Evolution of the thermotropic properties of large unilamellar vesicles (LUV)

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Summary. The evolution of the thermotropic properties of large unilamellar vesicles (LUV) made by the reverse-phase evaporation technique has been studied by differential scanning calorimetry (DSC) and by fluorescence polarization of the diphenylhexatriene probe inserted in the lipid phase. Lipid fluidity and transition temperatures of DL-α-dimyristoyl-and DL-α-dipalmitoylphosphatidylcholine vesicles were practically not modified at room temperature (19–20 °C), even after several days. Because a better knowledge of the physico-chemical properties of LUV seems essential for its use as a model membrane or as a carrier of exogenous material into cells, we compare it with the stability of the widely used multilamellar (MLV) and sonicated unilamellar vesicles (SUV).

Some lipids, when adequately dispersed in saline solution, form lipid vesicles (liposomes)[1], which can be used as models of biomembranes or as carriers to introduce exogenous material into cells[2–4]. According to the mode of dispersion used, a large variety of liposomes can be prepared[5]. There is much interest in a new generation of liposomes, the large unilamellar vesicles (LUV) which have an internal aqueous volume sufficiently large to allow the encapsulation of large molecules, such as genetic material[6–11]. However, they must remain stable for a long time to allow the transfer of their contents into the cell. Although the stability and physico-chemical characteristics of multilamellar vesicles (MLV) and sonicated unilamellar vesicles (SUV) has been extensively studied, little is known about LUV. It is the purpose of this paper to evaluate the thermotropic properties of LUV and their evolution as a function of time.

Material and methods. DL-α-dimyristoylphosphatidylcholine and DL-α-dipalmitoylphosphatidylcholine (DPPC) were purchased from Sigma (St. Louis, MO.). LUV were prepared by the reverse-phase evaporation technique[12]. 60 μM of phospholipid were dissolved in 6 ml of chloroform-diethylether (1:1, v/v). 1 ml of aqueous phase (Tris-HCl 0.02 M–pH 7.4, NaCl 0.15 M) was added to that mixture. This preparation was then sonicated (Branson sonifier) at 60 W for a short time (2–3 min).

Organic solvents were evaporated slowly under reduced pressure in a rotary evaporator. The temperature was maintained at 30 °C with a water bath. The resulting turbid suspension was submitted to centrifugation at 600 × g, 10 min to eliminate a possible residue of multilamellar vesicles. Preliminary experiments showed that the internal aqueous volume of our LUV (11.3 μl/mg) corresponds to the data in the literature (not shown). Multilamellar vesicles were obtained by vortexing a dry film of lipid (vacuum overnight) in the buffer, above the

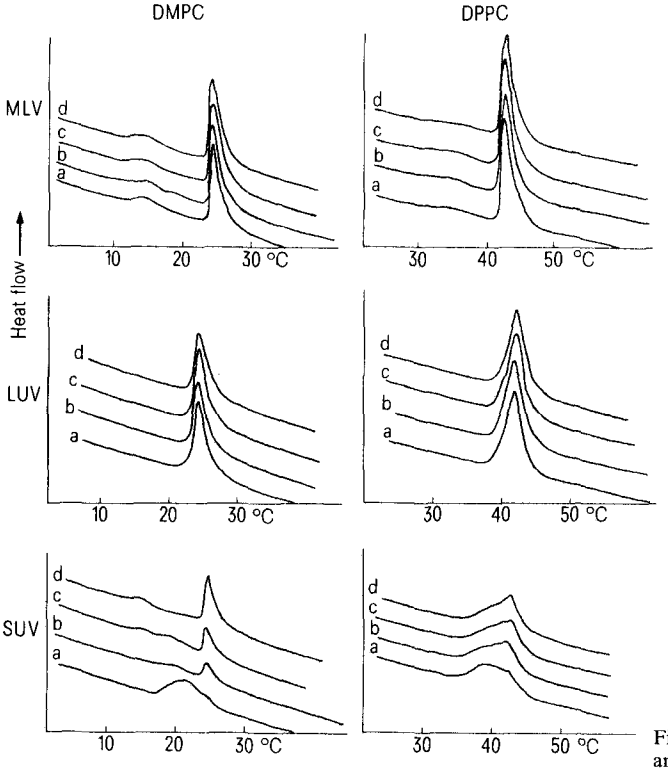

Figure 1. Evolution of the DSC spectra of DMPC and DPPC vesicles with time. Incubation time (h): a = 0; b = 24; c = 72; d = 144. Lipid concentration: 60 mM. Heating rate: 0.5 °C/min.

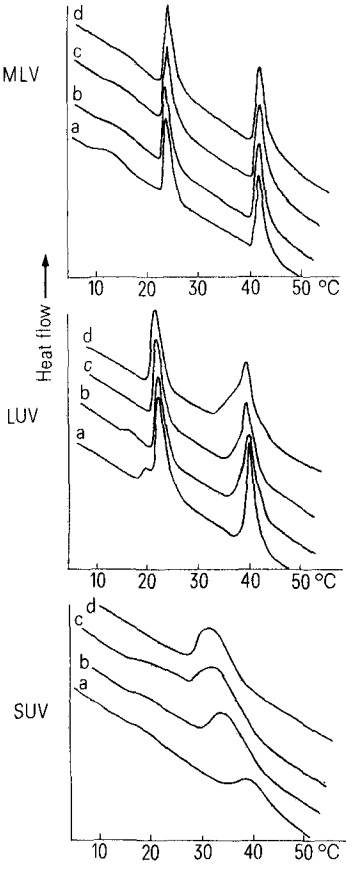

Figure 2. Evolution of the DSC spectra of a mixture of DMPC vesicles and DPPC vesicles (1/1 molar ratio). Incubation time (h): a = 0; b = 24; c = 72; d = 144. Lipid concentration: 60 mM. Heating rate: 0.5 °C/min. Note that the DMPC peak of SUV, which is somewhat difficult to identify, is situated around 19 °C in curve a.

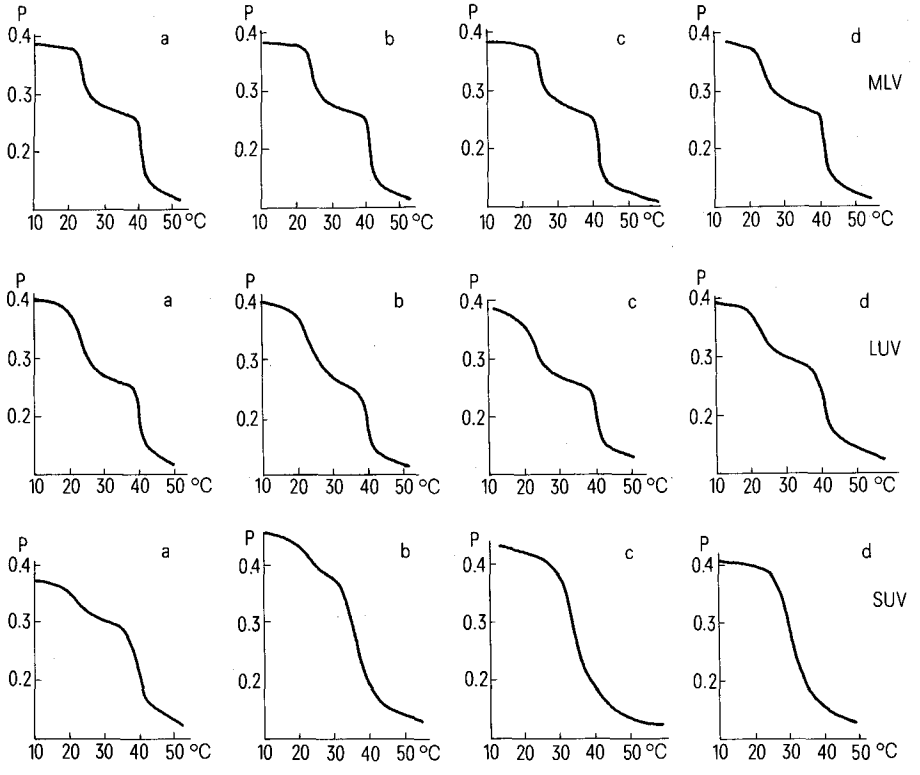

Figure 3. Evolution of the fluorescence polarization of a mixture of DMPC vesicles and DPPC vesicles (1/1 molar ratio). Incubation time (hours): a = 0; b = 24; c = 72; d = 144. Lipid concentration: 60 mM.

main transition temperature. Sonicated unilamellar vesicles were made by sonication of multilamellar vesicles at 60 W with a titanium probe (Branson sonifier) for 30 min, 10 °C above their main transition temperature. They were centrifuged for 10 min at 600 × g in order to remove titanium particles. Differential scanning calorimetry (DSC) experiments were performed on a programmed differential calorimeter (Setaram DSC 111) with a heating rate of 0.5 °C/min for each experiment. Fluorescence polarization measurements were performed on a Elscint MV1a microviscosimeter equipped with a 200-W high pressure mercury lamp. The fluorescent probe (1,6 diphenylhexatriene-DPH-Sigma) dissolved in ethanol was added to the liposomes at a molar ratio of 1:750 (DPH:lipids). The final ethanol concentration was never higher than 1%.

Results and discussion. Evolution of the thermotropic properties of LUV as a function of time. Differential scanning calorimetry (DSC) is a preferred tool for the evaluation of the stability of liposomal structures as a function of time[13]. The profile of the DSC spectrum characterizes the liposomal structures and their occasional transformation into new liposomal structures. DSC spectra of DMPC and DPPC have been compared to the DSC spectra of MLV and SUV made of the same lipids (fig. 1). Measurements were performed directly after liposome formation, after 24, 72 and 144-h incubation at room temperature (19–20 °C) (fig. 1). Comparison of the calorimetric spectra demonstrated for DMPC and DPPC that the large unilamellar vesicles made by reverse-phase evaporation are highly stable; in contrast to SUV (fig. 1), they do not display any significant evolution of transition temperature (DMPC = 24.0 °C; DPPC = 41.5 °C) or any evolution of the profiles of the calorimetric curves with time, and behave much like MLV. Thus, even 6 days after their preparation, LUV have not undergone any modification which could significantly affect their thermotropic properties. During all that time, LUV cannot be centrifuged at 600 × g (10 min). The presence of a pre-transition in MLV only (there is no well-defined pre-transition in LUV, and none at all in SUV) could result from the interaction between adjacent lipid bilayers in the multilayered structure of MLV. Indeed, it has been shown by electron diffraction studies that in contrast to single bilayers, multibilayers formed the P'_β phase[14], which seems to be involved in the pre-transition phenomenon. We must also point out that LUV possess a broader transition temperature than MLV even if the area of the peak remain approximately the same.

Study of a mixture of 2 populations of LUV. The interaction between 2 populations of LUV (one made of DMPC and the other of DPPC) was monitored by DSC and fluorescence polarization of the DPH probe inserted into the lipid bilayers. As has been shown previously by others[13], the change in thermotropic properties and lipid fluidity observed with 2 types of liposomes allows us to detect lipid transfer or fusion between the 2 populations of liposomes (at least for 2 lipid species which do not induce lateral phase separation when they are mixed). The lipid transfer induces a broadening and a shift of the lipid transition temperature towards a new temperature which is intermediate between the transition temperature of the 2 lipid species. In contrast, fusion of the 2 populations causes the formation of a new vesicle population with a transition temperature which is intermediate between the transition temperature of the 2 lipid species, as would be obtained by direct mixing of the 2 lipid species[13]. In the last case, there is no shift in the transition temperature of the vesicle populations. Measurements have been performed after 24, 72 and 144 h of incubation at room temperature (19–20 °C). The spectra (figs 2 and 3) characterizing the mixture of 2 LUV populations are not strikingly modified with time. However, there is a slow but significant broadening of the DSC peak of DPPC with a slight shift of the maximum towards lower temperatures, which indicates a slow transfer of DMPC to DPPC vesicles. These results are also given, but to a lesser extent, by fluorescence po-

larization data (fig. 3), the step corresponding to the DPPC transition becoming higher with time. The transfer process is however much more striking in the case of SUV (figs 2 and 3), in which a lipid transfer from DMPC to DPPC is well documented[15].

In conclusion, LUV are closer to MLV in the stability of their thermotropic properties, and closer to SUV in the absence of a well-defined pre-transition. We think that the above results could contribute to a better understanding of the general properties and stability of a very promising new type of lipid vesicle, the LUV.

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0014-4754/84/070715-03$1.50 + 0.20/0

Isoenzymes of glutamate-oxalacetate transaminase in the larvae of silkworms *Bombyx mori* infected with nuclear polyhedrosis virus

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Summary. Nuclear polyhedrosis virus infection induced changes in the activity and isoenzyme pattern of glutamate-oxalacetate transaminase in the larvae of silkworm, *Bombyx mori.*

Nuclear polyhedrosis is characterized by the formation of polyhedral-shaped inclusion bodies within the nuclei of susceptible cells. Studies of insects with this type of disease have shown that large amounts of proteins are synthesized for the formation of the virus particles and inclusion bodies[2-4]. The host organism, therefore, will be affected especially in its protein and amino acid metabolism. Since transaminases are the key enzymes involved in protein and amino acid metabolism, comparative measurements of the activities of these enzymes in healthy and diseased tissues may help in understanding the physiological changes that arise from the disease. Earlier work by other investigators[5,6] indicated an increase in the glutamate-oxalacetate transaminase activity during infection. However, there is no information concerning the changes in the isoenzyme pattern of this enzyme in the infected tissues. The present work deals with a comparative study made on the activity and isoenzyme profile of glutamate-oxalacetate transaminase in healthy and diseased silkworm larvae.

Materials and methods. Healthy silkworm larvae belonging to the pure Mysore variety were obtained from the Central Sericultural Research and Training Institute (CSRTI), Mysore. The larvae were infected just after the 4th ecdysis by feeding them on mulberry leaves smeared with a nuclear polyhedrosis virus suspension. On the 5th day after inoculation, the larvae showed the typical symptoms of the disease. On the 6th day after inoculation hemolymph, fat body and intestine were collected separately from about 30 larvae, and those of control larvae fed on virus-free leaves were also obtained on the same day. The collection of hemolymph was done as described by Martignoni and Milstead[7]. Fat bodies and intestines were quickly removed and homogenized in ice-cold 0.1 M phosphate buffer pH 7.4 (1:1 w/v) in a Potter-Elvehjem tissue

Effect of nuclear-polyhedrosis on glutamate-oxalacetate transaminase activity in the larvae of *Bombyx mori**

Tissue	Group	Specific activity (μmoles/g protein/h)
Fat body	Control	72.23 ± 1.53
	Diseased	119.23 ± 1.12
Hemolymph	Control	56.73 ± 1.86
	Diseased	159.23 ± 4.55
Intestine	Control	47.73 ± 3.22
	Diseased	49.5 ± 4.42

*Values represent means ± SE for 3 batches.

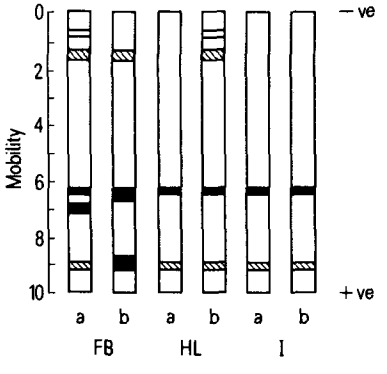

Fig. Zymogram showing the electrophoretic patterns of glutamate-oxalacetate transaminase in the fat body (FB), hemolymph (HL), and intestine (I) of healthy and infected larvae of *Bombyx mori.* a, control; b, diseased. □, Low intensity; ▨, moderate intensity; ■, high intensity.