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On the Interpretation of the 1100 cm^{-1} Raman Band in Phospholipids and Other Alkyl-Containing Molecular Entities

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The long-standing issue on the interpretation of a Raman band at 1100 cm^{-1} is discussed. By combining observations from studies on lipid bilayers, alkanes, and polyethylenes, one can now definitely assign this band to the presence of isolated gauche bonds. In addition, we discuss the use of an order parameter S_{trans} in lipid bilayer structures.

Introduction

Membranes are essential components of any living cell and therefore a continuous subject of scientific investigation. Membranes, in principle, consist of well-ordered (aligned) molecular chains, constituting the cell wall among other components such as protein channels. Apart from their mechanical function as a cell wall, (small) guest molecules may diffuse through the membrane. As this will distort the membrane structure, characterization tools that enable us to study the structure (ordering) of the membrane also provide information on the penetration of other species into the membranes.

Raman spectroscopy is an analytical technique that allows the characterization of the structure of (packed) alkyl chains as present in alkanes, polyolefins (in particular polyethylene), and, in the contact of the present contribution, to the structure of lipid double layers[1−4] as present in most biomembranes. In this communication, we address two issues related to the interpretation of the Raman spectra of (phospho)lipid systems. Phospholipids are often studied systems in view of their relation to biomembranes. Whereas very useful information can be retrieved from these Raman spectra, including the detection of very subtle effects due to minute quantities of additives,[4] the interpretation of the 1100 cm^{-1} spectral feature in many of the spectra has not yet been clearly settled, at least not in papers on lipid bilayer Raman spectra. Second, the definition of the trans parameter S_{trans} by Peticolas et al.[2] may need further comment. This parameter relates to the amount of C−C bonds in a trans conformation and therefore with the order in the membrane. A system with high order will have most C−C bonds in their trans conformation. We feel that the definition applied by Peticolas et al. does not follow the general definition of an order parameter, and therefore, we want to discuss this issue in order to state more precisely what this parameter means here for readership studying phospholipids and biomembranes. Generally, an order parameter is a quantity that takes a nonzero value for complete order and 0 for complete disorder.[5] These two states are opposite each other in terms of order. This parameter is used, e.g., in the context of spatial order such as molecular orientation order or magnetic spin order. In terms of the discussion between all-trans and chains with gauche content, the all-trans chain must be seen as the fully ordered state and is thus characterized by $S = 1$, whereas the disordered chain as it appears in the melt and in amorphous polyethylene and alkanes has $S = 0$. In practice, the order parameter is defined such that maximum order corresponds to an order parameter value of 1.

Discussion

Let us start discussing the order parameter definition. Order parameters are very useful to characterize, in a quantitative way, the amount of (dis)order in a system. While studying phospholipid systems, Gaber and Peticolas have defined what they have called the trans parameter (eq 2 in ref 2)

$$S_{trans} = \frac{(I_{1133}/I_{reference})_{observed}}{(I_{1133}/I_{reference})_{solid\ DPPC}} \quad (1)$$

The 1090 cm^{-1} band was suggested as a suitable reference, although the 722 cm^{-1} band [we assume that the "772 cm^{-1}" is a typo on page 266 of ref 2, and that what is meant is the 722 cm^{-1} band attributed to the choline headgroup CN vibration, viz. page 262 of ref 2] was suggested for very precise work on DPPC (dipalmitoyl phosphatidylcholine). The general definition of an order parameter implies that $S = 1$ for complete order and $S = 0$ for complete disorder. Following that common definition, eq 1 suggests (see also the right-hand side of Figure 6 in ref 2) $S_{trans} = 1$ for an all-trans DPPC structure. For this to be true, solid DPPC should have all alkyl segments in the all-trans conformation. It is known[6−8] that alkane and alkyl chains exhibit the following characteristic Raman bands: 1060 cm^{-1} and 1130 cm^{-1} for all-trans sequences (from a certain length onward, see ref 9); a broad feature centered around 1080 cm^{-1} and intensity (very broad feature) in the 800−950 cm^{-1} spectral range are both characteristic for the disordered (high gauche content) chain. The spectrum for solid DPPC reported by Gaber and Peticolas, however (viz. Figure 1 in ref 2), has significant Raman intensity in the 1100 cm^{-1} spectral range and in the

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800−900 cm^{-1} spectral range, whereas it is hard to see whether in the 1295−1305 cm^{-1} range intensity is only present at 1295 cm^{-1} (all-trans) and none at 1305 cm^{-1} (disordered chain). It is, therefore, simply not possible that solid DPPC has *only* all-trans alkyl chains, and therefore, S_{trans} as defined by eq 1 does not provide a measure for which $S = 1$ when the alkyl chains are all-trans. Now, Gaber and Peticolas did stress that S_{trans} is a relative measure of the number of trans bonds, and in that sense, the definition eq 1 may be well-used. However, when they state (page 269 of ref 2) that vesicles have a significantly lower probability for occurrence of all-trans conformations, we fear that confusion may arise among readers of this literature by thinking that $S = 1$ does correspond to all-trans alkyl chains. This, however, is certainly incorrect, as we just argued by referring to Figure 1 in ref 2 in combination with the well-known interpretation of the 1000−1300 cm^{-1} spectral range of alkanes and alkyl chains.[7,8] Because the intensity of the Raman signal at 1130 cm^{-1} does not seem to be proportional to the length of the trans sequence,[9] it is not clear how much $S_{trans} = 1$ defined according to eq 1 deviates from the true all-trans chain. Gaber and Peticolas stated (page 269 in ref 2) that S_{trans} is a relative measure of the number of trans bonds in a sequence of three or more trans bonds. Perhaps this was the consensus at the time (1977), but more recently, it has been argued that only trans sequences longer than some eight C−C bonds contribute significantly to the intensity of the Raman bands centered near 1060 cm^{-1} and 1130 cm^{-1} that are also found in 100% crystalline polyethylene.[9]

In conclusion, the definition of the order parameter according to eq 1 does not refer to the generally accepted interpretation and definition of an order parameter in physical chemistry. However, the order parameter, eq 1, might be used exclusively to obtain a relative measure for disorder in specific phospholipids.

Having said this, we turn to the interpretation of the 1100 cm^{-1} spectral feature observed in quite a few lipid bilayer Raman spectra, e.g., refs 2, 4, 10, and 11. As referred to already, it is well-known that the 1060 and 1130 cm^{-1} bands are characteristic of all-trans alkyl chains. Amorphous or melt alkanes or polyethylene reveal a broad band centered at 1080 cm^{-1}. Thus, for example, a semicrystalline polyethylene sample exhibits all-trans bands at 1060 and 1130 cm^{-1} and a broad band at 1080 cm^{-1} representing the amorphous fraction. A band in the 1090−1100 cm^{-1} spectral range shows up in the Raman spectrum of pure, solid DPPC and persists in the low-temperature aqueous phase, and is also interpreted as being due to structures containing gauche bonds, as was already reported by Peticolas et al.[2,10] The nature of this band does not, however, seem to have been determined unambiguously. A Raman band at this vibrational frequency was also reported to show up in solid nonadecane,[11] in solution-crystallized cyclic alkanes,[12] and in the low-temperature spectra ($T = 90$ K) of solid oleic acid (*cis*-9-octadecenoic acid) and elaidic acid (*trans*-9-octadecenoic acid).[13] It is interesting to note that, as was shown for *n*-nonadecane,[11] this band is replaced by the broad band centered at 1080 cm^{-1} when the material is brought into the melt (the statement by Zerbi et al., ref 11, page 3181, issue (*z*), that upon melting *n*-nonadecane the 1090 cm^{-1} band becomes strong rather than the 1080 cm^{-1} is at variance with the actual spectrum they reported, Figure 11 in ref 11, as can be corroborated after careful inspection of that figure).

When an alkane or alkyl chain exhibits significant disorder, significant Raman intensity in the form of a broad feature comes up in the 850 cm^{-1} spectral range.[7,8] As this is not the case for *n*-nonadecane in the solid phases, the 1090 cm^{-1}, assuming its origin is the same for the systems mentioned, does not just arise from entirely statistically disordered chains. The latter is further supported by the observation that the 1090 cm^{-1} is comparatively sharp compared to the 1080 cm^{-1} band profile. The clear and intense presence of the 1090 cm^{-1} band in solution-crystallized cyclic alkanes suggests attribution to isolated gauche bands or, possibly, isolated trans bonds connecting the gauche turns at the 180° turn present in the cyclic alkanes. This interpretation is further supported by a theoretical study reported by Zerbi and Gussoni.[14] Lattice dynamical calculations revealed that, each time a gauche bond is introduced into an all-trans hydrocarbon, a vibrational mode near 1095 cm^{-1} appears.

In conclusion, the interpretation due to Zerbi and Gussoni[13] of the Raman band near 1100 cm^{-1} as being due to isolated gauche bonds is in agreement with the bandwidth of this band in the various systems in which it was observed (see above), and the only acceptable interpretation suggested thus far. The 1090 cm^{-1} should, therefore, not simply be interpreted as the equivalent of the 1080 cm^{-1} band in amorphous, disordered alkane and alkyl systems. On the other hand, it needs further studies, involving other characterization techniques, to explain why such a single gauche defect occurs in, e.g., the solid forms of oleic and elaidic acid.

Finally, when we now turn to the DPPC model membrane system, we have observed a shift from a band centered at 1100 cm^{-1} toward one centered near 1080 cm^{-1} when DPPC goes from the (rippled) gel phase $P_{\beta'}$ into the liquid crystalline phase L_α. The named phase transition is near 40 °C,[4] and it is near that temperature that the mentioned shift in band position has been observed, viz. Figure 1 (taken from ref 4). At the same time, the all-trans band at 1130 cm^{-1} decreases in magnitude, whereas the band near 1160 cm^{-1} is more or less retained. The observation made here that intensity of the all-trans peak at 1130 cm^{-1} drops more quickly than for the accompanying band at 1060 cm^{-1} is in full accordance with earlier reports regarding this phenomenon on melts of long alkanes and polyethylene[8] and ab initio calculation.[9] So, in this respect, the alkyl chains of DPPC do seem to behave quite similarly to alkanes and polyethylene.

Let us now return to the transformation of the 1100 cm^{-1} band into a band centered more near 1080 cm^{-1}. When gauche bonds are introduced, it makes a difference for the Raman spectra whether the gauche bond can appear *anywhere* in the chain or (preferably) near a chain end. Because the alkyl chains have a length of only 16 carbon atoms, with a maximum sequence of 13 C−C bonds that can potentially be characterized as trans, a gauche in the middle would induce such short all-trans sequences that only a strongly reduced all-trans intensity is retained at the 1060 cm^{-1} and 1130 cm^{-1} spectral positions.[9] It seems, however, from recent work that there is a preference for the gauche bonds to start to be formed near the end of the alkyl chain.[15,16] When going from the (rippled) gel phase $P_{\beta'}$ into the liquid crystalline phase L_α, the gauche content strongly increases as was concluded from IR spectra on DPPC.[17] Very interestingly, a recent molecular dynamics study[18] revealed that the rippled gel phase shows tails of the lipid molecules being highly disordered, showing many gauche defects. Thus, the conclusion of these observations is that our experimental observations and the simulation data from ref 18 lend mutual support to each other. It also points out once more that the 1100 cm^{-1} band is due to localized gauche defects, whereas the disordered chain has a very broad spectral feature centered near

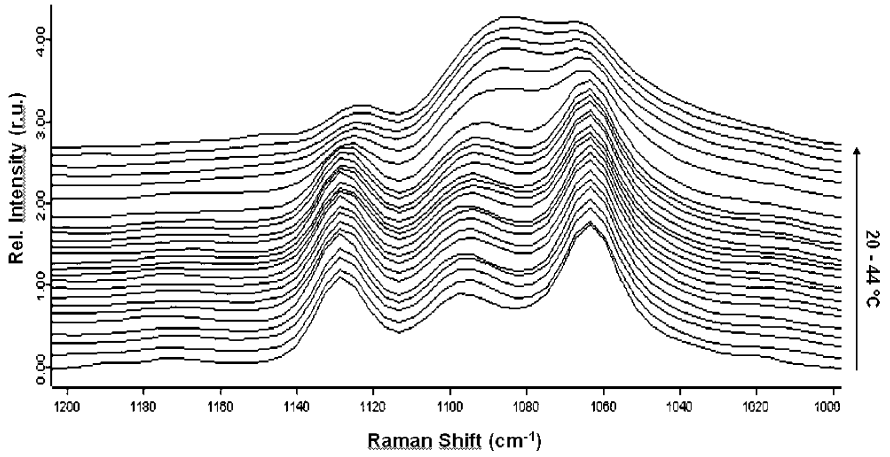

Figure 1. The Raman spectra of the DPPC/water system in the spectral range of C−C stretching region (1000−1200 cm^{-1}) from 20 to 44 °C (1 °C steps).

1080 cm^{-1}. When the liquid crystalline phase L_α is entered, the chain starts to exhibit disorder over its full length.

Finally, the presence of the 1100 cm^{-1} band in all the DPPC spectra in the different phases (Figure 1) indicates that pure DPPC does not contain only all-trans chains, in agreement with the preceding discussion regarding the definition of S_{trans} according to eq 1 and the presence of gauche bonds for $S = 1$. Second, the spectra in Figure 1 suggest that upon the transition into the liquid crystalline phase many more gauche bonds appear in the chain, i.e., various distributions of sequences of trans and gauche bonds as revealed by the broader spectral feature centered at 1080 cm^{-1}, rather than isolated gauche bonds as characterized by the 1090 cm^{-1} band.

Thus, on the basis of existing literature, the most supported interpretation of the 1090 cm^{-1} spectral feature is the presence of isolated gauche bonds. The presence of this band in the spectrum of solid DPPC then suggests the alkyl chains are not all-trans. What is needed to make definite statements are, we believe, the Raman spectra of comparatively short alkanes (C_{10}−C_{20}), both in the crystalline state as well as in the melt state, to study possible unknown effects in the spectra of alkanes with length comparative to alkyl chain in lipids. Reasons include the observation that for C_{24}[12] it is not quite clear from the experimental melt spectrum whether the band is centered at 1080 or 1090 cm^{-1}. Moreover, the spectra reported in refs 2 and 11 are of insufficient quality to allow for accurate analysis. Use of a modern spectrometer would most likely result in very high quality sharp line spectra.

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