans of DSPC and DSPG dispersions containing different concentrations of THP were presented in Figs. 2 and 3, respectively. As observed in Fig. 2, pure DSPC gave two endothermic peaks: individually centered at about 52.6 and 55.8 °C, corresponding to the pre and main transition temperatures; while in the heating scans of DSPG (Fig. 3), the main transition temperature (T_m) was around 55.3 °C. These values are in accordance with the literatures [22–25].

With the increasing amounts of THP, the peak width of the main transition of DSPC gradually broadened and T_m markedly shifted to lower temperature, which suggests that THP has a significant effect on the acyl chains of DSPC bilayers and its presence decreases the transition cooperativity of lipid acyl chains. The lowered temperature of main transition process of DSPC indicates that the incorporation of THP is more favorable to the formation of a disordered and loose state of the acyl chains. No obvious changes in the main phase behavior of DSPG were observed at the low concentrations of THP ranges from 0 to 15.0 mol%, whereas it is still affected at the high concentrations of THP,

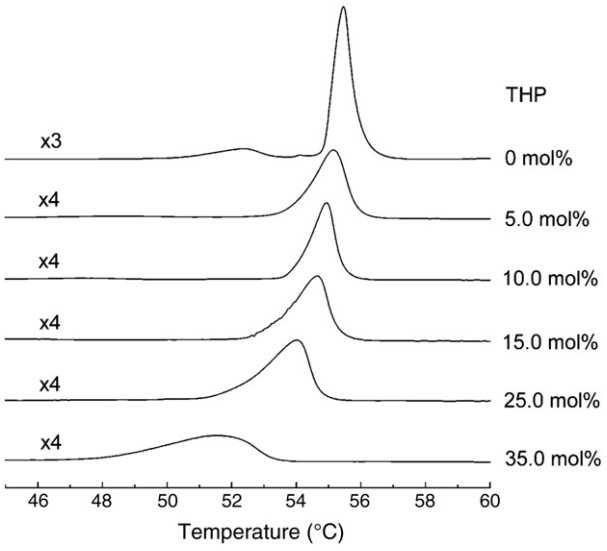

Fig. 2. DSC thermograms illustrating the effect of THP on the gel/liquid-crystalline phase transition of DSPC. The thermograms shown were required at the THP concentrations (mol%) indicated, and all had been normalized against the mass of DSPC used. *Y*-axis scaling factors were indicated on the left hand side of each thermogram.

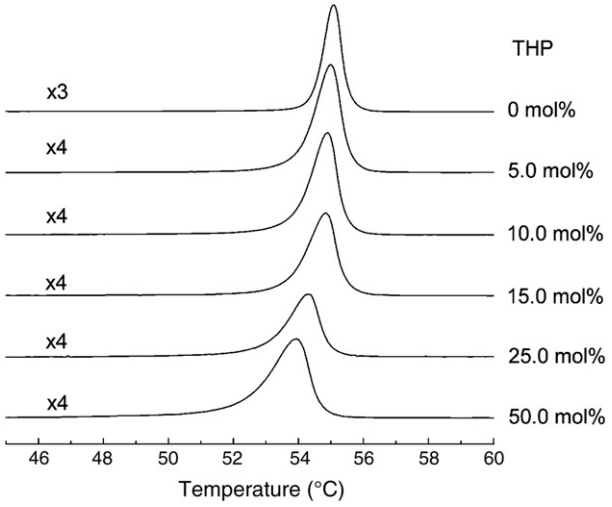

Fig. 3. DSC thermograms illustrating the effect of THP on the gel/liquid-crystalline phase transition of DSPG. The thermograms shown were required at the THP concentrations (mol%) indicated, and all had been normalized against the mass of DSPG used. *Y*-axis scaling factors were indicated on the left hand side of each thermogram.

and this suggests that the acyl chains of DSPG bilayers cannot be easily disturbed by THP.

The calorimetric parameters of main transition process, such as T_p (temperature of the peak maximum), T_{onset} (temperature at which the thermal effect starts), T_m (temperature at which the transition is half completed), are the most sensitive measures of the physical properties of phospholipids bilayers. And their changes can be attributed to the packing of exogenous molecules within the hydrophobic interior of phospholipids array [6,26,27]. In this paper, T_p and T_{onset} were selected as the characteristic parameters, and the corresponding curves of T_p and T_{onset} versus THP molar ratio were plotted in Figs. 4 and 5, respectively. The calorimetric parameter curves indicated that THP exhibited a more pronounced effect on the main phase behavior of DSPC by substantially reducing T_p and T_{onset}. These significant phenomena can further support the conclusion that DSPC bilayers can be more easily perturbed by THP than those of DSPG.

It is evidenced that THP has different effects on the thermotropic phase behavior of DSPC and DSPG, which can be explained as a consequence of the molecular shapes of phospholipids differed markedly in their head groups. Due to the electrostatic repulsion, the effective area of DSPG head group is considered larger to the predicted by its geometrical size, which almost matches its

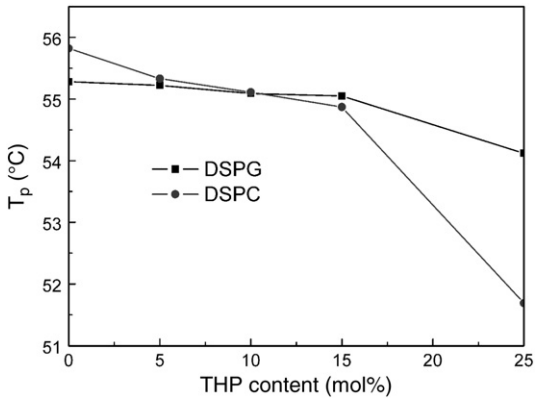

Fig. 4. Effects of increasing THP concentration on the T_p of DSPC and DSPG. Symbols of (–■–) and (–●–) represented the data from the DSPC–THP and DSPG–THP samples, respectively.

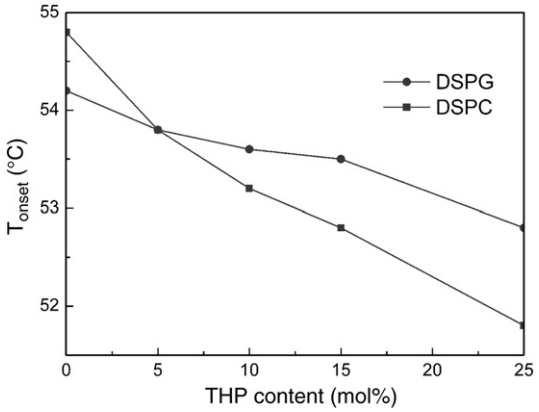

Fig. 5. Effects of increasing THP concentration on the T_{onset} of the main phase transition of DSPC and DSPG. Symbols of (–■–) and (–●–) represented the data from the THP-containing DSPG and DSPC samples, respectively.

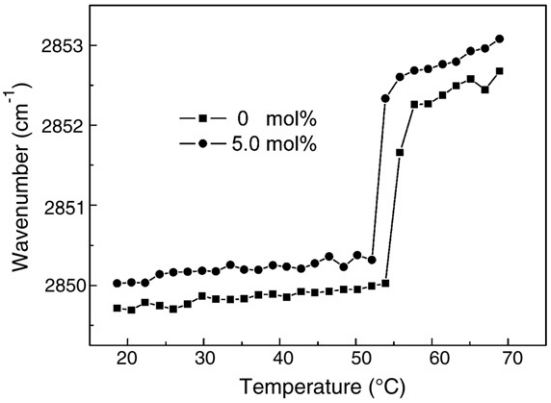

Fig. 6. Temperature-dependant changes in the frequency of the CH_2 symmetric stretching band from FTIR spectra, and it was exhibited by THP-free (–■–) and THP-containing (–●–) DSPC bilayers in D_2O, respectively. The molar ratio of THP in the mixtures was 5%.

hydrophobic cross-section area; while the head group area is much smaller for DSPC with the identical acyl chains [14,28]. Larger head can induce larger contact area, which might not be favorable to the access of THP into the DSPG bilayers.

THP can be incorporated into the lipid bilayers, which might be closely related to its unique structure: an anthraquinone group ("chromophore"), a hydrophilic aminosugar which can be easily protonated and positively charged, and a hydrophobic tetrahydropyranyl group, thus THP is an amphiphilic molecule and is soluble in water as well as in polar organic solvents. Lipid bilayers have hydrophobic acyl chains as well as the polar head group. Meanwhile, it has been noted in the literature [11] that there are electrostatic interaction and hydrophobic interaction between THP and phospholipids: the chromophore and tetrahydropyranyl parts contact with the acyl chains, whereas the aminosugar part interacts with the polar head group of phospholipids. Therefore, it can be inserted into the lipid bilayers, though the THP molecule is relatively large.

3.2. FTIR studies

DSC is indeed a powerful technique in investigating the lipids phase behavior. Besides, another proven experimental method in lipid chemistry is infrared spectroscopy. FTIR can be used to monitor subtle changes in the structure and function of the lipid assemblies by analyzing the frequency or the bandwidth changes of the different vibration modes representing the acyl chains, the interfacial region and the head group region of lipid molecules. Various kinds of information can be derived from these bands. For instance, the frequency shifts in different regions of corresponding bands can be used to extract information about various physicochemical processes taking place in the systems [29,30].

In the CH_2 stretching region of the Infrared spectrum (2800–3000 cm^{-1}), the hydrocarbon chains which contain *gauche* conformers absorb infrared radiation at higher frequencies than those that contain all-*trans* conformers. Thus, increase in hydrocarbon chain conformational disorder is accompanied by increases in the frequencies of infrared absorption bands arising from the symmetric and asymmetric stretching vibrations of lipid hydrocarbon chains. This property can thus be used as a probe of changes in lipid hydrocarbon chain conformational disorder and has frequently been used for the detection and/or monitoring of lipid hydrocarbon chain-melting phase transitions [31,32]. The absorption bands of ester C=O are sensitive to changes in the polarity of their local environments and are influenced by hydrogen bonding and other interactions. Therefore, changes in the contours of the ester C=O absorption band can often be interpreted in terms of structural and/or hydration changes of the bilayer polar/apolar interface [33]. The asymmetric stretching band of PO_2^- has been shown useful to monitor the hydration state of the polar head group of phospholipids. For instance, a "free" PO_2^- is characterized by a frequency of about 1240 cm^{-1}, whereas a frequency of about 1220 cm^{-1} characterizes a fully hydrated PO_2^- group [34,35].

In this paper, FTIR was chosen to further investigate the effects of THP on the organization of host lipids: DSPC and DSPG. For both lipids, the presence of THP made no discernable changes in the contour of the C=O stretching band at ~1730 cm^{-1}, indicating that THP had no obvious effects on the hydration degree of the glycerol backbone for DSPC or DSPG (data not shown). Therefore, we mainly focused on the following two aspects: the acyl chains of bilayers and PO_2^- in the polar heads of phospholipids.

3.2.1. The effects of THP on the acyl chain of DSPC or DSPG

In the experiments, effects of THP on the acyl chain of phospholipids were investigated by monitoring the frequency changes of CH_2 symmetric stretching band located at around 2850.0 cm^{-1} over the temperatures. In order to determine accurately the peak maxima, a Gaussian line was fitted on the upper half of the maximum position for the CH_2 symmetric stretching band of DSPC or DSPG. And the results were plotted as a function of temperature (shown in Figs. 6 and 7).

In the frequency curve of the CH_2 symmetric stretching band for DSPC (Fig. 6), it appeared that THP caused a slight frequency increase both in the gel and liquid-crystalline phases in the presence of THP,

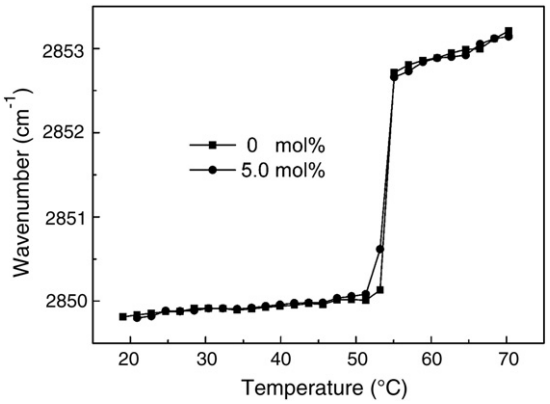

Fig. 7. Temperature-dependant changes in the frequency of the CH_2 symmetric stretching band from FTIR spectra, and it was exhibited by THP-free (–■–) and THP-containing (–●–) DSPG bilayers in D_2O, respectively. The molar ratio of THP in the mixtures was 5%.

though the shifts of the CH_2 stretching vibrational bands were all about 0.3 cm^{-1}. We might consider it as an error, if the shift of the CH_2 stretching vibrational band at one temperature is ~0.3 cm^{-1}; actually, the precision of frequency is better than 0.1 cm^{-1} in the FTIR experiments. However, the wavenumbers of the v_sCH_2 of DSPC in the presence of THP at all the temperatures were all higher than those of DSPC, and the shifts were all around 0.3 cm^{-1}. These phenomena should not be ignored, for they can truly reflect that THP has disturbed the conformation of the acyl chains but the disturbing degree is somewhat minor. Meanwhile, in the case of DSPG/THP (Fig. 7), the presence of THP caused no evident frequency changes, for its frequency curve was in good agreement with that of pure DSPG. This implies that the conformation of acyl chains cannot be easily disturbed by THP, which has been evidenced in the DSC section. This discrepancy can further imply that THP has a relatively more pronounced effect on the acyl chain of the DSPC bilayers than those of DSPG bilayers.

3.2.2. The effects of THP on the phosphate (PO_2^-) group in the polar head of DSPC or DSPG

It was observed that the wavenumber of PO_2^- band of pure DSPC was about 1215.0 cm^{-1}, while it was about 1235.0 cm^{-1} for pure DSPG (Figs. 8 and 9). This discrepancy might be ascribed to the unique polar heads of DSPC and DSPG. As the intermolecular hydrogen bonding interaction is impossible for DSPC, its PO_2^- in the polar head can only form hydrogen bonds with water; whereas the PO_2^- of DSPG might form hydrogen bonds with the OH group in the glycerol as well as water, thus reducing the hydration degree and making the wavenumber of PO_2^- in DSPG shift to a higher value.

However, it could also be noted that the shift of the wavenumber for the PO_2^- band went into different directions for DSPC and DSPG (Figs. 8 and 9). For DSPC, the presence of THP made the PO_2^- band shift to higher wavenumber, whereas THP made the wavenumber of the PO_2^- band of DSPG shift to lower values. This discrepancy should be attributed to the change of hydration degree induced by THP. Strong interactions (electrostatic interaction and hydrogen bonding interaction) between PO_2^- of phospholipids and NH_3^+ of THP should not be ignored, as they might play a vital part in the interactions of THP and phospholipids. The existence of $N(CH_3)_3^+$ group in the polar head of DSPC might prevent NH_3^+ of THP getting close to the PO_2^-, due to the electrostatic repulsive force between $N(CH_3)_3^+$ of DSPC and NH_3^+ of THP, thus weakening the interactions between PO_2^- and NH_3^+ of THP. Therefore, the wavenumber of the PO_2^- band of DSPC shifted to higher values. In the case of DSPG/THP, there might also be strong interaction between the OH group in the glycerol moiety of its polar head and

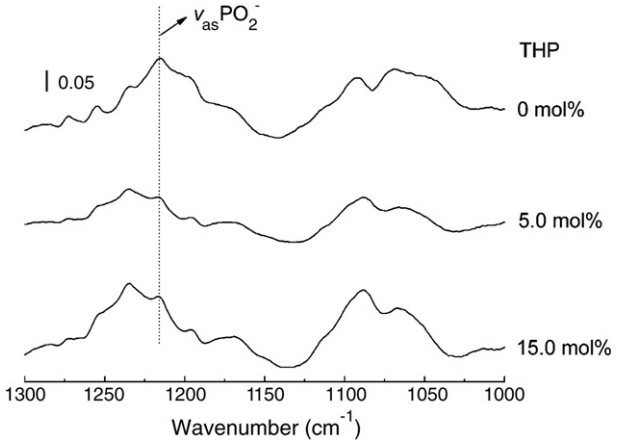

Fig. 8. FTIR absorbance spectra of the PO_2^- stretching regions for DSPC in H_2O in the absence and in the presence of different amounts of THP at room temperature, and the composition was expressed in terms of molar fraction of THP in the phospholipids mixtures. The scale of the *Y*-axis was 0.05.

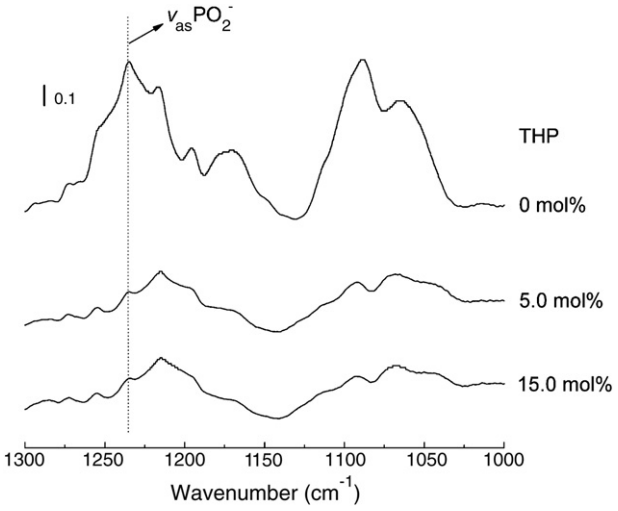

Fig. 9. FTIR absorbance spectra of the PO_2^- stretching regions for DSPG in H_2O in the absence and in the presence of different amounts of THP at room temperature. The composition was expressed in terms of molar fraction of THP in the phospholipids mixtures. The scale of the *Y*-axis was 0.1.

NH_3^+ of THP, which can make the NH_3^+ of THP get further close to its PO_2^- band, thus enhancing the interactions between PO_2^- and NH_3^+ of THP and making wavenumber of the PO_2^- band shift to lower values.

3.3. Quantum calculation based on the molecular simulation of interactions between NH_3^+ of THP and the phosphate (PO_2^-) group in the polar head of EthylPC or EthylPG

FTIR was indeed appropriate for studying the interaction between exogenous molecules and phospholipids. However, it will be very instructive for translating IR-spectroscopic data into a basic understanding of the underlying physical background. And an array of theoretical methods based on the molecular mechanics, such as MD, Monte Carlo simulations and *ab initio* quantum chemical calculations have often been exploited [13,36].

In this paper, molecular modeling between NH_3^+ of THP and PO_2^- in the polar heads of EthylPC and EthylPG were performed in the Gaussian 03W program. The optimized energy values (listed in Table 1) imply that the EthylPG–THP complex is more thermodynamic stable than EthylPC–THP complex. And the calculation results (listed in Table 1) showed that E_{int} of the interaction between EthylPG and THP was −78.2 kcal/mol, whereas this value was −36.2 kcal/mol for the interaction between EthylPC and THP. This directly suggests the NH_3^+ of THP will tend to associate with PO_2^- in the polar head of EthylPG. These phenomena should be ascribed to the consequence of structure discrepancy between EthylPC and EthylPG. In the case of EthylPG, the existence of glycerol moiety plays an indispensable role, for its OH group might also form hydrogen bonds with the NH_3^+ of THP, which will make the NH_3^+ get further close to the its polar head, thus promoting the interactions between PO_2^- and NH_3^+. These two catalogues of interactions might be enhanced by each other and thus making the whole complex more stable. While for EthylPC, its polar head has $N(CH_3)_3^+$ as well as PO_2^-, and this molecule should be very

Table 1
Minimum energy of THP, phospholipids and E_{int} of the stable complex of THP and EthylPC or EthylPG based on the quantum calculations.

THP	EthylPC	EthylPG	EthylPC–THP	EthylPG–THP	EthylPC–THP (E_{int})	EthylPC–THP (E_{int})
kcal/mol	kcal/mol	kcal/mol	kcal/mol	kcal/mol	kcal/mol	kcal/mol
−316.5	−384.0	−586.2	−736.7	−980.9	−36.2	−78.2

stable in terms of charge balance. Due to the electrostatic repulsive force between $N(CH_3)_3^+$ of EthylPC and NH_3^+ of THP, $N(CH_3)_3^+$ might prevent NH_3^+ of THP getting close to the PO_2^-, thus weakening the interactions between PO_2^- and NH_3^+ of THP. Meanwhile, there might also be electrostatic interaction between $N(CH_3)_3^+$ and PO_2^- for EthylPC, which will not be favorable to the interactions between PO_2^- of Ethyl PC and NH_3^+ of THP. Here, our quantum calculation results based on molecular modeling cannot only confirm the FTIR results, but only highlight that the influence of different polar head structures on the interactions between PO_2^- of phospholipids and NH_3^+ of THP. And the quantum calculation results well comprehend the calorimetric and spectroscopic phenomena from the underlying physical knowledge.

4. Conclusions

Based on the quantum calculation, calorimetric and spectroscopic studies, we could propose that THP can be easily incorporated into the DSPC bilayers, thus affecting its thermotropic phase behavior and IR spectra. The strong interactions between NH_3^+ of THP and PO_2^- in the polar head of DSPG limit the access of THP into the DSPG bilayers, thus the acyl chain order of DSPG is not easily disturbed and the mobility of bilayers is unchanged. The conclusions that anthracyclines would remain adsorbed on the polar head groups of the phospholipids in the presence of anionic DPPA and form a screen that limits a deeper penetration of other anthracycline molecules also support our hypothesis [37,38].

In the present study, we have investigated in detail the interaction of THP with DSPC or DSPG, using two noninvasive techniques: DSC and FTIR. Quantum calculation, as a complementary tool, was shown to be useful. Since DSPC and DSPG are frequently used phospholipids in the liposomal drug delivery system, this research can provide comprehensive insights and guidance for the safety mechanism elucidation as well as rational formulation design in the THP liposome for chemotherapy in future.

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