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ORIGINAL PAPER

Spontaneous transfer of stearic acids between human serum albumin and PEG:2000-grafted DPPC membranes

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Abstract Electron spin resonance (ESR) spectroscopy is used to study the transfer of stearic acids between human serum albumin (HSA) and sterically stabilized liposomes (SSL) composed of dipalmitoylphosphatidylcholine (DPPC) and of submicellar content of poly(ethylene glycol:2000)-dipalmitoylphosphatidylethanolamine (PEG:2000-DPPE). Protein/lipid dispersions are considered in which spin-labelled stearic acids at the 16th carbon atom along the acyl chain (16-SASL) are inserted either in the protein or in the SSL. Two component ESR spectra with different rotational mobility are obtained over a broad range of temperature and membrane composition. Indeed, superimposed to an anisotropic protein-signal, appears a more isotropic lipid-signal. Since in the samples only one matrix (protein or membranes) is spin-labelled, the other component accounts for the transfer of 16-SASL between albumin and membranes. The two components have been resolved and quantified by spectral subtractions, and the fraction, f_p (16-SASL), of spin labels bound non-covalently to the protein has been used to monitor the transfer. It is found that it depends on the type of donor and acceptor matrix, on the physical state of the membranes and on the grafting density of the polymer-lipids. Indeed, it is favoured from SSL to HSA and the fraction of stearic acids transferred increases with temperature in both directions of transfer. Moreover, in the presence of polymer-lipids, the transfer from HSA to SSL is slightly attenuated, especially in the brush regime of the polymer-chains. Instead, the transfer from SSL to HSA is favoured by the polymer-lipids much more in the mushroom than in the brush regime.

Keywords Human serum albumin · DPPC · PEG:2000-DPPE · Spin-label · Electron spin resonance · Lipid transfer

Abbreviations

HSA	Human serum albumin
16-SASL	Stearic acid spin label 2-(14-carboxytetradecyl)-4,4-dimethyl-2-ethyl-3-oxazolidinyloxy
DPPC	1,2-Dipalmitoyl-*sn*-glycero-3-phosphatidylcholine
PEG:2000-DPPE	1,2-Dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-*N*-poly(ethylene glycol):2000
SSL	Sterically stabilized liposomes
FA	Fatty acids
FABP	Fatty acid binding proteins
PBS	Phosphate buffer solution
ESR	Electron spin resonance

Introduction

The transport of amphiphiles and hydrophobic substances (such as phospholipids, cholesterol, fatty acids, drugs) across cell membranes is a topic of considerable multi-disciplinary research (Spector and Fletcher 1978; Hamilton 1998). It is essential for the understanding of many physiological, pathological and/or pharmacological processes.

An important class of molecules which are transported through cell compartments is represented by nonesterified

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fatty acids (FA) (Kleinfeld 2000; Zakim 2000; Pownall 2001). They are intermediates in lipid metabolism, represent fuel for cells, are in constant flux and enter and leave cells rapidly. A key role in the pathway of FA transport is played by fatty acid binding proteins (FABP) and membranes (Glatz and van der Vusse 1996; Zucker 2001; Weisiger and Zucker 2002). In particular, long-chain FA, besides bind to FABP, because of their hydrophobicity and low solubility in water (Vorum et al. 1992), they also have affinity for membranes (Luxon and Weisiger 1993). They spontaneously transfer between organized lipid structures, or between these and FABP. By these molecular mechanisms, the amount of fatty acids in the plasma is controlled and the supply to mammalian cells assured.

In this work, the binding and the spontaneous transfer of medium-long fatty acids having the acyl chain of 18 carbon atoms, namely stearic acids, between human serum albumin (HSA) and sterically stabilized liposomes (SSL) composed of dipalmitoyl-phosphatidylcholine (DPPC) and of sub-micellar content of the polymer-lipid poly(ethylene glycol:2000)-dipalmitoylphosphatidylethanolamine (PEG:2000-DPPE) have been studied by means of spin-label electron spin resonance (ESR) spectroscopy.

HSA, the most abundant plasma protein, shows a strong affinity to bind reversibly and non-covalently FA which are stored and transported through the plasma (Hamilton et al. 1984; Peters 1997; Curry et al. 1999). FA also insert in phospholipid membrane model systems accommodating the hydrocarbon chain in the hydrophobic region and the polar head toward the aqueous phase, respectively. SSL made of phosphatidylcholines and of PEG-lipids are long circulating drug carriers that avoid protein adsorption (Lasic 1993; Lasic and Martin 1995; Efremova et al. 2000; Bartucci et al. 2002). It is, therefore, of interest to investigate molecular aspects of the transport of fatty acids between PEG-grafted membranes and plasma proteins.

The transfer of stearic acids between HSA and SSL has been studied by conventional ESR utilizing spin-labelled stearic acids with the nitroxide moiety at the 16th carbon atom of the acyl chain (16-SASL). We have considered protein/lipid dispersions in which 16-SASL have been added either to the protein (in this case, HSA is the donor and the lipid bilayers the acceptor matrix, respectively) or to the membranes (in this case, the lipid bilayer is the donor and HSA is the acceptor, respectively). In any case, 16-SASL were incorporated at a content that does not induce conformational changes in HSA and does not perturb the structural organization of the lipid bilayers.

The study is based on the experimental evidence that 16-SASL gives raise to anisotropic spectra in the protein and to more isotropic spectra in the lipid bilayers. So that two components with different rotational mobility on the conventional ESR timescale are evidenced in the spectra of 16-SASL when mixed lipid/protein dispersions are considered. Since in these samples only one matrix (either the protein or the lipid bilayers) is spin-labelled, the fraction of 16-SASL which is spontaneously transferred from one environment (i.e., from the donor) to the other (i.e., to the acceptor) is readily evaluated by ESR difference spectroscopy. A similar approach has been previously used for studying the transfer of 16-SASL from HSA to DPPC liposomes by ESR on a different mixed protein/lipid dispersion preparation (Pantusa et al. 2005).

How the transfer of 16-SASL between HSA and SSL depends on the type of donor and acceptor, on the temperature and on the grafting density of the polymer-lipids is shown and discussed.

Materials and methods

Chemicals

Essentially fatty acid-free and globulin-free human serum albumin (HSA, type A-3782, purity approximately 99%), the synthetic lipid 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC), and the stearic acid spin label 2-(14-carboxytetradecyl)-4,4-dimethyl-2-ethyl-3-oxazolidinyloxy (16-SASL) were from Sigma/Aldrich (St. Luis, MO, USA). High-purity (>99%) PEG-lipid 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-*N*-poly(ethylene glycol) with PEG of average molecular mass 2,000 Da (PEG:2000-DPPE) was obtained from Avanti Polar Lipids (Birmingham, AL, USA). The reagent grade salts for the 10 mM phosphate buffer solution (PBS) at pH 7.2 were from Merck (Darmstadt, Germany). All materials were used as purchased with no further purification. Distilled water was used throughout.

Electron spin resonance

Sample preparation

Dispersions of HSA spin-labelled with 16-SASL at 1:1 molar ratio were prepared as follows. A volume of methanol containing the spin label was first evaporated under a flow of nitrogen gas and any residual solvent removed under vacuum. A buffered HSA solution (protein concentration $\sim$1 mM) was then added to the dried spin label. Finally, the dispersions were heated at 45°C, periodically vortexed for 30 min and stored at 4°C for 30 min. Spin-labelled handshaken polymer-grafted multibilayers were prepared by dissolving the required amounts of DPPC and PEG:2000-DPPE together with 1% molar concentration of 16-SASL in chloroform–methanol. The solvent was first evaporated in a nitrogen gas stream and then under vacuum

overnight. The dried lipid samples were fully hydrated with PBS (lipid concentration ~100 mM), by heating above the chain-melting transition temperature of the dispersions (i.e., 45°C), periodically vortexed for 30 min and stored overnight at 4°C.

Samples for measurements on lipid/protein complexes were prepared by mixing either preformed polymer-grafted multibilayers with spin-labelled HSA buffered solution or protein buffered solution with preformed spin-labelled PEG-grafted DPPC dispersions at a final protein/lipid ratio of 1:1 w/w. The mixed dispersions were finally transferred in a 1 mm (i.d.), 100 µl ESR glass capillaries and measured starting from low temperature (10°C).

ESR measurements

Conventional ESR spectra were acquired on a 9-GHz ESP 300 spectrometer (Bruker, Karlsruhe, Germany). The sample capillary was inserted in a standard 4 mm diameter ESR quartz tube containing light silicon oil for thermal stability and centred in a Bruker ER 4201 TE_{102} rectangular ESR cavity. Sample temperature was controlled with a Bruker ER 4111VT variable temperature control unit (accuracy ±0.5°C). Conventional, in-phase, absorption ESR spectra were recorded well below saturation at a microwave power of 10 mW using a 100 kHz field modulation frequency for phase sensitive detection and 1 G_{p-p} as amplitude of the magnetic field modulation signal.

Data analysis

The ESR spectra of 16-SASL either in HSA in the presence of unlabeled DPPC/PEG:2000-DPPE multilayers or in polymer-grafted DPPC membranes in the presence of unlabelled HSA consist of two spectral components corresponding to spin-label populations with different mobility on the conventional ESR time scale. In fact, in addition to an immobilized protein component, a second, sharper lipid component appears in the ESR spectra of 16-SASL in the mixed protein/lipid dispersions (see below in the text). The two spectral components are resolved and quantified by means of spectral subtraction and double integration of the spectra (Griffith and Jost 1976; Jost and Griffith 1976; Marsh 1982). Briefly, computer-aided subtraction of the digitized spectra are performed as follows. The first step is to take the composite spectrum and that of the immobilized (fluid) component to be subtracted. Then, the two spectra are adjusted for slanting baseline and offset and shifted horizontally relative to each other so that they are aligned at the central peak. Finally, incremental subtractions are carried out until an obvious endpoint is reached. In this way, the fluid (immobilized) component difference spectrum is obtained. Once that the two spectral components are resolved, by considering the double integral of the original spectra (i.e., the composite one and that used for the subtraction), the fraction of the spectrum that was subtracted from the total spectrum is obtained. In this way, the spin-label populations in the protein, f_p, and in the liposomes, $f_l = 1 - f_p$, can be determined.

The temperature dependence of the fraction of 16-SASL bound to the protein, f_p, was used to monitor the transfer of the stearic acids between HSA and SSL.

The ESR measurements were repeated to test their reproducibility.

Results and discussion

Transfer of 16-SASL between HSA and DPPC membranes

At 20°C, the ESR spectrum of 16-SASL in multibilayers of DPPC (Fig. 1a) is an axial, anisotropically averaged powder pattern typical of spin labels in the intermediate motional regime of conventional ESR time-scale, whereas the spectrum of 16-SASL bound non covalently to HSA (Fig. 1b) shows an high degree of motional anisotropy and corresponds to immobilized spin-labels (Marsh 1981).

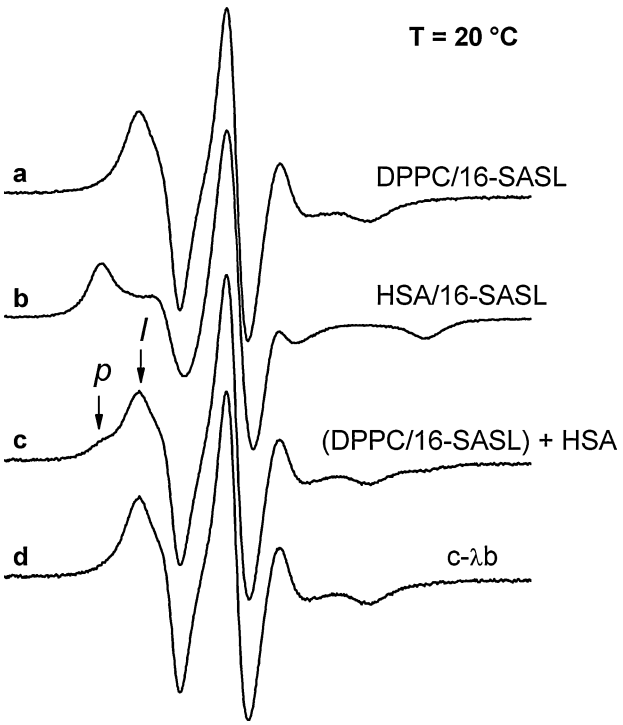

Fig. 1 ESR spectra at 20°C of 16-SASL **a** in DPPC multilayers; **b** bound non-covalently to HSA; **c** in DPPC multibilayers in the presence of unlabelled HSA. **d** Difference spectrum, i.e., the lipid mobile component, obtained by subtracting from spectrum c a percentage, λ, of the spectrum b. Total scan range = 100 Gs

The spectra are single-component without the presence of any free doxyl stearic acid spin-labels in the dispersion medium. This indicates the ability of both the DPPC membranes and the protein to bind spin-labelled fatty acids. At the same temperature, the spectrum of 16-SASL in DPPC bilayers in the presence of unlabelled HSA (Fig. 1c) shows two distinct components. Indeed, superimposed to an inner, isotropic, well defined lipid component, *l*, an outer, anisotropic, immobilized protein component, *p*, is evident in the outer wings of the spectrum.

Two components with different rotational mobility are also present in the spectrum of 16-SASL in HSA in the presence of unlabelled DPPC membranes (spectrum not shown).

On increasing the temperature, a decrease of the spectral anisotropy is seen in the ESR spectra of 16-SASL incorporated in the lipid and in the protein. This can be seen by comparing the spectra in Fig. 1a and b at 20°C with those reported in Fig. 2a and b at 32°C.

It is worthy to note that the spectrum in Fig. 2a of 16-SASL in DPPC multibilayers in the ripple phase at 32°C is the superposition of ordered- and disordered-type spectra, that are typical features of the nonhomogeneous structure in the ripple phase of DPPC liposomes, as has been established in the ESR studies of Tsuchida and Hatta (Tsuchida and Hatta 1988).

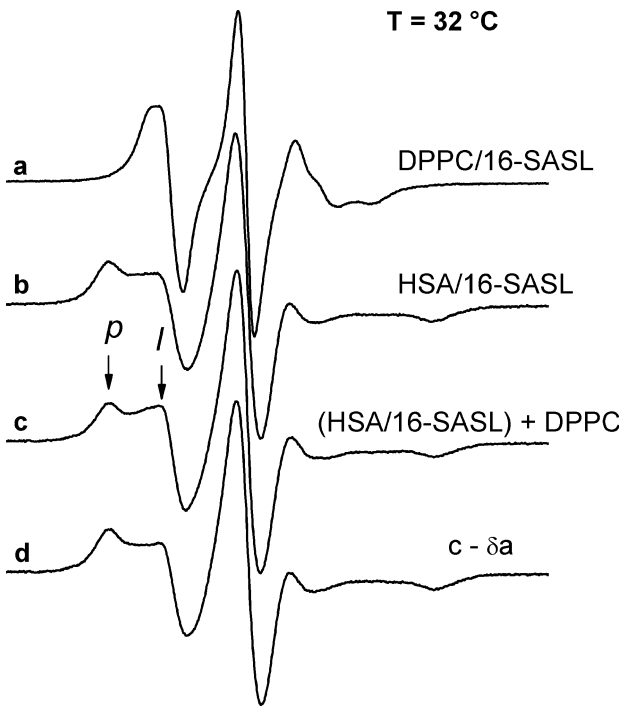

Fig. 2 ESR spectra at 32°C of 16-SASL **a** in DPPC multilayers; **b** bound non-covalently to HSA; **c** in HSA in the presence of unlabelled DPPC multibilayers. **d** Difference spectrum, i.e., the protein immobilized component, obtained by subtracting from spectrum c a percentage, δ, of the spectrum a. Total scan range = 100 Gs

In addition, the spin-labelled stearic acids, 16-SASL, continue to show different motional properties in the lipid and in the protein environments (compare Fig. 1a, b with Fig. 2a, b). Therefore, the immobilized protein component, *p*, and the more mobile lipid component, *l*, are still resolved in the ESR spectrum of 16-SASL in DPPC membranes in the presence of unlabelled HSA (not shown) and in that of 16-SASL in HSA in the presence of unlabelled DPPC at 32°C (Fig. 2c).

The coexistence of protein- and lipid-like spectral components is evidenced up to the highest temperature investigated of 60°C for spin-labelled HSA in the presence of DPPC membranes and for spin-labelled DPPC membranes in the presence of HSA (spectra not shown).

Taking advantage of the good resolution of the two components in the composite ESR spectra, it is possible to separate their contribution by performing spectral subtractions as described in "Materials and methods". As an example, in Fig. 1d is shown the difference spectrum at 20°C obtained by subtracting from the composite ESR spectrum in Fig. 1c an appropriate percentage, λ, of the protein component spectrum in Fig. 1b. The difference spectrum is contributed by the spin-labels in DPPC membranes and it is very similar to the experimental spectrum in Fig. 1a. Similarly, at 32°C, by subtracting from the composite ESR spectrum in Fig. 2c an appropriate amount, δ, of the lipid spectrum of Fig. 2a, the difference protein component is obtained (Fig. 2d). As in the mixed lipid/protein dispersions only the lipid membranes (case in Fig. 1c) or the protein (case in Fig. 2c) is initially labelled, the other component is due to the labels that spontaneously dislocate from one environment to the other, i.e., from DPPC to HSA when the label is initially in the bilayers (case in Fig. 1) and from HSA to DPPC when the label is initially in the protein (case in Fig. 2). In this way, the spontaneous transfer of 16-SASL between HSA and phospholipid membranes is readily studied by spin-label ESR and quantified by the fraction, f_p, (f_l) of labels in the protein (DPPC bilayers) at any temperature.

In Fig. 3 are reported the temperature dependences of f_p (16-SASL) when the label is initially in the protein (open squares) or in the DPPC membranes (solid squares).

When 16-SASL is non-covalently bound to HSA and the protein dispersions then mixed with preformed unlabelled DPPC membranes, it is possible to follow the transfer of stearic acids from the protein to the membranes. In these samples, a decrease of f_p indicates an enhancement of the transfer.

As can be seen in Fig. 3 (open squares), up to 25°C f_p keeps its maximum value. All the labels are bound to the protein and single component spectra are obtained. The passage of stearic acids from HSA to DPPC bilayers starts from 25°C onwards. The lipid fluid component appears in

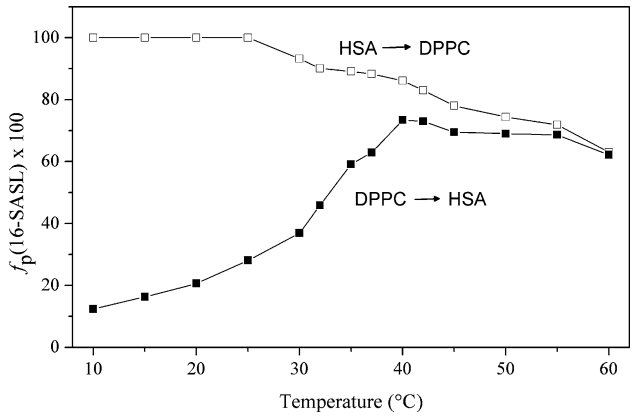

Fig. 3 Temperature dependences of the fraction, f_p (*16*-SASL), of 16-SASL bound to the protein for the transfer from HSA to DPPC membranes (*open squares*) and for the transfer from DPPC membranes to HSA (*solid squares*)

the composite ESR spectra and f_p decreases. The displacement towards the membranes increases at temperatures around that of DPPC pre-transition ($T_p \sim 30$°C) and even more on entering the DPPC main phase transition to the fluid phase state ($T_m \sim 41$°C). A further increase of transfer is seen at 60°C, i.e., close to the thermal unfolding of HSA ($T_d \sim 62$°C, Pantusa et al. 2008), where about 40% of stearic acid is transferred from the protein to the lipid bilayers.

When 16-SASL is incorporated in DPPC membranes and the samples then mixed with unlabelled HSA buffered dispersions, it is possible to follow the transfer of stearic acids from the membranes to the protein. In these samples, f_p directly reflects the transfer from DPPC to HSA and an increase of f_p indicates an enhancement of the transfer. From solid squares in Fig. 3, it comes out that the transfer of 16-SASL from DPPC liposomes to HSA is already evident at 10°C. Between 10 and 40°C, the percentage of 16-SASL that migrate from DPPC to HSA increases from 10 to 70% and then it slightly decreases at about 60% at 60°C.

The ESR data in Fig. 3 were used for determining the partitioning constant, k_p, for the partitioning of 16-SASL between HSA and DPPC multilayers by using the equation:

$$K_p = \frac{[\text{SAinDPPC}]}{[\text{DPPC}]} \times \frac{[\text{HSA}]}{[\text{SA in HSA}]}$$

From 10 to 60°C, the values of k_p vary between 0 and 6.4×10^{-3} for the transfer from HSA to DPPC whereas they pass from 77×10^{-3} to 6.6×10^{-3} for the transfer from DPPC to HSA. For DPPC bilayers in the fluid phase, they are comparable with those reported for the partitioning of fatty acids between vesicles of DMPC and BSA (Daniels et al. 1985) and between sonicated small unilamellar vesicles of palmitoyloleoylphosphatidylcholine (POPC) and HSA (Massey et al. 1997).

It is interesting to observe that at the highest temperature the f_p (and the K_p) values are almost coincident in both transfer directions. This indicates that partitioning of 16-SASL between DPPC and HSA is at equilibrium and the free energy, $\Delta G_p = -RT \ln K_p$, for the partitioning is 3.3 kcal/mol.

More generally, theoretical models have been proposed to describe the transport of fatty acids between intracellular membranes and/or between binding proteins and model membrane vesicles (Nichols 1988; Zucker 2001; Weisiger and Zucker 2002). Experimentally, different spectroscopic techniques have been used to study the transfer between a variety of both donors and acceptors (Brecher et al. 1984; Daniels et al. 1985; Storch and Kleinfeld 1986; Nichols 1988; Massey et al. 1997; Zucker 2001; Thomas et al. 2002; Abreu et al. 2003; Estronca et al. 2005; Pantusa et al. 2005). Among several physicochemical parameters (such as the incubation time of the protein/lipid bilayer mixtures, the concentration of the acceptors and donors, the pH value of the dispersion medium, the (multi)lamellarity of the bilayer structures), the transfer of fatty acids between fatty acid binding proteins and membranes is also influenced by the temperature and, hence, by the physical state of the phospholipid bilayers. Our present results, i.e., the increase of the fraction of stearic acid spin labels that leaves the donor (HSA, DPPC bilayers) and incorporates into the acceptor (DPPC bilayers, HSA) on a temperature increase, is consistent with the data reported in literature. In particular, the transfer of palmitic acid from DPPC or dimyristoylphosphatidylcholine (DMPC) multilamellar liposomes to albumin or fatty acid binding proteins is favoured in the fluid state of the lipid bilayers, although decreased binding was observed at temperatures corresponding to the main transition temperatures for those phospholipids (Brecher et al. 1984). The transfer of fluorescent lipid amphiphiles from bovine serum albumin (BSA) to unilamellar lipid vesicles of different composition shows highest levels in the case of liquid-disordered phases of pure DMPC and pure POPC at 30°C, well above their phase transition temperatures (Abreu et al. 2003). The fraction of transferred fatty acids from HSA to DPPC increases with increasing temperature when the bilayers pass from the gel to the liquid-crystalline phases (Pantusa et al. 2005).

Transfer of 16-SASL between HSA and PEG:2000 grafted DPPC membranes

Interesting features are evidenced when the transfer of 16-SASL between HSA and SSL of DPPC/PEG:2000-DPPE is studied. The content of the polymer-lipid mixed with DPPC was varied between 0 and 2.5 mol%, a submicellar concentration range that encompasses the transition from the low

density mushroom regime to the high density brush regime of the polymer chains. For gel phase DPPC bilayers and PEG:2000-lipids, this transition theoretically is predicted at 0.9 mol% of the polymer-lipid, whereas experimentally was found to occur at ~1.5 mol% (Bartucci et al. 2002; Marsh et al. 2003). Composite ESR spectra of anisotropic protein-like signal superimposed to a more mobile bilayer-like signal are also obtained when HSA/SSL dispersions are studied in which either the protein (SSL) or the SSL (protein) are the donor (acceptor) matrices for 16-SASL.

The plots of f_p (16-SASL) are given in Fig. 4 as a function of PEG:2000-DPPE concentration at different temperature for mixed HSA/SSL dispersions in which 16-SASL is initially in the protein (open symbols) or is in the SSL (solid symbols).

At 20°C (open squares), the passage of 16-SASL from the protein to the polymer-grafted membranes is suppressed in the whole polymer-lipid concentration range. At 32°C (open circles), a moderate migration of 16-SASL in the membranes occurs, i.e., f_p (32°C) < f_p (20°C). However, the transfer of 16-SASL from HSA toward PEG:2000-DPPE liposomes slightly decreases on increasing the concentration of the polymer-lipid in the brush regime (from 1 mol% onwards), i.e., relative to the absence of polymer-lipid, f_p undergoes a modest increase. A further increase in the fraction of 16-SASL transferred to the sterically stabilized membranes occurs at 45°C (open triangles), i.e., f_p (45°C) < f_p (32°C) < f_p (20°C). However, relative to the case in the absence of polymer-lipid, the transfer progressively reduces (i.e., f_p weakly grows) on going from the mushroom to the brush regime.

When 16-SASL is initially in the membranes, the solid symbols in Fig. 4 indicate the transfer from the SSL to the protein. Also in this direction, the fraction of spin-labels which migrates into the protein increases with temperature, from 20 (solid squares) to 32 (solid circles) and even more to 45°C (solid triangles). Moreover, the transfer of 16-SASL from SSL to HSA first markedly increases in the mushroom regime and then, on entering the brush regime of the polymer chains, progressively reduces to a value larger than or nearly equal to that in the absence of polymer-lipids. This holds at any temperature.

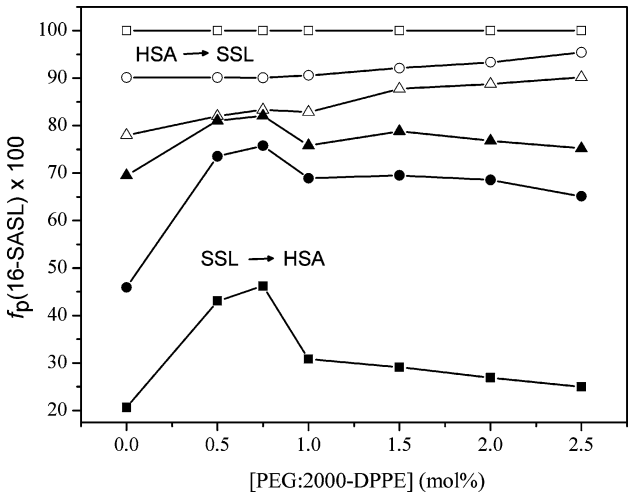

Fig. 4 Fraction, f_p (*16*-SASL), of 16-SASL bound to the protein vs. PEG:2000-DPPE concentration at 20 (*squares*), 32 (*circles*) and 45°C (*triangles*). *Open symbols* refer to the transfer from HSA to PEG:2000-grafted DPPC membranes and *solid symbols* to the transfer from PEG:2000-grafted DPPC membranes to HSA

The spin-label ESR data in the presence of polymer-grafted membranes suggest that the transfer of 16-SASL between HSA and SSL can be modulated by temperature and by the content of PEG:2000-DPPE incorporated in DPPC liposomes.

The reduction in the transfer of 16-SASL from the protein to the SSL by the presence of PEG:2000 chains, can be rationalized considering that the polymeric coating at the lipid/protein interface hinders the passage of fatty acids in the membrane matrix. This effect is reinforced by the steric stabilization due to the polymeric brush which has a protein repellent effect (Efremova et al. 2000; Bartucci et al. 2002). For the increase in the transfer of 16-SASL from SSL to HSA, it should be considered that at low grafting density in the mushroom regime, the lateral pressure exerted by the PEG:2000-DPPE polymer-lipids in DPPC bilayers results in a loosening of the lipid packing density which reduces the SSL main phase transition temperatures (Montesano et al. 2001; Pantusa et al. 2003). Likely, this favours the displacement of 16-SASL from the membranes toward the protein. At high grafting density in the brush regime, the steric stabilization comes into play and attenuates the passage of 16-SASL to the protein.

Conclusions

Mixing buffered solution of HSA spin-labelled with 16-SASL with unlabelled SSL of DPPC/PEG:2000-DPPE or labelled SSL with unlabelled HSA we have shown the transfer of stearic acids between HSA and SSL by conventional ESR spectroscopy.

It is found that the stearic acids show more affinity to bind reversibly and non-covalently to the protein and that the fraction of SA transferred between HSA and SSL can be modulated by the physical state of the lipid bilayers (i.e., by the temperature) and by the grafting density of the polymer-lipids at the protein/lipid interface.

In both directions of transfer (i.e., from HSA to SSL and from SSL to HSA), the fraction of transferred stearic acids increases with temperature and reaches the maximum value when the lipid membranes undergo the phase transition from the gel to the fluid states. This is much more evident in the passage from the lipid membranes to the protein. In the presence of polymer-lipids, the transfer is attenuated from HSA to

SSL in the fluid phase both at low and at high content of PEG:2000-DPPE. Instead, the transfer from SSL to HSA is increased by the polymer-lipids much more markedly at low grafting density than at high grafting density at any temperature.

The present results confirm the ability of albumin to bind, store and transport stearic acids and indicate parameters, such as temperature and amount of PEG-lipids mixed with DPPC that can be varied for controlling the amount of fatty acids both free in the blood plasma and transported trough cell membranes.

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