Affinity of Phosphatidylcholine Molecular Species for the Bovine Phosphatidylcholine and Phosphatidylinositol Transfer Proteins. Properties of the *sn*-1 and *sn*-2 Acyl Binding Sites†

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ABSTRACT: Both the phosphatidylcholine transfer protein (PC-TP) and the phosphatidylinositol transfer protein (PI-TP) act as carriers of phosphatidylcholine (PC) molecules between membranes. To study the structure of the acyl binding sites of these proteins, the affinity of 32 distinct natural and related PC molecular species was determined by using a previously developed fluorometric competition assay. Marked differences in affinity between species were observed with both proteins. Affinity vs lipid hydrophobicity (determined by reverse-phase HPLC) plots displayed a well-defined maximum indicating that the acyl chain hydrophobicity is an important determinant of binding of a phospholipid molecule by these transfer proteins. However, besides the overall lipid hydrophobicity, steric properties of the individual acyl chains contribute considerably to the affinity, and PC-TP and PI-TP respond differently to modifications of the acyl chain structure. The affinity of PC-TP increased steadily with increasing unsaturation of the *sn*-2 acyl moiety, resulting in high affinity for species containing four and six double bonds in the *sn*-2 chain, whereas the affinity of PI-TP first increased up to two to three double bonds and then declined. These data, as well as the distinct effects of *sn*-2 chain double bond position and bromination, indicate that the *sn*-2 acyl chain binding sites of the two proteins are structurally quite different. The *sn*-1 acyl binding sites are dissimilar as well, since variation of the length of saturated *sn*-1 chain affected the affinity differently. The data are discussed in terms of the structural organization of the *sn*-1 and *sn*-2 acyl binding sites of PC-TP and PI-TP. The major physiological implication of the present study is that in vivo all the major mammalian PC species should be transferred by PC-TP and PI-TP albeit at greatly variable rates. This conclusion may be extended also to the molecular species of PI, since the acyl chains of PC and PI probably share common binding sites in PI-TP [van Paridon, P. A., Gadella, T. W. J., Somerharju, P. J., & Wirtz, K. W. A. (1988) *Biochemistry* 27, 6208-6214].

The phosphatidylcholine-specific transfer protein (PC-TP)[1] from bovine liver and PI-TP from bovine brain are two of the phospholipid transfer proteins whose mode of action has been investigated most extensively (Helmkamp, 1985; Wirtz et al., 1986; Wirtz & Gadella, 1990). Both proteins function as carriers, but structural analyses have failed to demonstrate any significant sequence homologies between PC-TP and PI-TP (Dickeson et al., 1989). Bovine PI-TP has a distinct preference for PI, but also transfers PC (Helmkamp et al., 1974; DiCorleto et al., 1979). Competition experiments showed that bovine PI-TP has a 16-fold higher affinity for PI than for PC (Van Paridon et al., 1987a). A similar order of affinity has been recently found for PI-TP from yeast (Szolderits et al., 1989).

A number of investigations have dealt with the influence of the acyl chain structure of PC on the transfer activity of PC-TP and PI-TP (Kamp et al., 1978; Welti & Helmkamp, 1984; Kasper & Helmkamp, 1981; Zborowski & Demel, 1982; Kuypers et al., 1986; van Loon et al., 1986). These studies have demonstrated that the structure of the acyl chains can have a marked influence on the rate of PC transfer. However, the number of molecular species tested is too limited to allow one to obtain a coherent picture of the interactions of natural PC species with PC-TP and PI-TP.

Recent studies have provided clear evidence that bovine PC-TP and PI-TP have distinct binding sites for the *sn*-1 and *sn*-2 acyl chains (Berkhout et al., 1984; Van Loon et al., 1986; Somerharju et al., 1987; Van Paridon et al., 1988). Systematic probing of the acyl binding sites with PC analogues containing a pyrenylacyl or an unlabeled saturated chain of variable length demonstrated that the *sn*-1 and -2 sites of both proteins are distinct from each other as well from the corresponding site in the other protein (Somerharju et al., 1987; Van Paridon et al., 1988). Although these data suggested that PC-TP and PI-TP could have different specificities toward natural PC

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[1] Abbreviations: EDTA, ethylenediaminetetraacetic acid; HPLC, high-performance liquid chromatography; PI, phosphatidylinositol; PI-TP, phosphatidylinositol transfer protein; PC, phosphatidylcholine; PC-TP, phosphatidylcholine transfer protein; Pyr_8PC, 1-palmitoyl-2-(1-pyrenyl)octanoyl-*sn*-3-glycerophosphocholine; $Pyr_{10}PC$, 1-palmitoyl-2-(1-pyrenyl)decanoyl-*sn*-3-glycerophosphocholine; TNP-PE, 2,4,6-trinitrophenylphosphatidylethanolamine; Tris, tris(hydroxymethyl)aminomethane; 12:0, dodecanoyl; 14:0, myristoyl; 15:0, pentadecanoyl; 16:0, palmitoyl; 17:0, heptadecanoyl; 17:1, 10-heptadecenoyl; 18:0, stearoyl; 18:1(6,7), 6-octadecenoyl; 18:1(9,10), 9-octadecenoyl; 18:1(11,12), 11-octadecenoyl; 18:2, 9,12-octadecadienoyl; 18:3, 9,12,15-octadecatrienoyl; 18:4(cPnA), *c*9,*t*11,*t*13,*c*15-octadecatetraenoyl; 18:4(tPnA), *t*9,*t*11,*t*13,*t*15-octadecatetraenoyl; 20:4, 5,8,11,14-eicosatetraenoyl; 22:6, 4,7,10,13,16,19-docosahexaenoyl; $18(6,7Br_2)$, 6,7-dibromostearoyl; $18(9,10Br_2)$, 9,10-dibromostearoyl; $18(11,12Br_2)$, 11,12-dibromostearoyl.

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species, this as well as the actual nature of such specificities remained obscure.

In the present study, we have determined the relative affinity of PC-TP and PI-TP toward a variety of natural and related PC species by employing a competition assay developed previously (Somerharju et al., 1987; van Paridon et al., 1987a). The major results are that (1) both the hydrophobicity and sterical properties of the individual acyl chains contribute to the affinity, (2) the affinity vs structure correlation is markedly different for the two proteins supporting distinct binding site structures, and (3) these proteins are capable of transferring all major mammalian PC species.

Experimental Procedures

Lipids. Natural fatty acids were purchased from Nu Chek (Elysian, MN), *cis*-parinaric acid was from Molecular Probes (Eugene, OR), and *trans*-parinaric acid was prepared from *cis*-parinaric acid (Sklar et al., 1977). The synthesis and purification of the parinaroyl and pyrenyl phospholipids have been described previously (Somerharju et al., 1981, 1987). Unlabeled phospholipids either were synthesized according to the method described by Mason et al. (1981), were obtained from Sigma, Avanti Polar Lipids (Birmingham, AL), or were provided by Prof. F. Paltauf (Technical University of Gratz, Austria). The origin of the lipids is indicated in Table I. Brominated phosphatidylcholines were prepared according to Dawidowicz and Rothman (1976) and purified by HPLC on silica gel with $CHCl_3$/MeOH/water (5:4:1 v/v) as the solvent. *N*-Trinitrophenylphosphatidylethanolamine (TNP-PE) was obtained from Avanti. The purity of each lipid, assessed by TLC on silica gel using $CHCl_3$/methanol/25% ammonia/water (90:55:5.5:5.5 v/v) as the solvent, was estimated to be higher than 97%. The positional distribution of the fatty acyl residues was not determined, but the method of synthesis used typically produces lipids with a high (>97%) positional purity (Mason et al., 1981).

Proteins. Phosphatidylcholine-specific transfer protein (PC-TP) and the phosphatidylinositol transfer protein (PI-TP) were purified from bovine liver and brain, respectively, according to published methods (Westerman et al., 1983a; van Paridon et al., 1987b). The proteins were stored in a buffer containing 50% glycerol at <−20 °C.

Determination of Phospholipid Hydrophobicity. The relative hydrophobicity of each PC molecular species was determined by reverse-phase chromatography using an Ultrasphere octadecylsilica column (Beckman). The lipids were eluted with methanol/acetonitrile/water (90:3:4 v/v) containing 20 mM choline chloride at a speed of 0.4 mL/min and detected by absorbance at 210 nm (Patton et al., 1982). $Pyr_{10}PC$ was cochromatographed with each lipid as a retention time reference and was detected with a fluorescence detector.

Fluorescence Measurements. All fluorescence measurements were carried out with a Hitachi F-4000 spectrofluorometer equipped with a thermostated cuvette holder. Normally the excitation and emission wavelengths were 344 and 385 nm and the slits 1.5 and 20 nm, respectively, but for donors containing parinaroyl species, the emission wavelength was set to 378 nm and the slit to 3 nm. All measurements were carried out at 37 °C.

Determination of the Relative Binding Constants. This was accomplished by using the competition assay developed previously (Somerharju et al., 1987; van Paridon et al., 1987a). First, vesicles were prepared by injecting the lipids dissolved in ethanol (Batzri & Korn, 1973) into 2 mL of a Tris-HCl/NaCl/EDTA (20:100:1 mM, pH 7.4) buffer at 37 °C. The vesicles consisted of 1 nmol of Pyr_8PC (PC-TP) or $Pyr_{10}PC$ (PI-TP), an unlabeled PC species (0–0.8 nmol in the case of PC-TP, 0–1.3 nmol in the case of PI-TP; eight different concentrations), and 10 mol % TNP-PE. After equilibration for 1 min, the quenched vesicles were titrated by adding three 10-μL aliquots (0.02–0.05 nmol) of the transfer protein solution diluted 5–10-fold from the stock. The fluorescence intensity was measured after each addition and plotted as a function of the amount of protein added. The increase in pyrene monomer fluorescence per unit of transfer protein (F), which is proportional to the amount of pyrenyl-PC bound, was then plotted against the unlabeled PC to pyrenyl-PC ratio (R). The binding affinity of the transfer protein for the unlabeled PC species relative to pyrenyl-PC (K_{rel}) was obtained by parameter fitting using the equation:

$$F = F_{max}/(1 + K_{rel}R)$$

where F_{max} is the increase of monomer fluorescence in the absence of the competing (unlabeled) PC species (Van Paridon et al., 1987a).

To exclude that the observed differences in K_{rel} values would be due to differences in transbilayer distribution of the species rather than in their affinity for the protein binding site, K_{rel} was determined for a limited number of species under conditions where labeled and unlabeled lipids were (initially) in separate vesicles. Variable amounts (0–0.8 nmol) of the unlabeled lipid vesicles (containing 10 mol % TNP-PE) were mixed with the pyrenyl-PC (1 nmol) vesicles (also containing 10 mol % TNP-PE) and PC-TP (0.04 nmol) and the mixtures incubated for 10 min at 37 °C in the dark. The intensity of pyrenyl fluorescence was then measured, and K_{rel} values were determined as described above.

Results

The Affinity Assay. The relative affinities of a number of natural and related PC species have been determined by employing the competition assay developed previously (Somerharju et al., 1987; van Paridon et al., 1987a). This assay involves (1) titration of quenched vesicles containing pyrene-labeled PC and an unlabeled, competing PC species in a variable ratio with a transfer protein, (2) determination of the increase of the pyrenyl monomer fluorescence (F) as a function of added protein for each ratio, and (3) estimation of the unlabeled to pyrenyl lipid affinity ratio (K_{rel}) from a plot of the slope vs the unlabeled to pyrenyl lipid ratio (R) by a curve-fitting procedure.

Figure 1 displays examples of how addition of increasing amounts of an unlabeled PC species to quenched Pyr_8PC vesicles influences the pyrenyl monomer fluorescence intensity upon addition of PC-TP. Incorporation of the 16:0/22:6 or 17:1/17:1 species diminishes the pyrenyl lipid fluorescence in an efficient (40 and 60% reduction at 10 mol %, respectively) and concentration-dependent manner, indicating that these lipids have a high affinity for the lipid binding site of PC-TP. K_{rel} values of 10.5 ± 0.85 and 15.5 ± 1.13 were obtained for the 16:0/22:6 and 17:1/17:1 species, respectively. In contrast, addition of 18:0/18:0-PC to the Pyr_8PC vesicles had much less influence on the fluorescence intensity, suggesting that this lipid has a low affinity for the lipid binding site of PC-TP (K_{rel} = 0.9 ± 0.06).

The shape of the plot obtained for 18:0/18:0-PC appears anomalous, as there is little, if any, change in the slope at low R values (<0.2), whereas a moderate decrease is observed at higher values. Similar behavior was observed for the other high-melting (i.e., 16:0/18:0 and 17:0/17:0) species (not shown). It is likely that this decrease not only is due to competition for the lipid binding site by these species but also

Table I: Relative Binding Constants of Phosphatidylcholine Species for PC-TP and PI-TP

PC species	source[a]	hydrophobicity,[b] log (*RRT*)	PC-TP K_{rel}[c]	SD	*n*	K_{rel}[d]	PI-TP K_{rel}[e]	SD	*n*
12:0/12:0	a	0.363	1.1	0.30	4	0.9	1.69	0.17	3
14:0/14:0	a	0.794	2.9	0.58	4	2.3	5.67	0.66	4
14:0/16:0	a	0.998	2.5	0.41	4		4.44	0.40	2
14:0/18:0	a	1.199	1.7	0.11	4		1.69	0.13	2
14:0/18:1	a	1.013	3.8	1.05	4		3.29	0.24	2
15:0/15:0	s	0.995	3.7	0.69	4		7.12	0.38	4
16:0/16:0	s	1.186	3.0	0.49	4		5.25	0.30	2
16:0/18:0	s	1.386	1.6	0.29	4		1.05	0.13	2
16:0/18:1(6,7)	p	1.255	5.8	0.57	4		1.80	0.03	2
16:0/18:1(9,10)	p	1.219	4.3	0.55	5		1.61	0.28	2
16:0/18:1(11,12)	p	1.202	3.9	0.47	4		2.44	0.21	2
16:0/18:2	p	1.066	7.5	1.47	4		4.54	0.17	2
16:0/18:3	p	0.943	6.1	2.15	4		4.11	0.00	2
16:0/18:4(cPnA)	p	0.925	0.6	0.18	2		0.64	0.07	2
16:0/18:4(tPnA)	p	0.987	0.5	0.06	2		0.40		1
16:0/20:4	p	1.031	9.3	0.51	4		2.40	0.61	2
16:0/22:6	p	1.000	10.5	0.85	4	15.0	0.94	0.15	4
17:0/17:0	s	1.386	1.7	0.17	5		1.90	0.22	4
17:1/17:1	p	1.025	15.5	1.13	3	20.8	9.22	0.47	3
18:0/18:0	s	1.583	0.9	0.06	4		0.71	0.46	4
18:0/18:1	s	1.402	1.6	0.39	4		0.61	0.00	2
18:0/18:2	s	1.273	3.6	0.24	4		2.95	0.83	4
18:0/18:3	d	1.135	3.0	0.65	4		2.92	0.29	2
18:0/20:4	d	1.271	3.6	0.06	2		1.28	0.09	2
18:0/22:6	d	1.200	4.3	0.03	2		0.55	0.02	2
18:1/18:1	s	1.242	5.4	0.21	4		2.60	0.11	3
18:2/18:2	s	0.941	11.7	0.85	4		8.09	1.38	5
18:4/18:4(cPnA)	p	0.647	1.5	0.48	2		0.63	0.12	2
18:4/18:4(tPnA)	p	0.769	0.8	0.44	2		0.48		1
16:0/18(6,7Br_2)	p	1.288	1.5	0.30	4		1.19	0.14	2
16:0/18(9,10Br_2)	p	1.242	1.2	0.32	4	0.7	2.28	0.17	2
16:0/18(11,12Br_2)	p	1.211	2.0	0.34	4		2.06	0.12	2

[a] Source of species: a, Avanti Polar Lipids; s, Sigma Chemical Co.; d, gift from Prof. Paltauf; p, synthesized. [b] Hydrophobicity was calculated from the retention time on octadecylsilica and normalized against the value obtained for 16:0/22:6-PC. [c] Affinity relative to Pyr_8PC. [d] Affinity relative to Pyr_8PC when the labeled and unlabeled lipids were in separate vesicles. [e] Affinity relative to $Pyr_{10}PC$. *n* is the number of experiments; SD is standard deviation.

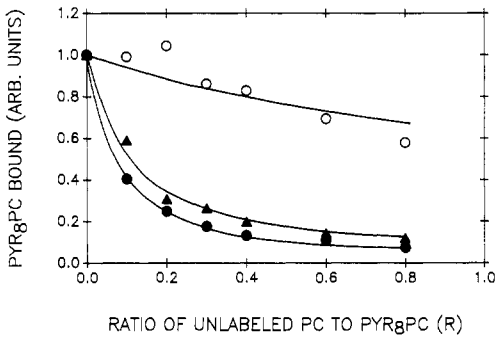

FIGURE 1: Influence of unlabeled PC species on the binding of Pyr_8PC to PC-TP. Vesicles consisting of 1 nmol of Pyr_8PC: 0–1.8 nmol of 18:0/18:0-PC (○), 17:1/17:1-PC (●), or 16:0/22:6-PC (▲); and 10 mol % of TNP-PE were titrated with PC-TP. The binding of Pyr_8PC, considered equal to the increase of monomer fluorescence intensity per unit of protein, was plotted as a function of the molar ratio (*R*) of the unlabeled PC species to Pyr_8PC. The curves represent best fits to the data (see Experimental Procedures).

results from association of the protein with the vesicle surface, where the bound probe is quenched by proximal TNP-PE molecules. Such association could occur at boundaries between coexisting lipid phases, which seem to exist in bilayers consisting of a pyrenyl-PC and a saturated PC below the phase transition temperature of the latter (Somerharju et al., 1985). Due to this artifact, the K_{rel} values obtained for the high-melting disaturated species should be considered as the maximal values.

Affinities of PC Molecular Species for PC-TP and PI-TP. The K_{rel} values for a variety of PC species are given in Table I. It is obvious that there are large differences in the relative affinity between species with both proteins. In the case of PC-TP, high affinities were found for the 17:1/17:1, 18:2/18:2, 16:0/22:6, and 16:0/20:4 species, whereas the 18:0/18:0, 17:0/17:0, 16:0/18:0, and 12:0/12:0 as well some species containing a brominated or a parinaroyl acyl chain(s) had low affinities. PI-TP displayed high affinity toward the 17:1/17:1, 18:2/18:2, and 15:0/15:0 species, but in contrast to PC-TP, 16:0/20:4 and especially 16:0/22:6 were poorly bound by this protein. Similarly to PC-TP, PI-TP had low affinity for the species containing parinaroyl residues (Table I). To determine the major factors contributing to the affinity, K_{rel} values were plotted as a function of several parameters including lipid hydrophobicity and the length and/or unsaturation of the individual (*sn*-1 or -2) acyl chains.

Influence of Lipid Hydrophobicity. This was investigated by plotting the K_{rel} value of each PC species vs its apparent hydrophobicity determined by reverse-phase chromatography (Massey et al., 1984). From the plots obtained for PC-TP (Figure 2A), one can see that the species with the highest affinities (species 1–6: 17:1/17:1, 18:2/18:2, 16:0/22:6, 16:0/20:4, 16:0/18:2, and 16:0/18:3) fall in a narrow hydrophobicity range (0.94–1.07). In agreement with this notion, the data obtained for the dihomosaturated species (species 8–13) show that 15:0/15:0-PC (species 10) with an apparent hydrophobicity of 0.995 has the highest affinity and that either a decrease or an increase in hydrophobicity (i.e., species 8 and 9 and species 11–13, respectively) leads to diminished binding. This strongly suggests that a certain hydrophobicity is required for optimal binding to PC-TP. It is, however, important to note that in this narrow range of optimal hydrophobicity K_{rel} varies from 15.5 for 17:1/17:1-PC to 3.7 for 15:0/15:0-PC to

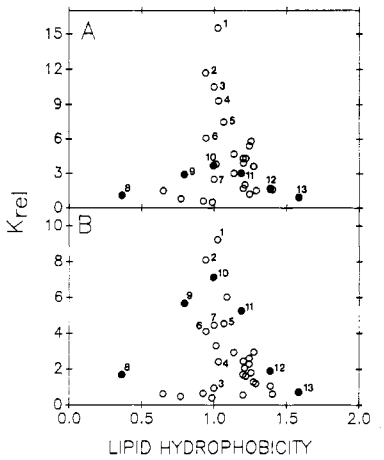

FIGURE 2: Relative binding constants (K_{rel}) of PC-TP (A) and of PI-TP (B) for the various molecular species of PC plotted as a function of lipid hydrophobicity. The numbered species are (1) 17:1/17:1-PC, (2) 18:2/18:2-PC, (3) 16:0/22:6-PC, (4) 16:0/20:4-PC, (5) 16:0/18:2-PC, (6) 16:0/18:3-PC, (7) 14:0/16:0-PC, (8) 12:0/12:0-PC, (9) 14:0/14:0-PC, (10) 15:0/15:0-PC, (11) 16:0/16:0-PC, (12) 17:0/17:0-PC, and (13) 18:0/18:0-PC. Homodisaturated species (●) and the remaining species of Table I (○). Error bars have been omitted for clarity; see Table I for SD values. For further details, see Experimental Procedures.

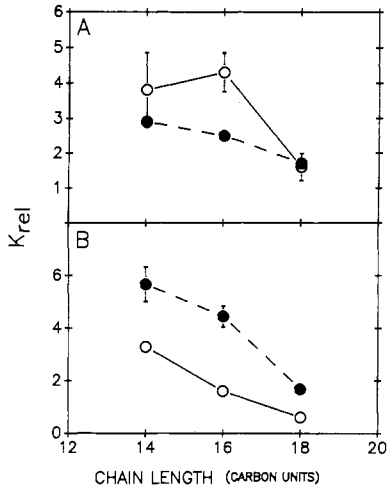

FIGURE 3: Effect of the length of a saturated *sn*-1 or -2 chain on the affinity of PC-TP (A) and PI-TP (B). K_{rel} values are plotted for the PC species containing a saturated chain of indicated length in the *sn*-1 position (○) or *sn*-2 position (●). The 18:1(9,10) or 14:0 chain is present in the *sn*-2 or *sn*-1 position, respectively. When error bars are not visible, they are smaller than the symbols.

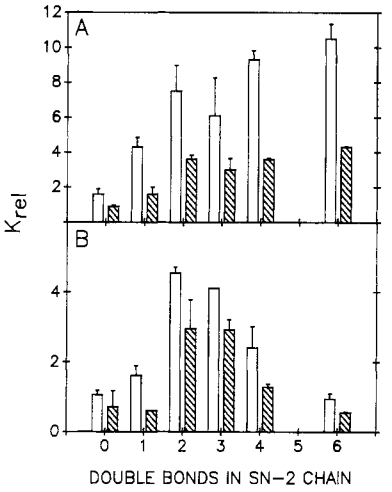

FIGURE 4: Influence of *sn*-2 acyl chain unsaturation on the affinity of PC-TP (A) and PI-TP (B). K_{rel} values are plotted for PC species containing an increasing number of double bonds in the *sn*-2 acyl chain. This acyl chain has a length of 18 C atoms for the species with 0, 1, 2, and 3 double bonds, and a length of 20 C atoms and of 22 C atoms for the species with four and six double bonds, respectively. The *sn*-1 acyl chain is either 16:0 (open bars) or 18:0 (hatched bars).

even lower values for species like 14:0/16:0 (species 7), 16:0/18:4(cPnA), and 16:0/18:0(tPnA) (Table I). This clearly indicates that besides hydrophobicity, other, most likely sterical, factors contribute markedly to the affinity.

Similar to what was observed for PC-TP, the K_{rel} vs hydrophobicity plots obtained for PI-TP yielded a distinct maximum close to the apparent hydrophobicity value of 1. The 17:1/17:1, 18:2/18:2, and 15:0/15:0 PC species displayed the highest affinities in this case (Figure 2B). In contrast to PC-TP, high K_{rel} values were found for the disaturated species in relation to the unsaturated ones, suggesting more favorable interaction of saturated acyl chains with the corresponding binding sites as compared to unsaturated chains of similar hydrophobicity.

Length of a Saturated sn-1 or -2 Chain. PC species with the 14:0, 16:0, and 18:0 chain in the *sn*-1-position (18:1 in the *sn*-2 position) or in the *sn*-2 position (14:0 in the *sn*-1 position) were used to test the effect of the length of a saturated acyl chain on the affinity. With PC-TP, there is an optimum for the 16:0 chain in the *sn*-1 position while the affinity decreases monotonously with increasing length of the *sn*-2 chain (Figure 3A). With PI-TP, the affinity decreases more strongly with increasing chain length for both sets of species, suggesting that both the *sn*-1 and -2 acyl binding sites are less hydrophobic than the corresponding sites of PC-TP.

Unsaturation of the sn-2 Chain. The number of double bonds in the *sn*-2 acyl chain has a major effect on the affinity of the PC species (Figure 4). For the species containing a *sn*-1 palmitoyl chain, it is found that with PC-TP the affinity increases steadily with unsaturation up to the 22:6 chain (Figure 4A). The same trend was observed for the series of species containing a *sn*-1 stearoyl chain. With PI-TP, the affinity of both sets of species increases markedly with unsaturation up to the 18:3 chain whereafter it declines strongly with species carrying a 20:4 and 22:6 chain (Figure 4B). The large difference between the two proteins in the affinity for the species carrying a 20:4 and 22:6 chain is notable and may relate to differences in the volume of the *sn*-2 acyl binding sites.

The Postion of a Single Double Bond and Bromines in the sn-2 Chain. To obtain further information on the properties of the *sn*-2 chain binding site of the proteins, we tested the effect of the position of a single double bond in an 18-carbon chain on the affinity as well the effect of bromination of this double bond (Figure 5). In the case of PC-TP (Figure 5A), the affinity is highest when the double bond is located between carbons 6 and 7 and decreases gradually when it is shifted toward the methyl end of the chain. Bromination of the 6,7 and 9,10 double bonds causes a remarkable (4-fold) decrease of affinity, while bromination of the 11,12 double bond results in somewhat smaller (2-fold) decrease.

Also with PI-TP (Figure 5B) the position of the double bond influences the affinity somewhat, but now there is no clear trend to be observed. Quite distinctly from PC-TP, bromination of the double bond either decreases (positions 6,7 and 11,12) or increases (position 9,10) the affinity, and the effects are much smaller than with PC-TP.

Effect of Transbilayer Distribution on K_{rel}. To study whether the obtained K_{rel} values are biased by dissimilar transbilayer distributions of the unlabeled PC species, a limited number of titrations were carried out with the pyrenyl lipid (Pyr_8PC) and the competing unlabeled species 12:0/12:0,

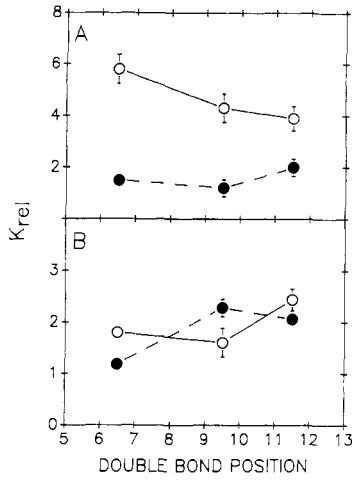

FIGURE 5: Effect of double bond position and bromination of the *sn*-2 acyl chain on the affinity of PC-TP (A) and PI-TP (B). K_{rel} values are plotted for PC species containing an *sn*-2 18-carbon acyl chain with a double bond (○) or bromines (●) in positions 6,7; 9,10; or 11,12. The chain in the *sn*-1 position was 16:0 in each case.

14:0/14:0, 16:0/22:6, and 16:0/18(9,10$_{Br}$) being (originally) in separate vesicles. The K_{rel} values thus obtained for PC-TP are similar to the ones found for the mixed vesicles (Table I), and thus differences in the transbilayer distribution of the species cannot be responsible for the observed major differences in K_{rel}.

Discussion

In this study, we have determined the relative affinities of 32 natural and related PC species for bovine PC-TP and PI-TP (see Table I). Species were selected according to their potential to provide information on (i) the importance of the hydrophobicity of the phospholipid molecule on the affinity, (ii) the structural properties of the *sn*-1 and -2 acyl binding sites, and (iii) the question whether these proteins bind preferentially to particular natural PC species, which could provide information on the physiological role of the transfer proteins.

Lipid Hydrophobicity. The data of Figure 2 show that the PC species displaying the highest affinities for PC-TP and PI-TP fall into a fairly narrow hydrophobicity range, indicating the importance of hydrophobic interactions. The optimal binding of species with acyl chains of medium hydrophobicity, which is most evident from the data obtained for the homodisaturates, strongly suggests that the affinity is critically dependent on the hydrophobicity of the lipid acyl chains matching that of the corresponding binding sites of the protein. Accordingly, the low affinity of short-chain lipids like 12:0/12:0-PC (species 8, Figure 2) could be due to the failure of the acyl chains of this lipid to provide enough hydrophobic surface for a stable association. Possibly some hydrophobic amino acid side chains at the binding site remain exposed as suggested by Welti and Helmkamp (1984), thus preventing the release of the lipid–protein complex. The low affinity of the long-chain disaturates, i.e., 17:0/17:0-PC (species 12) and 16:0/18:0- and 18:0/18:0-PC (species 13), on the other hand, would be due to that there is not enough hydrophobic surface available in acyl binding sites to compensate for the increased membrane affinity of these species.

As shown by Table I and Figure 4, addition of double bonds to a 18:0 chain (in either *sn*-1 or the *sn*-2 position) results in a considerable increase in the affinity both for PC-TP and for PI-TP. This can be explained by assuming that the double bonds weaken the hydrophobic interactions of an acyl chain within the membrane to a greater extent than those with the binding site in the protein. Addition of double bonds increases markedly both the molecular surface area (Stubbs & Smith, 1984) and the rate of spontaneous transfer of phospholipid molecules (Massey et al., 1982, 1984; Patton et al., 1985), suggesting that the hydrophobic interactions with the membrane are strongly reduced. On the other hand, addition of a double bond influences binding to protein sites in an unpredictable manner, since the identity and spatial arrangement of the amino acid side chains at the binding sites are not known. However, it seems likely that, unless critically located, addition of a double bond destabilizes less the interaction with the protein site than those with the membrane site(s), thus shifting the equilibrium toward the protein-bound state.

The lack of strict correlation between the lipid hydrophobicity and K_{rel} values (Figure 2; Table I) demonstrates that, besides hydrophobicity, other factors, such as steric constraints, determine the affinity of an acyl chain for its binding site in PC-TP and PI-TP. These factors will be discussed in the following.

The Acyl Binding Sites of PC-TP. Transfer studies with PC positional isomers (van Loon et al., 1986) as well as time-resolved fluorescence studies (Berkhout et al., 1984) originally suggested that PC-TP has separate, structurally distinct binding sites for the *sn*-1 and -2 chains of PC. The present data (Figure 3A) show that among saturated chains, 16:0 has the highest affinity for the *sn*-1 acyl binding site of PC-TP and there is a 2.7-fold decrease in K_{rel} upon replacement of the 16:0 chain with a 18:0 one (Table I). These findings confirm the previous results obtained with pyrenyl-PC species (Somerharju et al., 1987). The fact that the 18:1/18:1 and 18:2/18:2 species display higher affinities than the 16:0/18:1 and 16:0/18:2 species, respectively (Table I), indicates that limited hydrophobicity rather than the length of this site is responsible for the greatly reduced affinity of the 18:0 acyl chain as compared to the 16:0 one. However, also the volume of the *sn*-1 site is probably limited as indicated by the poor accommodation of the longer pyrenylacyl chains by this site (Somerharju et al., 1987).

The *sn*-2 binding site of PC-TP can obviously accommodate a variety of different acyl chains as indicated by Figure 2A and Table I. The data given in Figure 4 strongly suggest that the affinity for this site correlates positively with the number of double bonds. As discussed above, this is most likely due to that when the number of double bonds increases, the affinity of a chain for the membrane decreases more than its affinity for the site in the protein. However, the high affinities observed for the polyunsaturated 20:4 and 22:6 chains for PC-TP in contrast to PI-TP (Figure 4) indicates that also the volume of the chain contributes to the affinity. In line with this, the affinities of 16:0/Pyr$_{10}$- and 16:0/Pyr$_{12}$-PC species were found to be very similar to those observed for the 16:0/22:6 species (not shown). That the volume, rather than the length, of the 20:4 and 22:6 chains accounts for the high affinity is supported by the fact that in their energetically most favorable conformations these acyl chains probably have a much shorter effective length than indicated by the number of carbon atoms. This is due to the presence of several (chain-twisting) double bonds (Stubbs & Smith, 1984; Applegate & Glomset, 1986).

Concerning the shape of the *sn*-2 acyl binding site, it is of interest to note that bromination of the double bonds at positions 6,7 or 9,10 causes a strong reduction in affinity, while bromination of the 11,12 double bond has a smaller effect (Figure 5A). Thus, bromines, which are hydrophobic by nature and have a size comparable to that of a methyl group (McIntosh & Holloway, 1987), are not well tolerated in the

region corresponding to carbons 6–10 of the *sn*-2 acyl chain. This, as well as the low affinity of the Pyr_6 and Pyr_8 acyl chains (Somerharju et al., 1987), suggest a "bottle" shape for this site, the neck of the "bottle" extending to carbons 9–10. Alternatively, the low affinity of Pyr6 and Pyr8, as compared to the Pyr10 chain, could be due to bending of the binding site in the region corresponding to carbons 6–10 of the *sn*-2 acyl chain as has been found for the acyl binding site of a fatty acid binding protein (Sacchettini et al., 1988). The aromatic ring system of pyrene is essentially inflexible and would not thus fit well to a region where bending is required. The presence of a bend would explain the low affinity of species containing a *cis*- or *trans*-parinaroyl moiety in the *sn*-2 position (Table I), since also the chromophore of the parinaroyl moieties, extending from carbon 9 to carbon 16, represents an essentially rigid structure (Wolber & Hudson, 1981).

Properties of the Acyl Binding Sites of PI-TP. The *sn*-1 acyl binding sites of PI-TP have properties different from those of the corresponding site in PC-TP. First, it appears to be less hydrophobic, since the affinity decreases monotonously from the 14:0 to the 18:0 chain (Figure 3B), while PC-TP shows highest affinity for the 16:0 chain (Figure 3A). Supporting this, the previous pyrenyl lipid data showed that among saturated chains of 10–20 carbons the 12:0 one displayed the highest affinity (van Paridon et al., 1988). The 2-fold higher affinity of 18:2/18:2 as compared to 16:0/18:2 (Table I) indicates that limited hydrophobicity of this binding site rather than steric factors (limited length) is responsible for the poor binding of the medium and long saturated chains.

Also, the *sn*-2 sites of PI-TP have properties clearly different from those of PC-TP. Most remarkably, the 22:6 chain, which has high affinity for the *sn*-2 site of PC-TP, is poorly accommodated (Figure 4B). This as well as the relatively low affinity of the 20:4 chain indicates that the volume of this site is more limited than that of PC-TP. In line with this, the affinities of all the 16:0/Pyr_x-PC species were found to be over 10-fold lower than those of the best bound unlabeled species (not shown). Previously, a conspicuous reduction of affinity upon replacing a Pyr_8 chain with a Pyr_6 one was noted (van Paridon et al., 1988). This suggests a "bottle" shape for the *sn*-2 binding site of PI-TP, too. The two-carbon shift in the binding optimum (as compared to binding to PC-TP) implies that the "neck" is somewhat shorter for PI-TP, i.e., extends to carbon 7. Supporting this bromination of a 18:1 chain diminished the affinity for PI-TP when a 6,7 double bond was involved, but had little effect when the double bonds were at positions 9,10 or 11,12 (Figure 5B). On the other hand, the very low affinity of PI-TP for species carrying a parinaroyl chain (Table I) suggests that the *sn*-2 acyl binding site of PI-TP may have a curved shape as well.

In conclusion, both the *sn*-1 and the *sn*-2 acyl binding sites of bovine PC-TP and PI-TP seem to have quite distinct binding specificities. As yet, the molecular details responsible remain unresolved. However, it was reported recently that the primary structure of rat brain PI-TP, which most likely is very similar to that of bovine brain PI-TP, has no significant homology with the sequence of bovine PC-TP (Dickeson et al., 1989). Moreover, in contrast to PC-TP (Akeroyd et al., 1982; Westerman et al., 1983b), rat PI-TP lacked extended hydrophobic peptide segments. Thus, different evolutionary origins may be responsible for the distinct structural properties of the acyl binding sites of PC-TP and PI-TP.

As to other proteins containing fatty acyl binding sites, it is of interest to note that α-fetoprotein binds preferentially the polyunsaturated 20:4 and 22:6 chains, while serum albumin prefers saturated and monounsaturated fatty acids (Hsia et al., 1980, 1986). Thus, the acyl binding site(s) of these two proteins may be structurally similar to the *sn*-2 and -1 acyl binding sites of PC-TP, respectively.

Affinity versus the Rate of Transfer. Studies with pyrenyl phospholipids have demonstrated that, in general, there is a reasonable correlation between affinity and the rate of transfer between phospholipid vesicles (Somerharju et al., 1987; van Paridon et al., 1988). As to the natural species, only a limited amount of information is available for comparison with the present affinity data. Welti and Helmkamp (1984) found the following order for the rate of transfer by PC-TP: 18:1/18:1 > 18:2/18:2 > 17:1/17:1 > 14:0/14:0, which is different from the order of affinities as determined in the present study, 17:1/17:1 > 18:2/18:2 > 18:1/18:1 > 14:0/14:0 (Table I). The fact that PC-TP transferred 17:1/17:1-PC and 18:2/18:2-PC slower than 18:1/18:1-PC could result from a sluggish release of the former, high-affinity species from the protein. In support of this, the pyrenyl species with the highest affinity for PC-TP was transferred somewhat slower than PC species of lower affinity (Somerharju et al., 1987). This lack of correlation could also in part result from the fact that, unlike binding, the transfer is sensitive to factors influencing kinetics such as membrane fluidity (Helmkamp, 1980; Kasper & Helmkamp, 1981).

Kuypers et al. (1986) have investigated the influence of matrix (bulk) lipid species on the rate of PC-TP-mediated transfer of various PC species, present as a minor component, from PC/cholesterol vesicles to intact erythrocytes. They found that the rate of transfer of the 16:0/18:1 species decreased in the following order of matrix lipids: 16:0/16:0 > 16:0/18:1 = 16:0/18:2 > 16:0/20:4. Assuming that matrix lipid influenced the rate of transfer of the minor component by competing for the lipid binding site of the transfer protein, the order found by Kuypers et al. corresponds to the order of affinities found in the present study (Table I).

In a previous study, evidence was provided that the *sn*-2 (and possibly also the *sn*-1) chain of PC and PI binds to the same site in PI-TP (van Paridon et al., 1988). From this we infer that the order of affinities for the natural PI species is most likely very similar to that of found here for the natural PC species (Table I).

Physiological Implications. It is clear from the present data that PC-TP and PI-TP are able to bind and, consequently, also transfer all major mammalian PC species. However, estimation of the relative rates of protein-mediated transfer in vivo is not possible mainly due to lack of (1) a strict correlation between the affinity and the rate of transfer, (2) information on the relative abundances of the various PC species on the cytoplasmic surfaces of different organelle membranes as well as the rates of transbilayer movement, and (3) acyl chain dependent interactions of PC molecules with other membrane lipids and proteins. Yet some general predictions can be made. First, it seem feasible that the transfer of the minor species with low affinity (12:/12:0, 14:0/18:0, 16:0/18:0, and 18:0/18:1) is slow, since the probability that they would become bound to the protein should be low due to the competition by major species of much higher affinity (e.g., 16:0/18:2). Second, the lower affinity of the species containing the 18:0 chain in the *sn*-1 position as compared to those containing the 16:0 chain in that position (Figure 4) indicates that the former are, on the average, transported more slowly.

At this point, it remains to be established whether the different affinities of the various PC species are connected to their metabolic and/or functional properties. In this respect,

it is intriguing that PC-TP displays a high affinity for the PC species containing the 20:4 acyl chain in the *sn*-2 position (Figure 4A). Such species probably serve as a major source of arachidonic acid released upon stimuli by a phospholipase A_2 (Wijkander & Sundler, 1989). One of the functions of PC-TP could be the maintenance of adequate levels of these species at the site(s) of arachidonic acid release.

Since the acyl chains of PC and PI probably share common binding sites in PI-TP (see above), the data of Table I imply that among PI species, this protein should display the highest affinity toward the minor 16:0/18:2- and 18:2/18:2-PI species. Interestingly, these particular species are the metabolically most active ones according to the recent data of Nakagawa et al. (1989).

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Registry No. 12:0/12:0, 18194-25-7; 14:0/14:0, 18194-24-6; 14:0/16:0, 69525-80-0; 14:0/18:0, 76343-22-1; 14:0/18:1, 80863-06-5; 15:0/15:0, 3355-27-9; 16:0/16:0, 63-89-8; 16:0/18:0, 59403-51-9; 16:0/18:1(6,7), 128163-04-2; 16:0/18:1(9,10), 26853-31-6; 16:0/18:1(11,12), 128163-05-3; 16:0/18:2, 128114-92-1; 16:0/18:3, 128114-93-2; 16:0/18:4(cPnA), 83349-96-6; 16:0/18:4(tPnA), 82188-63-4; 16:0/20:4, 68883-73-8; 16:0/22:6, 83306-24-5; 17:0/17:0, 70897-27-7; 17:1/17:1, 128114-94-3; 18:0/18:0, 816-94-4; 18:0/18:1, 56421-10-4; 18:0/18:2, 27098-24-4; 18:0/18:3, 128114-95-4; 18:0/20:4, 128114-96-5; 18:0/22:6, 128134-51-0; 18:1/18:1, 4235-95-4; 18:2/18:2, 998-06-1; 18:4/18:4(cPnA), 88547-62-0; 18:4/18:4(tPnA), 90581-91-2; 16:0/18(6,7-Br_2), 81138-96-7; 16:0/18(9,10-Br_2), 81124-54-1; 16:0/18(11,12-Br_2), 96110-16-6.

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