Effect of cholesterol on structural and dynamic properties of tripalmitoyl glyceride

A high-pressure infrared spectroscopic study

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ABSTRACT The infrared spectra of tripalmitoyl glyceride confirm the tuning fork configuration previously attributed to trilauroyl glyceride (Small, D. M. 1986. Handbook of Lipid Research. Vol. 4). The acyl chains in solid tripalmitoyl glycerol, either within each molecule or between neighboring molecules, are oriented parallel to each other with the sn-3 acyl chains extended toward the opposite direction of the sn-1 and sn-2 chains. The presence of cholesterol increases the orientational disorder of the tripalmitoyl glyceride molecules in terms of increased reorientational fluctuations and twisting/torsion motions of the acyl chains. In the solid mixture, cholesterol is embedded in the tripalmitoyl glyceride lattice which results in a reorientation of the acyl chains within each molecule from a parallel packing to a nonparallel packing. No evidence was found for hydrogen bond formation between the OH group of cholesterol and any of the three C=O groups of tripalmitoyl glyceride.

INTRODUCTION

In our laboratory in Ottawa, we have made extensive use of vibrational spectroscopy under high pressure to gain new insights into the physical properties of biomembranes and model membrane systems (Auger et al., 1987, 1988; Siminovitch et al., 1988; Wong, 1984, 1986, 1987*a–c*; Wong et al., 1988; Wong and Mantsch, 1988*a* and *b*). Pressure is now well recognized as an essential variable in the study of the dynamic structure of membrane systems (Wong, 1984, 1986, 1987*a–c*; Wong et al., 1988). A continuous variation in intermolecular distances can be achieved by pressure, and thus the intermolecular interactions in such systems can be studied more readily.

Triacyl glycerides are the most important forms of stored biological lipids in higher animals and most plants. They are also implicated in various biomedical conditions. For instance, hydrogen bonding between triacyl glycerides and cholesterol has been suggested as one of the factors affecting the plaque formation in atherosclerosis (Brooks et al., 1966; Parker and Bhaskar, 1968; Steel et al., 1966). Moreover, one of the mechanisms for the alcohol-induced liver cirrhosis is known to be an increase in the accumulation of triacyl glycerides (Brindley, 1988; French, 1989). Despite the importance of triacyl glycerides in nature, to our knowledge, only one triacyl glyceride, triacetin, has been investigated so far by high-pressure vibrational spectroscopy (Mushayakarara et al., 1986). Because the fatty acid composition of tissue triglycerides consists mainly of myristic, palmitic, oleic, and linoleic acid (Hübscher, 1970), in the present work we have investigated the structural and dynamic properties of tripalmitoyl glyceride, as well as its interaction with cholesterol by high-pressure infrared spectroscopy.

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EXPERIMENTAL

High purity (>99%) tripalmitoyl glyceride (TPG) was obtained from Nu Check Prep, Inc., Elysian, MN, and was used as such. Cholesterol (CHOL) was from Sigma Chemical Co., St. Louis, MO, and was recrystallized from acetone, then kept under vacuum for 48 h to remove any residual acetone and water. A 1:1 (wt/wt) mixture of TPG/CHOL was prepared by two separate methods. In a first case, the solid components were codissolved in chloroform, vortexed, and the solution was dried with nitrogen gas; the sample was then kept under vacuum for 48 h. In a second case, the TPG/CHOL mixture was heated above the melting point of the solid mixture, vortexed, and then cooled to room temperature.

The samples were placed at room temperature in a 0.37-mm-diameter hole on a 0.23-mm-thick stainless gasket mounted on a diamond anvil cell, along with powdered α-quartz, used as internal pressure calibrant (Wong et al., 1985). Because the solid TPG/CHOL mixture easily adsorbs moisture if exposed to air, the diamond cell was placed in the sample compartment of the infrared spectrophotometer and purged with dry nitrogen for at least 48 h. The resulting anhydrous sample was then sealed by closing up the diamond anvils. Water

removal during purging was followed by monitoring the decrease in intensity of the water OH stretching band (in the 3,000–4,000 cm^{-1} region), relative to that of the lipid CH stretching band (in the 2,800–3,000 cm^{-1} region).

Infrared spectra were measured on a model DA3.02 Fourier transform spectrophotometer (Bomem, Vanier, Quebec) with a liquid nitrogen cooled mercury cadmium telluride detector. Duplicate runs were obtained for each sample. A sodium chloride lens system was used to condense the infrared beam onto the sample in the diamond anvil cell. 512 interferograms were coadded for each spectrum at a spectral resolution of 4 cm^{-1} and zero-filled once to yield on encoding interval of 2 cm^{-1}. Data reduction was performed using software developed in this laboratory. In all figures with spectra, the *y*-scale is absorbance.

RESULTS AND DISCUSSION

Under ambient conditions TPG is in the solid state. Infrared spectra of pure TPG, and of the 1:1 (wt/wt) mixture of TPG and CHOL were measured as a function of pressure up to 45 kbar. The infrared spectra of pure TPG and TPG with cholesterol, and their pressure dependences are very different. Consequently, the structural and dynamic properties of these systems are expected to be different, as will be shown below.

Tripalmitoyl glyceride

Fig. 1 *A* illustrates pressure profiles of the infrared spectra in the frequency region of the CH_2 bending mode (δCH_2) of the acyl chains in TPG. The δCH_2 mode appears as a single narrow band up to the measured pressure of 34 kbar, indicating that the vibrational frequencies of the CH_2 bending modes of the sn-1, sn-2, and sn-3 acyl chains in TPG are the same, and that the δCH_2 bands of the three acyl chains in TPG overlap to produce a single infrared band. Consequently, the environment and the interchain interactions of the three acyl chains in TPG must be the same. The δCH_2 band does not exhibit a pressure-induced correlation field splitting (Wong et al., 1988). However, as shown in Fig. 2 *A*, pressure induces a gradual shift of the δCH_2 band toward higher frequencies. It is known that correlation field splitting is the result of transition moment interactions among neighboring chains of nonequivalent orientations. However, a pressure-induced frequency shift is the result of pressure-enhanced transition moment interactions among equivalently oriented chains (Boerio and Koenig, 1970; Wong 1984). Therefore, the present infrared spectral results suggest that the acyl chains in solid TPG pack parallel to each other, and that the intra- and interchain packing of the

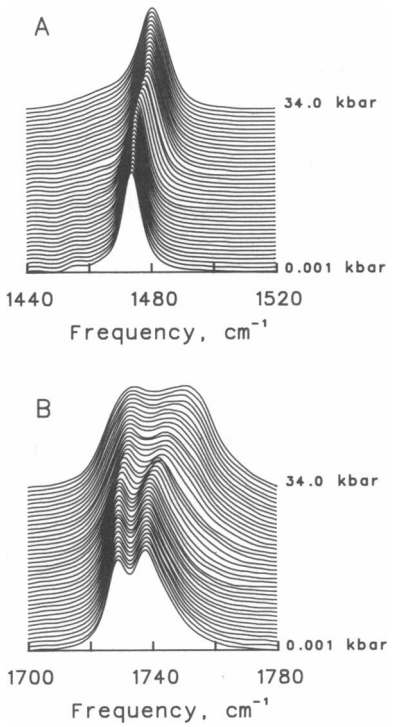

FIGURE 1 Infrared spectra of pure tripalmitoyl glyceride as a function of pressure in the region of the CH_2 bending mode (*A*) and the C=O stretching mode (*B*).

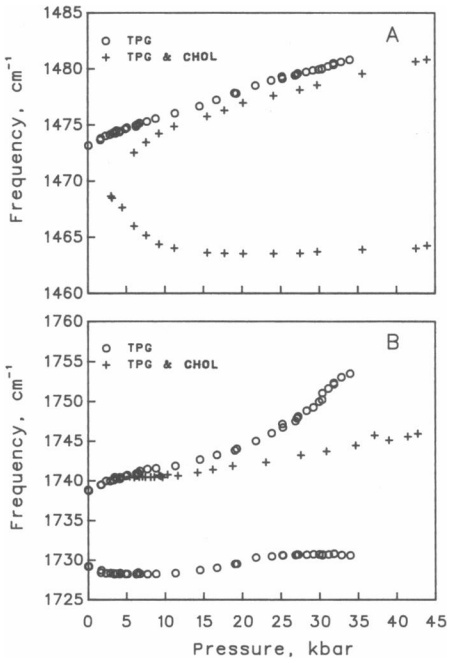

FIGURE 2 Pressure dependences of the frequencies of the CH_2 bending mode (*A*) and the C=O stretching mode (*B*) in pure TPG and in a 1:1 (wt/wt) mixture of TPG and cholesterol.

three palmitoyl chains is similar to that of the lauroyl chains in trilauroyl glyceride, which was shown to have a triclinic parallel subcell belonging to the space group P_1^- (Small, 1986 and references therein).

Fig. 1 *B* shows the pressure profiles of the C=O stretching mode (νC=O) of the ester C=O groups of TPG. The νC=O mode appears as a doublet up to 34 kbar, with peak positions at 1,729.2 and 1,739.5 cm^{-1} (frequencies at ambient conditions). The component band at 1,739.5 cm^{-1} is more intense and broader than the 1,729.2-cm^{-1} component band and is composed of two overlapping bands. There are three carbonyl groups in each TPG molecule. It is known from x-ray studies (Small, 1986) that in solid triglycerides each triglyceride molecule exhibits a tuning fork configuration in which the sn-1 and sn-2 acyl chains are arranged as parallel, nearly straight chains, whereas the sn-3 acyl chain projects out at right angles to the direction of the sn-1 and sn-2 chains and folds over at the ester carbonyl carbon, in a manner similar to that of the sn-2 chains in 1,2-diacyl phospholipids. Thus the sn-3 acyl chain is also oriented parallel to the sn-1 and sn-2 chains but in the opposite direction. In such a structure, the environment of the sn-3 carbonyl group certainly differs from that of either the sn-1 or the sn-2 chains, and thus the νC=O frequency of the sn-3 carbonyl group is expected to be different from that of the sn-1/sn-2 carbonyl groups. In fact, the presence of two νC=O bands in the infrared spectrum of TPG nicely confirms the tuning fork configuration of this triglyceride. In anhydrous 1,2-diacyl phospholipids, the νC=O frequency of the straight sn-1 acyl chain is at ~1,740 cm^{-1}, whereas that of the bent sn-2 acyl chain is at ~1,730 cm^{-1} (Wong and Mantsch, 1988). Therefore, it is reasonable to assume that in TPG the νC=O band at 1,739.5 cm^{-1} is due to the stretching vibration of the sn-1 and sn-2 C=O groups, whereas the band at 1,729.2 cm^{-1} is due to the stretching vibration of the sn-3 C=O group. This assignment is consistent with the intensity ratio of the two νC=O bands.

The frequencies of the two νC=O bands of TPG are plotted against pressure in Fig. 2 *B*. Both frequencies increase nonlinearly with increasing pressure, however, the frequency of the combined sn-1/sn-2 νC=O band shifts faster with pressure than that of the sn-3 νC=O band. This indicates that the interaction between neighboring C=O groups in the lattice is stronger for the sn-1 and sn-2 C=O groups than for the sn-3 C=O group, although the interchain interactions of the three methylene chains in TPG are similar, as was shown above. Space-filling models (Small, 1986) show clearly that the sn-1 and sn-2 C=O groups of neighboring triglyceride molecules are oriented in the same direction, whereas the sn-3 C=O groups are oriented in a different direction. In such an arrangement, the intermolecular interactions among neighboring sn-1 and sn-2 C=O groups are expected to be stronger than those among neighboring sn-3 groups which is consistent with the pressure dependences of the νC=O frequencies of the carbonyl groups in TPG observed in the present work.

Mixture of tripalmitoyl glyceride and cholesterol

The infrared band of the CH_2 bending mode, δCH_2, of the TPG/CHOL mixture (solid trace) is compared with the δCH_2 band of pure TPG (broken trace) in Fig. 3, which also shows the spectrum of pure cholesterol in the same frequency region (dashed trace). The δCH_2 band of the mixture is much broader and shifted towards lower frequencies with respect to the δCH_2 band of pure TPG. Such dramatic changes in the δCH_2 band indicate that the cholesterol molecules are partitioned into the TPG lattice, which in turn perturbs the interchain coupling of the CH_2 bending mode.

The most striking effect of cholesterol on the δCH_2 mode of TPG is that the δCH_2 band exhibits a pressure-induced correlation field splitting in the presence of cholesterol. The frequencies of the correlation field component bands of the δCH_2 mode are plotted against pressure in Fig. 2 *A*. The correlation field splitting appears at a pressure of ~6 kbar. Only a single δCH_2 band is observed at pressures below ~6 kbar, which reflects the fact that below 6 kbar the orientation of the methylene chains is disordered due to significant reorientational fluctuations and torsional/twisting motions or the acyl chains with respect to their equilibrium positions (Auger et al., 1988; Wong, 1984; Wong et al., 1988). An increase in pressure leads to the dampening of these acyl chain motions and to an increase in interchain interactions which give rise to the correlation field splitting (Auger et al., 1988; Wong, 1984; wong et al., 1988). In 1,2-diacyl phospholipids the correlation field splitting pressure is

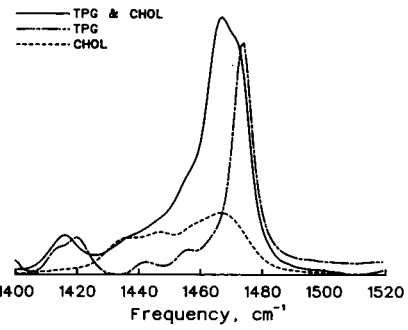

FIGURE 3 Infrared spectrum of pure TPG, pure cholesterol, and of the mixture TPG/CHOL in the frequency region 1,400–1,520 cm^{-1} at ~5 kbar.

usually below 3 kbar (Auger et al., 1988; Wong and Mantsch, 1985) and increases by ~1 kbar in the presence of cholesterol (Auger et al., 1988). In the TPG/CHOL mixture, the correlation field splitting pressure is as high as 6 kbar, indicating that at ambient conditions the orientations of the acyl chains of TPG are highly disordered.

These spectroscopic results suggest that the presence of cholesterol in the TPG matrix results in a rotation of the zig-zag planes of neighboring acyl chains of TPG, from a parallel packing to a nearly perpendicular packing. The partition of cholesterol molecules into the TPG lattice is also evident from changes in other vibrational modes. Thus, the frequency of the symmetric CH_2 stretching mode decreases drastically in the presence of cholesterol (Fig. 4 *A*), indicating that the TPG molecules are kept apart by the cholesterol molecules, and thus the intermolecular coupling of this vibrational mode is reduced. Because in the TPG/CHOL mixture the TPG molecules are isolated by cholesterol molecules, the observed correlation field splitting of the δCH_2 band of TPG must be the result of correlation field interactions among the three acyl chains within each TPG molecule rather than among the acyl chains of neighboring TPG molecules. If so, in the TPG/CHOL mixture the acyl chains of each TPG molecule must pack nearly perpendicular to each other, instead of parallel to each other as in the tuning fork configuration of pure TPG. One way to achieve such a nearly perpendicular packing of the acyl chains in each TPG molecule is to rotate the sn-2 chain by 180°, so that the three acyl chains in each TPG molecule orient towards the same direction, similar to that found in the perpendicular configuration of tristearoyl glyceride at the air-water or oil-water interface (see Fig. 10-1 in Small, 1986).

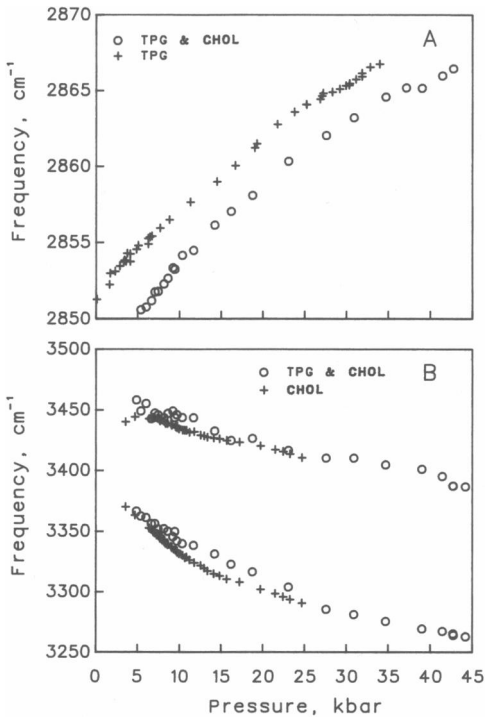

FIGURE 4 (*A*) Pressure dependency of the frequency of the symmetric CH_2 stretching mode of the palmitoyl chains in pure TPG and in the TPG/CHOL mixture. (*B*) Pressure dependency of the frequency of the OH stretching bands of cholesterol in pure cholesterol and of cholesterol in the presence of TPG.

Such a molecular configuration is consistent with the band shape of the $\nu C{=}O$ mode of TPG in the TPG/CHOL mixture, as illustrated in Fig. 5. The $\nu C{=}O$ band at 1,729.2 cm^{-1} in pure TPG shifts to higher frequencies in the spectrum of the TPG/CHOL mixture and thus overlaps with the $\nu C{=}O$ band at 1,739.5 cm^{-1}. In the spectrum of the mixture, the three $\nu C{=}O$ modes produce a single broad band which cannot be resolved into component bands by deconvolution techniques using reasonable deconvolution parameters (Mantsch et al., 1986). In the perpendicular configuration, the three C=O groups in each TPG molecule are in a similar environment, although the individual orientations of the three C=O groups in each TPG molecule differ slightly. The nonequivalent orientations of the C=O groups in each molecule, plus the random distribution of the cholesterol molecules in the TPG lattice result in the broadening of the $\nu C{=}O$ band in the spectrum of the TPG/CHOL mixture.

At atmospheric pressure the center frequency of the single $\nu C{=}O$ band of the mixture is at 1,740.4 cm^{-1}, and increases slightly with increasing pressure (Fig. 2 *B*). Because it is well established that the stretching frequency of a hydrogen-bonded ester C=O group falls into the region 1,710–1,720 cm^{-1}, and that it decreases with increasing pressure (Wong, 1987*b*; Wong et al., 1988; Wong and Mantsch, 1988), the present results show unambiguously that there is no hydrogen bonding between any of the C=O groups of TPG and the OH

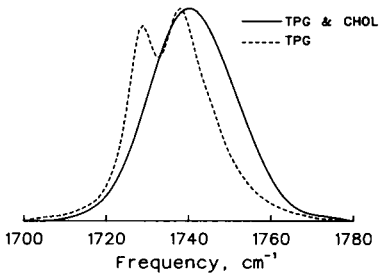

FIGURE 5 Infrared spectra of the C=O stretching band in pure TPG and in the TPG/CHOL mixture.

group of cholesterol. This conclusion is further supported by the frequencies of the cholesterol OH stretching bands and their pressure dependences in the TPG/CHOL mixture. The spectrum of pure cholesterol contains two overlapping bands at 3,451 and 3,363 cm^{-1} (Wong et al., 1989) which arise from two sets of vibrationally inequivalent hydroxyl groups of anhydrous self-associated cholesterol. Fig. 4 *B* compares the pressure dependences of the OH stretching frequencies of pure cholesterol with that of cholesterol in the TPG/CHOL mixture. It is evident that the OH stretching frequencies, as well as the pressure-induced shifts are the same, within experimental error, for pure cholesterol and for cholesterol in the presence of TPG. Moreover, it has been shown that when the OH group of cholesterol forms hydrogen bonds with the ester C=O groups of lipids (Wong et al., 1989), the OH stretching frequency (at atmospheric pressure) decreases to ~3,200 cm^{-1}. In the TPG/CHOL mixture the OH stretching frequencies of cholesterol are practically indistinguishable from those of pure cholesterol and there is no evidence for hydrogen bond formation between the OH group of cholesterol and the C=O groups of TPG.

It has been suggested that one of the mechanisms of cholesterol deposition in atherosclerotic plaques is through hydrogen bond formation between the OH group of cholesterol and the C=O groups of triglycerides (Brooks et al., 1966; Parker and Bhaskar, 1968; Steel et al., 1966). In view of the above results, and because 26-hydroxy cholesterol has been detected as a constituent of human atherosclerotic plaques (Brooks et al., 1966; Steel et al., 1966), we suggest that if there is hydrogen bonding with the C=O groups of triglycerides in atherosclerotic plaques, it occurs via the 26-OH group of 26-hydroxy cholesterol, rather than via the 3-OH group of cholesterol.

CONCLUSIONS

From an analysis of such infrared spectral parameters as the δCH_2 and $\nu C{=}O$ modes, and their behavior under pressure, it is concluded that the molecular structure adopted by solid tripalmitoyl glyceride is that of a tuning fork configuration, similar to that shown to exist in solid trilauroyl glyceride (Small, 1986). The orientation of the acyl chains in solid TPG, either within each molecule or between neighboring TPG molecules, is parallel to each other. Although in the tuning fork configuration the sn-3 acyl chain extends toward the opposite direction of the sn-1 and sn-2 acyl chains, the environment of all three acyl chains is the same, except for the ester C=O group moiety, for which the environment of the sn-3 C=O group differs significantly from that of the sn-1 and sn-2 chains.

In the tripalmitoyl glyceride/cholesterol mixture the cholesterol molecules are embedded in the TPG lattice which results in a reorientation of the zig-zag planes of the acyl chains within each molecule from a parallel packing to a nonparallel packing. Moreover, cholesterol induces more orientational disorder of the TPG molecules in terms of an increase of the reorientational fluctuations of the molecules, and twisting/torsion motions of the acyl chains. Although the cholesterol molecules are embedded in the TPG lattice, no hydrogen bonds are formed between the OH group of cholesterol and any of the three C=O groups of the TPG molecules.

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