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DETECTION BY HIGH PRESSURE INFRARED SPECTROMETRY OF HYDROGEN-BONDING BETWEEN WATER AND TRIACETYL GLYCEROL

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SUMMARY: The barotropic behavior of neat and aqueous 1,2,3-triacetyl glycerol was investigated by FT-IR spectroscopy over the pressure range 0.001 to 35 kbar. The infrared spectrum in the presence of water shows bands characteristic of hydrogen bonded carbonyl groups. An increase in hydrostatic pressure leads to a strengthening of the intermolecular hydrogen bond between water and the lipid ester C=O groups. The pressure-induced formation of ice VI at 9 kbar does not affect this hydrogen bond, however, the formation, at 20 kbar, of ice VII in which the water/water hydrogen bonds are stronger than the lipid C=O/water hydrogen bonds, frees the lipid carbonyl groups from the hydrogen-bonding to water.

The hydrogen bond is a ubiquitous and major determinant feature in biological interactions. The interfacial zone of membrane lipids is well endowed with proton-accepting groups such as carbonyl oxygens. It is therefore attractive to assume that molecules which possess proton-donating groups such as cholesterol and water are anchored in biomembranes by hydrogen-bonding to the interfacial regions of the membrane lipids (1,2). However, while certain nuclear magnetic resonance and infrared spectroscopic studies suggest the existence of such hydrogen-bonding in lipid associations (3-6), other reports discount direct hydrogen-bonding on recombinant lipid systems (7-10). Thus, this significant biological phenomenon is not yet well understood.

The presence in membrane lipids of functional groups suitable for hydrogen-bonding such as phosphate, choline, ethanolamine or serine groups, complicates the investigation of lipid/water/cholesterol associations in the interfacial region of these lipids. Therefore we have chosen a triacyl glycerol in which such functional groups are absent and which possesses only

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the ester carbonyl moiety at the lipid interface. Triacyl glycerols are key molecules in lipid metabolism (11) and major components of plasma lipoproteins (12), and thus are physiologically relevant lipids. We have chosen triacetyl glycerol (triacetin) for its solubility in water. The only functional groups in this lipid water assembly capable to form hydrogen bonds are the water hydroxyl groups and the lipid ester carbonyl groups. Both OH and C=O bands are infrared active and thus the interaction between water and the ester segment of the lipid can be investigated conveniently and non-invasively by infrared spectrometry. An elevation of pressure converts water to its various ice forms (13). Since water water hydrogen bondings are known to change with the conversion to the solid states, the pressure parameter accords the opportunity to examine the competition between lipid water and water water associations.

EXPERIMENTAL

Materials: 1,2,3-triacetyl glycerol, also known as triacetin, was obtained from Aldrich Chemical Co. at a stated purity of greater than 99%. Triacetin/water mixtures were prepared by dissolving 1 part triacetin in 15 parts double distilled H_2O or 99.8% D_2O (Merck, Sharp and Dohme, Montreal). The 1:15 (v/v) triacetin/water sample is equivalent to 0.353 m. Approximately 5µl of aqueous or neat triacetin, 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 details of the pressure calibration see ref. 14).

Spectra: Infrared spectra were measured 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 (15). For each sample approximately 25 spectra were recorded at different pressures up to 35 kbar. For each spectrum, 1024 scans were co-added; the spectral resolution was 4 cm^{-1}. Data reduction was performed using software developed in this laboratory.

RESULTS AND DISCUSSION

Effect of hydrogen-bonding on the C=O stretching band of triacetin: At ambient pressure and temperature (22°C) the infrared spectrum of neat (liquid) triacetin shows a single band in the region of the C=O stretching modes at 1748 cm^{-1}. This strong band is assigned to a free ester carbonyl stretching vibration. In contrast, when D_2O or H_2O is added, the C=O stretching vibration decreases considerably. The infrared spectra of

triacetin/H_2O and triacetin/D_2O show this band at 1732 and 1728 cm^{-1}, respectively. The decrease in the frequency of the C=O stretching vibration in aqueous triacetin is undoubtedly the consequence of hydrogen-bonding. As the ester carbonyl is the only polar group in triacetin, the solubility of triacetin in water is solely a consequence of hydrogen bond formation between this polar ester group and water. Thus, in the aqueous solution of triacetin all ester carbonyl groups are hydrogen-bonded.

There are two factors that can affect the frequency of the C=O stretching band when the carbonyl group is hydrogen-bonded, namely a mechanical factor and an electronic factor. The bonding of the oxygen atom of a free carbonyl group to an OH group mechanically restricts the free vibration of the oxygen atom with respect to the carbon atom on the C=O group and thus the C=O stretching 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, at least partially, towards the hydrogen atom on the OH group; this partial electron withdrawal from the oxygen atom on the C=O group leads to a stronger C=O bond and consequently to a larger C=O stretching force constant (16,17). The result of this electronic effect is an increase of the C=O stretching frequency. Thus, the mechanical and electronic factors have opposite effects on the C=O stretching frequency. At atmospheric pressure the mechanical effect is larger than the electronic effect and thus the net effect observed in aqueous triacetin is a $\sim$ 20 cm^{-1} decrease of the C=O frequency upon-hydrogen bonding. The existence of these two opposing effects also explains the larger decrease in the C=O frequency when bonded to an OD rather than an OH group since the increase in mass enhances the mechanical factor in D_2O over that in H_2O.

Effect of pressure on the C=O stretching band of triacetin: Figure 1 illustrates the frequency dependence of the free and hydrogen bonded C=O stretching bands of triacetin on pressure. The non-hydrogen bonded C=O stretching band of pure triacetin is only slightly affected by a change in hydrostatic pressure. At first the frequency of this C=O stretching band

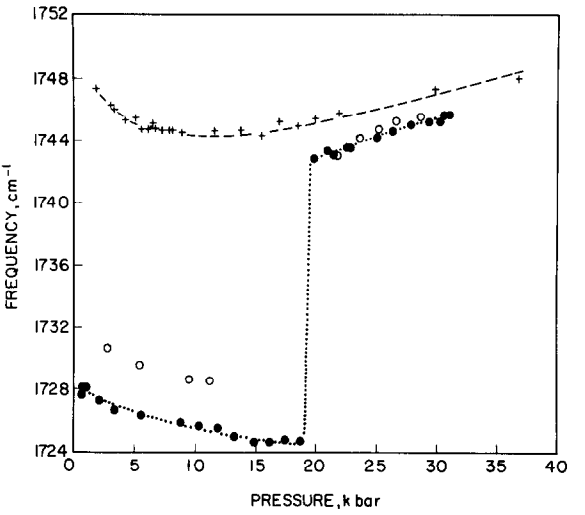

Figure 1. Pressure dependence of the C=O stretching frequencies of neat triacetyl glycerol (+) and of its solutions in D_2O (●) and H_2O (o).

decreases to 1744 cm^{-1} at 10 kbar, after which it increases slightly and reaches 1748 cm^{-1} at 35 kbar. These shifts reflect small conformational changes in neat triacetin that will be discussed elsewhere.

The frequency-pressure dependency of the hydrogen bonded C=O stretching bands, however, differs considerably from that of the free C=O stretching band. As shown by the detailed frequency-pressure profile of the carbonyl band of triacetin in D_2O (Fig. 1) the frequency of the associated C=O group decreases from 1728 cm^{-1} at atmospheric pressure to 1725 cm^{-1} at 15 kbar. This indicates that pressure further enhances the mechanical effect on the C=O group and that an increase in pressure is associated with a strengthening of the hydrogen bond. However, at a hydrostatic pressure of about 20 kbar the frequency of the C=O stretching band shows a sharp increase to 1744 cm^{-1}, a frequency value characteristic for a free C=O stretching band. The further slight increase in this frequency between 20 and 30 kbar parallels that observed for the free C=O band. The frequency-pressure profile of the hydrogen bonded C=O band of triacetin in H_2O is similar to that of triacetin in D_2O, except that the discontinuity occurs around 21 kbar. In Fig. 1 only a few points are shown for H_2O below and above 21 kbar.

The shift in the frequency of the C=O band at 20 kbar (D_2O) respectively 21 kbar (H_2O) indicates that water is withdrawn from the interfacial region at these pressures as the C=O frequency now corresponds to that of a free C=O group. These pressure points, i.e., ~ 20 kbar for triacetin/D_2O and ~ 21 kbar for triacetin/H_2O correspond to the release of the C=O group from the hydrogen bond and therefore will be termed "release points" and/or "release pressure". The further small increase in the frequency of the C=O groups in triacetin/D_2O and triacetin/H_2O at pressures above the release pressure is mainly the result of the compression of the C=O bond by external pressure and is similar to that of the free C=O bond in neat triacetin.

Pressure-induced dehydration of the lipid interface: As mentioned above, the decrease in the frequency of the associated C=O groups (up to about 15 kbar) reflects a strengthening of the hydrogen bond due to the increase in pressure. However, the process of strengthening the bonding of water to triacetin is abruptly interrupted at ~ 20 kbar. The abrupt increase in the frequency of this hydrogen bonded carbonyl group, which literally "snaps back" to the value of a free C=O bond, demonstrates that water is withdrawn from the interfacial zone by the suction effect of the stronger water/water association. That is, at the release point water preferrentially binds to itself and thus withdraws the bound water molecules from the interfacial region of triacetin. The release points in H_2O and D_2O correspond to the pressure at which ice VII is formed. The macroscopic structure of ice VII is body-centered cubic with each molecule hydrogen-bonded to four of its neighbours and not hydrogen-bonded to the other four (18). Thus the water molecules experience a large increase in hydrogen-bonding to the neighbouring water molecules upon ice VII formation. This observation supports our conclusion that at the release pressure the bonding to water becomes stronger than the bonding to the interfacial C=O.

At room temperature liquid water transforms to ice VI at ~ 9 kbar (19). The hydrogen bond strength between water molecules in ice VI is weaker than that in ice VII due to the bending of some O-H ... O hydrogen bonds in

ice VI (20). Interestingly, there is no discontinuity at 9 kbar in the pressure dependence of the C=O stretching band of triacetin in water (H_2O or D_2O) and the frequency of the hydrogen bonded C=O band continues to decrease with increasing pressure above 9 kbar. This indicates that the ester carbonyl groups of triacetin are still hydrogen-bonded to water molecules at 9 kbar, although as shown by our infrared spectra, the excess water molecules have been converted into ice VI at this pressure. Thus we conclude that the hydrogen bond between the C=O ester groups of triacetin and water is stronger than that between water molecules in ice VI, but weaker than that between water molecules in ice VII.

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