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COMBINATION OF DSC AND X-RAY DIFFRACTION IN THE STUDY OF THE PHASE BEHAVIOUR OF LIPIDS
Mini-review

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Abstract

The phase behaviour and phase stability of lipids are of importance in an understanding of the biological functions of cell membranes. Among a variety of physical techniques employed to study the phase behaviour and structural properties of polar lipids, differential scanning calorimetry and X-ray diffraction have proved to be successful and are the most frequently used methods. Applications involving a combination of the two techniques, particularly when synchrotron radiation is used as the light source of X-ray diffraction, are reviewed in this article.

Keywords: DSC, lipid, phase behaviour, synchrotron radiation, X-ray diffraction

Introduction

It is well known that biological membranes contain a great diversity of lipids. To understand their biological functions, the physical and chemical properties of lipids have been the subject of investigation for several decades. Particularly the phase structures and their stability have been at the centre of the research interests of many scientists [1–3]. Similarly to the coupling of different spectroscopic techniques with thermal analysis to reveal details of chemical reactions, X-ray diffraction and differential scanning calorimetry (DSC) have been complementary methods in studies of the structural properties of lipid assemblies. This mini-review discusses the progress made in the applications of a combination of thermal analysis methods and X-ray diffraction techniques in studies of phospholipid systems, where they may be used simultaneously or separately.

Phase identification of lipid dispersions

Lipids dispersed in aqueous solutions or other solvents exhibit different phase behaviour at different temperatures. The most common phase is the lamellar phase with a two-dimensional structure, i.e. a stacked bilayer structure with intervening layers of solvent between them. This long-range order can be identified by using an X-ray diffraction method, where the Bragg spacings give a ratio of 1:1/2:1/3:1/4: etc. Depending on the ways in which the acyl chains arrange themselves, they can form lamellar liquid-crystal phases (L_α), lamellar-gel phases (L_β), or ordered lamellar crystalline phases (L_c). As concerns the lamellar liquid-crystal and gel phases, wide-

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angle Bragg diffraction reveals a broad diffraction maximum at around 0.46 nm and a sharp maximum at 0.42 nm, respectively. More ordered lamellar crystalline phases usually give patterns involving more than one sharp diffraction maximum in the wide-angle region. In addition to the lamellar phases, the long-range order of a lipid assembly can also display non-bilayer structures, such as an inverted hexagonal phase (H_{II}), where the ratio of the diffraction spacings is $1:1/\sqrt{3}:1/\sqrt{4}:1/\sqrt{7}:1/\sqrt{9}$:etc. The hexagonal phase is also a liquid-crystal phase.

Conventional X-ray diffraction with DSC

Conventional X-ray diffraction uses a light source from X-ray tubes or generators. It usually takes several minutes to hours or even longer to collect qualified diffraction data from lipid dispersions, which are multilamellar vesicles in nature [4]. It is therefore very difficult to run experiments in the scale of real time and to learn dynamic properties of phase transitions. Nevertheless, structural information of lipid assemblies can be collected at individual temperatures across phase transitions. Phase diagrams can then be constructed combining the thermal data from DSC and the structural data from X-ray diffraction [4–6]. In fact, most of the phase diagrams of lipid systems collected in the book compiled by Marsh [7] were constructed in this way. The information that can be retrieved from the phase diagrams includes lipid phase separation properties, which are closely related to the localization of certain lipid species to fulfil certain biological functions [8]. Phase diagrams of lipids have also been employed in defining conditions for membrane protein crystallization [9].

The conventional static method can also be used to construct electron density profiles of lipid bilayers [10, 11]. This is very useful in separating the span of a lipid bilayer from that of the solvent layer [11, 12]. When hydrophobic or amphiphilic guest molecules are incorporated in the dispersion, the electron density profiles can help to locate the guest molecules inside the membrane bilayer region [11]. One point that should be kept in mind is the thermal history dependence of the phases. Different modes of treatment of a sample may result in different lipid structures, even at a definite temperature, due to the existence of many metastable phases. Special attention should be paid when a set of X-ray diffraction data is correlated to the DSC results.

X-ray diffraction using a synchrotron radiation source with DSC

The brightness of synchrotron radiation is at least 10^3 times that of the characteristic X-rays from the best conventional generator, and 10^6 times that of a continuous X-ray spectrum [13]. This means that, in comparison with a conventional method using X-ray tubes, the time resolution can be improved by some 3–4 orders of magnitude in recording diffraction data, to a range as short as milliseconds [14]. Temperature scanning of the specimen can therefore be exploited to study phase transitions and the dynamic phase behaviour of lipid systems. The structural information obtained in this way can then be compared with the results from DSC. Due to the excellent properties of the synchrotron beam in its brightness and collimation, the separation between sample holder and detector can be extended to several metres [14, 15], and even to as long as 10 m [16]. This will greatly improve the angle-reso-

lution of the diffraction data. In this case, a separate detector can be installed to collect the diffraction data simultaneously at wide angles [15, 17, 18].

The major drawback in using a synchrotron radiation source is that the light beam is so bright that samples could be damaged or locally overheated if radiation lasts for a too long time. Although the sample damage problem can be verified by using a variety of analytical methods such as thin-layer chromatography, a simpler way to address the question is to examine the reproducibility or the time-dependence of the scattering pattern of the samples. Actually, what an examiner cares about most is whether the potential damage or overheating is significant enough to cause different scattering patterns.

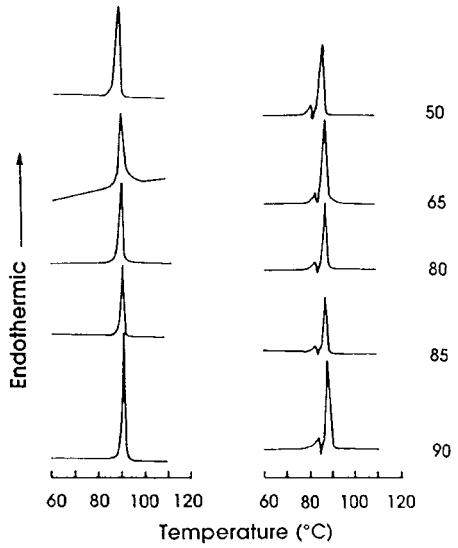

Fig. 1 DSC heating scans of dispersions of distearoyl-phosphatidyl-ethanolamine in glycerol with different solvent contents (wt%) as indicated on the right side of the curves. The thermoanalytical curves on the left were recorded during the initial heating of samples equilibrated for 30 min at 20°C, while those on the right were recorded during reheating immediately after cooling from 110 to 60°C (scanning rate 2°C min^{-1})

As an example, Fig. 1 shows DSC heating curves of dispersions of distearoyl-phosphatidyl-ethanolamine in glycerol in different ratios. The aim of the study was to justify the effect of the popular cryoprotectant glycerol on the phase behaviour of a model membrane lipid [18]. All the thermoanalytical curves in the left panel demonstrate a single endotherm during the initial heating, where the sample had been kept at 20°C for 30 min before measurement. To understand the nature of the transition, synchrotron X-ray diffraction experiments were run; the data on a dispersion with equal mass ratio are shown in Fig. 2. The phase transition was thereby identified as a transition from a highly-ordered crystalline phase (L_c) to a disordered inverted hexagonal phase (H_{II}).

Differently from the initial heating, the thermoanalytical curves of the reheating scans immediately after cooling demonstrated an interesting pattern of endotherm-

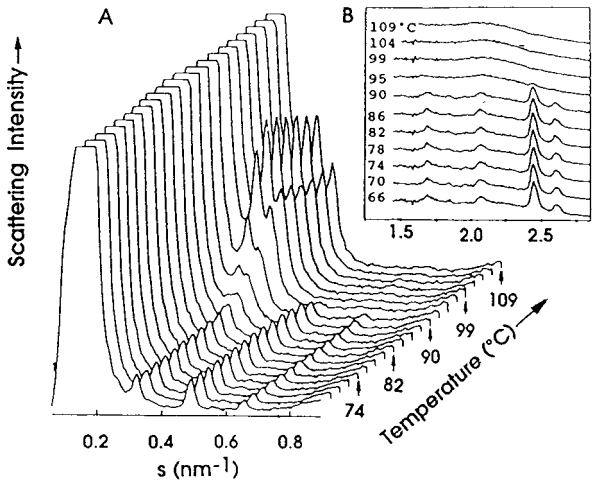

Fig. 2 Real-time X-ray diffraction patterns of distearoyl-phosphatidyl-ethanolamine in glycerol (50 wt%) showing the lamellar-crystalline to inverted hexagonal phase transition. Data were collected continuously during an initial heating scan at a rate of $5^{\circ}C\ min^{-1}$. Sample was prepared as in Fig. 1. Each pattern represents scattering intensity accumulated during 24 s. A – Small-angle scattering region; first-order maxima are truncated to emphasize higher-order diffractions; B – corresponding wide-angle pattern scattering intensity

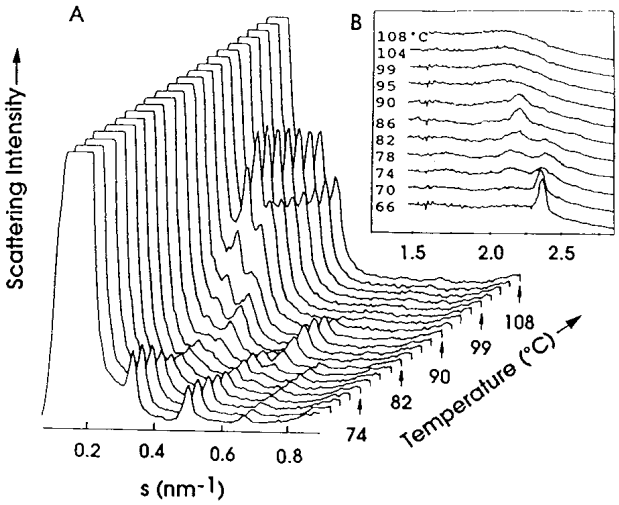

Fig. 3 Real-time X-ray diffraction patterns of distearoyl-phosphatidyl-ethanolamine in glycerol (50 wt%) showing the sequence of lamellar-gel to tilted lamellar crystalline to inverted hexagonal phase transitions. Data were acquired on reheating the same sample as shown in Fig. 2 immediately after cooling to $60^{\circ}C$. See the caption to Fig. 2 for other details

exotherm-endotherm, as depicted in the right panel of Fig. 1. The corresponding X-ray diffraction data on the dispersion with 50 wt% glycerol are shown in Fig. 3. In this way, the exotherm was assigned as the transition from a metastable lamellar-gel (L_β) phase to a more ordered lamellar crystalline ($L_{c'}$) phase. The second endotherm was the transition to the disordered hexagonal phase (H_{II}). Finally, the first endotherm was believed to reflect partial melting of the gel phase when it transformed to the crystalline phase at almost the same time. The partial melting of the gel phase most probably helped the lipid molecules to rearrange themselves and to relax to a more stable phase.

The complicated thermal features during reheating are due to the non-equilibrated nature of the sample, which needs some time to relax to a more stable phase. There was more evidence to support the idea that the relaxation process, corresponding to the exotherms in Fig. 1, was accelerated at elevated temperatures. When the sample was held at an appropriate temperature, the time-dependence of the relaxation was monitored successfully by using a DSC technique [18]. We would like to point out that this phenomenon is very similar to that of the crystallization process of polymers after the glass transition upon heating. Such a transition has similarly been observed for small molecular systems such as the water–dimethylsulphoxide binary system [19, 20], where it is best known as devitrification.

In addition to the identification of lipid phases, synchrotron X-ray diffraction has also been employed to monitor the temperature-dependence of the span of lipid bilayers. It has been demonstrated that the small cosolute dimethylsulphoxide is able to cause a shrinkage of the length of phospholipids in the lamellar liquid-crystal phase, which might be relevant to its ability to function as a membrane permeability enhancer [21].

Simultaneous measurements of X-ray diffraction and DSC

The best way to relate a thermal event and the underlying structural changes in a lipid system is to collect both data at the same time under identical conditions. This will avoid the miscorrelation of X-ray diffraction data to DSC results when samples have been subjected to different thermal histories. For this purpose, simultaneous X-ray diffraction and DSC measurement techniques have been developed by a few groups [22–26]. Again, only the synchrotron radiation source is bright enough for the time-resolved experiments. The thermal instrumentation must be designed so that the incident X-ray beam can reach the specimen and scattering light can be collected. There is commercially available apparatus such as the DSC (Model FP84) from Mettler Instrument Corp., which was developed originally for coupling with polarized optical microscopy. Sample pans such as that made of thin aluminium plate can be used directly in the measurement, although modification has been made to increase the transmittance of X-ray beams [24, 27]. In a more recent study, a thin glass capillary has been used successfully to hold very small amount of samples of 1–20 nl in simultaneous DSC-X-ray diffraction measurements [26].

Conclusions

DSC and X-ray diffraction are two powerful tools in studies of lipid phase behaviour. DSC alone cannot identify phase structures relating to the thermal events. It is also difficult to detect phase transitions with very low enthalpies. On the other hand, X-ray diffraction is unable to provide thermodynamic information relating to phase transitions, which could be important for an understanding of the nature of the transition. When the conventional method is used, more problems will arise from the thermal history of the samples. Joint application of the two techniques, particularly DSC and synchrotron X-ray diffraction, will be ideal in studies of lipid phase transitions, their mechanisms, and the time relaxation of the phase structures.

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