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Determination of the Acyl Chain Specificity of the Bovine Liver Phosphatidylcholine Transfer Protein. Application of Pyrene-Labeled Phosphatidylcholine Species†

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ABSTRACT: The phosphatidylcholine transfer protein from bovine liver has specific binding sites for the *sn*-1 and *sn*-2 acyl chains of the phosphatidylcholine molecule [Berkhout, T. A., Visser, A. J. W. G., & Wirtz, K. W. A. (1984) *Biochemistry 23*, 1505–1513]. In the present study, we have investigated the properties of these binding sites by determining both binding and transfer of several sets of pyrenylphosphatidylcholine species. These sets consisted of positional isomers in which the length of the pyrene-labeled acyl chain (i.e., 5–13 methylene units) or of the unlabeled saturated acyl chain (i.e., 9–19 methylene units) was varied in either the *sn*-1 or the *sn*-2 position. Binding studies showed that there was a considerable discrimination between positional isomers with the higher affinity observed for those lipids that carry the pyrenyl chain in the *sn*-2 position. In addition, the affinity is markedly dependent on the length of the acyl chains; pyrenyl acyl chains of 9 and 11 methylene units and the palmitoyl chain provided the most efficient binding. The affinity of the transfer protein for the strongest bound pyrene lipid was approximately 2.5 times higher than for an average egg phosphatidylcholine molecule. In general, the transfer studies were in agreement with the binding data. However, with some short-chain derivatives, transfer rates were faster than expected on the basis of the binding data. This emphasizes the importance of kinetic factors (i.e., activation energy) in the transfer process. The rates of spontaneous transfer decreased monotonically with increasing chain length and were very similar for all positional isomer pairs studied. This strongly suggests that the discrimination between isomers in the protein-mediated transfer reflects the structure of the lipid binding site rather than differences in the physical properties of the isomers. In conclusion, the present data strongly support the presence of separate binding sites in the transfer protein for the *sn*-1 and *sn*-2 chains of phosphatidylcholine and indicate that these binding sites have considerably different acyl chain specificity.

Phospholipids and cholesterol are known to transfer between membranes and lipoproteins (Bell, 1978; Norum et al., 1983). Transfer may take place spontaneously by diffusion of monomers through the aqueous phase (Martin & MacDonald, 1976; Duckwitz-Peterlein et al., 1977; Doody et al., 1980; Roseman & Thompson, 1980; McLean & Phillips, 1981; Nichols & Pagano, 1981) or is mediated by specific proteins found in cells (Wirtz, 1982; Kader et al., 1983; Zilversmit, 1984) and serum (Ihm et al., 1980; Morton & Zilversmit, 1982; Tall et al., 1983; Abbey et al., 1985). The mode of action of some of these lipid transfer proteins has been studied in considerable detail [for reviews, see Wirtz (1982), Helm-

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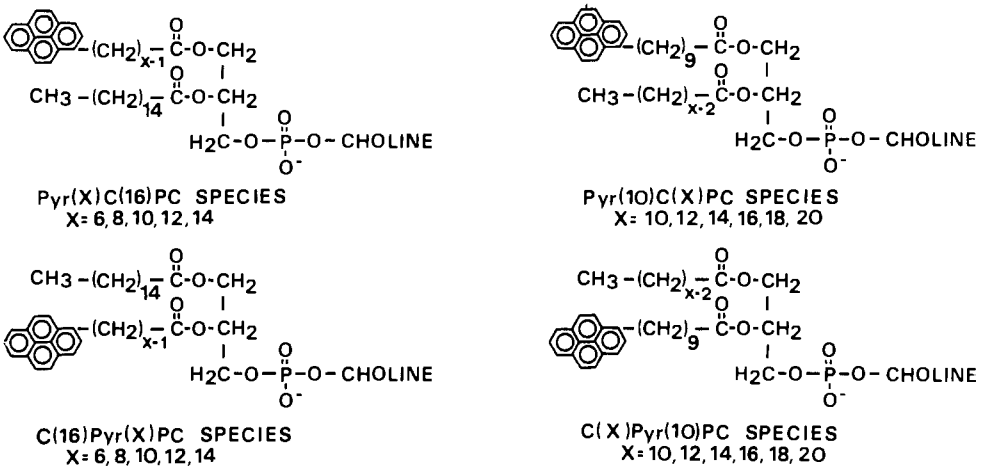

FIGURE 1: Structures and nomenclature of the pyrenyl-PC species. *x* indicates the total number of carbon units (including the carbonyl one) in the aliphatic chain.

kamp (1986), and Tall (1986)]. For instance, the phosphatidylcholine-specific transfer protein from bovine liver (PC-TP)[1] and the phosphatidylinositol transfer protein from bovine brain and heart act as lipid carriers; i.e., they contain a bound phospholipid molecule, which can be exchanged for another molecule upon collision of the protein with a membrane interface. It has been clearly demonstrated that both the physical state of the membrane and the acyl chain structure of the phospholipid molecule to be transferred strongly influence the rate of transfer. In general, slow rates of transfer have been observed for gel-state bilayers as compared to fluid ones, and unsaturated lipids are transferred faster than saturated ones (Kasper & Helmkamp, 1981; Bozzato & Tinker, 1982; Welti & Helmkamp, 1984).

Recent studies have provided evidence that PC-TP has separate binding sites for the *sn*-1 and *sn*-2 acyl chains of PC (Berkhout et al., 1984). Its ability to discriminate between PC isomers suggested that these two binding sites have different properties (Van Loon et al., 1986). Here we have further investigated the properties of these acyl binding sites by employing several sets of PyrPC species. The results support the existence of specific, individual binding sites for the two acyl chains and suggest that the *sn*-2 acyl binding site can more readily accommodate bulky (unsaturated) chains than the site for the *sn*-1 chain.

Experimental Procedures

Materials

Didecanoyl-, dilauroyl-, dimyristoyl-, dipalmitoyl-, and distearoyl-PC species and phospholipase A_2 from *Crotalus adamanteus* were obtained from Sigma. Saturated fatty acids were products of Fluka. Egg yolk PC was purified according to Singleton et al. (1965), and phosphatidic acid (PA) was prepared from this lipid by phospholipase D catalyzed hydrolysis. 8-Pyrenyloctanoic acid was a generous gift from Dr. J. Virtanen from KSV Chemicals (Helsinki, Finland). Other pyrenyl fatty acids were synthesized essentially as described by Galla and Hartmann (1981) and were purified by silica gel column chromatography. Diarachidoyl-PC and diPyrPC species were prepared from the glycerophosphocholine–cadmium adduct and the corresponding fatty acids (Patel et al., 1979). Lysophosphatidylcholines were prepared from the diacyl-PC species by phospholipase A_2 treatment and purified by repeated acetone precipitation (Kates, 1972). Mixed-chain PyrPCs (for formulas, see Figure 1) were synthesized from lysophosphatidylcholines and fatty acid anhydrides according to Gupta et al. (1977) and purified by HPLC silica column chromatography. Some lipids were further purified by reverse-phase HPLC chromatography employing a Beckman Ultrasphere ODS 4.6 × 25 cm column (Patton et al., 1982). Phosphatidylethanolamine was obtained from egg PC as described previously (Somerharju et al., 1985). TNP-PE was prepared from phosphatidylethanolamine as described by Gordensky and Marinetti (1973) and was purified by silica gel column chromatography. All lipids appeared homogeneous by thin-layer chromatography, and the molecular species purity of the PyrPC species, as analyzed by reverse-phase HPLC, was higher than 95%. Analysis of the positional distribution of the pyrenyl acyl chains in the PyrPC species by phospholipase A_2 degradation indicated that acyl chain migration was less than 5%.

PC-TP was purified from bovine liver according to the method of Westerman et al. (1983a) and stored at −20 °C in 50% glycerol.

Methods

Fluorescence Measurements. Measurements were carried out by using either a Hitachi-Perkin Elmer MPF 3 or a Hitachi F-3000 fluorometer equipped with a thermostated cuvette holder. Excitation was set at 343 nm (transfer experiments) or 346 nm (binding studies). The slit was 2 nm. Pyrene monomer emission was monitored at 378 nm (slit 20 nm).

Preparation of Phosholipid Vesicles. Single-bilayer donor vesicles for the transfer and binding assays were prepared in the cuvette by injecting the appropriate lipids dissolved in ethanol (aliquot of 5–10 μL) into 2 mL of 20 mM Tris-HCl/5 mM EDTA buffer, pH 7.4 (Batzri & Korn, 1973). A 2–5-min equilibration period was allowed before the measurements were started. To prepare the acceptor vesicles for the transfer assay, the lipids were dried down from a chloroform solution, dispersed in Tris/EDTA buffer, and sonicated for 10 min on ice with a Branson probe sonicator (60-W output). Titanium particles and any undispersed lipid were removed by centrifugation (8000*g* for 20 min).

Phospholipid Binding Assay. Binding of PyrPC species to PC-TP was determined by titrating quenched vesicles consisting of PyrPC, egg PC, and TNP-PE (18:73:9, mol %) with

[1] Abbreviations: C(20:4), arachidonate; C(22:6), docosahexanoate; PC, phosphatidylcholine; PA, phosphatidic acid; PyrPC, pyrenylphosphatidylcholine (see Figure 1 for the nomenclature of the PyrPC species); TNP-PE, 2,4,6-trinitrophenylphosphatidylethanolamine; EDTA, ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)aminomethane; PC-TP, phosphatidylcholine transfer protein; HPLC, high-pressure liquid chromatography; K_{rel}, relative binding constant.

PC-TP. Upon addition of PC-TP, PyrPC molecules incorporate into the lipid binding site, resulting in a considerable increase of the pyrene monomer fluorescence. In a typical experiment, donor vesicles (5.5 nmol total phospholipid) were titrated with PC-TP, and the increase of pyrene monomer fluorescence was plotted as a function of PC-TP concentration. The initial slope of such a plot is a measure for the relative affinity of PC-TP for the respective PyrPC species. Correct relative affinities are obtained by this procedure only if the quantum yield of the various PyrPCs bound to PC-TP is identical. To test this, vesicles were prepared from each C(16)Pyr(x)PC species (20 nmol) and PA (10 nmol) in Tris-HCl/NaCl/$MgCl_2$ (20:60:5 mM, pH 7.4) using the ethanol injection method. Upon addition of 5 nmol of PC-TP, the mixture was incubated for 10 min at 35–40 °C and then passed through a small column of DEAE-cellulose. The eluate contains the transfer protein with the incorporated PyrPC species whereas all vesicles are bound at the top of the column due to their high negative charge density (Van Paridon et al., 1987). The relative quantum yields were obtained by dividing the pyrene monomer intensity of the eluted PyrPC/PC-TP complexes by the amount of PyrPC bound. PyrPC was quantified by measuring either the absorbance at the pyrene long-wavelength maximum (346–347.5 nm) or the fluorescence intensity after release of the bound PyrPC into egg PC/PA (97:3, mol %, 200 nmol) vesicles. The quantum yields of the different PyrPC/PC-TP complexes were found to be very similar (less than 10% difference). Hence, the initial slope of the plots of the fluorescence intensity vs [PC-TP] could be taken as a measure for binding.

Binding of PyrPC Relative to Egg PC. To determine how the affinity of PC-TP for the pyrene lipids compares with that for egg PC (the matrix lipid), vesicles containing a constant amount of PyrPC and variable amounts of egg PC (molar ratios from 1:0 to 1:9) were prepared. These vesicles were titrated with PC-TP, and the increase in the monomer emission intensity (ΔF) was plotted as a function of the amount of PC-TP added. A maximal fluorescence increase (ΔF_{max}) is observed for vesicles that consist of only PyrPC. The relative binding constant (K_{rel}) for the PyrPC species was derived from the equation:

$$\Delta F = \frac{-1}{K_{rel}} \frac{[L_0]}{[L_1]} \Delta F + \Delta F_{max}$$

where $[L_0]/[L_1]$ represents the egg PC:PyrPC molar ratio. By plotting ΔF as a function of $([L_0]/[L_1])\Delta F$, we obtained a straight line with a slope of $-1/K_{rel}$. For a derivation of the equation, see Van Paridon et al. (1987).

Phospholipid Transfer Assay. This assay is based on the increase of pyrene monomer fluorescence intensity resulting from transfer of PyrPC molecules from donor vesicles containing quencher molecules (TNP-PE) to unquenched acceptor vesicles. The donor vesicles consisting of PyrPC, egg PC, and TNP-PE (18:73:9 mol %, 5.5 nmol) were mixed with a 50–100-fold excess of sonicated acceptor vesicles (egg PC/egg PA, 97:3 mol %) in 2 mL of Tris/EDTA buffer, and the increase of pyrene monomer fluorescence was recorded as a function of time. The initial slope of the progress curve corresponds to the relative spontaneous transfer rate of PyrPC. Subsequently, PC-TP (0.2–1 μg) was added, and the initial rate of fluorescence increase was recorded and, after subtraction of the blank (i.e., rate of spontaneous transfer), taken as a measure for the rate of protein-mediated transfer.

Other Analytical Methods. Concentrations of the PyrPC species were determined by measuring the absorbance at 342 nm in ethanol and using the molar abosrption coefficient of 42000 mol^{-1} cm^{-1} (Somerharju et al., 1985). Concentrations of other lipids were determined by a phosphorus assay (Rouser et al., 1970).

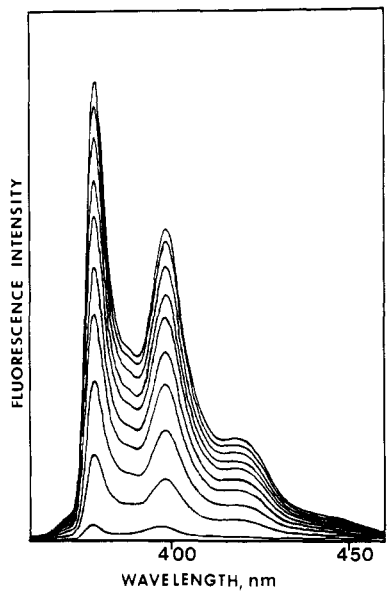

FIGURE 2: Titration of C(16)Pyr(10)PC vesicles with PC-TP. Quenched vesicles consisting of C(16)Pyr(10)PC/egg PC/TNP-PE (1:4:0.5 nmol) were titrated with PC-TP (aliquot of 0.1 nmol) at 30 °C. See text for further details.

RESULTS

Binding of PyrPC Species by PC-TP. Binding of PyrPC species by PC-TP was determined by titrating vesicles consisting of PyrPC/egg PC/TNP-PE (18:73:9 mol %) with PC-TP. In such vesicles, the pyrene fluorescence is strongly quenched (>95%) due to energy transfer to the TNP-PE acceptor. Addition of PC-TP results in a marked increase of pyrene monomer emission (Figure 2) because of the incorporation of PyrPC into the transfer protein. This bound PyrPC molecule is not accessible to quenching by TNP-PE since, at any time, most of the protein molecules are not associated with the membrane. Hence, the average distance between the protein-bound fluorophore and the membrane-embedded quencher exceeds the limits of energy transfer. Since PC-TP contains a single endogenous PC molecule (Kamp et al., 1975), incorporation of a PyrPC molecule actually involves a coupled exchange event rather than a simple binding phenomenon.

We first studied the effect of the length of the pyrenyl acyl chain on the binding affinity. Two sets of PyrPC species (see Figure 1) were used: the lipids of the first set contained palmitic acid in the *sn*-1 position and a pyrenyl chain of variable length in the *sn*-2 position [i.e., C(16)Pyr(x)PC species]; the second set consisted of the positional isomers of the first set [i.e., Pyr(x)C(16)PC species]. The results are shown in Figure 3A. First, it is obvious that the affinity is dependent on the length of the pyrenyl chain whether located in the *sn*-1 or *sn*-2 position. However, this chain length dependency is much more pronounced for the *sn*-2 position; the large increase in affinity when the Pyr(8) acyl chain is replaced by a Pyr(10) acyl chain is especially noteworthy. Second, with the exception of the two short-chain lipids, PC-TP displays a much higher affinity toward C(16)Pyr(x)PC species than toward the positional isomers. These findings suggest (i) that PC-TP contains specific binding sites for the acyl chain of PC and (ii) that the site for the *sn*-2 chain can more readily accommodate the relative bulky pyrene moiety as compared to the site for the *sn*-1 chain.

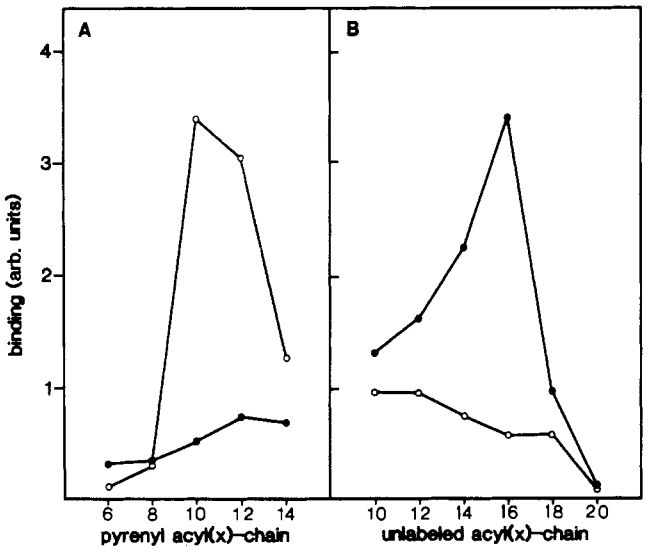

FIGURE 3: Effect of acyl chain length on binding of PyrPC species by PC-TP. (A) Binding of C(16)Pyr(x)PC (○) and Pyr(x)C(16)PC (●). (B) Binding of C(x)Pyr(10)PC (●) and Pyr(10)C(x)PC (○). The relative affinity of PC-TP for PyrPC species was determined by titration of quenched PyrPC/egg PC/TNP-PE (1:4:0.5 nmol) vesicles by PC-TP as described under Methods. Temperature was 30 °C.

We also tested the effect of the length of the unlabeled chain on the binding of the PyrPC species by PC-TP. Again, two sets of lipids were used: in the first set, a Pyr(10) chain was located in the *sn*-2 position while a normal saturated chain of variable length occupied the *sn*-1 position [i.e., C(x)Pyr-(10)PC species]; the second set consisted of the positional isomers [i.e., Pyr(10)C(x)PC species]. It is obvious (Figure 3B) that the affinity is also dependent on the length of the unlabeled chain and that different responses are observed depending on whether the length of the chain is varied in the *sn*-1 or *sn*-2 position. When the chain length increases in the *sn*-1 position, the affinity gradually increases until a sudden decrease is observed for acyl chains longer than 16 carbon atoms; a very low affinity is found for the C(20) acyl chain derivative. For the lipids with the unlabeled chain in the *sn*-2 position, a different behavior is found: the affinity decreases rather monotonically from the C(10) to the C(18) acyl chain and then abruptly for the C(20) acyl chain derivative. Altogether, the binding data strongly support the presence of separate, individual binding sites for the two acyl chains of PC in PC-TP.

Since the data in Figure 3 only give the affinities of the PyrPC species relative to each other, it was considered important to assess how these affinities compare with that for natural PC species. This was accomplished by titrating vesicles prepared from a constant amount of C(16)pyr(8)PC (a low-affinity lipid) or C(16)Pyr(10)PC (a high-affinity lipid) and variable amounts of egg PC. Dilution of the former lipid with egg PC caused a marked decrease in the pyrene monomer intensity vs [PC-TP] plot [as compared to undiluted C(16)-Pyr(8)PC vesicles] while dilution of C(16)Pyr(10)PC with egg PC had much less of an effect on the pyrene monomer intensity (data not shown). The affinity constants relative to egg PC (K_{rel}), estimated as described under Experimental Procedures, were found to be 0.38 for C(16)Pyr(8)PC and 2.7 for C-(16)Pyr(10)PC. Thus, the affinity of PC-TP for the PyrPC species used in this study appears to be in the same order as that for natural PC species.

Transfer of PyrPC Species by PC-TP. The relative rates of PyrPC transfer by PC-TP were obtained from the initial increase of pyrene monomer emission intensity upon addition

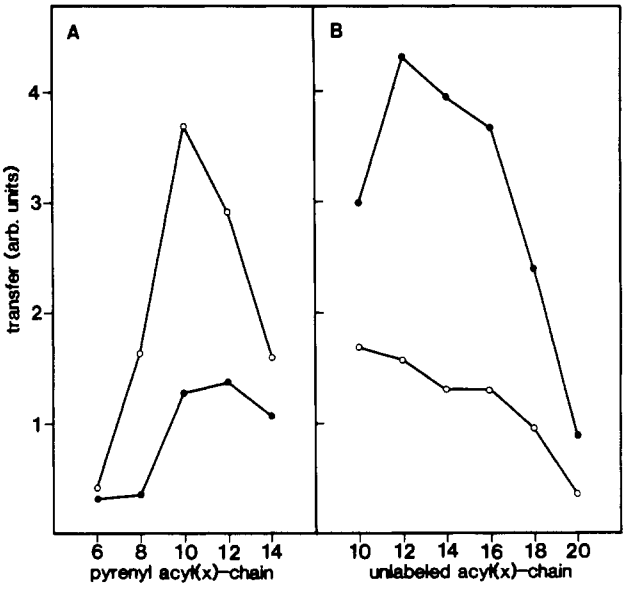

FIGURE 4: Relative rates of transfer of PyrPC species by PC-TP as a function of acyl chain length. (A) Transfer rates for C(16)Pyr(x)PC (○) and Pyr(x)C(16)PC (●). (B) Transfer rates for C(x)Pyr(10)PC (●) and Pyr(10)C(x)PC (○). The initial rates of transfer of PyrPC species from PyrPC/egg PC/TNP-PE (1:4:0.5 nmol) donor vesicles to egg PC/PA (97:3 nmol) acceptor vesicles were determined as specified under Methods.

of the transfer protein to a solution containing PyrPC/egg PC/TNP-PE donor vesicles and an excess of egg PC/PA acceptor vesicles. The results are shown in Figure 4. Variation of the length of the pyrenyl chain (Figure 4A) affected the rate of transfer in much the same way as it affected binding; i.e., lipids containing chains of medium length gave the highest rate independent of the position, and C(16)-Pyr(x)PC species were transferred faster than their positional isomers. However, it is to be noted that the transfer rates of the C(16)Pyr(6)PC and C(16)Pyr(8)PC species were markedly higher than expected from the binding data (see Discussion).

In the case of Pyr(10)C(x)PC species, variation of the length of the unlabeled chain (Figure 4B) influenced the rate of transfer in a similar fashion as binding. On the other hand, the rate vs chain length profile for the C(x)Pyr(10)PC species was considerably different from the binding profile. Particularly, when the chain length decreases from 16 to 12 carbon atoms, the rate of transfer increases while the binding diminishes (Figure 3B). This apparent discrepancy can be explained by assuming that the rates of transfer are dependent not only on the affinity but also on the energy of activation. One expects that the activation energy decreases (i.e., the rate of transfer increases) with decreasing acyl chain length.

Discussion

In this study, we have attempted to elucidate the structural properties of the acyl chain binding site(s) of the PC-TP from bovine liver by determining how systematical modifications of the acyl chains of a PC molecule affect the binding and transfer by this protein. We have preferred to use pyrene-labeled lipids since both binding and transfer can be measured readily. Second, one can make use of the bulkiness of the pyrene moiety to probe the accommodative properties of the lipid binding site.

Binding of PyrPC Species. Binding data (see Figure 3) demonstrate that PC-TP is able to discriminate between positional isomers: the affnity vs chain length profiles were quite different for the isomeric series independent of whether

the length of the pyrenyl chain or of the unlabeled chain was varied. We feel that this discrimination is a strong indication for the presence of separate binding sites for the *sn*-1 and *sn*-2 chains of the PC molecule, in support of our earlier data (Berkhout et al., 1985; Van Loon et al., 1986).

We shall now discuss the properties of these binding sites assuming that the two sites are independent from each other; i.e., the binding properties of one site are not affected by the acyl chain occupying the other site. In the case of the *sn*-1 acyl binding site, the length of the unlabeled chain strongly influences the affinity: binding increases steadily up to the C(16) chain and then suddenly decreases and becomes very low with the C(20) chain (Figure 3B). The sudden drop of the binding profile suggests that space limitations may prohibit the accommodation of chains longer than 18–20 carbon atoms into the binding site. It is apparent that the affinity for PC species carrying the pyrenyl chain at the *sn*-1 position is low (Figure 3A). The formal length of the Pyr(10) chain is close to that of the C(16) aliphatic chain (i.e., the length of the pyrene moiety corresponds to six methylene units), yet the affinity of Pyr(10)C(16)PC is only a fraction of that observed for C(16)Pyr(10)PC. In general, it appears that the *sn*-1 acyl binding site has a limited capacity to accommodate the pyrene moiety.

When the length of the unlabeled chain in the *sn*-2 position increases (Figure 3B), the binding decreases gradually up to the C(18) chain and then abruptly for the C(20) chain. It is likely that this reduced binding is not due to space limitations but due to an unfavorable energy of interaction with the binding site; i.e., the increase of the acyl chain length stabilizes more the membrane-bound state than the protein-bound state. Variation of the pyrenyl acyl chain influences the affinity quite differently (Figure 3A) Binding is poor with the two shortest chains but improves remarkably with the Pyr(10) and Pyr(12) chains. We attribute this behavior to a favorable accommodation of the pyrene moiety of the Pyr(10) and Pyr(12) chains in the *sn*-2 binding site. Reduced binding of the Pyr(14) acyl chain may be due to the length of this chain since also the C(20) chain with a similar apparent length is poorly bound (Figure 3B).

The influence of acyl chain length and position has recently been studied by using radiolabeled PC species containing a saturated acyl chain of variable length in the *sn*-1 or *sn*-2 position and an oleoyl residue in the alternate position (Van Loon et al., 1986). It was found that an elongation of the saturated chain from 10 to 14 carbon atoms in the *sn*-1 position resulted in an increase in binding of approximately 50% while with the positional isomers (i.e., those having the saturated chain in the *sn*-2 position) an increase in chain length hardly influenced binding. These results are in good agreement with the ones obtained in this study and suggest that the presence of a pyrenyl moiety in one of the acyl binding sites does not markedly perturb the interactions of the unlabeled chain with the other binding site. Van Loon et al. (1986) also found that the lipids containing the oleoyl residue in the *sn*-2 position displayed a higher affinity for PC-TP than the positional isomers. Thus, it seems that PC species with a saturated chain in the *sn*-1 position and an unsaturated or bulky acyl moiety in the *sn*-2 position are preferred over their positional isomers (see below).

Transfer of PyrPC Species by PC-TP. In general, the transfer rate vs chain length profiles were quite comparable to those obtained in the binding studies. This suggests that the rate of tranfer of a particular PyrPC species is related to the binding affinity. However, this relationship does not hold for all species. For instance, the rate of transfer of the short-chain C(x)Pyr(10)PC species was faster than expected from the binding data (Figures 3 and 4). This can be explained by assuming that with a shortening of the chain the energy of activation for binding (and release) decreases. This will lead to a faster rate of transfer despite the somewhat diminished affinity. In agreement with our data, Massey et al. (1985) have found for C(x)Pyr(9)PC species that a shortening of the saturated chain from 18 to 14 carbon atoms increases the rate of transfer by PC-TP. When the affinity becomes to low, the rate of transfer decreases (Figures 3 and 5) despite the fact that such a species more readily desorbs from the membrane (see below). Low-affinity binding is also the most likely explanation for the poor transfer of dimyristoyl-PC and some other short-chain PC species by PC-TP (Welti & Helmkamp, 1984; Van Loon et al., 1986).

Massey et al. (1985) found that the addition of double bonds to the C(18) chain in the *sn*-1 position considerably improved the transfer of the PyrPC species. Several other investigators have also noticed that an increased unsaturation improves the rate of transfer (Kamp et al., 1977; Welti & Helmkamp, 1984; Kuypers et al., 1986). Presumably, this is primarily an effect on the energy barrier. Namely, the presence of double bonds introduces kinks in the acyl chain, thereby facilitating the "flipping" of the acyl chain from the bilayer into the binding site of the transfer protein (see below).

Spontaneous vs Protein-Mediated Transfer. We have observed that the spontaneous transfer rates of, for example, C(x)Pyr(10)PC and Pyr(10)C(x)PC species decrease monotonically with the acyl chain length; i.e., the logarithm of the rate is a linear function of the chain length as previously found for C(x)Pyr(9)PC species (Massey et al., 1982, 1984). This is in sharp contrast to the data obtained for the PC-TP-mediated transfer where the chain length dependence is much more complicated (Figure 4). Furthermore, we observed that there are no significant differences in spontaneous transfer rates between the position isomer pairs. This strongly suggests that the discrimination between the isomers in the protein-mediated transfer reaction is not due to differences in the physicochemical properties which determine the rate of desorption from the bilayer but reflects the structural properties of the acyl chain binding sites on PC-TP. Such a conclusion is strongly supported by the observation that the bovine brain phospholipid transfer protein, which is also a carrier protein for PC, displays a completely different pattern of isomer preference (Van Paridon, 1987). Furthermore, the nonspecific lipid transfer protein which probably is not a carrier (Thompson, 1982; Nichols & Pagano, 1982) facilitates the transfer of positional isomers at identical rates (A. van Amerongen, unpublished experiments).

Model for the Acyl Chain Binding Sites. On the basis of the data obtained, we suggest a model for the *sn*-1 and *sn*-2 acyl binding sites of PC-TP (Figure 5). The site for the *sn*-1 chain is depicted as narrow and straight based on the poor binding of PC species with a pyrenyl chain in the *sn*-1 position (Figure 3A) and the favorable interaction with saturated chains up to 16 carbon atoms (Figure 3B). The site for the *sn*-2 chain is depicted as bottle-shaped because the short-chain pyrenyl chains are poorly accommodated while the longer Pyr(10) and Pyr(12) chains show high affinities (Figure 3A). In addition, the elongation of the saturated chain in the *sn*-2 position leads to diminished binding, suggesting that long saturated chains do not interact favorably with this site (Figure 3B).

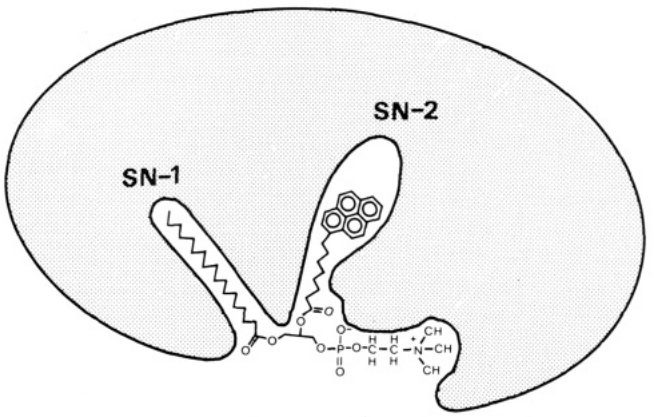

FIGURE 5: Schematic model for the lipid binding site of PC-TP. *sn*-1 and *sn*-2 refer to the corresponding acyl residues of PC.

We emphasize that what is depicted as space in the binding sites should be taken as a measure of conformational flexibility. In addition, we cannot exclude that a good accommodation of the pyrene moiety in certain regions of the *sn*-2 binding site is due to an energetically favorable interaction with some aromatic amino acid side chains in a fashion analogous to the stabilization of protein tertiary structure through aromatic–aromatic interactions (Burley & Petsko, 1985). Aromatic residues have been shown to be present in the *sn*-2 acyl chain binding site of PC-TP (Westerman et al., 1983b).

Previous time-resolved fluorescence measurements employing parinaroyl-PC species have been interpreted to indicate that the long axes of the two binding sites make an angle of 60–90° (Berkhout et al., 1984). We have recently found that PC species containing pyrenyl acyl chains of variable length at both the *sn*-1 and *sn*-2 position (nine different lipids were studied) do not show any detectable excimer formation when bound to PC-TP (P. J. Somerharju, unpublished results). This confirms the spatial separation of the binding sites.

Biological Implications. A majority of mammalian PC species contain saturated fatty acyl residue in the *sn*-1 position (Montfoort et al., 1971). The variety of saturated residues in mammalian phospholipids is quite limited. For instance, in rat liver, palmitic and stearic acids represent the bulk of the saturated chains (Patton et al., 1982). As shown in Figure 3B, the *sn*-1 acyl binding site of PC-TP has the highest affinity for a palmitoyl chain.

In contrast to the *sn*-1 position, most of the acyl residues found in the *sn*-2 position are unsaturated (Holub & Kuksis, 1978), polyunsaturated C(20:4) and C(22:6) residues being the most common ones in rat liver (Patton et al., 1982) and many other tissues. Recent theoretical studies suggest that the double-bond region of the C(22:6) acyl chain (and probably also other polyunsaturated chains) adopts a helical or an angle iron-shaped conformation, which makes the chain much shorter and bulkier than a saturated chain of the same number of carbon atoms (Stubbs & Smith, 1984; Applegate & Glomset, 1986). Therefore, the shape and dimensions of these natural fatty acids may not be too different from those of the Pyr(10) and Pyr(12) residues, the pyrenyl chains with the highest affinity for the *sn*-2 acyl binding site.

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Registry No. Pyr(6)C(16)PC, 110419-98-2; Pyr(8)C(16)PC, 110419-99-3; Pyr(10)C(16)PC, 110420-00-3; Pyr(12)C(16)PC, 110420-01-4; Pyr(14)C(16)PC, 110420-02-5; C(16)Pyr(6)PC, 103625-33-8; C(16)Pyr(8)PC, 110420-03-6; C(16)Pyr(10)PC, 95864-17-8; C(16)Pyr(12)PC, 110420-04-7; C(16)Pyr(14)PC, 110420-05-8; Pyr(10)C(10)PC, 110420-06-9; Pyr(10)C(12)PC, 110420-07-0; Pyr(10)C(14)PC, 110420-08-1; Pyr(10)C(18)PC, 110420-09-2; Pyr(10)C(20)PC, 110420-10-5; C(10)Pyr(10)PC, 110420-11-6; C(12)Pyr(10)PC, 110420-12-7; C(14)Pyr(10)PC, 110420-13-8; C(18)Pyr(10)PC, 110420-14-9; C(20)Pyr(10)PC, 110420-15-0.

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Thiophilic Adsorption: A Comparison of Model Protein Behavior†

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ABSTRACT: A newly recognized type of protein–ligand interaction phenomenon has resulted in the preparation of simple, nonionic, and highly specific gel derivatives for selective adsorption chromatography. The essential structure of the immobilized ligand can be represented as agarose–$CH_2CH_2SO_2CH_2CH_2SCH_2CH_2OH$, which was prepared by using mercaptoethanol to derivatize [0.9–1.0 mmol (g of dry gel)$^{-1}$] divinyl sulfone activated agarose (thiophilic or T-gel). Proteins interacting with this ligand are provisionally termed "thiophilic" to recognize their affinity for the definitive sulfone–thioether constituents. To better understand the experimental variables affecting adsorption efficiency and selectivity, several well-characterized proteins with diverse physicochemical features have been evaluated for thiophilic properties. Thiophilic interaction chromatography was investigated as a function of pH as well as the type and concentration of water-structure-forming salts required to promote adsorption. The model proteins characterized varied distinctly in their individual thiophilic affinities. At acidic pH values, a salt-independent adsorption process was observed. Furthermore, a minimum in the salt-promoted thiophilic adsorption tendency at pH 5–6 was found, with varying magnitude, for each of the model proteins evaluated. Recovery of adsorbed proteins routinely varied from 90% to 100%. There does not appear as yet to be any easily recognized physicochemical property associated with either thiophilic or nonthiophilic behavior. These results suggest that thiophilic interaction chromatography is a process that utilizes a previously unrecognized protein–ligand interaction mechanism. We suggest that salt allows the protein into close proximity with the sulfone–thioether group where short-range forces are effective. An electron donor–acceptor or proton-transfer mechanism may be involved. The demonstrated potential for modification of experimental conditions to vary T-gel selectivity makes thiophilic adsorption a powerful new alternative for the noncovalent immobilization and purification of biologically significant macromolecules.

Important molecular (ligand–ligate) interactions can occur entirely in free solution, but in biology these interactions often include interfacial recognition events involving immobilized ligands or acceptor sites which constitute the essence of cellular and subcellular infrastructure. For proteins in an aqueous environment, organized water structure is a characteristic of the protein–solvent interface and may be considered an imporant extension of the protein's molecular surface properties. Indeed, discussions of both the thermodynamic and kinetic limitations of protein structural dynamics (Karplus & McCammon, 1983) as well as the solvent accessibility of individual functional groups (Eisenberg, 1984) are focused (in

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