ELSEVIER

International Journal of Pharmaceutics 195 (2000) 207–218

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

The interaction of human serum albumin and model membranes

Rita Galántai *, Irén Bárdos-Nagy

Institute of Biophysics and Radiation Biology, Semmelweis University of Medicine, P.O.B. 263, Budapest, H-1444 Hungary

Received 19 May 1999; accepted 9 November 1999

Abstract

It is frequently observed that the interaction of human serum albumin (HSA) with different lipid membranes may affect molecular transport both in vivo and in vitro experiments. There was a lack of consensus however in the interpretation of results. Earlier studies on the serum albumin membrane association had different conclusions depending on the source of protein, the preparation and the composition of the membranes applied. In this work the change of heat capacity, a sensitive parameter of the interacting system, is compared for uni- and multilamellar liposomes (dimyristoyl-phosphatidylcoline/dimyristoyl-phosphatidylglycerol) at 0, 1×10^{-3}, 8×10^{-3}, 1.2×10^{-2} and 3.3×10^{-2} HSA–lipid ratios. The thermal properties of the sonicated and vortexed liposomes show remarkable differences. The presence of HSA in both types of liposomes also modified their thermal properties, providing clear evidence for protein–vesicle interaction, different in the uni- and multilamellar liposomes. In the case of unilamellar liposomes, two additional transitions were observed at lower temperature, independently of the HSA–lipid ratio, and the protein binding mode to smaller or larger sized liposomes was also distinguishable. The addition of HSA to the multilamellar liposomes resulted in an increase of the pretransition temperature only at the higher HSA–lipid ratio, but the main transition temperature was not affected.

Keywords: Liposome; Human serum albumin; Albumin–liposome interaction; Differential scanning calorimetry

1. Introduction

Distribution of mesoporphyrin and magnesium-mesoporphyrin between human serum albumin (HSA) and small unilamellar liposomes (SUV) composed of dimyristoyl-phosphatidylcoline–dimyristoyl-phosphatidylglycerol (DMPC/DMPG) 19/1 was found different as we recently reported (Bárdos-Nagy et al., 1998). We proposed that a plausible reason for this observation was that the HSA interacted with liposomes, which

Abbreviations: DMPC, 1,2-dimyristoyl-sn-glycero-3-phosphatidylcoline; DMPG, 1,2-dimyristoyl-sn-glycero-3-phosphatidylglycerol; DSC, differential scanning calorimetry; HSA, human serum albumin; LUV, large unilamellar vesicle; MLV, multilamellar vesicle; SUV, small unilamellar vesicle; T1/2, width of the half- maximum of the main phase transition peak; Tm, main thermal transition temperature; Tp, pretransition temperature.

* Corresponding author. Tel.: +36-1-2676261; Fax: +36-1-2666656.

E-mail address: galantai@puskin.sote.hu (R. Galántai)

0378-5173/00/$ - see front matter
PII: S0378-5173(99)00399-3

mostly took place in the surface region of the vesicles.

The effect of albumin or other serum components on the result of experiment carried out in the presence of serum is frequently referred in literature. When for example liposomes are used as carriers to introduce certain substances into cells, the entrapped substance may be rapidly lost after intravenous injection because of the interaction of serum components with the liposomes (Scherphof et al., 1978; Liu et al., 1990; Jones and Nicholas, 1991; Chonn et al., 1991). For this reason care was taken to protect the liposomes against serum components by using appropriate modifications of their composition and structure (van-Borssum-Waalkes et al., 1993; Hernandez-Caselles et al., 1993; Jizomoto et al., 1994; Mercadal et al., 1995). However, in the case of in vivo and in vitro experiments not only the liposomes but also the cellular surface as well as the properties of the cell lines may be affected by serum constituents, either intrinsic or added to the culture medium for optimal growth and maintenance. Since liposomal transport of the incorporated substances into the cells may take place in many ways, such as by intermembrane transfer, contact release, adsorption, fusion, or phagocytosis–endocytosis (New et al., 1990), the uptake of the carried molecules by the cells may be influenced in the presence of serum components (Zborowski et al., 1977; Hoekstra and Scherphof, 1979). Taking into account that the serum contains transport proteins (such as albumin), this raises the question as to whether the interaction of the liposome- or the cell membrane with the serum transport proteins is also involved in the transport and uptake mechanisms, i.e. whether this interaction influences the transport properties of the serum protein.

The above considerations prompted extensive investigation of albumin-liposome complexes with a view of generating a model for albumin-cell interactions as well. In the literature, the binding of serum albumin to liposomes is mostly focused on the study of bovine serum albumin (Sweet and Zull, 1969; Juliano et al., 1971; Lis et al., 1976; Jonas, 1976; Zborowski et al., 1977; Hoekstra and Scherphof, 1979; Daniels et al., 1985; Law et al., 1986, 1988, 1994). However no general consensus seems to be emerging as reports attest both to the binding and to non-binding of albumin to liposomes (see discussion below). A review of available experimental data suggests that results depend on lipid composition, preparation method, and also on experimental conditions such as pH, temperature, albumin–lipid molar ratio, as well as on chemical conditions including the homogeneity and the source of the protein. A reinvestigation of the albumin–liposome interaction was accordingly felt warranted.

In this work, we focus our attention on the interaction of HSA with the lipid membrane using differential scanning calorimetry (DSC), to study the thermodynamic characteristics and dynamic light scattering to investigate the aggregation and the fusion of liposomes. Until now, microcalorimetry has not yet been applied to the study of the binding of albumins to liposome, but it could be successfully used to qualify the phospholipid vesicles and their changes (Biltonen and Lichtenberg, 1993).

2. Materials and methods

2.1. Sample preparation

DMPC, DMPG and 1 x crystallized and lyophilized HSA were purchased from Sigma Chemical Co and were used without further purification. All solvents (chloroform, methanol) were obtained from Merck, and high purity grade. The concentration of HSA was calculated using $\varepsilon_{280} = 37\,400/\mathrm{M}$ per cm in phosphate-buffers (Reddi et al., 1987).

The samples were prepared in 10 mM sodium-phosphate buffer at pH 7.4. For light scattering measurements, the buffer was filtrated using AMICON filter.

Small unilamellar liposomes were prepared as previously described (Bárdos-Nagy et al., 1998).

Multilamellar lipid vesicles (MLV) were prepared by dissolving, DMPC/DMPG (19:1 w:w) in a chloroform–methanol mixture (9:1 w:w). The solvent was evaporated under a stream of nitrogen at room temperature, then buffer was added

to the sample. The samples were vigorously mixed for 30 min above the phase transition temperature of DMPC in a Vortex mixer. HSA was dissolved in buffer and added to the dry lipid film during vortexing.

2.2. Light scattering measurements

Light scattering measurements were performed using an ALV goniometer with a 35 mW He–Ne laser ($\lambda = 632.8$ nm) light source or using a BI-200SM goniometer with an Ar-ion laser (Omnichrome 543AP) operating at 488 nm. The arithmetic mean of the data acquired on parallel experiments performed on the same sample was calculated and used in assessing particle size. The contribution of the particles with different size (diameter) to the autocorrelation function was determined using the maximum entropy method. This contribution is proportional to the weight of the particles, therefore in the case of liposomes it is proportional to the relative surface area of the vesicles. This function (relative contribution vs. diameter) was used to characterise the particle size distribution.

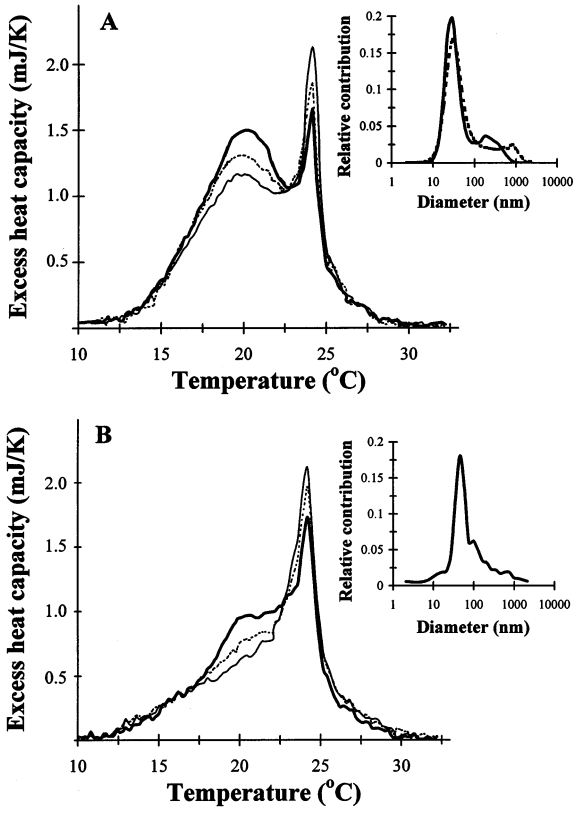

Fig. 1. Excess heat capacity curves of centrifuged (A) and uncentrifuged (B) SUVs (thick solid lines). The results of the first and of the second reheatings are shown by dotted and by thin solid lines, respectively. Inset: The size distribution of centrifuged (A) and uncentrifuged (B) SUVs before (solid line) and after (dotted line) recording of the DSC curves characterised by their relative contribution to the autocorrelation function.

2.3. Microcalorimetry

Microcalorimetry was performed on SUVs using a DASM-4 differential scanning microcalorimeter between 10 and 40°C at a heating rate of 1°C/min. 0.473 ml of the sample was cooled to ≈ 3°C, equilibrated and heated that required some 3 h. The MLVs were examined using a Du Pont Thermoanalyzer 990 (Barley Mill, Wilmington, Delaware, USA) at a heating rate of 5°C/min. 10 μl of the sample was closed in an aluminium capsule. The DSC measurements were performed using the same buffer in the reference cell with a protein content identical with that of the sample.

3. Results

Since relatively little data are available in the literature concerning the phase transition of SUVs, the DSC experiments were first performed on the liposome solution in the absence of HSA and the results compared with literature data reported of DPPC SUVs (Biltonen and Lichtenberg, 1993). In all DSC experiments, performed on SUVs, the lipid concentration was 1.5×10^{-3} M. The results were well reproducible when the samples in the parallel experiments were made from the same liposome preparation. New liposome preparations caused only slight changes in the relative contribution of the components.

The DSC curve acquired for the DMPC/DMPG 19/1 liposome solution after the first cooling process is shown in Fig. 1A (thick solid line). The curve consists of two phase transitions at

20.0 ± 0.2 and 24.10 ± 0.05°C, respectively. Although the peaks are not fully resolved, the width of their half maximum ($T_{1/2}$) were estimated as 6.0 ± 0.2 and 1.7 ± 0.2°C, corresponding to an increasing main thermal transition temperature (T_m). Dynamic light scattering on the freshly prepared liposome solutions, measured before the DSC experiments, showed an average particle diameter of 47.4 ± 3.3 nm. Further analysis of the light scattering data, showed the presence of a major population of liposomes with average diameter of ≈ 40 nm, but a small amount of larger liposomes was also detected (average diameter ≈ 150 nm) as shown in the inset of Fig. 1A (solid line). The size distribution function of the liposome solution after the DSC experiment showed a shift of diameter to the larger values as seen by the dotted line in the inset of Fig. 1A. Similarly to the results for DPPC liposomes (Lichtenberg et al., 1981; Biltonen and Lichtenberg, 1993), the lower phase transition temperature (Fig. 1A) has been attributed to the major SUV liposome component, and the higher one to a population of liposomes with larger size (LUV).

The effect of the cooling process—before heating—on the thermal transition curve of the liposome solution was studied by repeated recooling of the same sample. The DSC curves detected after the first and the second recooling are shown in Fig. 1A (dotted and thin solid lines, respectively). In between recooling steps, the sample was kept at ≈ 3°C in the DSC instrument. The peak corresponding to the population of smaller liposomes is observed to decrease, while the second peak increases. This observation is indicative of a change in the colloidal state of the liposome solution during the DSC experiments including the cooling process, and is in good agreement with the identification of two components in the phase transition peaks.

The validity of the attribution of the phase transition peaks to different liposomal populations was further examined by performing a series of experiments on an uncentrifuged sonicated liposome solution. In this case, the average size of the vesicles measured by light scattering experiment was found to be 75.3 ± 3.3 nm because the larger liposomes were not removed from solution. The size distribution, shown in the inset of Fig. 1B, is reflected by the average diameter of the liposomes in the supernatant and in the remaining solution after centrifugation, i.e. 47.4 ± 3.3 and 132.7 ± 13.8 nm, respectively. The thermal transition curve obtained after the first cooling is shown in Fig. 1B (thick solid line). As was the case for the DSC curve of the centrifuged liposome solution, it consists of two components, but the contribution of the second one is more important and the maximum of the first peak appears at higher temperature, at 21.1 ± 0.2°C (cf. 20.0 ± 0.2°C in Fig. 1A).

The influence of the cooling process on this sample was also studied and the same effect was observed as with the centrifuged solution. The relative decrease of the contribution of the SUV component after the first and the second recooling process is shown in Fig. 1B (dotted and thin solid lines, respectively).

The effect of HSA addition at HSA–lipid 8×10^{-3} concentration ratio is shown in Fig. 2A. It clearly affects the phase transition curve: the first peak is shifted to lower temperature (18.4 ± 0.05°C) and its width becomes smaller corresponding to $T_{1/2} = 3.4 \pm 0.1$°C. The area under the second transition increases and becomes resolved into two peaks, with a new transition at 23.2 ± 0.05°C. These changes are indicative of the presence of at least three liposome populations after HSA addition at this HSA–lipid concentration ratio. Control DSC measurements, performed on an HSA sample showed no phase transitions in the studied temperature interval. The position of the 24.1°C peak corresponds to the intrinsic LUV population present before HSA addition. The new peaks at 18.4°C and at 23.2°C appearing only after HSA addition, are attributed to the phase transition of liposome populations that interacted with HSA. A more precise identification of the new components was achieved after repeated recooling of this sample. The modifications of the phase transition curves upon first and second recooling are shown in Fig. 2A (dotted and thin solid lines, respectively). The first peak decreases while the second increases and shifts slightly to lower temperature. This shift is, however, only apparent: in reality, the peak at 23.2°C

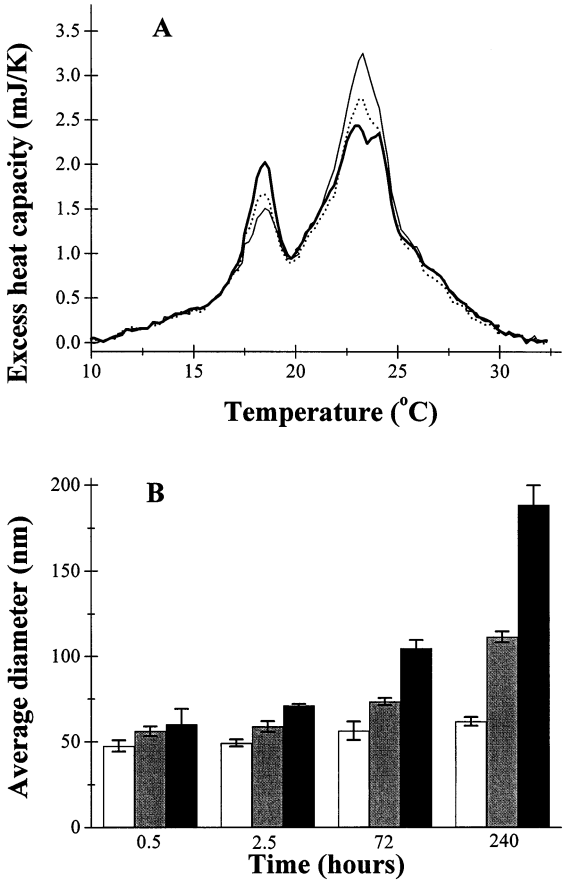

Fig. 2. A: The effect of HSA addition at a HSA–lipid molar ratio 8×10^{-3} (thick solid line). The results of the first and of the second reheatings are shown as thin and dotted lines, respectively. B: The average diameter of SUVs in the absence (first column) and in the presence of HSA (second and third columns) as a function of time measured by light scattering experiments. HSA–lipid molar ratios: 1×10^{-2} (second column), 2.5×10^{-2} (third column).

increases more than the peak at 24.1°C, which also increases upon recooling. The increase of the 24.1°C component is indicative of the presence of uncomplexed SUVs in solution (see Fig. 2). The differences in the observed extent of the increase of the respective peaks suggest more considerable aggregation–fusion in the presence of HSA.

The latter assumption was further investigated by measuring the average diameter of sonicated liposomes in the absence and in the presence of HSA as a function of time. Dynamic light scattering experiments were performed at 0, 1×10^{-2} and 2.5×10^{-2} HSA–lipid concentration ratios, with the lipid concentration 1×10^{-3} M. The incubation times were 0.5, 2.5 h, 3 and 10 days. The results are presented in Fig. 2B. The first column shows the diameter of liposomes in the absence of HSA, the second and third correspond to the results obtained with HSA–lipid solutions of different (1×10^{-2} and 2.5×10^{-2}) concentration ratios. The diameter of the liposomes increased in all solutions as a function of time. The presence of HSA definitely promotes the formation of larger size particles and higher HSA concentrations enhance the size increase.

The modification of the DSC curves with increasing HSA–lipid molar ratio is shown in Fig. 3A–C (thick solid lines corresponding to protein–lipid molar ratios as 0, 1×10^{-3} and 1.2×10^{-2}, respectively). The phase transition curve obtained in the absence of HSA was deconvoluted into two symmetrical components associated with the presence of two liposome populations with different average sizes, as shown in Fig. 3A (thin solid lines). Since in the case of the 8×10^{-3} HSA–lipid molar ratio (Fig. 2A) the repeated recooling showed a minimum of four liposome populations (SUV, LUV, SUV-HSA, LUV-HSA), the phase transition curves in Fig. 3B and C were deconvoluted into four components (thin solid lines). The fits were performed by fixing the fourth component to the parameters used for the second component obtained in the absence of HSA, and the other components were left to iterate freely. The relative areas under the fitted curves related to the total area of the phase transition curve in Fig. 3 are summarized in Table 1. (The best-fit components are shown in the figures by thin lines and the sum of the components are overlaid on the experimentally determined DSC curves (dotted line)). In Fig. 3B and C the second component associated with the first component in the lipid solution (Fig. 3A), decreases upon adding HSA and shifts to higher temperature. The contribution of the new components (1 and 3) increases and the half width of the first one becomes smaller with increasing amount of HSA. The deconvolution was repeated with the fourth component floating. In this case, a slight decrease of the

fourth component is observed upon increasing HSA–lipid concentration ratio, but the other effects are as previously described. The total area under the phase transition curves slightly increases with increasing amount of HSA. This shows that the molar enthalpy change of the HSA–liposome complex is higher than that of the pure lipid. The determination of accurate values was not possible, because the concentrations of the lipid molecules making up the different liposome populations were unknown.

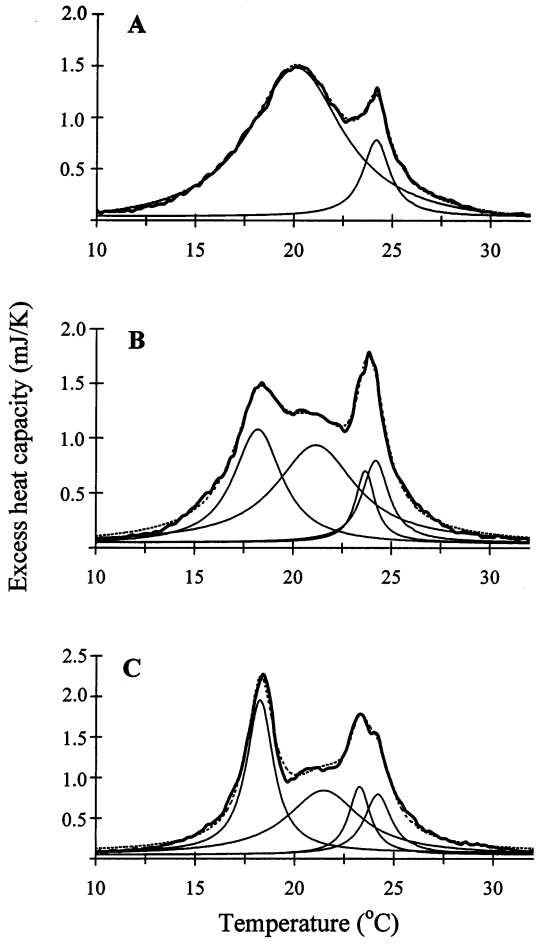

Fig. 3. Excess heat capacity curves of SUVs in the absence (A) and in the presence of HSA (B–C). HSA–lipid molar ratios: B, 1×10^{-3}; C, 1.2×10^{-2}.

Table 1
Relative contribution of the different liposomes and liposome-HSA components in function of the HSA–lipid ratio

HAS–lip	SUV-HSA	SUV	LUV-HSA	LUV
0	–	0.84	–	0.16
1.0×10^{-3}	0.34	0.50	0.10	0.16
1.2×10^{-2}	0.44	0.42	0.14	0.16

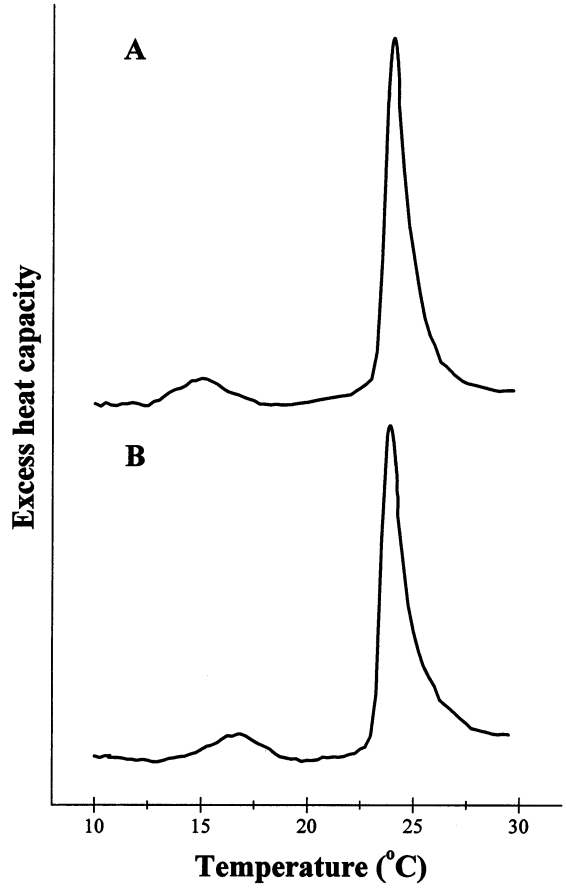

Fig. 4. Excess heat capacity curves of MLVs in the absence (A) and in the presence of HSA (B). HSA–lipid molar ratio: 3.3×10^{-2}.

We also examined the MLVs, more widely investigated by DSC in literature reports. For these measurements the lipid concentration was 125 mg/ml, more than 100 times higher than that of the previously described SUV solutions.

The phase transition curve in the absence of HSA is shown in Fig. 4A. T_p was observed at 15.0 ± 0.2°C and T_m at 24.0 ± 0.2°C. The HSA addition did not influence the DSC curve at a

molar ratio of 1×10^{-3} HSA–lipid (not shown). In the case of the 3.3×10^{-2} HSA–lipid molar ratio, T_p increased by 1.5°C to 16.5 ± 0.2°C as shown in Fig. 4B. Neither the position nor the width of the main transition peak was affected. The change in T_p is indicative of HSA and MLVs interaction.

4. Discussion

4.1. The phase transition of DMPC/DMPG 19/1 lipid systems

The MLV phase transition curve (Fig. 4A) shows homogenous mixing of the DMPC and DMPG components in agreement with the fact that the phase transition characteristics of the pure components are similar. Comparing this result with literature data for unmixed DMPC and DMPG, only slight broadening and asymmetry were observed upon mixing (Findlay and Barton, 1978).

Comparison of the excess heat capacity curve of sonicated liposomes (Fig. 1A) with that of MLVs shows different phase transition characteristics for unilamellar vesicles and MLVs. In the case of unilamellar liposomes, the phase transition that occurs at the same temperature as the main transition of MLVs (second transition in Fig. 1A) is preceded by a broader transition at lower temperature. This first transition is unambiguously different than the pretransition of MLV. A similar difference was observed in the DPPC SUV DSC measurement. The phase transition of smaller liposomes occurs at lower temperature and it shows a pronounced increase as a function of diameter in the 35–70 nm diameter range. Outside this interval, T_m is independent of size (Suurkuusk et al., 1976; Biltonen and Lichtenberg, 1993). This result is however not so straightforward, because during the DSC experiment, the colloidal state of the liposome solution is affected. Comparison of the change of the size distribution function during the DSC experiment (inset of Fig. 1A) with the increase in average diameter caused by a 'normal incubation' of 2.5 h ($47.4 \pm 3.3 \rightarrow 49.2 \pm 2.0$, Fig. 2B) shows that the effect of the DSC measurement is not limited to the usual aggregation occurring in a solution containing colloidal particles during storage. This observation is in agreement with the result obtained for DPPC where the tendency of the gel-phase SUV to fuse into larger liposomes was observed in the 'heating mode' of the DSC experiment involving a longer cooling process (Lichtenberg and Thomson, 1990). The presence of SUVs in coexistence with LUVs results in a heat capacity curve with two phase transitions; the first transition (maximum at lower temperature) corresponds to the SUVs, and the second one to the LUVs (Lichtenberg et al., 1981). The SUV transition occurs in a much broader temperature range than that observed for LUVs. This is due to the sensitivity of the phase transition temperature to even small variations in SUV diameter size. This conclusion is also supported by comparing the centrifuged SUV result (Fig. 1A) to the phase transition curve of a SUV-LUV mixture containing larger sized vesicles (Fig. 1B) The larger particles are responsible for the relative increase of the second component, and, in accordance with the size dependence of SUVs T_m, the maximum of the first transition also increases by ≈ 1°C.

Both the size increasing effect of the 'heating mode' and the different phase transition of SUVs and LUVs are observable by the repeated detection of the excess heat capacity curve. The higher amount of LUVs in the sample caused by cooling resulted in a decrease of the first component and an increase of the second component of the thermal transition curve.

4.2. The effect of HSA addition on the thermal characteristic of DMPC/DMPG 19/1 lipid systems

The appearance of two new transitions in the excess heat capacity curve of SUVs after HSA addition unambiguously shows that there is interaction between the liposomes and HSA. The recooling and the HSA concentration dependent experiments allowed identification of the new components.

The observation of the repeated detection of the DSC curve on the same sample may lead to

several conclusions. Taking into account the fact that recooling caused increase of the 24.1°C component associated to SUV-LUV transformation, we can infer that there is uncomplexed SUV in the HSA–lipid solution at 8×10^{-3} concentration ratio. Comparing the parallel decrease of the 18.4°C transition and the increase of the 23.2°C component, we conclude that the behavior of the first new component (at lower temperature) is similar to that of the SUV component, while that of the second new component is similar to that of the LUV upon repeated recooling: they are changing stepwise. The phase transition at 18.4°C was attributed to the SUV-HSA complexes. It is clear that — as was the case with the pure SUV solution — the transformation of SUV-HSA complexes into LUV-HSA complexes also occurs during the cooling process. The assignment of the second new transition, however is not so evident. The recooling experiments show that one part of this component must be the result of fusion–aggregation of SUV-HSA complexes. The different extent of the increase of the 23.2 and 24.1°C transitions suggests that the amount of HSA-LUV complex increases to a more significant extent than was the case with uncomplexed LUV. The time dependent dynamic light scattering experiments (Fig. 2b) are also indicative of the occurrence of a HSA induced size increase, however, the presence of a LUV-HSA complex caused by the interaction of intrinsic LUVs and the protein can not be excluded. This possibility is also not eliminated by the four-component deconvolution of phase transition curves. Both fixed and floating four-component deconvoluting were in good agreement with the experimental curve. When the fitted components were left floating, a small decrease of the fourth component was found with increasing amount of HSA, suggestive of a decrease in the LUV component caused by LUV-HSA complex formation. Although the possibility of LUV-HSA interaction can not be ruled out, it is clear that the HSA-SUV interaction is more significant than HSA-LUV binding.

The higher affinity of HSA for SUVs when compared to LUVs shows that the smaller liposomes are better candidates for interaction with HSA. This conclusion is also supported by the phase transition temperature value for SUV, which was higher in the presence (≈ 21°C) than in the absence of HSA (20°C). As already pointed out, in this range, a higher T_m is indicative of a grater diameter. This explains why, after the interaction, the uncomplexed SUV population yielded a higher T_m value (20 → 21°C). It is also probable that the higher curvature obtained with the smaller liposomes is favorable for interaction with HSA.

Since the enthalpy (ΔH_m) increases only slightly with increasing amount of HSA, while the T_m value is significantly lower for the SUV-HSA complex, the entropy change equal to $\Delta H_m/T_m$ increases. This means that the packing of lipids is disturbed within the bilayer, i.e. their order is reduced. We interpreted these changes as a kind of entering of HSA to the phospholipid layer. Similarly to our conclusion, in all cases where interaction was found to occur, surface binding, followed by partial penetration and/or deformation of the bilayer was proposed as a mechanism (Sweet and Zull, 1969; Juliano et al., 1971; Lis et al., 1976; Law et al., 1986, 1988, 1994) as it is obvious from the data in Table 2. where we survey the earlier results reported for albumin–liposome systems.

The interaction between MLVs and HSA molecules is less favourable as shown by the unaffected phase transition curve at 1×10^{-3} HSA–lipid concentration ratio. Interaction happens, but at higher HSA concentrations (3.3×10^{-2} HSA–lipid), as shown by the significant T_p increase. The pretransition phase is known to be particularly sensitive to the effect of small amounts of contaminants. In most cases the effect of contaminants on the pretransition is more pronounced than that is on the main transition (Biltonen and Lichtenberg, 1993). Furthermore the change observed in pretransition temperature shows that the surface of the MLVs is definitely involved in the HSA-liposome interaction. As concluded from the SUVs result it is again suggestive of surface binding and penetration.

Since the DSC technique has been used mostly for multilamellar liposomes as routine facility even in that cases, when the other experimental methods applied required the comparison with

Table 2
A survey of earlier experiments on albumin–liposome systems

Composition of liposomes	Method of liposome preparation	Source of albumin	Albumin/lipid concentration ratio	Method of measurement	pH	t (°C)	Interaction	Conclusion	Ref.
lec/chol/DCP = 7/1/2 (−)[a]	Mechanical shaking	Bovine from General Biochemicals, no purification	4×10^{-4} saturation $\approx 20\times 10^{-4}$	Diffusion of [^{14}C]glucose from liposomes measured by dialysis	5.5–3	22–23	Yes	Electrostatic and hydrophobic interaction suggested, the penetration of the protein molecule into the hydrocarbon matrix of the membrane occurred	Sweet and Zull, 1969
lec/chol/SA = 7/1/2 (+)					7		No		
lec/chol = 7/1 (0)					7 3.5 7; 3.5		No No No; no		
PS (−)	Sonication	Human from Miles Laboratories, no purification	0.3–0.6	Diffusion of $^{22}Na^{+}$ from liposomes measured by dialysis	4.5	25	Yes	See previous	Juliano et al., 1971
PC (0)					7.4 7.4		No No		
DMPC (0)	Dispersion	Bovine from Miles Laboratories, no purification	≈ 0.04	Change of Raman intensity ratio ($I2890/I2850$; $I2850/I2930$)	? (Water)	24	Yes	A conformational change of the lipid hydrocarbon chains in the presence of albumin(→)albumin penetrates the hydrocarbon chains	Lis et al., 1976
Egg PC (0)	Dispersion	Bovine from Sigma, no purification	$\approx 3\times10^{-3}$ -5×10^{-2}	^{14}C labeled liposomes; UV absorption	8	20–25	No	albumin is capable of binding only the separated lipid molecules but not lipid molecules included in liposomes	Jonas, 1976
Rat liver PC (0) PC/PA = 48/47 (−)	Sonication	Bovine from Sigma, no purification	100	Leakage of radioactive sucrose from liposomes;	7.4	37	Yes	drastic stimulation of the sucrose release by albumin in case of any liposome composition;	Zborowski et al., 1977

Table 2 (Continued)

Composition of liposomes	Method of liposome preparation	Source of albumin	Albumin/lipid concentration ratio	Method of measurement	pH	t (°C)	Interaction	Conclusion	Ref.
lec/chol = 67/33 (0)				Liposomes labeled radioactively			No	liposomes are the lipid donors for the formation of albumin–lipid complex;	
PC/chol/DCP = 7/2/1 (−)	Sonication MLV and SUV separated by gel filtration	Bovine from Sigma, no purification	≈100	Incubation of [^{14}C]PC liposomes with parenchymal cells;	7.4	37	Yes	The liposomal surface membrane or the cell surface or both are modified by the albumin;	Hoekstra and Scherphof, 1979
PC/chol/SA = 7/2/1 (+)				Protein assayed with fluorescent dye			Yes	Protein can be bound to the vesicle surface, the presence of albumin resulting in increased permeability of the MLV	
DMPC (0)	Sonication	Bovine from Sigma, no purification	3×10^{-5}	Fluorescence of albumin is measured	7.4	30 or 37	No	The liposomes themselves was no effect on the fluorescence of albumin	Daniels et al., 1985
PC/chol/SA = 1.6/1/0.2 (+)	Dispersion	Bovine from Sigma, no purification	$\approx10^{-4}$	Micro-electrophoresis	4–7	37	Yes	Most of the albumin molecules penetrates the phospholipid bilayer to the hydrocarbic region independently of the surface charge of the liposomes; hydrophobic interaction is the dominant force for adsorption	Law et al., 1986, 1988
PC/chol/DCP = 1.6/1/0.2 (−)							Yes		
PC/chol = 1.6/1 (0)							Yes		
PC	Sonication	Bovine from Sigma, no purification	$\approx10^{-4}$	Micro-electrophoresis	7.4	37	Yes	Albumin molecules may penetrate into the phospholipid bilayers and the nonpenetrated moiety coats on the liposomes	Law et al., 1994
DOPC							Yes		

[a] Surface charge of the liposomes.

SUVs, finally, we would like to emphasize that the application of DSC method in the studies of unilamellar vesicles and their interaction with proteins is advantageous in more respects. Our results present that the excess heat capacity curves of the SUVs are well understandable and more sensitive even at lower lipid and protein concentration. The study of the SUVs thermal transition makes possible not only the observation of the changes of the membrane structure but detects the alterations of the colloidal state of liposome solution as well. It serves reliable information about the size dependence of interaction between the agent components and liposome.

Acknowledgements

The authors thank Dr P. Závodszky for providing use of the DSC facilities at the Institute of Enzymology, Biological Research Center, Hungarian Academy of Science, Budapest and J. Kardos for help in the microcalorimetry measurements. Thank for help in the light scattering experiments Dr T. Gilányi and I. Varga at the Department of Colloid Chemistry of Eötvös L. University, Budapest. The professional help of R. Markács and É. Bányai in sample preparation is highly appreciated.

References

Bárdos-Nagy, I., Galántai, R., Kaposi, A.D., Fidy, J., 1998. Difference in the transport of metal and free-base porphyrins. Steady-state and time-resolved fluorescence studies. Int. J. Pharm. 175, 255–267.

Biltonen, R.L., Lichtenberg, D., 1993. The use of differential scanning calorimetry as a tool to characterize liposome preparations. Chem. Phys. Lipids 64, 129–142.

Chonn, A., Semple, S.C., Cullis, P.R., 1991. Separation of large unilamellar liposomes from blood components by a spin column procedure: towards identifying plasma proteins which mediate liposome clearance in vivo. Biochim. Biophys. Acta 1070, 215–222.

Daniels, C., Noy, N., Zakim, D., 1985. Rates of hydration of fatty acids bound to unilamellar vesicles of phosphatidylcholine or to albumin. Biochemistry 24, 3286–3292.

Findlay, E.J., Barton, P.G., 1978. Phase behavior of synthetic phosphatidylglycerols and binary mixtures with phosphatidylcholines in the presence and absence of calcium ions. Biochemistry 17, 2400–2405.

Hernandez-Caselles, T., Villalain, J., Gomez-Fernandez, J.C., 1993. Influence of liposome charge and composition on their interaction with human blood serum proteins. Mol. Cell. Biochem. 120, 119–126.

Hoekstra, D., Scherphof, G., 1979. Effect of fetal calf serum and serum protein fractions on the uptake of liposomal phosphatidylcholine by rat hepatocytes in primary monolayer culture. Biochim. Biophys. Acta 551, 109–121.

Jizomoto, H., Kanaoka, E., Hirano, K., 1994. pH-sensitive liposomes composed of tocopherol hemisuccinate and of phosphatidylethanolamine including tocopherol hemisuccinate. Biochim. Biophys. Acta 1213, 343–348.

Jonas, A., 1976. Interaction of phosphatidylcholine with bovine serum albumin. Specificity and properties of the complexes. Biochim. Biophys. Acta 427, 325–336.

Jones, M.N., Nicholas, A.R., 1991. The effect of blood serum on the size and stability of phospholipid liposomes. Biochim. Biophys. Acta 1065, 145–152.

Juliano, R.L., Kimelberg, H.K., Papahadjopoulos, D., 1971. Synergistic effects of a membrane protein (spectrin) and Ca2+ on the Na+ permeability of phospholipid vesicles. Biochim. Biophys. Acta 241, 894–905.

Law, S.L., Lo, W.Y., Pai, S.H., Teh, G.W., Kou, F.Y., 1986. The adsorption of bovine serum albumin by liposomes. Int. J. Pharm. 32, 237–241.

Law, S.L., Lo, W.Y., Pai, S.H., Teh, G.W., 1988. The electrokinetic behaviour of liposomes adsorbed with bovine serum albumin. Int. J. Pharm. 43, 257–260.

Law, S.L., Lo, W.Y., Lin, M., 1994. Increase of liposome stability by incorporation of bovine serum albumin. Drug Dev. Ind. Pharm. 20, 1411–1423.

Lichtenberg, D., Freire, E., Schmidt, C.F., Barenholz, Y., Felgner, P.L., Thompson, T.E., 1981. Effect of surface curvature on stability, thermodynamic behavior, and osmotic activity of dipalmitoylphosphatidylcholine single lamellar vesicles. Biochemistry 20, 3462–3467.

Lichtenberg, D., Thomson, T.E., 1990. In: Wischut J., Hoekstra D. (Eds.), Membrane Fusion, Marcel Dekker, New York, Basel, Hong Kong.

New, R.R.C., Black, C.D.V., Parker, R.J., Puri, A., Scherphof, G.L., 1990. In: New, R.R.C. (Ed.), Liposomes a Practical Approach. Oxford University Press, New York.

Lis, L.J., Kauffman, J.W., Shriver, D.F., 1976. Raman spectroscopic detection and examination of the interaction of amino acids, polypeptides and proteins with the phosphatidylcholine lamellar structure. Biochim. Biophis. Acta 436, 513–522.

Liu, D., Huang, L., Moore, M.A., Anantharamaiah, G.M., Segrest, J.P., 1990. Interactions of serum proteins with small unilamellar liposomes composed of dioleoylphosphatidylethanolamine and oleic acid: high-density lipoprotein, apolipoprotein A1, and amphipathic peptides stabilize liposomes. Biochemistry 29, 3637–3643.

Mercadal, M., Domingo, J.C., Bermudez, M., Mora, M., De-Madariaga, M.A., 1995. *N*-palmitoylphosphatidylethanolamine stabilizes liposomes in the presence of human serum: effect of lipidic composition and system characterization. Biochim. Biophys. Acta 1235, 281–288.

Reddi, E., Lambert, C.R., Jori, G., Rodgers, A.J., 1987. Photokinetic and photophysical measurements of the sensitized photooxidation of the tryptophyl residue in *N*-acetyl tryptophanamide and in human serum albumin. Photochem. Photobiol. 45, 345–351.

Scherphof, G., Roerdink, F.H., Hoekstra, D., Zborowski, J., Wisse, E., 1978. In: Allison, A.C., Gregoriadis, G. (Eds.), Liposomes in Biological Systems. John Wiley, London.

Suurkuusk, J., Lentz, B.R., Barenholz, Y., Biltonen, R.L., Thompson, T.E., 1976. A calorimetric and fluorescent probe study of the gel–liquid crystalline phase transition in small, single-lamellar dipalmitoylphosphatidylcholine vesicles. Biochemistry 15, 1393–1401.

Sweet, C., Zull, J.E., 1969. Activation of glucose diffusion from egg lecithin liquid crystals by serum albumin. Biochim. Biophys. Acta 173, 94–103.

van-Borssum-Waalkes, M., van-Galen, M., Morselt, H., Sternberg, B., Scherphof, G.L., 1993. In-vitro stability and cytostatic activity of liposomal formulations of 5-fluoro-2′-deoxyuridine and its diacylated derivatives. Biochim. Biophys. Acta 1148, 161–172.

Zborowski, J., Roerding, F., Scherphof, G., 1977. Leakage of sucrose from phosphatidylcholine liposomes induced by interaction with serum albumin. Biochim. Biophys. Acta 497, 183–191.