BIOPHYSICS

Differential Thermal Analysis of Phospholipids

For many years the melting points of both synthetic and natural phospholipids have been measured and recorded[1–3]. An examination of a given class of phospholipids reveals that these melting points are, to a large extent, independent of the fatty acid residues present. Thus, 2-oleoyl-3-stearoyl-L-1-phosphatidylcholine and 2,3-distearoyl-L-1-phosphatidylcholine both have recorded melting points[3] of 230°–231° C. The thermal transitions of phospholipids are difficult to observe with normal melting equipment, even when using a microscope. Occasionally some workers have observed 'sintering points', corresponding to thermal transitions, which occur below the normal melting point. However, the relevance of these points has never been demonstrated.

Recently we showed experimentally that melting of the hydrocarbon chains of the phospholipid, as revealed by infra-red spectroscopic evidence, takes place at much lower temperatures than the recorded melting points[4]. Furthermore, a feature of some biological interest is that the temperature required to melt the hydrocarbon chain decreases as the degree of unsaturation of the chains is increased. Above this transition temperature the material is in a liquid crystalline condition. Dervichian has recently inferred that this behaviour might occur[5].

Here we wish to point out the value of differential thermal analysis for detecting the thermal transitions and determining the transition temperatures. With modern differential thermal analysis equipment only small quantities of material are needed (some milligrams) and the technique is simple and rapid. Shown in Fig. 1 is an example taken from some of our recent investigations. This shows the differential heating curve for 2,3-dimyristoyl-DL-1-phosphatidylethanolamine (capillary m.p. 207°). The large heat change observed at 115° C corresponds to the melting of the hydrocarbon chains, while the small heat change observed near 214° C is perhaps related to the separation of ionic groups, or to the decomposition of the material. It can be seen that there is an additional transition at ~135° C and it is of interest to note that this is the temperature corresponding to a sintering point for this phospholipid. This transition probably corresponds to some lattice change and we are examining this with X-rays and nuclear magnetic resonance spectroscopy. There is no apparent change in the infra-red spectra above and below this temperature. The differential thermal analysis curves for this phospholipid sometimes show an additional transition near 100° C which we have shown corresponds to a polymorphic change.

If the phospholipid is heated to a temperature just above 135° C, cooled to room temperature and then heated up again, the curve shown by the dashed line is obtained. The endothermic transition at 115° C is now not observed. This effect can be related to the unusual super-cooling which occurs with these molecules. The hydrocarbon chains remain in a liquid condition for a considerable length of time unless the phospholipid is quenched to a low temperature.

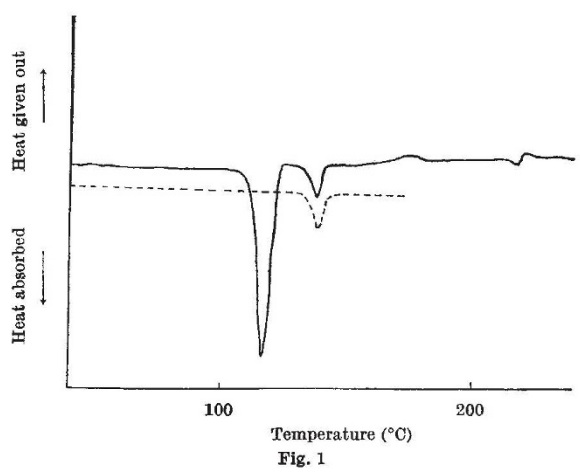

Fig. 1

Using this technique of differential thermal analysis we have now examined a whole range of phospholipids, both synthetic and natural, including saturated and mixed-saturated/unsaturated phosphatidylethanolamines, phosphatidylcholines (including egg yolk lecithin) and sphingomyelins, as well as white and grey matter from brain. We shall discuss the results in detail elsewhere. These results may help us to understand the reason why phospholipids in biological tissues are associated with a particular distribution of fatty acid residues.

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BIOCHEMISTRY

Uptake, during Absorption, of Free Fatty Acids by Phospholipids of the Intestinal Mucosa of Rats

Several authors have suggested that the phospholipids may participate in the absorption of fats[1–3]. However, an examination of the ^{32}P turnover in the phospholipids of the mucosa of dogs and rats led Zilversmit *et al.*[4,5] to conclude that these compounds are of little significance in this process. More recently, from the results of ^{32}P-turnover in experiments with everted intestinal sacs of golden hamsters, Johnston and Bearden[6] have suggested that the phosphatidic acid might be an intermediate in the transport of fatty acids during absorption. But these claims could not be confirmed by Gurr *et al.*[7], who studied the ^{32}P turnover of the intestinal phospholipids of living rats. It is clear that in all these experiments labelled ^{32}P was used and no attempt was made to investigate the uptake of free fatty acids by the mucosal phospholipids; we have found that the mucosal phospholipids rapidly take up the free fatty acid from the lumen of the small intestine.

The rats were treated and dosed as described earlier[8], excepting that 5·0 μc. of 1-^{14}C-stearic acid (22·7 mc./m.mole) was mixed with 0·5 g of each of the diets containing 5 or 25 per cent groundnut oil. The animals were killed 1 h after the dose by bleeding under ether anaesthesia, after which the intestinal contents, mucosa and muscles were quickly separated[9] and immediately transferred into conical flasks containing 10 vol. of chloroform : methanol (2 : 1). After 18 h the chloroform layer was separated and the aqueous layer, if any, was re-extracted with more chloroform. The mixed extracts were washed with 0·2 vol. of water to remove the non-lipid materials and were then dried over anhydrous sodium sulphate. The samples were evaporated to dryness under vacuum at temperatures kept below 35° C and the lipids were redissolved in a small amount of light petroleum ether. Separation of the glycerides and phospholipids was effected by chromatography on silicic acid–'Celite' (2 : 1) columns. The glycerides along with the free fatty acids were first eluted with 200 ml. of ether, after which the phospholipids were removed by 200 ml. of methanol. The ether eluate was further resolved into mono-, 1,2- and 1,3-di-, tri-glycerides and free fatty acids by thin-layer