Himmel, M. E., & Squire, P. G. (1981) *Int. J. Pept. Protein Res. 17*, 365–373.
Horiike, K., Tojo, H., Yamano, T., & Nozaki, M. (1983) *J. Biochem. (Tokyo) 93*, 99–106.
Imber, M. J., & Pizzo, S. V. (1981) *J. Biol. Chem. 256*, 8134–8139.
Jensen, P. E. H., & Sottrup-Jensen, L. (1986) *J. Biol. Chem. 261*, 15863–15869.
Jones, J. M., Creeth, J. M., & Kekwick, R. A. (1973) *Biochem. J. 127*, 187–197.
Larsson, L.-J., Lindahl, P., Hallén-Sandgren, C., & Björk, I. (1987) *Biochem. J. 243*, 47–54.
Larsson, L.-J., Holmgren, A., Smedsrød, B., Lindblom, T., & Björk, I. (1988) *Biochemistry 27*, 983–991.
Liu, D., Feinman, R. D., & Wang, D. (1987) *Biochemistry 26*, 5221–5226.
McConnell, D. J., & Loeb, J. N. (1974) *Proc. Soc. Exp. Biol. Med. 147*, 891–896.
Nishigai, M., Osada, T., & Ikai, A. (1985) *Biochim. Biophys. Acta 831*, 236–241.
Österberg, R., & Malmensten, B. (1984) *Eur. J. Biochem. 143*, 541–544.
Pochon, F., & Bieth, J. G. (1982) *J. Biol. Chem. 257*, 6683–6685.
Pochon, F., Amand, B., Lavalette, D., & Bieth, J. (1978) *J. Biol. Chem. 253*, 7496–7499.
Roberts, R. C. (1986) in *Reviews in Hematology*, Vol. II, pp 129–224, PJD Publications Limited, Westbury, NY.
Roche, P. A., & Pizzo, S. V. (1987) *Biochemistry 26*, 468–491.
Ruben, G. C., Harris, E. D., Jr., & Nagase, H. (1988) *J. Biol. Chem. 263*, 2861–2869.
Sjöberg, B., Pap, S., & Kjems, J. K. (1985) *Eur. Biophys. J. 13*, 25–30.
Sottrup-Jensen, L. (1987) in *The Plasma Proteins*, Vol. V, pp 191–291, Academic, Orlando, FL.
Starkey, P. M., & Barrett, A. J. (1982) *Biochem. J. 205*, 105–111.
Steiner, J. P., Bhattacharya, P., & Strickland, D. K. (1985) *Biochemistry 24*, 2993–3001.
Strickland, D. K., Steiner, J. P., Feldman, S. R., & Pizzo, S. V. (1984) *Biochemistry 23*, 6679–6685.
Travis, J., & Salvesen, G. S. (1983) *Annu. Rev. Biochem. 52*, 655–709.
Wilf, J., & Minton, A. P. (1986) *Biochemistry 25*, 3124–3135.

Kinetics and Mechanism of Transfer of Synthetic Model Apolipoproteins†

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ABSTRACT: The effect of hydrophobicity on the rate and mechanism of transfer of a synthetic amphiphilic peptide between phosphatidylcholine single bilayer vesicles has been evaluated. These peptides, which had the sequence C_n-SSLKEYWSSLKESFS (where C_n represents a saturated acyl chain of n carbons that is attached to the amino terminus of the peptide and n = 8, 12, or 16), were distinguished by the length of the saturated acyl chain of n carbons that was covalently bonded to the amino terminus. The transfer of the peptides was monitored by following the rate of change of the intrinsic tryptophan fluorescence that followed mixing of donor vesicles, which contained peptide, phosphatidylcholine, and a fluorescence quencher, with acceptors composed only of phosphatidylcholine. The transfer rates were independent of the structure and concentration of the acceptor. The kinetics were biexponential with the contribution of the fast and slow components being nearly equal. The rates of both components decreased with increasing acyl chain length; the respective free energies of activation were linear with respect to the acyl chain length. These results showed that, unlike lipid transfer, peptide transfer is not always a simple unimolecular process. However, like lipid transfer, the transfer rates are a predictable function of hydrophobicity. It is proposed that the peptides exist as dimers on the phospholipid surface and that the two components of transfer are due to sequential transfer of each molecule in a dimer.

In plasma lipoproteins, phospholipids, cholesterol, and proteins form a surface monolayer that separates the apolar core lipids from the surrounding aqueous phase (Shen, 1977). The surface components transfer among lipoprotein surfaces whereas the core lipids, mostly triglyceride and cholesteryl esters, require specific transfer proteins (Ihm, 1980; Zilversmit, 1975; Massey, 1985). The mechanisms of spontaneous phospholipid and cholesterol transfer are fairly well understood; transfer occurs via rate-limiting first-order desorption of the molecule into the surrounding aqueous phase followed by a rapid diffusion-controlled transfer to an acceptor lipoprotein (Lund-Katz, 1982; Massey, 1982). This process is regulated by the hydrophobicity of the transferring species with other structural features having only an incremental effect (Massey, 1982; Pownall, 1983). The mechanism of transfer of the apoprotein component of lipoproteins is important because some are ligands for receptor-mediated endocytosis (Gianturco & Bradley, 1987) and activators of lipolytic enzymes (Fielding, 1972; LaRosa, 1970; Zorich, 1985).

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Reijngoud and Phillips (1982) reported that apo[1] A-I is irreversibly bound in its complexes with dimyristoylphosphatidylcholine (DMPC) due to an unusually high energy barrier for the desorption of the partially unfolded protein. In subsequent work they reported that apo A-II and total apo C proteins reversibly desorb from DMPC complexes over a time interval of 1–15 min (Reijngoud & Phillips, 1984). Recently, we have used a spectroscopic stopped-flow method to measure directly the rates of transfer of each of the apo C proteins between single bilayer vesicles of POPC over a physiological temperature range (McKeone et al., 1988). The kinetics were complex, and it was difficult to obtain an unambiguous assignment of the reaction mechanism. In the present paper we have investigated the kinetics and mechanism of a series of acylated peptides to identify the rate-limiting structure. Unlike native proteins, synthetic model apolipoproteins can be tailored to determine the effects of a specific structural preturbation on transfer rates. The results of this work clarify the role of hydrophobicity in peptide transfer and permit an unambiguous interpretation of the transfer kinetics obtained with native apolipoproteins.

Materials and Methods

Materials

The acylated lipid-associating peptides (C_n-LAP), which were identical with those previously described by Ponsin et al. (1984), had the structure, C_n-SSLKEYWSSLKESFS, where C_n represents a saturated acyl chain of n carbons that is attached to the amino terminus of the peptide and n = 8, 12, or 16. *N*-[[5-(dimethylamino)-1-naphthalenyl]sulfonyl]-*N*,*N*-dioctadecylamine (DNS-DODA) was synthesized by mixing equimolar amounts of 5-(dimethylamino)-1-naphthalenesulfonyl chloride and dioctadecylamine in chloroform that contained a trace of triethylamine as a catalyst. Purification was achieved by chromatography over silica gel with a hexane to ethyl acetate gradient. Measurements of the rate of transfer of DNS-DODA between single bilayer vesicles and reassembled HDL showed that transfer half-times were greater than 12 h at 25 °C (results not shown).

Apolipoprotein A-II was isolated from human plasma as described by Massey et al. (1981). 1-Hexadecyl-2-(9-octadecenyl)phosphatidylcholine (POPC ether) was synthesized according to Pownall et al. (1985). All other phospholipids were obtained from Avanti Polar Lipids (Birmingham, AL), and their purity was verified by the appearance of a single spot when tested by thin-layer chromatography on silica gel eluted with chloroform, methanol, and water (65/25/4). Phospholipase A_2 (*Crotalus atrox*) was obtained from the Miami Serpentarium. Buffer salts, acids, and bases, which were of reagent grade, were purchased from Fisher Scientific (Houston, TX). A buffer of 100 mM NaCl containing 10 mM Tris, pH 7.4, was used throughout.

Methods

Analytical Methods. Fluorescence spectra of the peptides were measured on an SLM photon counting spectrometer operated at ambient temperatures. Peptide concentrations were calculated from the absorbance at 280 nm (a = 6850 M^{-1} cm^{-1}) measured on a Cary 14 absorption spectrophotometer. Lipid concentrations were determined by a phosphorus analysis (Bartlett, 1959).

Preparation of Donor and Acceptor Particles. Model lipoprotein complexes of human apolipoprotein A-II and DMPC with a lipid to protein ratio of 75/1 were prepared according to Massey et al. (1981). Single-bilayer vesicles of phospholipids were prepared by ultrasonic irradiation (Huang, 1969). Donor single-bilayer vesicles in which the outer density of lipids was reduced by 5% were prepared as follows. POPC ether (95%) and POPC (5%) were cosonicated to produce single-bilayer vesicles and were then treated with phospholipase A_2 in a lipid to enzyme weight ratio of 100. Fatty acid free bovine serum albumin (2%) was added to the mixture to bind the liberated lysolecithin and fatty acid. The reaction was conducted in a buffer composed of 5 mM $CaCl_2$ and 10 mM Tris, pH 7.4, at 37 °C for 1 h. Both complexes and vesicles were purified by chromatography over Sepharose CL-4B which separates vesicles of complexes from multilamellar species as well as fatty acid and lysolecithin, which are bound to albumin. Analysis of an aliquot of the lipids before chromatography was obtained by high-performance liquid chromatography on silica gel (Christie, 1986) with a Spectraphysics 8000 HPLC equipped with a radiolabel detector (Betacord, LKB Instruments, Bromma, Sweden). These analyses, which were performed in triplicate, showed that 63% of the total label was hydrolyzed. The ratio, 63/37, is the estimated outside/inside distribution of lipids in single-bilayer vesicles. We conclude that only the lipids in the outer leaflet have been hydrolyzed and that it is this population of hydrolysis products that is transferred to albumin and removed by chromatography.

Transfer Kinetics. The kinetics of peptide transfer were measured by a modification of the procedure of Plant et al. (1983) in which the rate of transfer was a direct function of the change in the fluorescence that accompanied the transfer of a fluorophore from donor vesicles composed of POPC to acceptor vesicles that contained a fluorescence quencher; in the present study, the fluorescence derived from the intrinsic fluorescence of the peptide. Donor single-bilayer vesicles of POPC were prepared by ultrasonic irradiation and purified by chromatography over Sepharose CL-4B. A solution of the peptide in buffer was added to the donor vesicles in the desired ratio. Acceptor vesicles were prepared by cosonication of POPC containing 5 mol % DNS-DODA. The transfer reaction was initiated by mixing equal volumes of donor and acceptor vesicles in a Hi-Tech stopped-flow accessory that was mounted on an SLM spectrofluorometer. The excitation wavelength was 280 nm, and the emission at wavelengths greater than 365 nm was isolated by a Corning 3-74 cutoff filter (arrow, Figure 1). The signal was transferred from the fluorometer to a Sperry personal computer where the data were stored for processing. Between 2000 and 4000 data points were collected in each kinetic experiment. Statistical tests were conducted to determine the best fit for second-order, single, or biexponential reactions. The latter, which always gave the best fit, had the form $I = A + B\exp(-k_1t) + C\exp(-k_2t)$, where I is the fluorescence intensity, t is time, A, B, and C are constants, and k_1 and k_2 are the respective rate constants of the two decay components. Other experiments, in which donor POPC single-bilayer vesicles were replaced by complexes of apo A-II and DMPC or POPC ether single-bilayer vesicles, were conducted and analyzed similarly.

Results

Preliminary fluorescence experiments were conducted to identify the optimal conditions for monitoring the transfer of peptides between the donor and acceptor single-bilayer vesicles.

[1] Abbreviations: apo, apolipoprotein; DMPC, dimyristoylphosphatidylcholine; LAP, lipid-associating peptides; DNS-DODA, *N*-[[5-(dimethylamino)-1-naphthalenyl]sulfonyl]-*N*,*N*-dioctadecylamine; POPC, 1-palmitoyl-2-oleoyl-*sn*-3-glycerophosphocholine; POPC ether, 1-hexadecyl-2-(9-octadecenyl)phosphatidylcholine.

Table I: Rates and Activation Parameters for the Transfer of C_n-LAP-15 at 37 °C

donor (μg/mg of POPC)[a]	k (s^{-1})[b]	E_a (kcal/mol)	ΔG^* (kcal/mol)[c]	% fast[d]	F_1/F_2 (t)
fast component					
C_8-LAP, POPC (0.05)	117.0	21.20	15.26	53	1.7 (10)
C_{12}-LAP, POPC (0.05	10.2	16.29	16.76	49	8.0 (25)
C_{12}-LAP, POPC (0.01)	3.25	17.95	17.47	51	
C_{16}-LAP, POPC (0.05)	0.0116	21.26	20.94	52	3.0 (50)
C_{12}-LAP, DMPC/apo A-II complexes	16.4	12.19	19.33	56	
slow component					
C_8-LAP, POPC (0.05)	38.1	23.05	15.95		
C_{12}-LAP, POPC (0.05)	3.23	15.62	17.47		
C_{12}-LAP, POPC (0.01)	0.62	21.10	18.49		
C_{16}-LAP, POPC (0.05)	0.0014	26.00	20.81		
C_{12}-LAP, DMPC/apo A-II complexes	2.46	14.31	17.64		

[a] Concentration ratio is for the donor. Acceptor vesicles were present in a 5-fold excess. [b] Average deviation was 10% or less. [c] Calculated from absolute rate theory. [d] Percent fast is the ratio of the preexponential factor for the fast component to that of the fast and slow component times 100%. [e] F_1/F_2 is the ratio of the F values for a biexponential fit of the data to that of a single-exponential fit of the same data; (t) indicates the temperature at which the ratio was calculated.

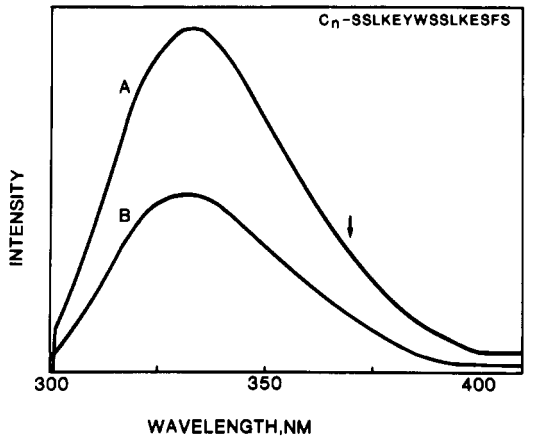

FIGURE 1: Fluorescence spectra of C_n-LAP in the presence of POPC (A) and DNS-DODA and POPC (B). In both curves, the concentrations of peptide and phospholipid were 0.03 and 1.30 mM, respectively. Excitation was 280 nm. Insert shows the structure of C_n-LAP, where n is the number of carbon units in a single saturated fatty acyl chain. The arrow indicates the cutoff wavelength of the filter used for kinetics.

The peptides were mixed with the donor vesicles, and the fluorescence spectrum was recorded. In each case the peptide exhibited a strong fluorescence maximum between 330 and 335 nm (Figure 1A). When the peptides were mixed with donor vesicles, which were composed of POPC plus 5 mol % of the nontransferable fluorescence quencher, DNS-DODA, the resulting spectra were similar but exhibited intensities that were reduced by 50% (Figure 1B). Independent kinetic experiments showed that the association of the acylated peptides with acceptor vesicles at similar concentrations was much faster than the limits of our system. Thus, the transfer of any of the C_n-LAP peptides from POPC vesicles to those containing a fluorescence quencher results in a reduction of the fluorescence intensity, and the rate of reduction can be used as a direct measure of the kinetics of peptide transfer. Other experiments (not shown), in which the quencher was in the donor and the acceptor was composed of 100% POPC, exhibited an increase in fluorescence intensity after mixing. However, the kinetics were identical with those observed with quencher in the acceptor.

The rates of peptide transfer were a regular function of the length of the acyl chain and of temperature. Some representative kinetic traces are given in Figure 2. The data were fitted to both a single and a biexponential fitting routine and the residuals plotted as a function of time; as the insert to the lower panel of Figure 2 shows, there is an uneven distribution of the residuals in the single-exponential fit that is consistent

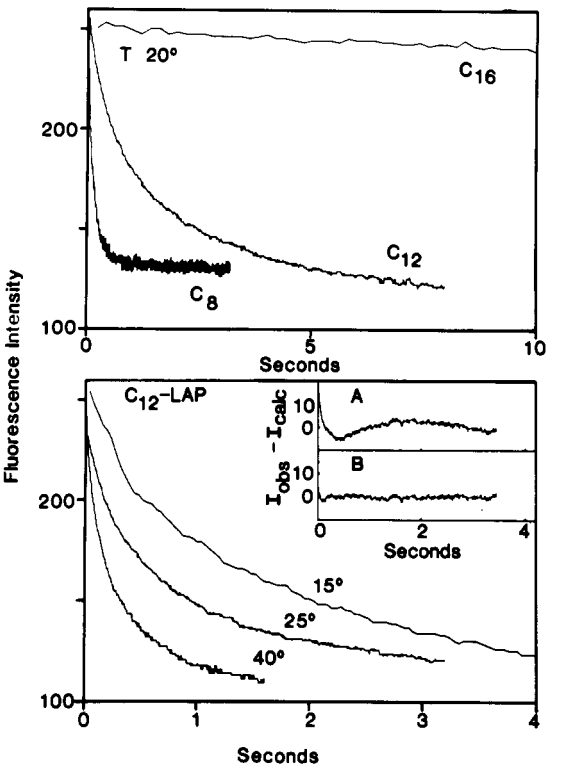

FIGURE 2: Transfer kinetics of C_n-LAP. (Upper panel) Transfer of C_n-LAP (n = 8, 12, or 16) at 20 °C. (Lower panel) Transfer of C_{12}-LAP at 15, 25, and 40 °C. The identity of the peptides (upper panel) and temperatures (lower panel) are labeled in the figure. The insert compares residuals observed when fitted to a single-exponential decay (A) to those obtained with two exponential components (B).

with the observed and calculated curves intersecting at two points. In addition, with a biexponential fit there is a stochastic distribution of the observed values about the calculated curve. Moreover, for these data we calculated an F value for both fits. For a single exponential, this value was 38 237; for the biexponential fit, the F value was 305 523. Since the F value is proportional to the significance level (Snedecor & Cochran, 1980), we expect that the function that yields the greatest F value is the one that provides the best description of the data. As the last column of Table I indicates, the ratio of the F value for a biexponential decay to that of a single-exponential decay was always greater than 1. Therefore, our mechanistic model for peptide transfer is more consistent with biexponential kinetics.

At constant temperature an increase in the acyl chain length of the peptide was accompanied by a decrease in the rate of

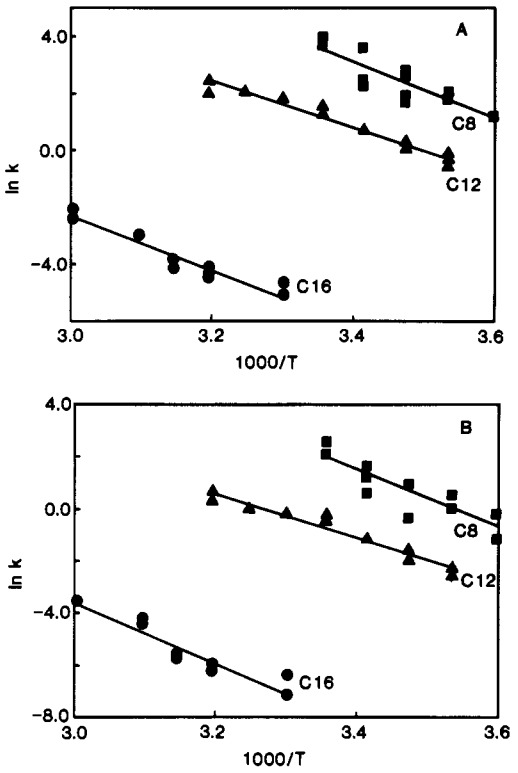

FIGURE 3: Arrhenius plots of C_n-LAP transfer. Fast (panel A) and slow (panel B) components of C_n-LAP transfer: (▲) C_8; (■) C_{12}; (●) C_{16}.

transfer (Figure 2). For a given peptide, an increase in the temperature led to an increase in the rate of peptide transfer. For all three peptides, the relative contributions of the long and short kinetic components to the total change in amplitude were nearly equal and independent of temperature. The rate constant for each component was measured as a function of temperature and plotted according to Arrhenius and the activation energies calculated from the slopes of the curves (Figure 3). The rates and activation energies are summarized in Table I which also lists the free energies of activation calculated from absolute rate theory. The most notable feature of these data was the variation in the free energy of activation with acyl chain length, which increased at the rate of about 700–800 cal per methylene unit for both the fast and slow components. However, the contribution of each of the methylene units between C_8 and C_{12} was less (375 cal per CH_2) than that observed between C_{12} and C_{16} (1100 cal per CH_2). The transfer rate constants were independent of the ratio of the donor to acceptor and the identity of the donor. Similar studies (Table I) of the transfer of an acylated peptide from the surface of a model lipoprotein composed of DMPC and apolipoprotein A-II (75/1) also exhibited two components, although the rates were faster than those observed with single-bilayer vesicles. Finally, the fast and slow rates of transfer of C_{12}-LAP between single-bilayer vesicles of POPC were identical (±10%) to those observed for the same donors using apo A-II (75/1) DMPC complexes as acceptors. In the latter case, increasing the acceptor to donor ratio from 5 to 15 had no significant effect on the fast and slow rate constants.

The effect of removing 5% of the phospholipids in the outer leaflet of the donor vesicles was also evaluated. This was achieved by using single-bilayer vesicles composed of 95% POPC ether and 5% POPC and treating them with phospholipase A_2. The phospholipid density of the donor was thus reduced to that of the POPC ether, which was not susceptible

Table II: Rates and Activation Energies for the Transfer of C_{12}-LAP-15 from Normal and Modified[a] POPC Ether Single-Bilayer Vesicles at 37 °C

	rate constant (s^{-1})		activation energy (kcal/mol)	
phospholipase A_2	fast component	slow component	fast component	slow component
−	0.52	0.10	21.86	26.82
+	0.58	0.08	21.85	23.18

[a] Donors were composed of POPC ether (95%) and POPC (5%) or POPC ether (95%) and POPC in which all of the POPC in the outer leaflet was removed by hydrolysis with phospholipase A_2 (see text for details).

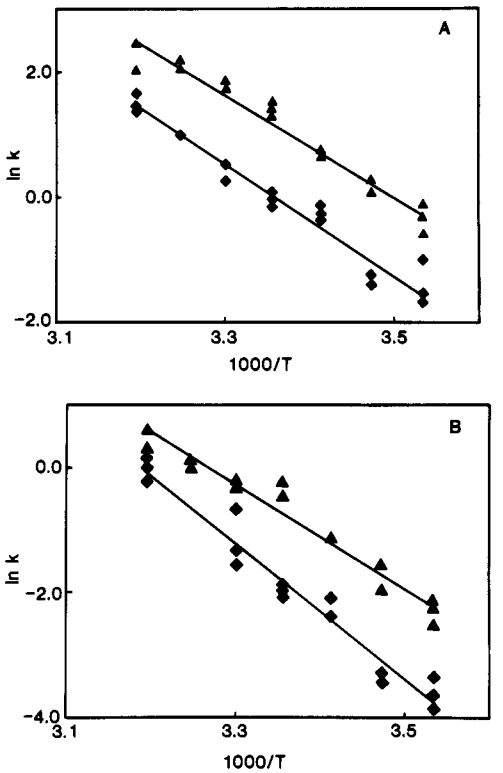

FIGURE 4: Effect of peptide/PC ratio on C_{12}-LAP transfer. Fast component (panel A) and slow component (panel B): 50 μg of peptide/mg of PC (▲); 10 μg of peptide/mg of PC (◆).

to hydrolysis. We observed (Table II) that the transfer rates for C_{12}-LAP were not greatly affected by treatment of the donors with phospholipase. Therefore, over this small range, the rate of peptide transfer was independent of the phospholipid density on the outer surface of the donor vesicles. Independent studies with a pyrene-labeled phospholipid indicated negligible migration of phospholipid across the bilayer membranes (Homan & Pownall, 1986).

To determine the effect of the lipid to peptide ratio on the reaction rates, we conducted one set of experiments at the lower limit of reliable detection on our stopped-flow system. The Arrhenius plots of these data are shown in Figure 4. Both fast and slow components were still observed although both rates were slower by a factor of about 3 at the lower peptide to lipid ratio. This ratio was 0.01 mg of peptide/mg of lipid, which corresponds to about 10 peptide molecules per vesicle if one assumes an aggregation number of 2500 lipids per vesicle.

Discussion

The kinetics and thermodynamics of transfer of the C_n-LAP-15 peptides had many similarities to those of a wide variety of lipids including alcohols, fatty acids and their methyl

esters, alkanes, and phospholipids (Pownall et al., 1983; Massey et al., 1982; Nichols, 1985; Lund-Katz et al., 1982). These earlier studies had shown that many sparingly soluble compounds transfer between lipid surfaces by a mechanism that involves rate-limiting desorption of the molecule from the surface of the lipid into the surrounding aqueous phase. This is succeeded by the very rapid diffusion-controlled association of the transferring species with the acceptor lipid surface. A major characteristic distinguishes this mechanism from one that involves transfer via collision of the donor and acceptor particles; the latter process is second order and dependent on the concentrations of the donor and acceptor. We observed no dependence on the acceptor concentration and conclude that the peptide transfer occurs through the aqueous phase. Other observations are also consistent with this assignment. The transfer times and free energies of activation increase with an increase in the hydrophobicity of the transferring species; moreover, there is a reasonably good correspondence between the incremental free energies of activation for the transfer of each methylene unit and the measured free energy of transfer of methylene units from a hydrocarbon phase to an aqueous phase (Tanford, 1973); both are between 700 and 800 cal per methylene unit. However, the average incremental free energy is smaller between 8 and 12 methylene units than it is between 12 and 16 methylene units. This is consistent with there being some penetration of water beyond the interfacial region to create a hydrophobicity gradient. The average magnitude of the values is sufficiently close to 800 cal per CH_2 to suggest that, in the activated state, the transferring species is in an environment that is similar if not identical with that of bulk-phase water.

The major distinction between the kinetics of lipid and peptide transfer is that the former is usually a single-exponential decay whereas the transfer of the acylated peptides is best described by a biexponential rate equation. This observation is consistent with certain earlier findings. The transfer of factor V and its phospholipid binding peptide between phospholipid vesicles is not exponential although a suitable rate expression was never reported (Pusey & Nelsestuen, 1984). Also, Leto et al. (1980) observed that the transfer of cytochrome b_5 between phospholipid vesicles is first order over only the first 65% of the reaction. They attribute the absence of a purely exponential decay to negative cooperativity in the association of the protein with the phospholipid vesicle. Moreover, independent measurements in our laboratory have shown that the kinetics of transfer of the individual C apolipoproteins and of melittin (H. Pownall, unpublished results) are also biexponential. Therefore, this type of behavior appears to be characteristic of protein transfer.

One possibility is that the two components are due to the difference in rates of transfer from the two leaflets of the phospholipid vesicle. However, our transfer experiments in which the phospholipid vesicles were replaced by model lipoproteins composed of apo A-II and DMPC also exhibited two components. Since this model lipoprotein has a micellar architecture in which only an outer leaflet is present, it is probable that the biexponential decay is a specific function of the interaction of peptides in the phospholipid surface.

Another possibility is that the different rate constants arise from differences in the packing of lipids and peptides on the donor surface. However, in the experiments in which 5% of the phospholipids had been removed by phospholipase treatment, the peptide transfer rates and activation energies were identical with those of the untreated sample. It is unlikely that PC and C_n-LAP are transferred as a complex because addition

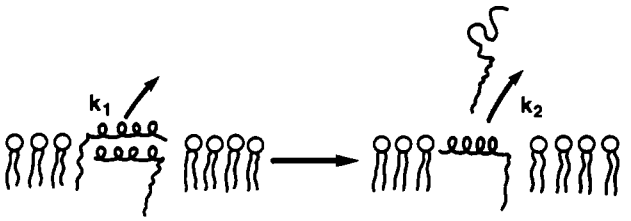

FIGURE 5: Schematic representation of the transfer of acylated peptides from a phospholipid surface into water. The peptides form dimers which, according to circular dichroic studies (Ponsin et al., 1984), are helical. The first component (k_1) is transfer of a peptide from the dimer. The second, slower component (k_2) is due to transfer of a monomer from a totally lipidic environment. The structure in water is represented as a random coil.

of the peptide to labeled single-bilayer vesicles did not affect the rate of desorption of pyrene-labeled PCs (data not shown). Therefore, the peptide transfer probably occurs by monomer desorption into the aqueous phase followed by rapid diffusion to acceptor particles. This conclusion is consistent with the report of Ponsin et al. (1984) that the acylated peptides were dialyzable over a time scale in which little lipid transfer was observed. The mechanistic details that lead to the biexponential decay remain to be established. One mechanism to explain our data is that the acylated peptides, like some glycosphingolipids (Brown et al., 1985), form a noncovalent dimer on the surface of a phospholipid vesicle. This is illustrated in Figure 5. The first component of the transfer, which is rapid, corresponds to the dissociation of one molecule from the dimer. The second component corresponds to the subsequent transfer of the second molecule from a smaller oligomer. Both components should be exponential, and each should be sensitive in a similar way to the peptide hydrophobicity, which is governed by the length of the saturated acyl chain. The assignment of a dimeric species to the physical state of the peptide on the lipid surface is based upon the observation that the fast and slow components contributed nearly equally to the total signal change (Table I). This effect did not disappear when the peptide to lipid ratio was reduced by a factor of 5. Instead, the rate constants for both components were smaller. This may be due to changes in the surface density of peptide molecules that perturb the system in a way that is different from that produced by lowering the phospholipid surface density. Thermodynamically, this model is identical with the negative cooperativity suggested by Leto et al. (1980). It suggests that, as the number of peptides on a membrane or lipoprotein surface is increased, the affinity of all of the peptides for the surface is reduced.

Physiological Insights. During lipolysis of chylomicron and very low density lipoproteins the fatty acid hydrolytic products are rapidly transferred to serum albumin via the aqueous phase. The removal of the triglyceride core and a portion of the phospholipids that compose the surface produces a net increase in the surface density of proteins. It is known that, following lipolysis, the C and E apoproteins are associated mainly with the high-density lipoproteins; the lower affinity of peptides for lipid surfaces under conditions where there is a high surface density of proteins could be an important determinant in the in vivo regulation of peptide transfer. However, the major determinant of the transfer rate is the hydrophobicity of the transferring species. In the case of the acylated peptides and of phospholipids, this is a predictable function of the length of the acyl chains. If this rule is to be extended to native proteins, the major determinant must be the hydrophobicity of the amino acid residues that are in contact with the phospholipid matrix.

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REFERENCES

Bartlett, G. R. (1959) *J. Biol. Chem. 234*, 466–468.
Brown, R. E., Sugár, J. P., & Thompson, T. E. (1985) *Biochemistry 24*, 4082–4091.
Christie, W. W. (1986) *J. Chromatogr. 361*, 396–399.
Fielding, C. J., Shore, V. G., & Fielding, P. E. (1972) *Biochim. Biophys. Acta 270*, 513–518.
Gianturco, S. H., & Bradley, W. A. (1987) *Plasma Lipoproteins* (Gotto, A. M., Jr., Ed.) pp 183–220, Elsevier Science, Amsterdam.
Homan, R., & Pownall, H. J. (1986) *Biophys. J. 49*, 517a.
Huang, C. (1969) *Biochemistry 8*, 344–351.
Ihm, J., & Harmony, J. A. K. (1980) *Biochem. Biophys. Res. Commun. 93*, 1114–1120.
LaRosa, J. C., Levy, R. I., Herbert, P., Lux, S. E., & Fredrickson, D. S. (1970) *Biochem. Biophys. Res. Commun. 41*, 57–72.
Leto, T. L., Roseman, M. A., & Holloway, P. W. (1980) *Biochemistry 19*, 1911–1916.
Lund-Katz, S., Hammerschlag, B., & Phillips, M. C. (1982) *Biochemistry 21*, 2964–2969.
Massey, J. B., Rohde, M. F., Van Winkle, W. B., Gotto, A. M., Jr., & Pownall, H. J. (1981) *Biochemistry 20*, 1569–1574.
Massey, J. B., Gotto, A. M., Jr., & Pownall, H. J. (1982) *Biochemistry 21*, 3630–3636.
Massey, J. B., Hickson-Bick, D., Via, D. P., Gotto, A. M., Jr., & Pownall, H. J. (1985) *Biochim. Biophys. Acta 835*, 124–131.
McKeone, B. J., Massey, J. B., Knapp, R. D., & Pownall, H. J. (1988) *Biochemistry 27*, 4500–4505.
Nichols, J. W. (1985) *Biochemistry 24*, 6390–6398.
Ponsin, G., Strong, K., Gotto, A. M., Jr., Sparrow, J. T., & Pownall, H. J. (1984) *Biochemistry 23*, 5337–5342.
Pownall, H. J., Hickson, D. L., & Smith, L. C. (1983) *J. Am. Chem. Soc. 105*, 2440–2445.
Pownall, H. J., Pao, Q., & Massey, J. B. (1985) *J. Biol. Chem. 260*, 11719–11723.
Pusey, M. L., & Nelsestuen, G. L. (1984) *Biochemistry 23*, 6202–6210.
Reijngoud, D.-J., & Phillips, M. C. (1982) *Biochemistry 21*, 2969–2976.
Reijngoud, D.-J., & Phillips, M. C. (1984) *Biochemistry 23*, 726–734.
Shen, B. W., Scanu, A. M., & Kezdy, F. J. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 837–841.
Snedecor, G. W., & Cochran, W. G. (1980) *Statistical Methods*, 7th ed., The Iowa State University Press, Ames, IA.
Tanford, C. (1973) *The Hydrophobic Effect: Formation of Micelles and Biological Membranes*, Wiley, New York.
Zilversmith, D. B., Hughes, L. B., & Balmer, J. (1975) *Biochim. Biophys. Acta 409*, 393–398.
Zorich, N., Jonas, A., & Pownall, H. J. (1985) *J. Biol. Chem. 260*, 8831–8837.

Homology-Dependent Underwinding of Duplex DNA in RecA Protein Generated Paranemic Complexes†

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ABSTRACT: RecA protein promoted formation of paranemic joints, in which a recA–ssDNA complex and a nicked circular dsDNA molecule are homologously aligned without net cross-strand interwinding, is accompanied by extensive underwinding of the dsDNA molecule. When the nick is sealed by DNA ligase, a highly negatively superhelical DNA molecule is formed. This underwinding has the following properties: (a) it occurs within 2 min; (b) it is completely homology dependent; (c) it does not require a homologous free DNA end. The resulting underwound DNA species is comprised of a heterogeneous population of topoisomers. The degree of unwinding exhibits a strong dependence on the fractional length of homology in the dsDNA molecule and indicates that paranemic joints can extend for at least 2900 base pairs. The rapid underwinding associated with paranemic joint formation is followed by a longer phase in which the dsDNA molecule is more extensively underwound.

The recA protein of *Escherichia coli* promotes homologous genetic recombination in vivo and in vitro [reviewed in Dressler and Potter (1982) and Cox and Lehman (1987)]. An early step in homologous genetic recombination is the homologous synapsis (pairing) of two DNA molecules (Holliday, 1964; Meselson & Radding, 1975). In vitro, a prerequisite step to pairing is the binding of recA protein to DNA (Shibata et al., 1979a; Cox & Lehman, 1981). RecA protein stoichiometrically binds (one recA monomer per four nucleotides) to single-stranded DNA (ssDNA)[1] to form a filamentous nu-

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