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HOMOGENEOUS CATALYTIC DEUTERATION OF FATTY ACYL CHAINS AS A TOOL TO DETECT LIPID PHASE TRANSITIONS IN SPECIFIC MEMBRANE DOMAINS: A FOURIER TRANSFORM INFRARED SPECTROSCOPIC STUDY

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Synthetic phospholipid molecules have been deuterated by using a water soluble catalyst and deuterium gas. The physical state of both deuterated segments and unaffected bulk part of the lipid molecules can be monitored simultaneously by Fourier Transform Infrared Spectroscopy. It is shown on multilamellar phospholipid systems that the deuterated segments can be used as structural probes. Whereas the ν(C-H) frequencies represent an average conformational order along all the alkyl chains present, by following changes in ν(C-D) vibrations, mobility of those membrane domains deuterium labeled at specific depths in the hydrocarbon core can be estimated. The potential importance of this new approach in the study of biological membranes is discussed.

The usefulness of the homogeneous hydrogenation catalyst, palladium di(sodium alizarine monosulphonate), $Pd(QS)_2$, for reducing fatty acyl chain double bonds in isolated native membranes and in intact cells in order to examine the consequences of the resulting reduced lipid flexibility on various cell functions has been well demonstrated (1-7).

This catalyst was shown to reduce isolated *cis* double bonds of free and glycerolipid-bound fatty acids, but not those present in chlorophylls, carotenoids and plastoquinone (8). Within a particular lipid class, the catalyst showed little specificity towards different molecular species (5,9,10,11). Transition from the bilayer to the inverted hexagonal phase appeared to slow the rate of hydrogenation appreciably, and even bilayer phase lipids, while themselves being very susceptible to hydrogenation, retarded the diffusion of the catalyst to inner regions of the multilamellar

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vesicles (5). Thus, with careful timing of the reaction, a selective surface membrane modulation could be obtained with both photosynthetic (12,13) and mammalian cells (3). Tight association of specific lipids with proteins, like in the case of protein-immobilized cardiolipins in rat liver mitochondria could also reduce the apparent accessibility of the substrates to the Pd-complex (10). Differential accessibility of the catalyst to specific acyl lipids in thylakoids derived from atrazine-resistant and -susceptible weed biotypes enabled the disclosure of subtle alterations in microheterogeneity of membranes, evidenced also by the fluorescence life-time distribution analysis, but shown to be apparently undetectable if tested on bulk fluidity level (14).

By further exploiting the fact that hydrogenation acts like a structural probe while it is able to reveal the microheterogeneous organization of biomembranes, here we introduce a new strategy to increase its selectivity. Unsaturated lipids of model membranes have been catalytically deuterated, and the thermotropic properties of the deuterium labelled pool of phospholipids were monitored by the non-perturbing, Fourier transform infrared (FTIR) spectroscopy. Since the FTIR spectroscopy can detect the gel-to-liquid-crystalline phase transition of the specifically deuterated lipid domains this procedure is a promising tool to measure hydrocarbon chain conformational order of *in situ* selectively labelled membrane regions even within complex biological systems.

Materials and Methods

The catalyst, palladium di (sodium alizarine monosulphonate), $Pd(QS)_2$, was purchased from Molecular Probes, Eugene, OR. Dioleoyl-phosphatidylcholine (DOPC) and dioleoylphosphatidylethanolamine (DOPE) were obtained from Serdary Research Laboratories and used after confirming their purity by TLC. Gas chromatographic analysis of the fatty acids was carried out after transesterification as described in (11). Catalytic deuteration of the lipid dispersion was conducted exactly as in (5) except that deuterium gas (from Linde, Germany) was used instead of hydrogen. Samples for FTIR measurements were prepared as follows. 3 mg phospholipid dissolved in chloroform was dropped onto a CaF_2 window; the solvent was evaporated and the resulting film was hydrated by adding 5 μl of double distilled water. The sample was closed by an other window using a 10 μm thick aluminium spacer. To ensure the constant level of hydration, edges of the windows were sealed with silicon grease. FTIR spectra were recorded on a Philips PU9800 Fourier transform infrared spectrometer, spectral resolution was 2 cm^{-1}, and 128 scans were collected. Spectra were calculated with the standard procedure of the spectrometer. No additional data manipulation was done. Temperature control was by means of a Peltier device, and sample temperature was recorded with a thermocouple within the sample window.

Differential scanning calorimetry was performed as in (15). The lipid sample was placed inside an airtight ampoule. Calorimetric scans were recorded with a high sensitivity differential scanning calorimeter (Hart DSC 7707 Series) between 25 to 85 °C, at a scan rate of 20 °C/hr.

Results and Discussion

In accordance with the mechanism of the action of Pd-catalyst (5-7), saturation reaction of phospholipid liposomes conducted under deuterium atmosphere

should give rise to the replacement of =CH- groups by -CHD- ones. Fig. 1 shows the ν(C-H) (2800-3000 cm^{-1}) and the ν(C-D) stretching (2050-2250 cm^{-1}) regions of the FTIR spectrum obtained from partially saturated DOPC (mol % of 18:0=80, *cis*-Δ^9-18:1=15 and *trans*-Δ^9-18:1=5). The C-D stretching region of the saturated DOPC FTIR spectrum is shown enlarged in the inset of Fig. 1. Two of the three bands of the region at 2179 and 2091 cm^{-1} can be assigned to symmetric and anti-symmetric CD_2 stretching modes, respectively (16). Although the reaction of an olefinic bond with D_2 formally results in the formation of CHD groups only, the presence of CD_2 units is not completely unexpected (17). However, understanding the origin of these CD_2 groups, obviously present in a significant quantity according to the FTIR spectra may offer a deeper insight into the reaction mechanism of the Pd-catalyst and awaits further studies. We assign the third band of the 2050-2250 cm^{-1} region at 2138 cm^{-1} to ν(C-D) stretching originating from -CHD- groups. Due to the large mass difference between H and D atoms the ν(C-D) vibration is not coupled to the ν(C-H) in these groups. In accordance with the expectations, νCD locates between $\nu_{as}CD_2$ and ν_sCD_2. In addition, the 2138 cm^{-1} band is the most intensive of the three as predictable from the catalyst action.

Stretching frequencies of both CH_2 and CD_2 groups of lipid acyl chains are known to be sensitive markers of lipid phase properties in biological membranes and model systems (18). Changes of these frequencies reflect the isomerization in the hydrocarbon chains that occurs as the lipids are transformed from straight chain, all trans conformation in the gel phase to fluid, predominantly gauche conformation in liquid crystalline phase (15). No data are available, however, on the sensitivity of νCD bands for gel-to-fluid phase transitions. The comparison between the tempera-

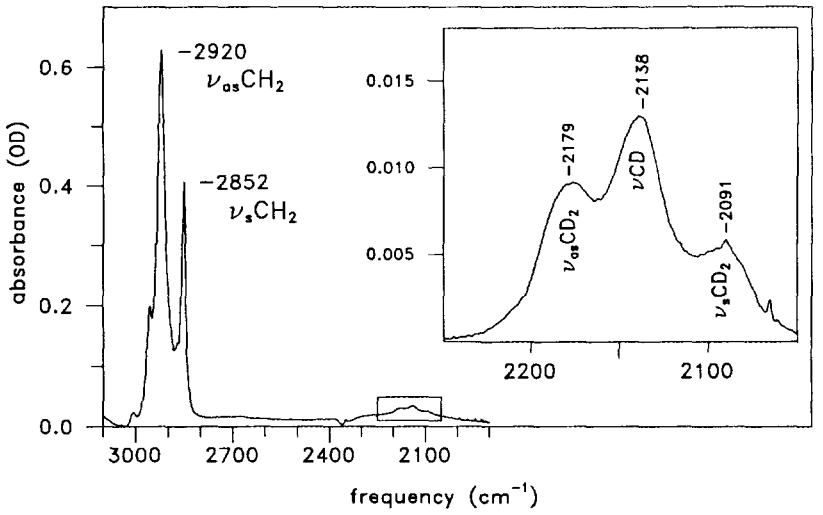


Fig. 1. Fourier Transform Infrared spectrum of 80% deuterated DOPC. Inset shows enlarged the ν(C-D) region of the spectrum.

ture dependence of the frequency of $\nu_s CH_2$ and νCD stretching band of highly deuterated DOPC (fatty acid composition is identical with sample used in Fig. 1.) is shown by Fig. 2. It can be seen that frequency shifts of both modes take place at the same temperature, i.e. the νCD is an equally good marker of lipid phase transitions as $\nu_s CH_2$. It is also interesting to note that the magnitude of frequency shift obtained upon the phase transition of partially saturated DOPC is about twice as large for νCD as detected for $\nu_s CH_2$. This observation is in agreement with the results of Cameron et al. (19). Analysis of their data shows that frequency shifts of both $\nu_s CD_2$ and $\nu_{as} CD_2$ modes observed upon gel-to-liquid-cristalline phase transition have a maximum at deuterium labelling in central segments of the acyl chains, i.e. in that particular core of the membrane which is being deuterated by the palladium complex in the present study. This phenomenon can apparently be attributed to the mobility gradients of alkyl chains within bilayer: the molecular motion is fairly constant for the first nine chain segments but rapidly increasing thereafter towards the methyl end (20,21).

With mixture of lipids having the same head group but very different fatty acyl chains, packing in the solid state will be different leading to solid-phase immiscibility. Like in natural membranes, such phase-separated lipids might be expected to melt independently on warming leading to low entalpy endotherms if tested by DSC (15). In fact, if the sample was carefully prepared by less advanced saturation of DOPE (mol % 18:0=55, *cis*-Δ^9-18-1=16, *trans*-Δ^9-18-1=29), the thermogram of the multilamellar system revealed at least three small endotherms (Fig. 3). In the frequency of the symmetric CH_2 stretching vibration measured in the same DOPE sample a slight increase was seen with a midpoint of about 45 °C (Fig. 4) corresponding to the relatively broad gel-to-liquid crystal phase transition of this mixture of various fatty acid molecular species. In the frequency of the C-H stretching modes no other characteristic change can be detected at further eleva-

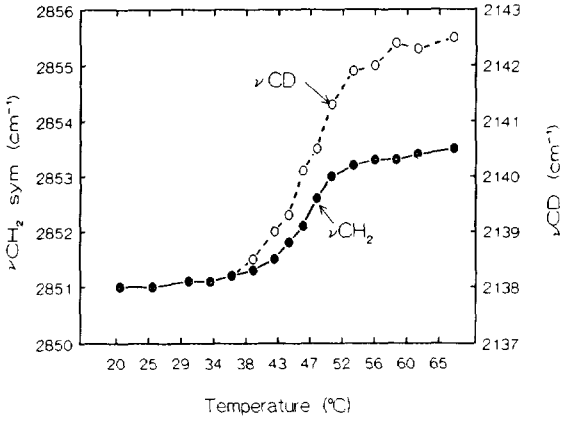
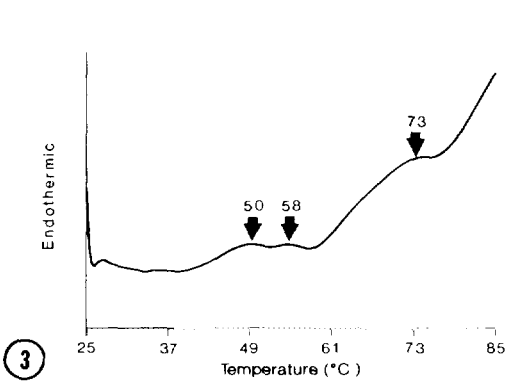


Fig. 2. Frequency shifts of the νCD and $\nu_s CH_2$ modes of 80% deuterated DOPC multilamellar system upon gel-to-liquid cristalline phase transition.



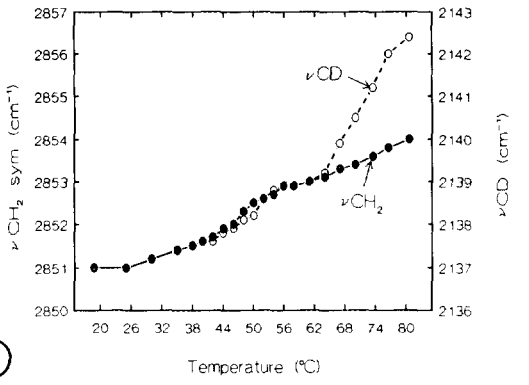


Fig. 3. Thermogram of partially, 55%, deuterated DOPE vesicles. Endotherms are indicated by arrows with the midpoint temperatures, respectively.

Fig. 4. Frequency shifts of the νCD and $\nu_s CH_2$ modes of partially , 55%, deuterated DOPE multilamellar system upon increasing the temperature from 20 to 80 °C. For details see the text.

tion of temperature as shown in other phosphatydilethanolamine systems (22), we conclude, therefore, that lamellar to inverted hexagonal phase transition did not take place in the temperature range investigated. In contrast to νCH_2, νCD exhibited a large further shift centered around 73 °C. Apparently this phase change was detectable also by DSC (Fig. 3.). The possible reason for this finding could be, that although a phase separated and deuterated domain melts independently within the lipid mixture it remains undetectable by testing through the frequency shift of the νCH_2 bands. The insensitivity of the apparent νCH_2 frequency derives from the fact that CH_2 groups all along the fatty acyl chains contribute to it. Thus, it reflects an average order of the chains being either saturated or unsaturated. In contrast, νCD in this case arises selectively from the C_9-C_{10} regions of the saturated fatty acyl chains where the chain mobility is still low. Moreover, the frequency shift of νCD observed upon gel-to-liquid-cristalline phase transition exceeds about twofolds νCH_2 frequency shifts. As a consequence of these factors, the presence and phase transition of such domains could be identified. Further studies with mixture of lipids also indicate (data not shown) that melting of segregated and deuterated phospholipid pools are characterized by such a low entalpy change that it is difficult to separate their endotherms from the noise in the DSC baseline, whereas they can be distinguished by recording C-D stretching vibrations.

In conclusion, we demonstrated that catalytic deuteration of phospolipids at certain positions of lipid acyl chains by using the water soluble Pd-catalyst and deuterium gas is a simple and rapid method. FTIR spectroscopy offers *selective* information about deuterated lipid molecules by monitoring simultaneously the unreacted species as well. Deuterating alkyl chains with double bonds at specific

positions the saturated molecules can be used as structural probes. In the present case the double bond between C_9 and C_{10} of the oleic acid alkyl chain was deuterated thus, the signals about the physical state of this specific membrane domain by FTIR could be selectively observed. It is usually inferred that biophysical characteristics measured on isolated membranes also apply to the membranes in live systems. In contrast, infrared spectroscopic study of the gel to liquid-crystal phase transition in live *Acholeplasma laidlawii* cells revealed, that the live mycoplasma is able to keep the fluidity of its plasma membrane at a much higher value than that of the isolated plasma membrane at the same temperature (23). By applying reaction conditions enabling selective saturation of surface membranes (3,6,7,12,13), the technique could directly be applied to live cells to measure the alterations in the conformational order and thermal characteristics of plasma membrane without its isolation.

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