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LYSOPHOSPHATIDYLCHOLINE-WATER SYSTEM: CALORIMETRIC STUDY OF THE LAMELLAR-MICELLAR TRANSITION BEFORE AND AFTER GAMMA-IRRADIATION

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ABSTRACT

Gamma-radiation induced modifications have been observed by differential scanning calorimetry (DSC) in 1-stearoyllysophosphatidylcholine in water excess. Analysis of gamma-irradiated samples showed that the lamellar-to-micellar transition displays substantial modifications. These are observed at much smaller doses than those for which modifications begin to appear in the main transition of distearoylphosphatidylcholine liposomes in water excess. Moreover, the enthalpy change associated with the 1-stearoyllysophosphatidylcholine transition progressively decreases with increasing dose, and the beginning and ending transition temperatures are shifted towards lower and higher values respectively.

INTRODUCTION

Our previous studies have described structural and chemical modifications produced by ionizing radiations in saturated diacylphospholipid multilamellar liposomes in water excess [1-7].

Experiments using DSC, ESR and fluorescence techniques showed that gamma radiation induces, either directly or indirectly, the production of new components, obviously derived from degradation and/or a cross-linking of the original lipid molecules.

An addition of a suitable amount of a foreign compound to the bilayer, would normally be expected to result in a change of the phase transition temperature. In the case of gamma-irradiated diacylphospholipid multilayers, however, these

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techniques show that the temperature at which the main transition begins does not undergo any appreciable change, whereas its final temperature increases with the dose absorbed. In spite of this broadening effect, the change of enthalpy associated with the main transition is not appreciably modified. Moreover, both NMR spectroscopy and TLC show that gamma-radiation products are mainly lysolecithins and fatty acids.

Investigation of the modifications that gamma-radiation produces in lysophospholipid dispersions is thus a matter of interest.

EXPERIMENTAL

Samples preparation

1-Stearoyllysophosphatidylcholine (1SPC) was purchased from Sigma Chemical Co. St. Louis, Mo. The 1SPC from a newly opened ampoule was checked for purity by TLC. Samples free from detectable impurities (<1%) were used without further purification. To prepare each sample for DSC analysis, the lipid powder was transferred to an aluminium pan and dried under a vacuum. Double-distilled water was then added to the pre-weighed lipid. All the samples were thus obtained, in open air, with a lipid concentration of about 6.4×10^{-2} mol L^{-1}. They were hermetically sealed, their weight was accurately checked and they were then left at room temperature until the first calorimetric analysis. After which, about half the samples was irradiated at room temperature by a ^{60}Co source to absorb increasing doses from 4 to 187×10^2 Gy (i.e. from 1.3 to 61.2×10^{21} eV lipid-mol^{-1} and from 2.5 to 116.9×10^{18} eV mL^{-1} of solution), with a dose rate of 8.2×10^{-2} Gy s^{-1}. The absorbed doses were measured by alanine dosimeters [8]. A second aliquot was left at room temperature to be used as a blank, on which calorimetric and TLC tests were performed to check whether spontaneous modifications could take place with time.

Differential scanning calorimetry

Before DSC analysis, both the non-irradiated and the irradiated specimens were weighed once more to check for seal fitness. The DSC analysis was carried out with a Perkin-Elmer DSC-2C calorimeter. Operational control of DSC-2C and data acquisition were computerized. The specimens were introduced into the calorimeter at 290 K and then heated to 340 K. They underwent not less than four thermal cycles between 340 and 240 K. At the end of each heating and cooling run, the sample was kept for 120 s at its final temperature. The scanning

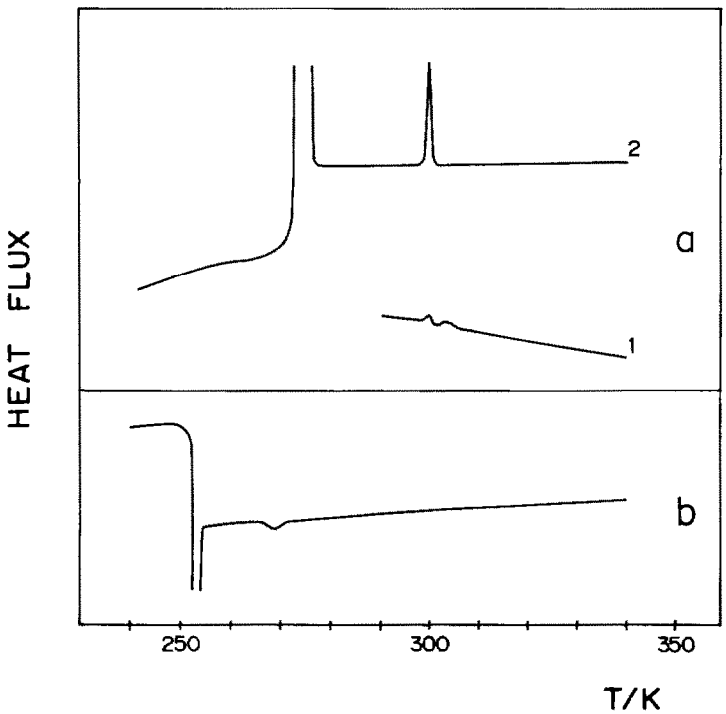

Fig.1. Typical DSC profiles for 1SPC samples before irradiation.
(a) Heating scans: (1) the peak of the lamellar-to-micellar transition is almost absent; (2) the peak on the left corresponds to the ice-melting transition; that on the right to the lamellar-to-micellar transition.
(b) Cooling scans: the peak on the left corresponds to water freezing; that on the right to a minor structural transition.

rate was 1.39×10^{-3} K s^{-1}. After this procedure, a few experiments were carried out isothermally. The desired isotherm temperature was reached by cooling the samples from 340 K.

RESULTS AND DISCUSSION

It is known that lysophosphatidylcholines in water excess undergo a thermotropic transition between a lamellar and a micellar configuration at a temperature depending on the length of their acyl chain [9-11]. To study this transition calorimetrically, we chose 1SPC, since it displays the transition at a temperature, T_{trs} (lamellar-to-micellar), high enough with respect to ice melting temperature to avoid any overlapping of the corresponding peaks.

Some typical calorimetric curves for a non-irradiated specimen are shown in Fig.1. Thermogram 1 refers to the first heating run carried out about one hour after preparation of the sample. The DSC peak of the lamellar-to-micellar transition is almost absent, whereas it is clearly observable in the subsequent runs. The process, by which 1SPC molecules (initially as a powder in water) organize into lamellar structures at room temperature is very slow and is still not complete after a few days after preparation of the samples. It is sufficient to keep samples for a few minutes at a temperature over T_{trs}, to obtain 1SPC molecules spontaneously organized into micellar structures [10,11]. In our experiments, the sample stayed at a temperature over T_{trs} for about 18 minutes, computing the scanning time from 300 to 340 K, the time isothermally spent at 340 K and the scanning time from 340 to 300 K. This time is certainly sufficient to obtain a spontaneous organization of the 1SPC into micellar structures.

Initially the lamellar-to-micellar transition becomes sharper and sharper from one run to the next. However, after four scannings the transition peak maintains a constant area and a fixed shape. The corresponding temperature (299.1 ± 0.2 K) and the associated change in enthalpy (29 ± 2 J mol^{-1}) are in good agreement with previous results [10].

Fig.1b shows a typical cooling thermogram for the same specimen. The DSC peak on the left corresponds to water freezing. At the selected scanning rate, the water supercools and then freezes suddenly at a temperature between 258 and 253 K. This noticeable supercooling is not unusual for other lipid-water systems as well [12]. In addition, our experiments carried out isothermally at various temperatures in the supercooling range show that it is often necessary to wait for a long time before freezing takes place, if the temperature is greater than about 258 K.

The area of the exothermic peak at 270 K is only about one-tenth of that of the endothermic peak obtained during heating marking the transition from the lamellar to the micellar phase. This suggested that this peak is not connected with the micellar-to-lamellar transition. This belief is strengthened by the fact that the heating scannings successively performed from 262 K upwards, fail to disclose any endothermic transition. This small peak is thus likely to be due to a minor structural transition, possibly connected with a packing change of the acyl chains in the micelles. These observations are consistent with results obtained by other authors [11] showing that this packing is tighter at supercooling temperatures than above T_{trs}. The same authors have also demonstrated that in some circumstances the process of packing rearrangements can be decoupled from the growing process of the lamellar structure. However, as can be seen in Fig.1a, the lamellar-to-micellar transition is always present in

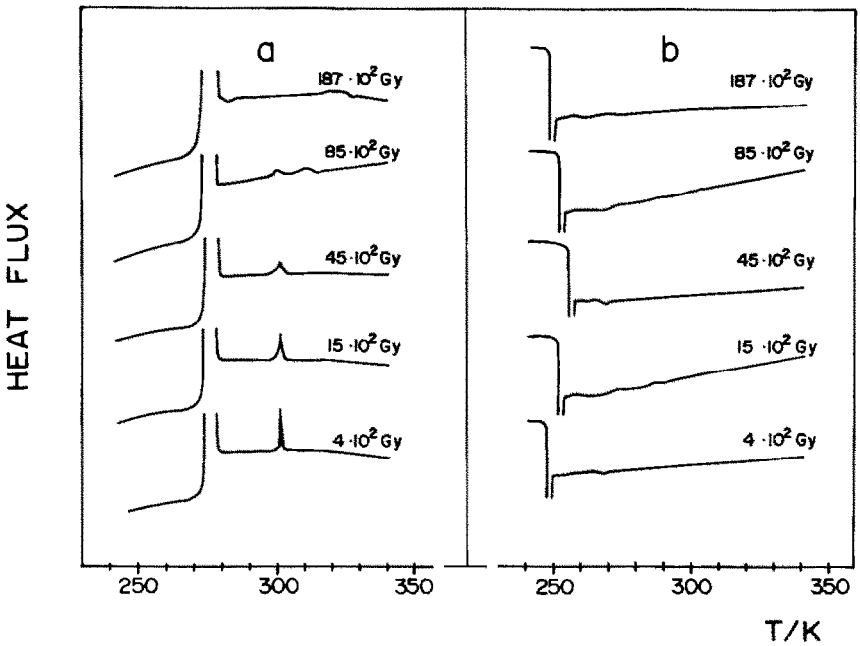

Fig. 2. DSC profiles of the fourth cycle for 1SPC samples after irradiation at various doses. Meaning of the peaks as in Fig. 1: (a) Heating scans; (b) Cooling scans.

the heating curves whenever the specimen has previously undergone a calorimetric cycle in which the water in the sample froze. It can thus be suggested that in our experiments the micellar-to-lamellar transition is correlated with water-ice transition. The behaviour described remains practically unchanged at least for a few weeks. Further experiments are in progress to better characterize the micellar-to-lamellar transition.

Figs. 2a and 2b show the thermograms in heating and in cooling respectively for the fourth cycle for 1SPC specimens irradiated with increasing gamma doses. Further scannings do not reveal changes in either the area or the shape of the peak. The curves of the first, second and third cycle have been omitted, because their evolution is similar to that of non-irradiated specimens. Fig. 2a shows that as the absorbed dose increases the endothermic peak associated with the transition undergoes progressive modifications. Comparison of the measurements before and after irradiation brings out the following picture. First, when the dose is increased, the peak becomes flatter and wider. Its profile reveals a secondary peak that becomes wholly resolved at the highest doses. The initial temperature of this complicated transition is slightly lower than that for

non-irradiated specimens, whereas its final temperature is appreciably higher, particularly when the dose is large. Furthermore, with increasing doses, the area subtended by the peak decreases, so that it becomes practically unmeasurable at the highest doses. It may be pointed out that these are in any event lower than that at which modifications of the main transition in specimens of distearoylphosphatidylcholine (DSPC) in water excess begin to appear [2]. The gamma-radiation effects on 1SPC described here are of a permanent nature, as demonstrated by measurements performed a few weeks after irradiation.

TLC tests are in progress to provide evidence of chemical modifications in the irradiated samples. Preliminary results show that there is a high probability for the acyl chains to be detached from the polar head and divide. Various other degradation products are also present.

The structural transitions in 1SPC samples can also be studied with spin-label techniques. In these samples in particular, the spin-label 2,2,6,6,-tetramethylpiperidine-1-oxyl (TEMPO) presents an EPR spectrum resulting from the superposition of two resonances, due to the TEMPO partitioning between the lipid and aqueous phase. A spectral parameter, whose behaviour vs. temperature permits the characterization of the lamellar-to-micellar transition, can be derived [13]. Measurements of 1SPC samples irradiated at the doses used in this calorimetric study confirm the progressive broadening of the transition [14].

On the other hand, when water is frozen, spin label motion is strongly reduced and its EPR spectrum assumes a shape known as "strongly immobilized". We observed that water freezing occurs at temperatures lower than 273 K as detected calorimetrically.

In addition to the possibility of DSPC radical cross-linking, previous studies on irradiated DSPC showed that there is a strong probability that one acyl chain will detach from the polar head, giving rise to 1SPC formation [3]. Our DSC analysis of irradiated 1SPC samples can be interpreted in terms of degradation and cross-linking processes. In particular, the progressive reduction in the area of the lamellar-to-micellar transition peak may be seen as due to a radiation induced decrease of the number of 1SPC molecules, leading to the formation of residues no longer capable of self-organization in lamellar or micellar phase.

These hypotheses must naturally be carefully checked. This will involve precise investigation of 1SPC molecule modifications and further characterization of the structural modifications of their assemblies. Chemical analyses and other suitable techniques will be required in an essential development (on which we are already working) of the DSC results refered in

this paper. Our present results indicate that lysophospholipids are more radiation-sensitive than diacylphospholipids. Therefore, when studying chemical and structural modifications induced by gamma-radiation on liposomes of diacylphosphatidylcholines, the evolution of the lysophosphatidylcholines produced would appear to be a subject of continuing interest.

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