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ORIGINAL PAPER

Kinetics of stearic acid transfer between human serum albumin and sterically stabilized liposomes

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Abstract The kinetics of the transfer of stearic acids between human serum albumin (HSA) and long circulating sterically stabilised liposomes (SSL) composed of dipalmitoylphosphatidylcholine (DPPC) and of submicellar content of the polymer-lipid poly(ethylene glycol:2000)-dipalmitoylphosphatidylethanolamine (PEG:2000-DPPE) have been studied by fluorescence spectroscopy. The study exploits the fact that HSA has a single tryptophan (Trp) residue and that the intrinsic Trp-emission intensity is quenched by the presence of doxyl spin-labelled stearic acids (SASL). Protein/lipid dispersions are considered in which SASL molecules are inserted either in the protein or in the SSL, and the transfer of SASL between the protein and SSL is conveniently monitored by the time variation of the inherent Trp-fluorescence intensity of HSA. It was found that the transfer of fatty acids between HSA and SSL depends on the type of donor and acceptor matrix, on the temperature (i.e., on the physical state of the lipid bilayers) and on the grafting density of the PEG-lipids at the lipid/protein interface. In the absence of polymer-lipids, the rate of transfer increases with temperature in both directions of transfer, and it is higher for the passage from DPPC bilayers to HSA. The presence of polymer-lipids reduces the rate of transfer both in the mushroom and in the brush regime of the polymer chains, especially at low grafting density and for lipid membranes in the fluid phase.

Keywords Human serum albumin · DPPC · PEG:2000-DPPE · Sterically stabilised liposomes · Fluorescence · Lipid transfer

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Introduction

Non-esterified fatty acids regulate a broad spectrum of metabolic activities and are involved in many physiological, pathological and/or pharmacological processes in living cells. They are transported through cell compartments and enter and leave cells rapidly. The comprehension of the molecular aspects underlying fatty acid transport across cell membranes has attracted the attention of multidisciplinary research (Spector and Fletcher 1978; Hamilton 1998; Pownall 2001).

A number of basic research studies investigate the fatty acid transfer between model systems of donors and acceptors and, in particular, the transfer between proteins capable of binding fatty acids and lipid vesicles has been investigated (Brecher et al. 1984; Storch and Kleinfeld 1986; Nichols 1988; Glatz and van der Vusse 1996; Pownall 2001; Zucker 2001; Thomas et al. 2002; Weisiger and Zucker 2002; Abreu et al. 2003; Estronca et al. 2005; Falomir-Lockhart et al. 2006, 2009; Carley and Kleinfeld 2009). Appropriate and interesting protein/lipid aqueous dispersions for studying fatty acid transfer are represented by human serum albumin (HSA) and sterically stabilised liposomes (SSL) of polymer-grafted lipid membranes. Indeed, HSA, the most abundant plasma protein, shows a strong affinity to bind reversibly and non-covalently fatty acids, which are stored, transported through plasma compartments and delivered to target sites (Hamilton et al. 1984; Peters 1997; Curry et al. 1999). SSLs made of mixtures of common conventional, saturated symmetrical chain dipalmitoylphostatidylcholines (DPPC) and an appropriate amount of the polymer-lipid poly(ethylene glycol:2000)-dipalmitoylphosphatidylethanolamine (PEG:2000-DPPE) are long-circulating liposome-based drug carriers that evade protein adsorption and reduce the

degradation by the immune system (Lasic 1993; Lasic and Martin 1995; Efremova et al. 2000; Bartucci et al. 2002). Phosphatidylcholines are the most diffuse lipids in cellular membranes and fatty acids; on the other hand, because of their low solubility in water and hydrophobicity, they also associate with lipid membranes as endogenous lipids.

In previous work, the transfer and the partitioning of spin-labelled stearic acids (SASL) between human serum albumin and SSL made of DPPC/PEG:2000-DPPE binary mixtures have been studied by conventional electron spin resonance (ESR) spectroscopy (Pantusa et al. 2005, 2009).

Herein, we consider the same mixed HSA/SSL dispersions in which 2 mol% of SASL are inserted either in the protein (in this case, HSA is the donor and the SSL the acceptor matrix, respectively) or in the lipid membranes (in this case, the SSLs are the donor and HSA the acceptor matrix, respectively). The content of 2 mol% of SASL (which in our samples corresponds to a protein/SASL molar ratio of 1:2) does not induce conformational changes in HSA and does not perturb the structural organisation of the lipid bilayers. Exploiting the fact that HSA has a single tryptophan residue at position 214 in domain II and that the intrinsic Trp-fluorescence signal of HSA is quenched by the nitroxide moiety of SASL, the kinetics of fatty acid transfer between HSA and polymer-grafted lipid membranes is studied by means of fluorescence. At the HSA/SASL molar ratio of 1:2, we observed a considerable quenching of inherent fluorescence, and by spin-label ESR we detected a maximum amount of fatty acids transferred from DPPC membranes towards the protein. It is therefore likely that at 2 mol% of stearic acids the highest affinity binding sites around the Trp-residue in the protein are populated. When the stearic acids are initially in the protein, the kinetics of transfer from the protein to the lipid membranes is followed by the time-dependent increase in Trp-fluorescence intensity that occurs during the transfer. When the stearic acids are initially inserted in the lipid membranes, the kinetics of transfer from the lipid bilayers to the protein are followed by the time-dependent decrease in fluorescent intensity that accompanies the transfer. Data on the kinetics of transfer of SASL between HSA and SSL are presented and discussed as a function of the temperature and PEG:2000-lipid concentration.

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 doxyl stearic acid spin label 2-(3-carboxytetradecyl)-4,4-dimethyl-2-ethyl-3-oxazolidinyloxy (5-SASL) were from Sigma/Aldrich (St. Louis, MO). 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). The reagent grade salts for the 10 mM phosphate buffer solution (PBS) at pH 7.2 were from Merk (Darmstadt, Germany). All materials were used as purchased with no further purification. Distilled water was used throughout.

Fluorescence

Sample preparation

The protein/lipid dispersion samples were prepared by mixing protein solutions with pre-formed SSL in which SASL were inserted either into the protein or into the lipid membranes. Dispersions of HSA with SASL were freshly prepared as follows. A volume of methanol containing the spin-labelled fatty acids was first evaporated under a flow of nitrogen gas and any residual solvent removed under vacuum. A buffered HSA solution (protein concentration 3.5 μM) was then added to the dried SASL. Finally, the dispersions were heated at 45°C, periodically vortexed for 30 min and equilibrated at 4°C. Hand-shaken polymer-grafted multibilayers were prepared by dissolving the required amounts of DPPC and PEG:2000-DPPE together with 2% molar concentration of 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 protein concentration), 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.

For fluorescence measurements, the preformed polymer-grafted multibilayers (with or without SASL) were rapidly mixed with the protein-buffered solution (without or with SASL) in the cuvette, equilibrated at the measuring temperature.

In all samples, the lipid-to-protein ratio was 1:1 w/w, and the SASL concentration was 2% of the lipids, which corresponded to a 2:1 molar ratio of fatty acids to protein.

Fluorescence measurements

All fluorescence data were collected on a LS 50B spectrofluorometer (Perkin-Elmer, Beaconsfield, UK) equipped with a Peltier Temperature Programmer PTP-1. The excitation and the emission wavelengths were 290 and 345 nm, respectively. Both the excitation and the emission slit

widths were set to 4 nm. The temperature was measured directly by an YSI thermistor dipped into a 1-cm path length quartz cuvette.

The fluorescence measurements were repeated three times on new samples. The experiments were reproducible, and the errors of the data points are smaller than the symbols.

Data analysis

The kinetic curves, i.e., the time variation in the fluorescence intensity that occurs during the transfer, were fitted by a single exponential function:

$$y(t) = y_\infty + Ae^{-kt} \quad (1)$$

or by a combination of two exponential functions:

$$y(t) = y_\infty + A_1 e^{-k_1 t_1} + A_2 e^{-k_2 t_2} \quad (2)$$

where $y_\infty$is the fluorescence signal intensity at equilibrium, A_i the amplitude of phase i and k_i the corresponding rate constants.

Half-times $t_{1/2}$ were calculated from k_i as:

$$t_{1/2} = (\ln 2)/k_i \quad (3)$$

Results and discussion

Transfer of stearic acids between HSA and DPPC membranes

Typical kinetics traces at different temperatures for the transfer of SASL from HSA to DPPC bilayers are shown in Fig. 1.

The Trp-HSA fluorescence intensity in the measured samples depends on the temperature. In order to compare the kinetic behaviour and without losing information, the plots reported in Fig. 1a (and in Fig. 2a) are shifted to the same starting point.

From the figure it is evident that when HSA containing 2 mol% SASL is in interaction with DPPC bilayers, the kinetic trace is almost flat at 25°C. This indicates that the stearic acids are not transferred from the protein toward the L_β' gel phase of DPPC bilayers, and they remain non-covalently bound to the protein. On increasing the temperature, a modest increase of the fluorescence intensity is seen in the kinetic trace at 30°C, i.e., a small amount of stearic acids is transferred from the protein to DPPC membranes. At 35°C, i.e., when the DPPC bilayers are in the ripple P_β' gel phase, the time-dependent increase of the Trp-fluorescence is fitted by a single exponential function (Eq. 1) with a rate constant $k = 0.003\ \mathrm{s}^{-1}$. From 35°C onwards, the kinetic curves are fitted by biexponential functions (Eq. 2) as shown in Fig. 1 by the solid line that is superimposed to the kinetic trace at 45°C when the DPPC bilayers are in the liquid-crystalline L_α state. The plot of the residuals in Fig. 1b shows the goodness of the fit.

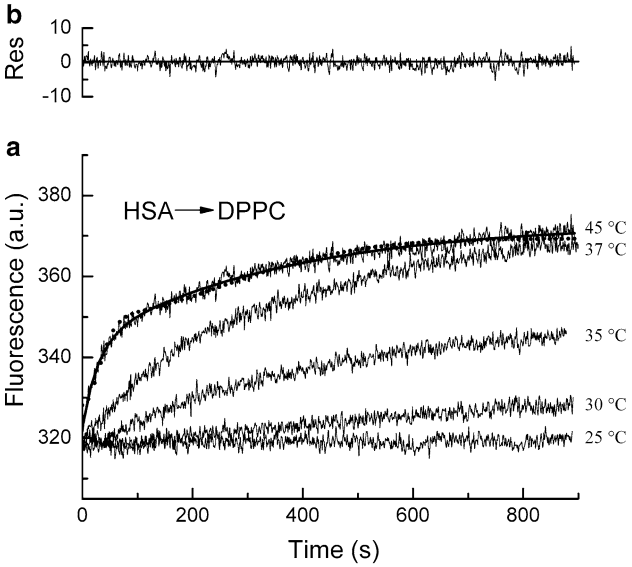

Fig. 1 **a** Time-dependent changes at different temperatures of Trp-fluorescence intensity of HSA in mixed HSA/DPPC dispersions in which the stearic acids are initially in protein. The kinetic curves refer to the transfer of stearic acids from HSA to DPPC bilayers. The *solid line* that is superimposed on the experimental curve at 45°C is a biexponential fit to the data according to Eq. 2. **b** Residuals of the fit to the data

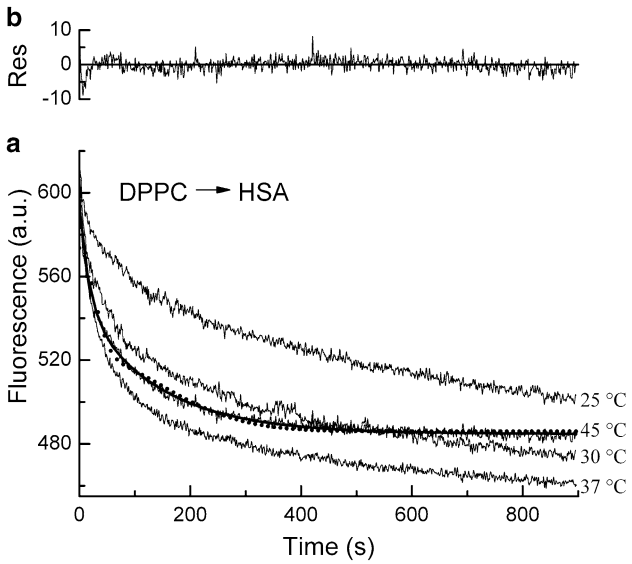

Fig. 2 **a** Time-dependent changes at different temperatures of Trp-fluorescence intensity of HSA in mixed HSA/DPPC dispersions in which the stearic acids are initially in the DPPC bilayers. The kinetic curves refer to the transfer of stearic acids from DPPC bilayers to HSA. The *solid line* that is superimposed on the experimental curve at 45°C is a biexponential fit to the data according to Eq. 2. **b** Residuals of the fit to the data

The plots of the time-dependent decrease of the Trp-fluorescence intensity, which occurs when the protein is in interaction with DPPC lipid membranes that contain 2 mol% SASL, are given in Fig. 2.

They indicate that the transfer of stearic acids from DPPC multibilayers to HSA already occurs at 25°C and persists over the whole temperature range investigated. Moreover, at any temperature, the kinetics traces are fitted by double-exponential functions according to Eq. 2. Also in this case, the plot of the residuals in Fig. 2b indicates that the fit describes the experimental results accurately.

The analysis of the kinetic curves suggests that the transfer of SASL between HSA and DPPC multibilayers is contributed by a population characterised by a fast rate constant, k_1, and by a population with a slow rate constant, k_2. From the fits to the kinetic data in Figs. 1 and 2, the values of k_1 and k_2, rate constants have been determined (see later Fig. 3) and used to calculate half-times, $t_{1/2}$, by means of Eq. 3. The rate of translocation, t_t, of 5-SASL across the bilayer is at least 34 s or faster (Yuann and Morse 1999). In both directions of transfer, $t_{1/2}$ values deduced from the fast rate constant k_1 are comparable to t_t, whereas $t_{1/2}$ derived from the slow rate constant k_2 is bigger than t_t. It is, therefore, likely that the fast population is due to stearic acids, which spontaneously transfer between the outer external bilayer leaflets of DPPC membranes and the protein. The slow population, instead, represents the spin-labelled amphiphiles that transfer between the internal layers of DPPC multilamellar vesicles and the protein.

Fluorescence data were used for determining the partitioning constant, K_p, for the partitioning of SASL between HSA and lipid bilayers by using the equation (Massey et al. 1997; Pantusa et al. 2009):

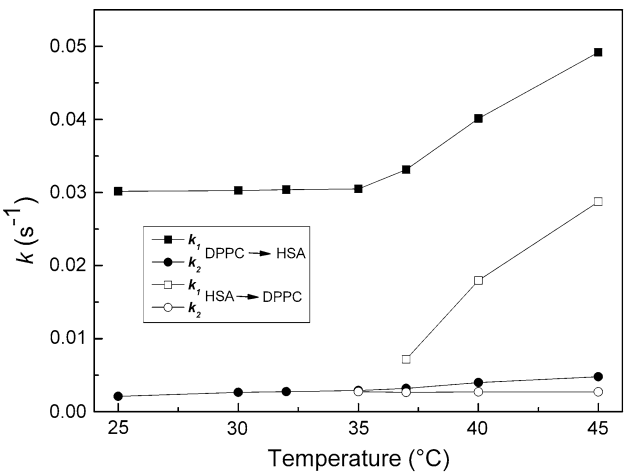

Fig. 3 Temperature dependences of the fast k_1 (*squares*) and slow k_2 (*circles*) rate constants for the transfer of stearic acids from HSA to DPPC membranes (*open symbols*) and from DPPC membranes to HSA (*solid symbols*)

$$K_p = \frac{[\text{SASLinBilayers}]}{[\text{DPPC}]} \times \frac{[\text{HSA}]}{[\text{SASL in HSA}]}$$

For any samples and at any temperature, the fraction, X, of SASL transferred to DPPC or to HSA is evaluated as $X = \frac{\Delta F}{\Delta F_{tot}}$, where $\Delta F = |(y_\infty - y_0)|$ is obtained from the kinetic curves in Figs. 1a and 2a, y_0 is the fluorescence intensity at $t = 0$ s, $\Delta F_{tot} = F_{max}(\text{HSA}) - F_{max}(\text{HSA} + 2\ \text{mol\%}\ \ \text{SASL})$ is calculated from fluorescence measurements carried out in the absence of lipid membranes as the difference between the maximum Trp-fluorescence intensity, F_{max}, of HSA alone and in the presence of 2 mol% SASL. ΔF_{tot} takes into account the efficiency of quenching the maximum amount of SASL on the inherent fluorescence of HSA.

With the above assumptions, for the transfer from the protein to the lipid membranes

$$K_p = \frac{X \times [\text{SASL}]}{[\text{DPPC}]} \times \frac{[\text{HSA}]}{(1-X) \times [\text{SASL}]} = \frac{X}{(1-X)} \times 10^{-2},$$

whereas for the transfer from the lipid membranes to the protein

$$K_p = \frac{(1-X) \times [\text{SASL}]}{[\text{DPPC}]} \times \frac{[\text{HSA}]}{X \times [\text{SASL}]} = \frac{(1-X)}{X} \times 10^{-2}.$$

For HSA → DPPC transfer, K_p increases progressively from 0.2×10^{-3} at 25°C to 1.7×10^{-3} at 35°C and to 4.4×10^{-3} at 45°C. For DPPC → HSA passage, instead, K_p decreases first rapidly from 10.6×10^{-3} to 3.5×10^{-3} on going from 25 to 35°C, and it is $\sim 4 \times 10^{-3}$ at 45°C, i.e., an increase is recorded on entering the fluid state of the DPPC bilayers. This behaviour is similar to that observed in samples composed of DMPC or DPPC and of albumin or other fatty acid-binding proteins, where a decreased binding of palmitic acid to the proteins was detected for the movement from membranes to protein at temperatures corresponding to the known main phase transition temperatures for those phospholipids (Brecher et al. 1984).

Almost the same K_p values are obtained when the partition coefficient is calculated by using the tritation method reported by Massey et al. (1997). On average, at 45°C, the free energy for the partitioning, $\Delta G_p = -RT \ln K_p$, is 3.5 kcal/mol. All the values are similar to those obtained previously on the same samples by spin-label ESR (Pantusa et al. 2009). 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 POPC and HSA (Massey et al. 1997).

On the whole, the results indicate that the transfer of SASL between HSA and DPPC multibilayers depends on the donor and acceptor matrix and on the temperature. The stearic acids preferentially are bound to the protein. Indeed,

at any temperature, K_p is lower for the transfer from the protein to the lipid bilayers than for the passage from the DPPC to HSA.

The process of transfer can also be studied considering the kinetic parameters deduced from the time variation of the fluorescence intensity in the different investigated samples.

The temperature dependences of the fast k_1 and slow k_2 rate constants for the transfer of stearic acids between the DPPC lipid bilayers, and the proteins are given in Fig. 3.

As can be seen, in both directions of transfer, the slow rate constants, k_2, keep a constant low value on the whole temperature range showing only a slight increase for the transfer from DPPC to HSA at the highest temperature (solid and open circles in Fig. 3). In contrast, k_1, for the transfer of fatty acids from DPPC lipid bilayers to HSA (solid squares in Fig. 3), increases considerably at temperatures corresponding to the DPPC main phase transition temperature to the fluid state. For the transfer from the protein to the lipid bilayers (open squares in Fig. 3), k_1 also increases considerably with temperature and has lower values relative to those corresponding to the DPPC → HSA passage. Indeed, k_1 values ($t_{1/2}$-values) for the transfer from DPPC membranes to HSA are in the range 0.03 ÷ 0.05 s^{-1} (23 ÷ 14 s), whereas the range of variation of the fast rate constant (half-times) for the transfer from HSA to DPPC multilayers is between 0.005 s^{-1} (96 s) and ≈0.03 s^{-1} (≈23 s).

More generally, both theoretical models (Nichols 1988; Zucker 2001; Weisiger and Zucker 2002) and experimental studies (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. 2009) have shown that the transfer of fatty acids between fatty acid binding proteins and membranes is influenced by several physicochemical parameters, such as the temperature, the incubation time of the protein/lipid mixtures, the concentration of the acceptor and donors, the type and content of the amphiphile, the pH and the ionic strength values of the dispersion medium, and the structure and lamellarity of the bilayers. In particular, in agreement with our observations, data reported in the literature show that the kinetic parameters of the transfer of amphiphiles depend on the donor/acceptor complexes and on the protein type and vesicular mesophase and composition (Massey et al. 1997; Zucker 2001; Thomas et al. 2002).

Transfer of stearic acids between HSA and sterically stabilised liposomes

The transfer of stearic acids between human serum albumin and sterically stabilised liposomes of DPPC/PEG:2000-DPPE has also been investigated. The sub-micellar concentration of the polymer-lipid mixed with DPPC was varied from 0 to 2 mol%, a content that encompasses the mushroom → brush transition of the polymer chains. For gel phase DPPC bilayers and PEG:2000-lipids, this transition theoretically is predicted at 0.9 mol% and experimentally was detected at ≈1.5 mol% of the polymer-lipid (Bartucci et al. 2002; Marsh et al. 2003).

Increase (decrease) of the fluorescence intensity is again obtained when HSA/SSL dispersions are measured in which either the protein (SSL) or the SSL (HSA) are the donor (acceptor) matrices for SASL. Even for these samples, the kinetic traces are fitted by single or double exponential curves.

The effects of the polymer coating at the lipid/protein interface on the spontaneous transfer of SASL between HSA and SSL are evaluated in the plots of k_1 given in Fig. 4 as a function of PEG:2000-DPPE concentration at 30 (squares), 37 (circles) and 45°C (triangles) for mixed HSA/SSL dispersions in which the SASLs are initially in the protein (open symbols) or in the SSL (solid symbols).

For the transfer of SASL from the protein to the polymer-grafted membranes, k_1 increases with the temperature increase (open symbols), whereas it decreases moderately on the whole PEG-lipid concentration range, from the low grafting density mushroom regime to the high grafting density brush regime of the polymer chains. The process of transfer is slightly slowed down by the polymer-lipids. This holds both at 37 and 45°C. The partition coefficient increases with the temperature increase at any PEG-lipid content, especially at low concentration. On increasing the concentration of the PEG-lipids, instead, K_p keeps a constant value at 30°C (≈0.55 × 10^{-3}), and it decreases significantly at the mushroom-brush transition of the polymer chains at 37 and 45°C. Indeed, going on from 0 to 2 mol% PEG:2000-DPPE, it varies from 6.6 × 10^{-3} to

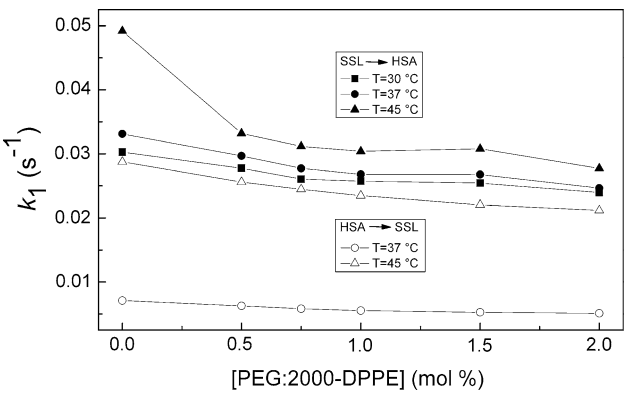

Fig. 4 Fast rate constants, k_1, for the transfer of stearic acids between HSA and sterically stabilised liposomes as a function of PEG:2000-DPPE concentration at 30 (*squares*), 37 (*circles*) and 45°C (*triangles*). *Open symbols* refer to the transfer from HSA to PEG:2000-grafted DPPC membranes and *solid symbols* to the transfer from PEG:2000-grafted DPPC membranes to HSA

1.6×10^{-3} at 37°C and from 4.4×10^{-3} to 1.5×10^{-3} at 45°C. It is likely that the stearic acids are bound to the protein, and the steric stabilisation exerted by the polymer chains reduces both the amount of fatty acids transferred to the SSL and the rate of transfer.

For the transfer of SASL from the PEG-grafted liposomes to the protein, k_1 increases with temperature from 30 to 37 to 45°C and, as a consequence of the presence of the polymer chains at the SSL/HSA interface, k_1 reduces with the PEG:2000-DPPE concentration, especially at 45°C and at low grafting density in the mushroom regime of the polymer chains (solid symbols in Fig. 4). It is therefore clear that the presence of the polymer chains slows down the transfer of SASL towards the protein. In the same direction of transfer, i.e., from SSL → HSA, at any PEG-lipid concentration, the partition coefficient K_p first decreases and then increases on approaching temperatures where the lipid bilayers are in the fluid phase. For instance, at 1 mol% of PEG:2000-DPPE, K_p is 4.2×10^{-3} at 30°C, reduces to 0.6×10^{-3} at 37°C and becomes 9.6×10^{-3} at 45°C. The behaviour of the partition coefficient as a function of the PEG-lipid concentration is the following: at 30°C, K_p decreases from 6.6×10^{-3} to 4×10^{-3} between 0 and 0.5 mol% of PEG:2000-DPPE and then it increases slightly up to 4.5×10^{-3} at 2 mol% of PEG-lipid; at 37°C, K_p sensibly decreases from 2.9×10^{-3} at 0 mol% to 0.6×10^{-3} at 1 mol%, and it is 1.2×10^{-3} at 2 mol%; finally, at 45°C, K_p increases progressively with the concentration of the polymer-lipids from 4×10^{-3} to 9.5×10^{-3} from 0 to 2 mol% of the polymer-lipids. This behaviour can be rationalised by considering that at low concentration of PEG:2000-DPPE, 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 chain-melting transition temperature (Montesano et al. 2001; Pantusa et al. 2003). This favours the transfer, i.e., the increase of the fraction of stearic acids transferred from SSL to HSA. At high concentration in the brush regime, instead the polymer steric stabilisation is predominant and inhibits the transfer.

The fluorescence results for the kinetics of the spontaneous transfer of stearic acids between human serum albumin and PEG-grafted DPPC membranes have shown that stearic acids are preferentially bound to HSA and partition between the protein and the sterically stabilised liposomes. The kinetics of partitioning are modulated by the temperature and by the presence of polymeric chains at the lipid/protein interface.

The results obtained on protein/lipid membrane model complexes are relevant for establishing parameters that can be varied to control the amount of fatty acids free in the blood plasma and transported trough cell membranes, and for establishing relations with the parameters observed in vivo for plasma membranes covered by their glycocalyxes.

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References

Abreu MSC, Estronca LMBB, Moreno MJ, Vaz WLC (2003) Binding of a fluorescent lipid amphiphile to albumin and its transfer to lipid bilayer membranes. Biophys J 84:386–399

Bartucci R, Pantusa M, Marsh D, Sportelli L (2002) Interaction of human serum albumin with membranes containing polymer-grafted lipids: spin label ESR studies in the mushroom and brush regimes. Biochim Biophys Acta 1564:237–242

Brecher P, Saouaf R, Sugarman JM, Eisenberg D, LaRosa K (1984) Fatty acid transfer between multilamellar liposomes and fatty acid-binding proteins. J Biol Chem 259:13401–13995

Carley AN, Kleinfeld AM (2009) Flip-flop is the rate-limiting step for transport of free fatty acids across lipid vesicle membranes. Biochemistry 48:10437–10445

Curry S, Brick P, Franks NP (1999) Fatty acid binding to human serum albumin: new insights from crystallographic studies. Biochim Biophys Acta 1441:131–140

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

Efremova NV, Bondurant B, O'Brien DF, Leckband DE (2000) Measurements of interbilayer forces and protein adsorption on uncharged lipid bilayers displaying poly(ethylene glycol) chains. Biochemistry 39:3441–3451

Estronca LMBB, Moreno MJ, Laranjinha JAN, Almeida LM, Vaz WLC (2005) Kinetics and thermodynamics of lipid amphiphile exchange between lipoproteins and albumin in serum. Biophys J 88:557–565

Falomir-Lockhart LJ, Laborde L, Kahn PC, Storch J, Corsico B (2006) Protein-membrane interaction and fatty acid transfer from intestinal fatty acid-binding protein to membranes. J. Biol. Chem 281:13979–13989

Falomir-Lockhart LJ, Burgardt NI, Ferreyra RG, Ceolin M, Ermàcora MR, Corsico B (2009) Fatty acid transfer from *yarrowia lipolytica* sterol carrier protein 2 to phospholipids membranes. Biophys J 97:248–256

Glatz JFC, van der Vusse GJ (1996) Cellular fatty acid-binding proteins: their function and physiological significance. Prog Lipid Res 35:243–282

Hamilton JA (1998) Fatty acid transport: difficult or easy? J Lipid Research 39:467–481

Hamilton JA, Cistola DP, Morriset JD, Sparrow JT, Small DM (1984) Interaction of myristic acid with bovine serum albumin: a ^{13}C NMR study. Proc Natl Acad Sci USA 81:3718–3722

Lasic DD (1993) Liposomes: from physics to applications. Elsevier, Amsterdam

Lasic DD, Martin F (1995) Stealth liposomes. CRC Press, Boca Raton

Marsh D, Bartucci R, Sportelli L (2003) Lipid membranes with grafted polymers: physicochemical aspects. Biochim Biophys Acta 1615:33–59

Massey JB, Bick DH, Pownall HJ (1997) Spontaneous transfer of monoacyl amphiphiles between lipid and protein surfaces. Biophys J 72:1732–1743

Montesano G, Bartucci R, Belsito S, Marsh D, Sportelli L (2001) Lipid membrane expansion and micelle formation by polymer-grafted

lipids: scaling with polymer length studied by spin-label electron spin resonance. Biophys J 80:1372–1383
Nichols JW (1988) Kinetics of fluorescent-labeled phosphatidylcholine transfer between nonspecific lipid transfer protein and phospholipid vesicles. Biochemistry 27:1889–1896
Pantusa M, Bartucci R, Marsh D, Sportelli L (2003) Shifts in chain-melting transition temperature of liposomal membranes by polymer-grafted lipids. Biochim Biophys Acta 1614:165–170
Pantusa M, Sportelli L, Bartucci R (2005) Transfer of stearic acids from albumin to polymer-grafted lipid containing membranes probed by spin-label electron spin resonance. Biophys Chem 114:121–127
Pantusa M, Stirpe A, Sportelli L, Bartucci R (2009) Spontaneous transfer of stearic acids between human serum albumin and PEG:2000-grafted DPPC membranes. Eur Biohys J. doi:10.1007/s00249-009-0442-0
Peters T (1997) All about albumin: biochemistry, genetics and medical applications. Academic Press, San Diego
Pownall HJ (2001) Cellular transport of nonesterified fatty acids. J Mol Neurosci 16:109–115
Spector AA, Fletcher JE (1978) Transport of fatty acids in the circulation. In: Dietschy JM, Gotto AM, Ontko JA (eds) Disturbances in lipid and lipoprotein metabolism. Am Physiol Soc, Bethesda, pp 229–249
Storch J, Kleinfeld AM (1986) Transfer of long-chain fluorescent free fatty acids between unilamellar vesicles. Biochemistry 25:1717–1726
Thomas RM, Baici A, Werder M, Schulthess G, Hauser H (2002) Kinetics and mechanism of long-chain fatty acid transport into phosphatidylcholine vesicles from various donor systems. Biochemistry 41:1591–1601
Weisiger RA, Zucker SD (2002) Transfer of fatty acids between intracellular membranes: roles of soluble binding proteins, distance and time. Am J Physiol Gastrointest Liver Physiol 282:105–115
Yuann JMP, Morse RPDII (1999) Determination by photoreduction of flip-flop kinetics of spin-labeled stearic acids across phospholipid bilayers. Biochim Biophys Acta 1416:135–144
Zucker SD (2001) Kinetic model of protein-mediated ligand transport. Influence of soluble binding proteins on the intermembrane diffusion of a fluorescent fatty acid. Biochemistry 40:977–986