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LIPID REORGANIZATION IN BIOLOGICAL MEMBRANES

A STUDY BY FOURIER TRANSFORM INFRARED DIFFERENCE SPECTROSCOPY*

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Summary

The first application of infrared difference spectroscopy to the study of a natural biological membrane is described. Perdeuterated palmitic acid was incorporated biosynthetically into the lipids of the plasma membrane of *Acholeplasma laidlawii* and the temperature-induced structural rearrangement of the endogenous lipids monitored via their C—^{2}H vibrational modes. Changes in infrared parameters were studied between 0 and 50°C and contrasted with those occurring in the model membrane system of 1,2-diperdeuteropalmitoyl-*sn*-glycero-3-phosphocholine. The phase transition of the biomembrane occurs over a 20°C range with the temperature of the maximum rate of change of absorbance coinciding with that of the sharp phase transition of the model membrane.

There is a growing interest in the state of lipids in natural membranes, particularly in the nature of their packing. This is important for their biological function, but difficult to measure directly [1]. Most spectroscopic studies have been restricted to model membranes and so far only ESR of spin labels [2] and ^{2}H NMR [3,4] have had some success with natural membranes. We report here the first study by vibrational spectroscopy of a thermal perturbation to the lipid acyl chains in a natural membrane, the plasma membrane of the microorganism *Acholeplasma laidlawii*.

High purity samples of 1,2-diperdeuteropalmitoyl-*sn*-glycero-3-phos-

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Abbreviation: DPPC-d_{62}, 1,2-diperdeuteropalmitoyl-*sn*-glycero-3-phosphocholine.

phocholine (DPPC-d_{62}) were obtained from Lipid Specialities Inc., Boston. Thin-layer chromatography gave a single spot and polarimetry measurements indicated that the natural *sn*-3 stereochemistry was retained. Multibilayer dispersions of DPPC-d_{62} for infrared spectroscopy were obtained as previously described [5,6]. Palmitic acid-d_{31} was incorporated biosynthetically into the glycerolipids of *Acholeplasma laidlawii* plasma membrane according to a procedure described earlier [7]. Samples for infrared spectroscopy were prepared as follows: 5 mg lyophilized *A. laidlawii* plasma membranes were hydrated with 1 ml double distilled water. After centrifugation at 8000 × *g*, the supernatant was removed and the fully hydrated membranes were placed in the middle of a CaF_2 window which was assembled into a temperature-controlled 50-μm thick cell. Spectra were collected between 0 and 50°C using a Nicolet 7199 Fourier transform infrared spectrometer, equipped with a deuterated triglycine sulphate detector. Difference spectra were generated by subtracting lower temperature spectra from higher temperature spectra; this procedure leads to positive peaks if the absorption increases with temperature.

Natural membranes are complex structures composed of different classes of biomolecules and likewise their infrared spectra are extremely complex. The characteristic vibrational frequencies of the membrane lipids are normally obscured by either the intense water absorption or those of other membrane constituents. In order to overcome this handicap with natural membranes, we studied the membrane lipids of *A. laidlawii* grown on fatty acid-free broth supplemented with perdeuteropalmitic acid. Since the integrity of the acyl chains is preserved by the microorganism we could monitor the C—^{2}H stretching vibrations located in the relatively unobstructed 2300—2000 cm^{-1} window (Fig. 1). The strong absorption bands at 2200 and 2090 cm^{-1} due to the antisymmetric and symmetric C^2H_2 stretching modes of the palmitoyl chains [5,8] are good probes for investigating the thermal lipid reorganization in this natural membrane. Both bands show temperature dependent variations in peak position, peak height, half-bandwidth and integrated intensity. The upper trace in Fig. 1 shows a typical infrared difference spectrum which was used to quantitate the change occurring in the vibrational pattern of *A. laidlawii* membrane lipids. From such individual difference spectra, a gradient parameter, $\Delta A/°C$, was obtained by measuring the peak to peak height for a given band in the difference spectrum as exemplified in Fig. 1. The ΔA value was normalized by dividing it by the temperature increment over which the individual difference infrared spectra were recorded. This parameter simultaneously reflects changes in peak height, peak position and bandwidth.

Fig. 2 shows a plot of $\Delta A/°C$ versus temperature for the *A. laidlawii* membrane lipids (solid line) compared to that for DPPC-d_{62} multibilayers (broken line). The profile for the DPPC-d_{62} is typical of model membranes, showing very little change with temperature except for a small increase in the region of the pretransition [9,10] around 30°C and a very sharp increase around the main gel to liquid crystal phase transition. We have shown earlier by peak height measurements [5] that the main rearrangement of

the deuterated lipid acyl chain packing in DPPC-d_{62}, which occurs at 37.5°C [5,9,10], is essentially complete within 0.7°C.

In the case of the *A. laidlawii* plasma membrane, several features are evident. The rearrangement of the endogenous lipid pool takes place between 22 and 42°C, that is over a wide temperature range (about 20°C) compared to that of DPPC-d_{62}, in agreement with earlier calorimetry [11] and ^{2}H NMR [3] observations. It is interesting, however, that after a steady increase of the rate of change, it reaches a maximum at a temperature coinciding with that of the phase transition of the corresponding model membrane. In the temperature range below the phase transition the rate of change in *A. laidlawii* membranes is similar to that in the model membrane. A common feature of the natural and model membrane is that after the main lipid reorganization is completed, the $\Delta A/°C$ values decrease abruptly and become quite similar.

The model generally proposed for the rearrangement of the lipid packing in model systems is an increase with temperature of the gauche rotamer population in the mainly trans lipid acyl chains, culminating in a condition where the chains have relatively free rotation about individual

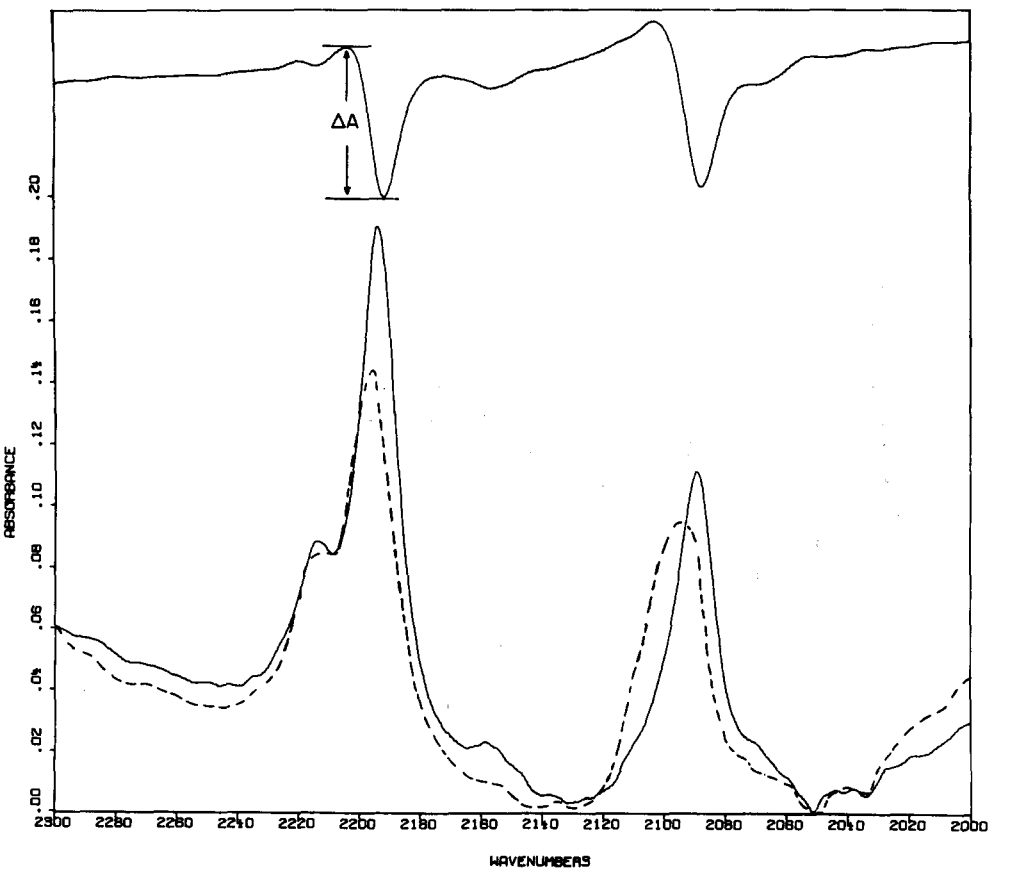

Fig. 1. Bottom: Fourier transform infrared absorbance spectra of *A. laidlawii* plasma membranes (grown on media supplemented with perdeuteropalmitic acid), in the C—^{2}H stretching region at 15°C (—) and at 42°C (- - - -). Top: Infrared difference spectrum obtained by subtracting the 15°C spectrum from that obtained under identical conditions at 42°C.

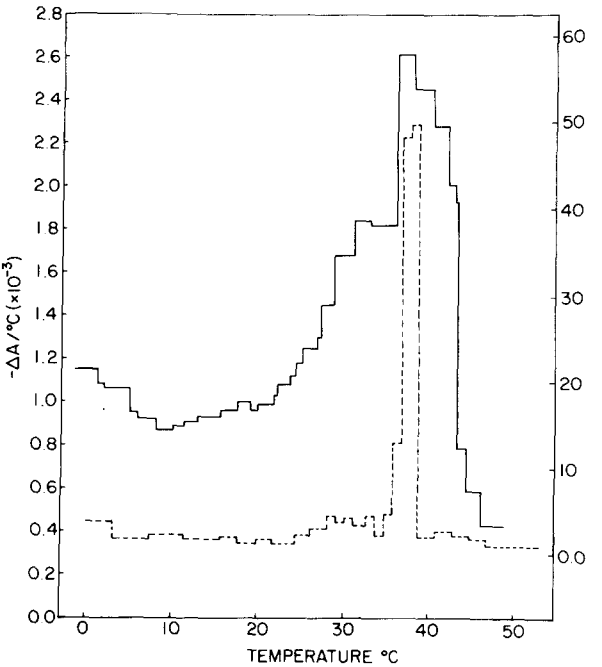

Fig. 2. Temperature dependence of the infrared spectral parameter ΔA (rate of change of the infrared spectrum) for the antisymmetric C^2H_2 stretching vibration in *A. laidlawii* plasma membranes (solid line and left hand scale) and in DPPC-d_{62} multibilayers (broken line and right hand scale).

C—C bonds and a high gauche/*trans* ratio. This model is confirmed by recent infrared studies with model membranes which showed that a number of bands characteristic of the solid state disappear completely during the phase transition [6]. The similarity of the two curves above 45° C (Fig. 2) indicates that after the phase transition the degree of disorder in the two types of lipids is very similar. The rate of change, that is the rate of increase of disorder in the lipid acyl chains at the phase transition reflects a strongly cooperative effect, which in the case of the natural membrane, is modulated by the other membrane components.

Recent ^2H NMR studies [3] have indicated that in the region of the phase transition of *A. laidlawii* membranes there is an equilibrium between temperature-dependent populations of liquid-crystalline and gel-state lipid. Spin label ESR studies [12] have suggested that some rigid lipid is specifically associated with membrane protein. We are presently isolating the various lipid classes from the *A. laidlawii* membranes to compare the widths of their thermal transitions with those of the intact membrane.

In concluding the comparison of the lipid reorganization in model and natural membranes we note that the coincidence of the two maxima in Fig. 2 demonstrates that, despite the broadening of the phase transition, a considerable proportion of the lipid is still highly ordered and sufficiently isolated from possible perturbing molecules that it behaves similarly to the lipids in the model system, undergoing a rapid conversion to the disordered liquid crystal state at approximately the same temperature. The relative proportions of gel and liquid-crystalline lipid in the membrane of *A. laidlawii*

are apparently very important determinants of the viability of the organism [13].

The present investigation demonstrates the viability of infrared spectroscopy for the study of biological membranes. The selective lipid absorption in the infrared spectrum can be achieved by a high level (80%) of biosynthetic ^{2}H fatty acid incorporation, while the lack of sensitivity of ordinary infrared spectroscopy can be overcome by the use of Fourier transform techniques.

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