Biochimica et Biophysica Acta 857 (1986) 259–264
Elsevier

BBA 73077

The effect of pressure on the hydrogen bonding between carbonyl and hydroxyl moieties in 1,2-dipalmitoylglycerol: a Fourier-transform infrared spectroscopy study *

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(Received November 13th, 1985)

Key words: Lipid-lipid interaction; Hydrogen bonding; Dipalmitoylglycerol; High pressure; Fourier-transform infrared spectroscopy

The barotropic behavior of solid 1,2-dipalmitoyl-*sn*-glycerol was investigated by Fourier-transform infrared spectroscopy over the pressure range 0.1–4250 MPa. At atmospheric pressure, the infrared spectrum of this lipid shows bands characteristic of a hydrogen-bonded hydroxyl group and of two carbonyl groups, one hydrogen-bonded, the other free. An increase in pressure leads to a considerable increase in the strength of the intermolecular hydrogen bond. The application of an external pressure of 4250 MPa increases the strength of the hydrogen bond by about 2/3 relative to that at atmospheric pressure. The stretching frequency of the proton-donating group (OH) is more sensitive to pressure-enhanced hydrogen-bonding than is the proton-accepting group (C=O). A structural phase transition was detected around 2500 MPa. In the infrared spectra recorded above this pressure, the OH and C=O stretching modes are split into two and four bands, respectively. For the high-pressure phase, there are at least two non-equivalent molecules in each unit cell with two types of hydrogen bonds.

Introduction

The presence of both proton donors and proton acceptors in biomembrane lipids presents an attractive set-up for models incorporating hydrogen bonding in lipid associations. Besides, the hydrogen bond is an ubiquitous and major determinant feature in biological interactions. Evidence for hydrogen bonding in biomembrane associations of cholesterol and phospholipids has been presented [1–5]. However, reports to the contrary [6–8] discount direct hydrogen-bonding on recombinant lipid systems. Thus, this important biological phenomenon is not yet fully understood.

In the model lipid, 1,2-dipalmitoyl-*sn*-glycerol (DPG), there are proton-donating (i.e., OH) and proton-accepting (i.e., C=O) groups; since the single hydroxyl of the glycerol backbone is known to be hydrogen-bonded intermolecularly to one of the interfacial acyl-chain ester carbonyls [9–11]. DPG is an attractive model for the study of hydrogen-bonding in lipids. Furthermore, DPG has been implicated as a second messenger in cellular response to receptor activation [12] and is therefore a physiologically relevant lipid. DPG also accords the advantage of investigating the spectral characteristics of the interfacial C=O group in the absence of H_2O. Both the OH and C=O stretching vibrations are infrared-active. Therefore, carbonyl and hydroxyl associations can be examined conveniently, speedily and non-invasively by FT-IR. On the other hand, pressure has been demon-

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* Issued as NRCC publication No. 25019.
Abbreviations: DPG, dipalmitoylglycerol; FT-IR, Fourier-transform infrared spectroscopy.

strated to induce, on lipid dispersions, considerable conformational changes which can be monitored by vibrational spectroscopy (Refs. 13,14 and references therein). Whereas an increase in temperature leads to the disruption of hydrogen-bonding due to the increased kinetic energy of the system (Mushayakarara, E.C. and Levin, I.W., unpublished results), a change in pressure allows the study of the influence on the hydrogen-bonding of changes in conformation and intermolecular distance.

In the present study, we have used high pressure, which enhances hydrogen-bonding, to investigate the infrared spectral characteristics of the free and associated hydroxyl and carbonyl moieties in DPG. The results from this basic study will be utilized to help with the interpretation of data on lipid-water and lipid-cholesterol interactions.

Experimental

Materials. 1,2-Dipalmitoyl-*sn*-glycerol (DPG) was obtained from Nu-Chek Prep. Inc. at a stated purity of greater than 99%. The solid lipid, together with some powdered α-quartz and KRS-5 were placed in a 0.34 mm diameter hole on a 0.23 mm thick stainless-steel gasket that was mounted on a diamond anvil cell. The KRS-5 served as substrate, the α-quartz was used as internal pressure calibrant (for detials of the pressure calibration see Ref. 15).

Spectra. Infrared spectra were measured at room temperature on a Bomem Model DA3.02 Fourier-transform infrared spectrometer equipped with a liquid-nitrogen-cooled mercury cadmium telluride detector. The infrared beam was condensed by a sodium chloride lens system onto the pin hole on the diamond anvil cell [16]. A total of 44 spectra were recorded at different pressures up to 4250 MPa. For each spectrum, 1000 scans were co-added; the spectral resolution was 4 cm^{-1}. Data reduction was performed using software developed in this laboratory.

Results

The infrared spectra of DPG shown in Fig. 1 for the C=O stretching (part A) and the OH stretching modes (part B) were measured between ambient pressure and 4250 MPa. Inspection of the spectral regions 1600–1660 and 3550–3650 cm^{-1} (not shown in Fig. 1) indicates the absence of infrared bands due to water and of free hydroxyl groups [17–19].

The two infrared bands at 1708 and 1733 cm^{-1} in Fig. 1A (frequencies at 0.1 MPa) are assigned to the hydrogen-bonded C=O and to the free carbonyl group, respectively [9]. The low-frequency C=O stretching band is broader than the high-frequency band. The broadening and the shifting characteristics of the low-frequency component are clearly associated with hydrogen-bonding [9,19]. As the pressure is raised above 2500 MPa, both bands

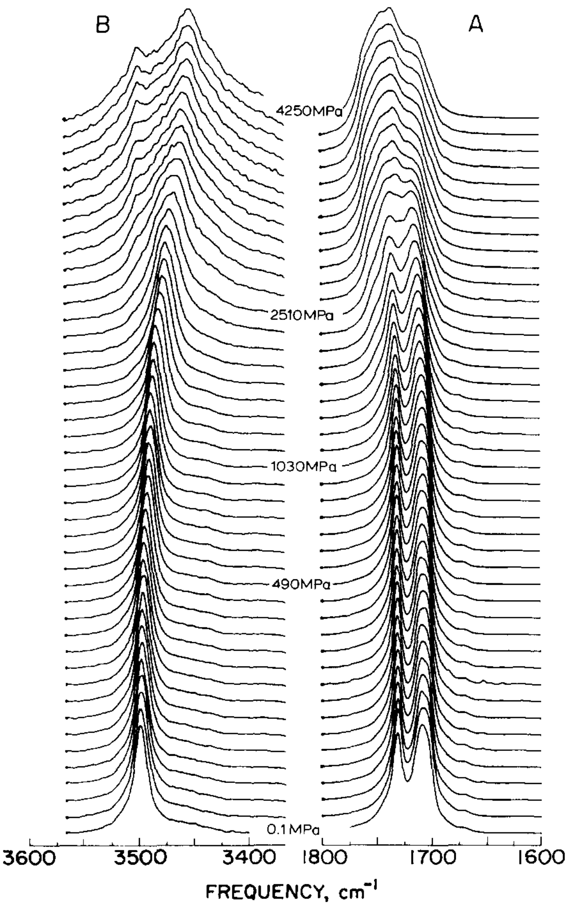

Fig. 1. Infrared spectra of DPG in the region of the C=O stretching modes (A) and in the region of the hydroxyl stretching modes (B) at several pressures between 0.1 MPa and 4250 MPa.

undergo a considerable broadening and then two new C=O stretching bands appear which increase in intensity with increasing pressure (Fig. 1A).

The strong hydroxyl band at 3498 cm^{-1} (at ambient pressure) is assigned to an associated OH moiety [9,19]. The infrared spectra in Fig. 1B show that this band is extremely sensitive to pressure and that it shifts significantly to lower frequencies as the pressure increases. At pressures greater than 2500 MPa, the OH band first broadens and then a new shoulder band appears at 3502 cm^{-1}; the intensity of this band increases with increasing pressure.

Pressure profiles of the carbonyl and hydroxyl bands that detail the dependences of the individual C=O and OH stretching vibrations on pressure are shown in Figs. 2 and 3. The data-points above 2500 MPa in Fig. 2 were obtained from deconvoluted infrared spectra as illustrated in Fig. 4 (for a description of this computational band-narrowing procedure, see Ref. 20). A closer examination of these pressure profiles indicates that the C=O bands are complex, but not as sensitive to pressure as is the OH band. The two C=O stretching bands (A and B) show a slight increase in frequency from ambient pressure to about 2500 MPa. Between 2500 and 3000 MPa, band A decreases while band B increases in frequency in a discontinuous way. Within this pressure range, bands C and D emerge. At higher pressure, band B shows a loss in intensity while the two components C and D increase in intensity.

The OH band, on the other hand, shows a progressive shift to lower frequency as pressure increases (Fig. 3). Between ambient pressure and 4250 MPa, the frequency of the OH band shifts from 3498 to 3431 cm^{-1}. At about 2500 MPa, a gradual band-broadening commences leading to the appearance of a shoulder (3502 cm^{-1}) at about 3000 MPa. This shoulder eventually becomes a well-defined band which is much narrower than the now broad main band. While the main band continues to decrease in frequency as the pressure is raised up to 4250 MPa, the shoulder band remains essentially constant at 3502 cm^{-1} but appears to increase in intensity at the expense of the main OH stretching band.

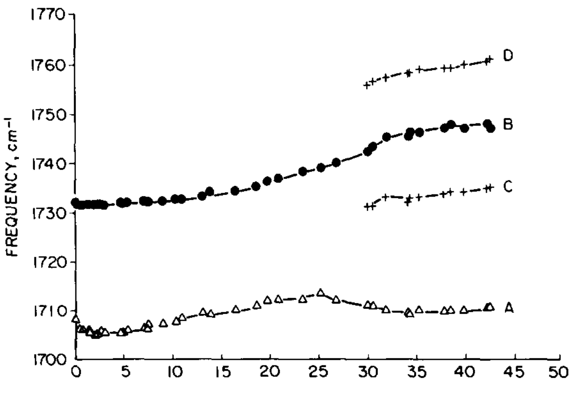

Fig. 2. Pressure dependence of the individual C=O stretching bands of DPG.

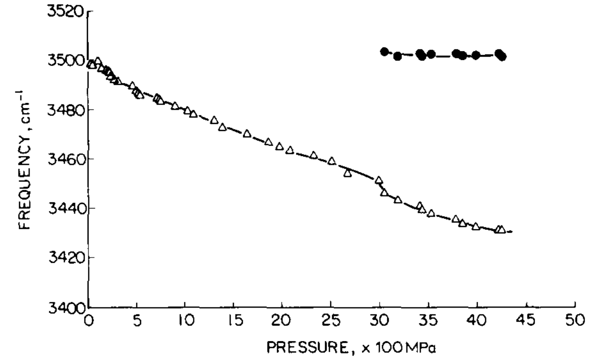

Fig. 3. Pressure dependence of the OH stretching bands of DPG.

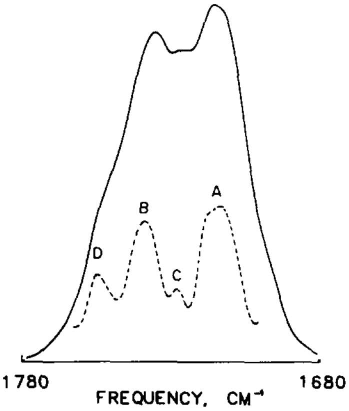

Fig. 4. Illustration of band-narrowing via deconvolution of the infrared spectrum of DPG. Original aspectrum at 2510 MPa (solid curve) and spectrum after deconvolution (broken curve).

Discussion

Effect of pressure on the hydroxyl group

Generally, the OH stretching band in a free (unassociated, nonhydrogen-bonded) hydroxyl group is at a frequency of or greater than 3600 cm^{-1}. This value however, decreases drastically when the OH group is associated (hydrogen-bonded) with a proton acceptor such as a C=O group [17,19,21]. Since the difference in frequency ($\Delta\nu_{OH}$) between the OH band of the associated (hydrogen-bonded) molecule and that of a free molecule is related to the enthalpy of association (hydrogen-bonding), $\Delta\nu_{OH}$ can be used as an index of hydrogen bond formation and strength [21,22]. The single OH stretching band observed in the infrared spectrum of DPG at 3498 cm^{-1} (at atmospheric pressure), is 102 cm^{-1} lower than the 3600 cm^{-1} value of a free hydroxyl group; this suggests that the OH moiety in DPG is completely hydrogen bonded. The only possible site for hydrogen-bonding in DPG is that between the OH group on the lipid glycerol backbone and the C=O group on one of the acyl chains of a neighboring molecule; this intermolecular hydrogen-bonding is consistent with the crystal structure of diacylglycerols derived from X-ray diffraction [10,11].

As the external pressure increases, the OH stretching frequency decreases monotonically (see Fig. 3). This indicates that the hydrogen bond between the OH and the C=O group of neighboring molecules is strengthened and that the distance between these two groups also becomes shorter with increasing pressure. However, the simple monotonic relationship between $\Delta\nu_{OH}$ and pressure holds only for pressures less than 2500 MPa. Between 2500 and 3000 MPa a discontinuity occurs in the pressure dependence of the OH stretching frequency. Although the ν_{OH} frequency decreases further with increasing pressure, the slope of the pressure dependence of the ν_{OH} frequency changes and the ν_{OH} band becomes broader. Moreover, a new OH stretching band appears at 3502 cm^{-1} when the pressure reaches about 3000 MPa, the intensity of which increases with increasing pressure. An additional association between the OH and the C=O group with weaker hydrogen-bonding, which is different from that observed in the low-pressure phase, appears to account best for this additional ν_{OH} band with a frequency comparable to that of the ν_{OH} band at atmospheric pressure.

At 4250 MPa, the frequency of the original ν_{OH} band is 67 cm^{-1} less than that observed at atmospheric pressure. This decrease in the ν_{oH} frequency indicates that the application of an external pressure of 4250 MPa has increased the hydrogen bond strength in DPG by about 2/3 relative to that at atmospheric pressure.

Effect of pressure on the carbonyl group

It is well-known that upon hydrogen-bonding the frequency shifts of the proton-acceptor groups are much smaller than the frequency shifts of the corresponding proton-donor groups. Indeed, upon hydrogen-bonding at atmospheric pressure, the shift in frequency of the hydroxyl group in DPG ($\Delta\nu_{OH}$) is about 100 cm^{-1}, whereas the shift of the corresponding carbonyl group ($\Delta\nu_{C=O}$) is only about 20 cm^{-1}. The presence of both free and associated C=O groups in DPG allows one to monitor the effect of pressure on both hydrogen-bonded and free carbonyl moieties.

The pressure-induced shift of the stretching frequency of the free carbonyl groups is extremely small up to pressures of about 1000 MPa; as the pressure increases further, the $\nu_{C=O}$ frequency also increases and shows a change of slope at 2500 MPa. The small pressure-induced shift of the free C=O stretching frequency indicates that (i) the pressure-induced compression of the C=O double bond is extremely small and (ii) shows that the pressure-induced interaction between the free C=O group and the neighboring molecules is insignificant.

There are two factors that can affect the $\nu_{C=O}$ frequency when the C=O group is hydrogen-bonded to a hydroxyl group, namely a mechanical and an electronic factor. The bonding of the oxygen atom of a free C=O group to a neighboring OH group mechanically restricts the free vibration of the carbonyl oxygen atom, and thus the C=O frequency is reduced. On the other hand, upon hydrogen-bonding, the lone pair electron density on the oxygen atom of the C=O group migrates towards the hydrogen atom on the OH group, and this partial electron withdrawal from the carbonyl oxygen atom leads to a tighter C=O bond and a larger C=O stretching-force constant [23,24]. The result of this electronic effect will certainly increase the C=O stretching frequency. At atmospheric pressure, the mechanical effect is larger than the electronic effect and thus the net result is a 20 cm^{-1} decrease in the carbonyl frequency upon hydrogen-bonding. The existence of the electronic effect explains the smaller decrease in the C=O stretching frequency compared to the decrease in the OH stretching frequency, because in this case the mechanical effect as well as the electronic effect favor a decrease in the OH stretching frequency upon hydrogen-bonding, as the migration of the electron density from the oxygen atom of the C=O group towards the hydrogen atom of the OH group repels the electron density on the OH bond and thus weakens the OH bond.

As the pressure increases, the $\nu_{\mathrm{C=O}}$ frequency of the associated C=O group decreases slightly at first (up to about 200–300 MPa), but then it increases at a rate comparable to that of the free C=O group. This implies that at pressures below about 300 MPa the mechanical effect is stronger than the electronic effect. At pressures above 300 MPa, the pressure enhancement of the mechanical effect and that of the electronic effect are comparable and compensate each other. The increase in the frequency of the associated C=O group at pressures above 300 MPa is then mainly the result of the compression of the C=O bond by external pressures similar to that of the free C=O bond.

The splitting of the C=O stretching bands commences at the same pressure at which a new OH stretching band appears. The simultaneous response to high pressure by both the C=O and the OH moieties demonstrates the common origin of these changes.

A pressure-induced phase transition in DPG

The discontinuities of the pressure dependences of various vibrational frequencies in DPG at 2500 MPa, and the appearance of additional ν_{OH} and $\nu_{\mathrm{C=O}}$ infrared bands above 2500 MPa, strongly suggest that a structural phase transition takes place at this pressure.

Two OH stretching bands are found in the high-pressure phase. The frequency of the new ν_{OH} band at 2500 MPa is about 45 cm^{-1} higher than that of the original ν_{OH} band, but is still about 100 cm^{-1} below that of a free OH group. Consequently, in this phase of DPG all OH groups are hydrogen bonded, and two types of hydrogen bonds with slightly different strength must co-exist in this new phase. Since each DPG molecule possesses only one OH group, the presence of two types of hydrogen bonds in this high-pressure phase indicates that there are at least two non-equivalent DPG molecules in each unit cell in the lattice. This is consistent with the splitting of both the free and associated $\nu_{\mathrm{C=O}}$ bands. The $\nu_{\mathrm{C=O}}$ frequency of one of the new bands (C) is lower than that of the free C=O band (B) but is higher than that of the originally hydrogen-bonded C=O band (A). Therefore, this band is most likely due to the weaker hydrogen-bonded C=O group that occurs only in the high-pressure phase. The $\nu_{\mathrm{C=O}}$ frequency of the second free C=O group (D) in the high-pressure phase is slightly higher than that of the original free C=O group (B). However, all three C=O frequencies show similar pressure dependences in the range from 3000 to 4250 MPa.

The data in Fig. 3 demonstrate that the strength of the original hydrogen bond is further enhanced in the pressure range above 2500 MPa, while the strength of the new hydrogen bond in the high-pressure phase is not affected by pressure. X-ray diffraction studies indicate that at atmospheric pressure the OH group in each DPG is hydrogen-bonded to the *sn*-1 C=O group of a neighboring molecule and that the *sn*-2 C=O group is the free carbonyl [10,11]. Thus, the ester segments are forced to adopt a *trans* conformation at the *sn*-1 ester linkage. This conformational arrangement is favorable to hydrogen-bonding by shortening the distance between the OH and the *sn*-1 C=O group of adjacent molecules.

At high pressure, the molecules in DPG are forced to pack more closely. One way to achieve this is to change the conformation at the interfacial region of some molecules so that the C=O groups re-orient differently. Because of this change in conformation, the distance between the OH groups and the hydrogen-bonded C=O groups of some neighboring molecules in DPG increases; this leads to the second, weaker hydrogen bond in the high-pressure phase.

Above 2500 MPa, the infrared bands in both the C=O and OH stretching region are broadened. Moreover, the intensities of the new ν_{OH} and $\nu_{C=O}$ bands increase with increasing pressure from 2500 to 4250 MPa. These pressure effects suggest that the transition into the high-pressure phase of DPG is sluggish as it takes higher pressure and longer time to complete the transition.

To our knowledge, this is the first study to report the effect of pressure on hydrogen-bonded hydroxyl and carbonyl moieties in a lipid, and demonstrates that a combination of the high-pressure technique with FT-IR spectrometry provides a means to investigate the hydrogen bond formation in the interface regions of biomembranes.

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