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Interaction of dehydroepiandrosterone with phospholipid membranes: an infrared spectroscopy investigation

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Abstract

The interaction between dehydroepiandrosterone (DHEA) and its sulfate metabolite (DHEA-S) with deuterated dimirystoylphosphatidylcholine (DMPC-d_{54}) was investigated by FTIR spectroscopy. DHEA, as cholesterol, induces some conformational order in the liquid-crystalline phase of DMPC-d_{54}. Attenuated total reflectance (ATR) measurements performed on oriented DMPC-d_{54}/steroids samples have shown that in the gel phase, the acyl chains of DMPC-d_{54} become more normal to the bilayer surface in the presence of DHEA or cholesterol. On the other hand, DHEA-S increases the number of *gauche* conformers along the hydrocarbon chains of DMPC-d_{54}. No evidence for the presence of hydrogen bond was found between both steroids and the ^{13}C labeled carbonyl group of hydrated DMPC. © 1998 Elsevier Science B.V.

Keywords: Infrared spectroscopy; Orientation; Phospholipid; Steroid; Dehydroepiandrosterone; Cholesterol

1. Introduction

Dehydroepiandrosterone (DHEA) and its sulfate derivative (DHEA-S) are adrenal steroids precursors of sex steroids such as testosterone, dihydrotestosterone and estradiol. DHEA-S circulating concentration in adult men and women is higher than any other steroid except cholesterol [1]. Serum concentration of DHEA and DHEA-S decreases by about 60% in men from age 40 to 80 years [2]. Moreover, replacement doses of DHEA in men and women of advancing age have been reported to be beneficial [3]. Interestingly, the DHEA/cortisol ratio was found to be lower in female Alzheimer's disease patients compared to age matched female controls [4]. DHEA is also considered as a neurosteroid since it has been shown to be synthesized in the brain [5,6].

Cholesterol was shown to play an important role in membrane structure [7]. Since the structure of DHEA is similar to that of cholesterol (Scheme 1) and since the circulating concentration of DHEA-S is high, we hypothesized that similarly to cholesterol, these steroids may influence membrane structure, more specifically phospholipid organization. To our knowledge, there are no physico-chemical studies in the literature on the interaction of DHEA with lipid membranes.

The aim of the present study was to investigate, by infrared spectroscopy, whether phospholipids are one

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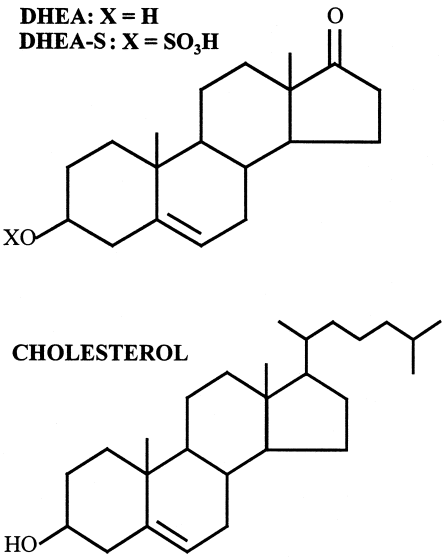

Scheme 1.

of the target sites of DHEA and DHEA-S action and to characterize the nature of the interactions that take place between the steroids and lipids in membranes. For that purpose, we have used as model membranes liposomes and supported multilayers of dimirystoylphosphatidylcholine (DMPC).

2. Materials and methods

2.1. Materials

All phospholipids used in this study were obtained from Avanti Polar Lipids (Alabaster, AL). Deuterated lipids were used to avoid spectral interferences between the C–H stretching vibrations of the steroids and those of the lipids acyl chains. For the same reason, DMPC with ^{13}C labeled ester groups (purity $> 99\%$) was used in order to distinguish the vibration of the ketone group of DHEA and DHEA-S from those of the phospholipid carbonyl groups. Cholesterol, DHEA and DHEA-S were purchased from Sigma (St. Louis, MO). Appropriate amounts of lipids and steroids (cholesterol or DHEA) stock solutions in chloroform were mixed and the solvent was evaporated under a stream of nitrogen gas, then dried overnight under vacuum to remove any trace of chloroform. The samples were dispersed in H_2O or D_2O with vigorous vortexing. The final concentration of lipids in water was 10% w/vol and the steroid/lipid molar ratio was 25%. To obtain complete hydration, the samples were heated at 20°C above the gel to liquid-crystalline phase transition temperature and quenched in liquid nitrogen at 77 K. This step was repeated thrice. The samples were vortexed after each thermal treatment cycle. The DHEA-S/lipid samples were prepared by dissolving the steroid in methanol and treated as described above. For measurements under anhydrous conditions (at room temperature), samples were dissolved in chloroform and spread on the internal reflection element as described below.

2.2. Infrared spectroscopy

2.2.1. Transmission measurements

20 μl of samples was spread on BaF_2 windows separated by a 12 μm mylar spacer. The temperature of the home-built sample holder was regulated thermoelectrically. Spectra were recorded with a Nicolet Magna 550 Fourier transform infrared spectrometer (Nicolet Instrument, Madison, WI). A narrow-band liquid nitrogen cooled Mercury–Cadmium–Telluride detector was used. For each spectrum, 250 scans were averaged at $2\,cm^{-1}$ resolution using Happ–Genzel apodization. Data treatment was performed using Spectra Calc software (Galactic Industries, Salem, NH). The frequency of the CD_2 stretching vibrations was determined as the gravity center at 80% of the height of each band [8]. The second derivative of the spectra was used in order to determine the frequency of the carbonyl and hydroxyl stretching bands because the components of these bands are not well resolved. Since data obtained using the second derivative procedure depend somewhat on the parameters used, the absorption maxima are not very accurate. The Fourier deconvolution technique was used to unravel the components of the $^{13}C{=}O$ band using a narrowing parameter γ of 2.11 and an apodization filter F of 0.31 [9].

2.2.2. Attenuated total internal reflectance (ATR) measurements

The ATR internal reflection element used was a germanium parallelogram ($50 \times 20 \times 2$ mm) cut at

45° angle. Before each experiment, the ATR crystal was cleaned by immersion in concentrated chromic acid for 15 min, rinsed with distilled water and then cleaned with a plasma cleaner (Harrick, NY). Oriented lipid films were obtained by spreading an aqueous solutions of the samples with a Teflon bar on one side of the ATR reflection plate until the solvent was completely evaporated. For measurements under anhydrous conditions, excess of solvent (chloroform) was removed overnight under vacuum. The polarization measurements were performed with a motorized polarizer (Specac, Orpington, UK) placed in front of the ATR unit (Harrick, NY). Dichroic ratios (R) were obtained from the peak height intensity of the bands measured with the incident radiation polarized parallel and perpendicular with respect to the normal of the ATR crystal. The order parameter $f(\theta)$ relating the chain axis and the normal to the ATR crystal was calculated using the formula:

$$f(\theta) = (3\cos^2\theta - 1)/2,$$

where θ is the angle between the chain axis and the bilayer normal. The angle between the transition moment of the CD_2 symmetric stretching vibration and the molecular chain was set at 90°. Since the measurements were made in the gel phase, the conformation of the hydrocarbon chains was considered as all *trans*. The angle θ was thus calculated assuming a narrow orientation distribution as described elsewhere [10]. Data acquisition and data analysis were performed as described above.

3. Results and discussion

3.1. CD_2 stretching modes region (2000–2300 cm^{-1})

This region is characterized by two strong absorption bands around 2193 and 2089 cm^{-1} due to the antisymmetric and symmetric CD_2 stretching vibrations, respectively. These modes are sensitive to temperature induced changes of the conformation of the acyl chains and to *trans-gauche* isomerization [11]. They are also slightly affected by interchain vibrational coupling and librotorsional motions [12]. Since both bands display qualitatively the same behavior, only the data relative to the 2089 cm^{-1} band will be presented here. The temperature dependence of the acyl chain CD_2 symmetric stretching vibration of DMPC-d_{54} with and without steroids are shown in Fig. 1. For both the gel and the liquid-crystalline phases, the frequency of the 2089 cm^{-1} band decreases in the presence of cholesterol and DHEA, while it increases for the sulfate derivative of DHEA. These results suggest that the presence of DHEA-S induces some conformational disorder along the acyl chain of DMPC-d_{54} whereas DHEA and cholesterol reduce the number of *gauche* conformers and thus improve the degree of ordering of the hydrocarbon part of DMPC-d_{54}. In addition, both DHEA and cholesterol considerably broaden the gel to liquid-crystalline phase transition of DMPC-d_{54} while DHEA-S has a much smaller effect. Finally, Fig. 1 demonstrates clearly that cholesterol is much more efficient than DHEA to order the liquid-crystalline phase of DMPC-d_{54}. The similar effect of DHEA and cholesterol on the thermotropic behavior of DMPC-d_{54} can partly be attributed to the common structural features of these molecules. The absence of the hydrocarbon chain at the 17-position is consistent with the fact that DHEA is less effective than cholesterol to inhibit the formation of *gauche* conformers. In their studies on sterol/DPPC interactions, Senak et

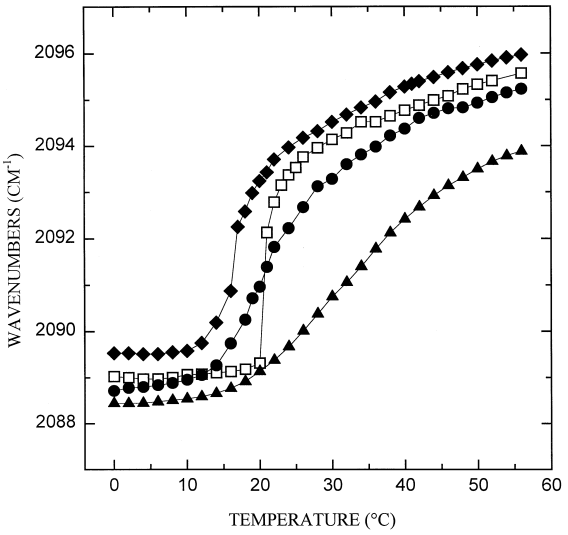

Fig. 1. Temperature dependence of the frequency of the acyl chains CD_2 symmetric stretching vibration of pure DMPC-d_{54} (□); DMPC-d_{54}/DHEA (●); DMPC-d_{54}/DHEA-S (♦) and DMPC-d_{54}/cholesterol (▲).

al. [13] and Chia et al. [14] have found that androstene has a smaller ordering effect on the hydrocarbon chains of the phospholipid compared to cholesterol. They attributed this behavior to the absence of C-17 hydrocarbon chain in androstene. We concur that, for similar reasons, the number of the acyl chain gauche conformers in DMPC in the presence of either DHEA or DHEA-S is higher compared to cholesterol.

In the presence of DHEA, the hydrocarbon chains of DMPC-d_{54} start to melt at a lower temperature than those of the pure lipid (Fig. 1). Similarly, a decrease of the gel to liquid-crystalline phase transition temperature (T_m) of DMPC-d_{54} occurs in the presence DHEA-S. These observations can be partly explained by a decrease of the van der Waals interactions. The concept of ''hydrophobic mismatch'', proposed by Nezil and Bloom [15], could also be used to interpret this finding. The shift of the T_m of a lipid in the presence of a steroid is dependent on the mismatch between the length of the hydrophobic chain of the sterol molecule and that of the lipid acyl chain. For a constant acyl chain length, sterols with a longer hydrocarbon chain stabilize the bilayer gel phase [14]. Fig. 1 shows that cholesterol shift up the transition temperature of DMPC-d_{54}. The stabilization of the lipid bilayer in the presence cholesterol was predicted by the concept of ''hydrophobic mismatch'' since the cholesterol molecule has about the same length as the DMPC acyl chains [16]. As DHEA and DHEA-S do not have hydrocarbon chains, both steroids are expected to destabilize the bilayer gel phase and shift the gel to fluid phase transition temperature downward. Le Grimellec et al. [17] observed a similar decrease of the transition temperature of DPPC in the presence of cholesteryl sulfate. They have associated this effect to the non specific interaction which takes place between cholesteryl sulfate and the lipid.

It is generally admitted that cholesterol disorders lipid acyl chains and particularly of DPPC in the gel phase [7,18]. However, the results presented in Fig. 1 seem to indicate that in the gel phase, cholesterol and DHEA induce some ordering on the acyl chains of DMPC-d_{54}. A similar observation has been made by McMullen et al. [19]. In addition to the ordering effect, the variation of the frequency of the methylene stretching bands may also be affected by other factors. It has been reported that for the gel state, the frequency of the CH_2 stretching modes of saturated PCs decreases as the acyl chain length increases [20], probably due to an increase of the van der Waals interactions. Furthermore, a decrease of the intermolecular vibrational coupling induces a shift toward higher frequencies of the methylene stretching mode of the lipid acyl chains [12,21]. On the other hand, a progressive increase of the frequency of the CH_2 symmetric stretching band has been observed from DPPE to DPPC as the three hydrogens of the ethanolamine head group are progressively substituted with methyl groups [22]. We have also observed that the frequency of the CD_2 symmetric band is about 1 cm^{-1} higher for DMPC-d_{54} compared to DMPE-d_{54} (results not shown). It is well known that, as the cross-sectional area increases, the acyl chains become tilted with respect to the bilayer normal [22,23] in order to maximize the van der Waals interactions. In light of these observations, it appears that a decrease of the acyl chain tilt angle also induce a decrease of the frequency of the methylene symmetric and antisymmetric stretching modes. We believe that this effect is due to the increase of the intermolecular coupling or the van der Waals interactions when the acyl chains are more normal to the bilayer.

The order parameter of the acyl chains of oriented multilayers of DMPC-d_{54} with and without steroids, was calculated from polarized ATR measurements of the CD_2 symmetric stretching vibration in the gel phase. The comparison of the acyl chain order parameters and the corresponding average chain tilt angle (θ) of DMPC-d_{54} with those of the DMPC-d_{54}/DHEA and DMPC-d_{54}/cholesterol complexes is presented in Figs. 2 and 3, respectively. The variations of the order parameter displayed in Fig. 2 may be interpreted in the light of the data shown in Figs. 1 and 3. A variation of the order parameter can either be due to a change of the conformational order or to a reorientation of the acyl chain axis. Fig. 1 shows that below 14°C, DHEA and cholesterol only slightly shift the frequency of the CD_2 symmetric stretching vibration of DMPC-d_{54}, thus showing that the conformation of the lipid is not very much affected by the presence of both steroids. On the other hand, Fig. 2 shows that both steroids significantly increase the order parameter of the acyl chain of DMPC-d_{54}. As seen in Fig. 3, this results in a decrease of about 6 and 10° of the tilt angle of the lipid acyl chains in the

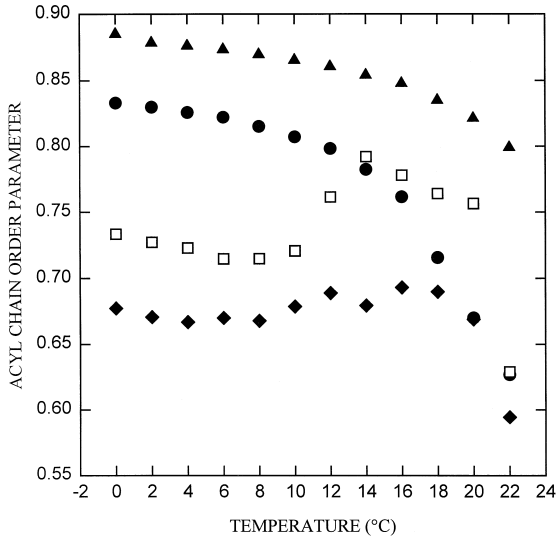

Fig. 2. Acyl chain order parameter calculated from the CD_2 symmetric stretching vibration of DMPC-d_{54} (□); DMPC-d_{54}/DHEA (●); DMPC-d_{54}/DHEA-S (◆) and DMPC-d_{54}/cholesterol (▲) in the gel phase.

presence of DHEA and cholesterol compared to the pure lipid, respectively. Assuming that the variation of the acyl chain tilt angle is correlated with the frequency of the stretching vibration, the slight but reproducible frequency decrease observed in the presence of DHEA or cholesterol (Fig. 1) may be attributed to the decrease of the hydrocarbon chain tilt angle of DMPC-d_{54}/steroids samples. However, the frequency decrease can also result in a slight ordering effect of the sterol on the lipid acyl chains. Since the acyl chains in the gel phase are thought to be in the all-*trans* conformation, this latter effect is unlikely, unless the bend in the *sn*-2 chain is affected by the straightening of the chain. Our results also show that, the decrease of the frequency of the antisymmetric CD_2 stretching vibration is more pronounced than that of the symmetric mode (data not shown), as observed by Kodati et al. [12] for the CH_2 stretching modes. These authors have proposed that the Fermi resonance is at the origin of the frequency shift due to the intermolecular coupling. Moreover, Figs. 2 and 3 show that the effect of cholesterol on the order parameter and on the acyl chains tilt angle of DMPC-d_{54} is more pronounced than for DHEA. Since cholesterol has the same length as the DMPC acyl chains [16], its ordering effect on this lipid is thus maximal, as opposed to the shorter DHEA molecule.

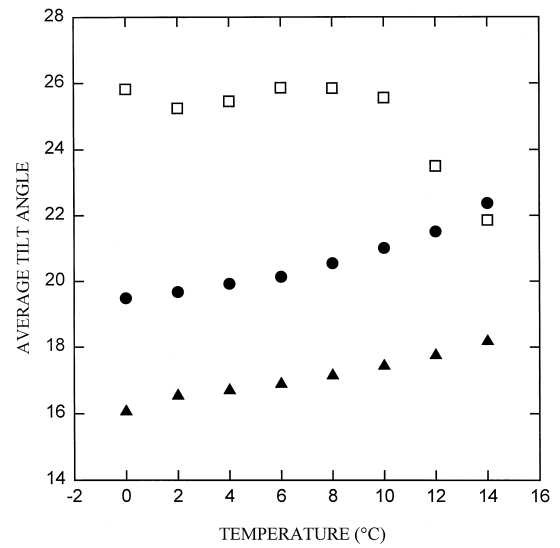

Fig. 3. Average chain tilt angle of the acyl chains of DMPC-d_{54} (□); DMPC-d_{54}/DHEA (●) and DMPC-d_{54}/cholesterol (▲) in the gel phase.

On the other hand, the presence of DHEA and cholesterol molecules in the phospholipid matrix can disrupt the intermolecular coupling. This should result in an increase of the methylene stretching frequency. However, isotopic dilution experiment using 20 mol% DMPC-d_{54} in DMPC have revealed that the effect of DHEA or cholesterol on the CD_2 symmetric stretching vibration of DMPC-d_{54}/DMPC mixture is similar to that observed with pure DMPC-d_{54}. This result suggests that the frequency decrease observed for the gel phase in the presence of the steroids is independent of the coupling between the acyl chains of the different molecules of the phospholipids matrix. However, the decrease of the CD_2 stretching frequencies due to an increase of the intermolecular coupling between the two hydrocarbon chains of a same phospholipid molecule cannot be excluded.

As described above, DHEA-S seems to disorder the acyl chain of DMPC-d_{54}, while DHEA exhibits the opposite effect. This is most likely related to the presence of the sulfate group, instead of an hydroxyl group, at the 3-position. Assuming that, similar to cholesterol, DHEA and DHEA-S are oriented perpendicular to the surface in phospholipids bilayers [7,16,24], we believe that because of its larger head group area, the insertion of DHEA-S into the bilayer

leaves more space between the lipid molecules than DHEA or cholesterol. Therefore, the lipid acyl chains are not in close contact with the sterol ring, thus reducing the van der Waals interaction, allowing the acyl chains to have more motional freedom and conformational disorder. This interpretation is in line with several NMR studies on similar systems. Faure et al. [25] reported that cholesterol sulfate is less effective than cholesterol in ordering the acyl chain of DMPC. Likewise, Kitson et al. [26] found that the presence of the bulky sulfate group in cholesterol sulfate is responsible for the lowering ability of this steroid to order the hydrocarbon chains of phospholipids. Moreover, the order parameter calculated from the methylene stretching vibration of the lipid further support the hypothesis that DHEA-S disorder the lipid acyl chain. Indeed, Fig. 2 show that DHEA-S decreases the order parameter of the DMPC-d_{54} acyl chains for the gel phase. The average chain tilt angle was not calculated for DMPC-d_{54}/DHEA-S mixture because of the presence of conformational disorder in the acyl chains. On the other hand, the increase of frequency of CD_2 band in the presence of DHEA-S may be interpreted as a breaking of the intermolecular coupling.

Fig. 2 also show that the order parameter of the acyl chain of the pure DMPC-d_{54} is fairly constant in the gel phase of the lipid between 0 and 10°C and increase significantly at the pretransition of the lipid between 10 and 14°C. Since the pretransition is not accompanied by a shift of the frequency of the CD_2 symmetric stretching mode, the increase of the order parameter of the DMPC-d_{54} is due to the reorientation of the lipid acyl chains toward the bilayer normal as the ripple phase ($P_{\beta'}$) appears. Fig. 3 indicates that the chain tilt decreases from ca. 26° to ca. 22° at the pretransition. Above 20°C, Fig. 2 shows that the order parameter decreases significantly as the acyl chains start to melt and thus to become conformationally disordered. In addition, it is seen in Fig. 2 that all three steroids inhibit the pretransition of DMPC-d_{54} since no discontinuity is observed between 10 and 14°C. A similar observation was made in several investigations of cholesterol/PC and androstene/PC complexes by differential scanning calorimetry [19,27]. A detailed study on the phospholipids pretransition has been made in our laboratory and will be published soon [28].

3.2. Carbonyl stretching mode region (1640–1760 cm^{-1})

The ester carbonyl stretching mode of hydrated DMPC is characterized by a broad band that after Fourier deconvolution or second derivative appears to be composed of two components. The frequency and relative intensity of these two components are known to be sensitive to hydrogen bonding. Fig. 4 displays the inverted second derivative spectra of the carbonyl bands of ^{13}C labeled DMPC, DMPC/DHEA and DMPC/DHEA-S samples in the gel and liquid-crystalline phases. In the gel phase, the two components of the DMPC $^{13}C{=}O$ absorption band appear near 1685 and 1700 cm^{-1} while they are observed near 1687 and 1701 cm^{-1} in the liquid-crystalline phase. These bands are assigned to hydrogen bonded and free carbonyl groups, respectively [29,30]. The ratio of the intensities of the free and hydrogen bonded components increases in the presence of both steroids for the gel phase, suggesting that the ester carbonyl groups of ^{13}C labeled DMPC are either less hydrated in the presence of DHEA [31] or hydrogen bonded to

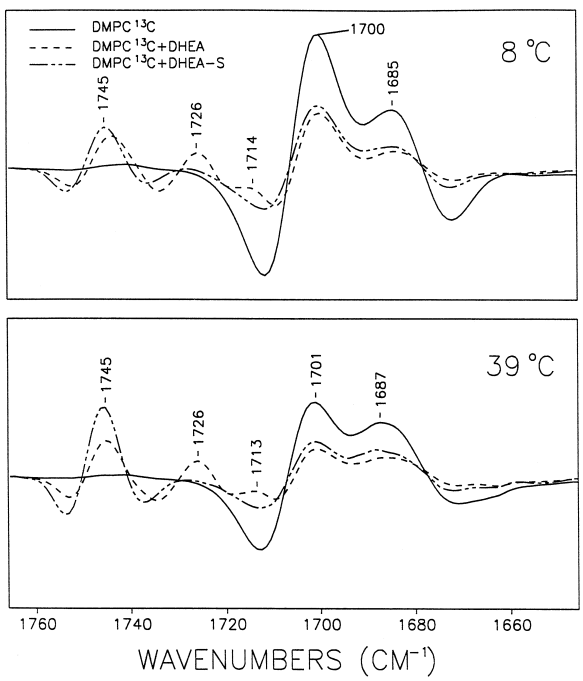

Fig. 4. Second derivative infrared spectra of carbonyl stretching mode region of ^{13}C labeled DMPC and DMPC/steroids mixtures in the gel (8°C) and liquid-crystalline (39°C) phases.

the steroid. Wong et al. [31] have proposed that the cholesterol OH group forms a stronger hydrogen bond than the water does with the *sn*-2 chain carbonyl groups of dry DMPC and DPPC [32]. Our results show that the steroids do not induce any frequency shift of the hydrated DMPC carbonyl bands. A similar observation has been made for phosphatidylserine/cholesterol mixture [33]. Since in hydrated samples, the hydrogen bonding strength of the cholesterol hydroxyl group is similar to that of water [18], it is not possible from our results to determine whether the C=O groups of DMPC are hydrogen bonded to water or to the OH group from the steroid. The second possible site for hydrogen bonding in DMPC is the phosphate group. Wong et al. [32] have proposed that in hydrated phosphatidylcholine/cholesterol samples, the phosphate group preferentially form hydrogen bonds with water. Furthermore, Umemura et al. [18] reported that almost no interaction occurs between DPPC phosphate group and cholesterol. As for the carbonyl bands, we did not observe any shift of the frequency of symmetric or antisymmetric stretching bands of the PO_2^- group in the presence of DHEA (results not shown). In light of these results, we cannot infer unambiguously that the DHEA hydroxyl group is hydrogen bonded with the carbonyl and/or the phosphate group of hydrated DMPC. However, for anhydrous DHEA/DMPC mixtures, the band due to the O–H stretching vibration of DHEA is partly shifted toward low frequencies and considerably broadens (result not shown), suggesting that some sterol OH group is involved in hydrogen bonding with either the carbonyl or the phosphate groups of the lipid [34].

Similarly to DHEA, the presence of DHEA-S does not shift the frequency of the DMPC carbonyl band. Since the sulfate group is ionized, it cannot form hydrogen bonds with the lipid carbonyl or phosphate groups. In this case, only water can form hydrogen bonds with the carbonyl group of hydrated DMPC.

Fig. 4 also shows the bands due to the DHEA carbonyl group at 1743 and 1726 cm^{-1}. The corresponding bands for DHEA-S are higher in frequency by about 2 cm^{-1}. As for the lipid carbonyl band, the high frequency component is assigned to the free carbonyl groups, while the low frequency component is associated to the steroids C=O group hydrogen bonded to water molecules. The presence of the latter band suggests that all the steroids molecules are not deeply embedded in the bilayer and that some C=O groups are exposed to the water environment. The third band observed in the spectrum of the DHEA/DMPC-d_{54} complex near 1714 cm^{-1} has not yet been clearly identified. Finally, the spectrum of the carbonyl stretching region of anhydrous DHEA-S displays one band near 1741 cm^{-1} while that of DHEA has two bands around 1740 and 1724 cm^{-1} (results not shown). These bands correspond to the frequencies of the free and the hydrogen bonded carbonyl groups respectively, indicating a self association of the molecules in crystalline DHEA. Indeed, investigation of the crystal structure of DHEA revealed the presence of four molecules in the unit cell [35].

4. Conclusion

In the present study, we have investigated for the first time the interaction of dehydroepiandrosterone (DHEA) and its sulfate derivative (DHEA-S) with dimyristoylphosphatidylcholine (DMPC). Our results reveal that in the presence of DHEA or cholesterol, the frequency of the CD_2 stretching band is lower in the gel phase, due to either a small ordering effect or the decrease of the DMPC-d_{54} average acyl chain tilt angle with respect to the bilayer normal. DHEA increases the conformational order of the DMPC-d_{54} acyl chains in the liquid-crystalline phase but at a lower extent than cholesterol. On the other hand, DHEA-S induces some conformational disorder along the lipid hydrocarbon chains in both the gel and liquid-crystalline phases. Furthermore, a larger population of free C=O was observed as compared to hydrogen bonded carbonyl groups in the presence of both DHEA or DHEA-S.

Our results clearly showed that DHEA and DHEA-S have a non negligible effect on DMPC membranes. These effects should be considered to elucidate the physiological action mechanism of these sterols.

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