BRIEF COMMUNICATION

MODIFICATIONS INDUCED IN PHOSPHATIDYLCHOLINE MULTILAYERS BY Co-60 γ-RAYS

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ABSTRACT Using differential scanning calorimetry it was observed that γ-radiation induced modifications in dimyristoyl phosphatidylcholine (DMPC) multilayers in excess water. It was observed that, with the increase of the absorbed dose, the peak associated with the pretransition disappeared gradually, while the peak associated with the main transition became wider and flatter. The enthalpy change associated with the pretransition was found to be 4.4 ± 0.3 kJ/mol of DMPC before irradiation and that associated with the main transition was found to be 26.0 ± 1.3 kJ/mol of DMPC before and after irradiation. Moreover from our measurements, it seems that the trapped water becomes stable free water, because of the effect of the γ-radiation.

The calorimetric analysis of the hydrated phospholipid multilayers shows some of their well-known transitions (1–3) and, at the same time, gives useful information on the role of the water in their structure (1). In fact, various experimental results have shown that the observed transitions depend on their degree of hydration (1,4,5).

It is also known that, in phosphatidylcholine multilayers in excess water, it is possible to distinguish between bound water, trapped water, and free water (6–8). It has been reported (8) that all the water in these dispersions does not freeze on cooling nor melt on subsequent heating. From this fact it was proposed (8) that the water molecules not involved in the ice-melting transition are structured around the polar head of the phosphatidylcholine molecules or bound to them. Calorimetric curves of these multilayers in an excess of water also showed that freezing of the water occurs in two stages (8). A portion of water supercools and then freezes suddenly as pure water usually does between 258° and 253°K. This has been called free water. Another portion (trapped water) undergoes supercooling and then freezes at lower temperature, but melts at ~273°K (8), as does free water, thus leading to only one broadened peak. These trapped water molecules should be in an intermediate state, between that of bound water and that of free water external to lamellae (7,8). Recently, using differential scanning calorimetry, we observed that the absorbed γ-radiation dose induces modifications in these different forms of water.

Synthetic dimyristoyl phosphatidylcholine (Sigma Chemical Co. St. Louis, Mo.) was used in our studies. Following are some preliminary results.

The lipid from a newly opened ampoule was checked for purity by thin-layer chromatography (TLC). The lipid samples showing no TLC-detectable impurities (<1%) were used with no further purification.

To prepare the samples for calorimetric analysis, lipid powder was transferred to a glass tube and dried for 3 d under vacuum. The appropriate amount of bidistilled water was then added to the weighed lipid. Dispersion was carried out by mechanical shaking on a Vortex mixer (Heidolph, W. Germany) at a constant temperature of 343°K. No lipid decomposition was detected (by TLC) after vortexing. The dispersions thus obtained, with lipid-weight content ranging from 31.9 to 33.5%, were immediately frozen to the temperature of liquid nitrogen and divided into samples from 5 to $8 \cdot 10^{-6}$ kg. These were hermetically sealed in aluminum pans and calorimetric analysis was carried out with a Perkin-Elmer DSC-1B calorimeter (Norwalk, Conn.); their weight had been accurately checked previously.

After a first analysis, a portion of the samples was maintained at room temperature and, at the same temperature, another portion was exposed to a γ-ray flow of a Co-60 source of about 66 TBq to absorb increasing doses from 3 to $\sim 7 \cdot 10^4$ Gy with a dose rate of $5 \cdot 10^{-2}$ Gy $\cdot$ s^{-1}.

A second calorimetric analysis was carried out only on the samples of both portions with no weight change. Thus, it was seen that the nonirradiated samples repeated the initial curve, while the curves of irradiated samples showed substantial modifications, not associated with aging but with the absorbed dose of radiation.

Figs. 1 and 2 show, as an example, typical curves of a sample before and after irradiation. The same characteristics were obtained for all the irradiated multilayers that absorbed the same radiation dose.

The heating curve in Fig. 1 (nonirradiated sample) shows all the known transitions i.e., the ice-melting transition (A_1) in which only the trapped and free water take part (6,8), the

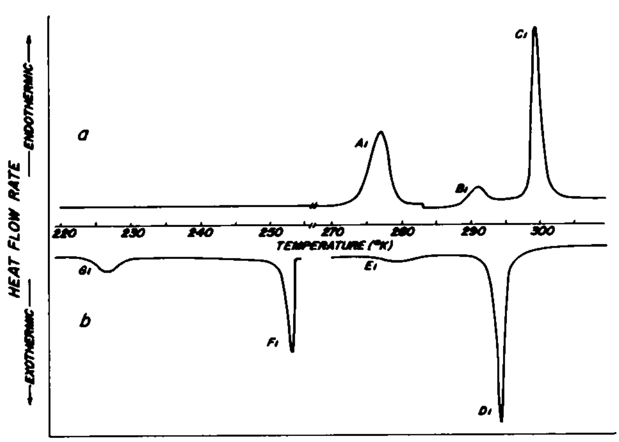

FIGURE 1 Differential scanning calorimetry curves for a DMPC sample, before irradiation. The analysis was carried out over the temperature range of 215° to 330°K, in both heating and cooling runs. A scan speed of 0, 13°K $\cdot$ s^{-1} was used. (*a*) Heating curve. Peak A_1 refers to the ice-melting transition, peak B_1 to pretransition and peak C_1 to main transition. Peak A_1 is scaled 20 times less than the other two. (*b*) Cooling curve. Peak D_1 refers to the main transition, peak E_1 to pretransition, peak F_1 to free water freezing and G_1 to the trapped water transition. Peak F_1 and G_1 are scaled 20 times less than the other two.

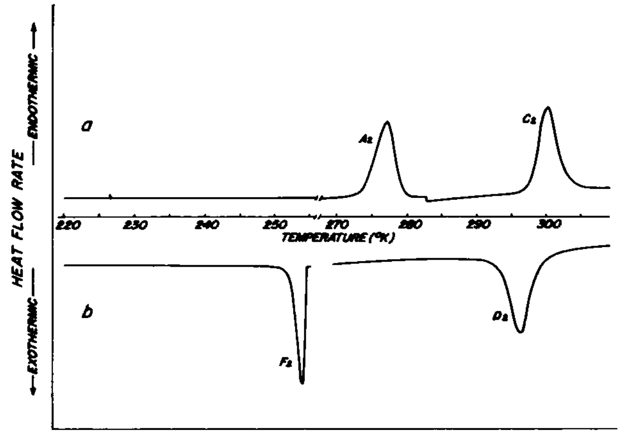

FIGURE 2 Differential scanning calorimetry curves after irradiation for the same sample as in Fig. 1. (*a*) Heating curve. Peak A_2 refers to the ice-melting transition and peak C_2 to the main transition, while the pretransition peak is absent. Peak A_2 is scaled 20 times less than the other. (*b*) Cooling curve. Peak D_2 refers to the main transition and peak F_2 to the free water freezing, while the pretransition and trapped water peaks are absent. Peak F_2 is scaled 20 times less than the other.

pretransition (B_1) and the main transition (C_1). In the cooling curve of the same figure, F_1 and G_1 refer to the freezing of free and trapped water, respectively (6,8); D_1 refers to the main transition and E_1 to the pretransition.

In the analogous curves of Fig. 2 (irradiated sample which absorbed a dose of $\sim 7 \cdot 10^4$ Gy), the pretransition and trapped water transition disappear, while the main transition peak becomes wider and flatter in both the heating (C_2) and cooling (D_2) runs.

Since the area of F_2 was equal to the sum of the areas of F_1 and G_1, it can be deduced that the radiation transforms trapped water into free water. This could also explain the disappearance of the pretransition and the widening of the main transition peak. Both of these effects have been observed in the absence of trapped water (1,5).

The heat associated with the ice-melting transition was calculated assuming that all the water in each sample had been frozen in the previous cooling run down to 215°K. From the area of the peak associated with this transition, the heat effectively absorbed was calculated. Thus, from the difference between the two, we derived the number of water molecules which had not participated and therefore were to be considered bound (8) to the lipid molecules.

From the areas of peaks A_1 and A_2, which are equal, it can be deduced that the bound water is unaffected by the radiation-absorbed dose up to $\sim 7 \cdot 10^4$ Gy. Moreover, since the number of lipid molecules in each sample was known, it was found that, on the average, there are 10 molecules of bound water for every phospholipid molecule.

The area of the peak associated with the trapped water transition decreases progressively to zero, as the absorbed dose increases, showing a pattern as in Fig. 3, where S/S_0 is the ratio of the peak areas after and before irradiation, respectively. Since the area of the free water peak increases progressively as the absorbed dose increases, it can be deduced that an increasing quantity of trapped water assumes the characteristic of free water, because of the effect of the radiations. The ratio S/S_0 for the pretransition peak is also given in Fig. 3.

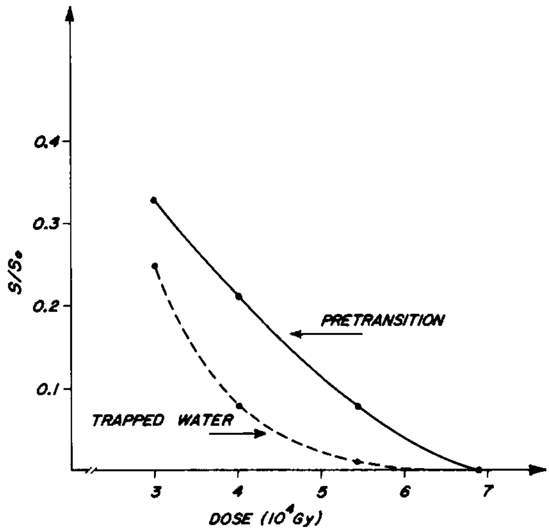

FIGURE 3 Decrease in area of the peaks relative to the pretransition and to the trapped water vs. the absorbed dose, S/S_0 being the ratio of the area of the peaks after and before irradiation, respectively.

The enthalpy change, associated with the pretransition before irradiation, confirms the value of 4.4 ± 0.3 kJ/mol of DMPC (2) and the change associated with the main transition before and after irradiation always gives the value of 26.0 ± 1.3 kJ/mol of DMPC.

The observed effect is irreversible even within a period of time (7 d) greater than necessary for phospholipids, immersed in water, to reach total spontaneous hydration. Because, on cessation of irradiation, there is no inverse passage from free water to trapped water, it seems that the absorbed radiation dose induces some structural modifications in the multilayers. Further studies are being carried out on this matter.

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