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Partitioning Thermodynamics of Selected Endocrine Disruptors between Water and Synthetic Membrane Vesicles: Effects of Membrane Compositions

JUNG-HWAN KWON,‡
HOWARD M. LILJESTRAND,*
LYNN E. KATZ, HIROSHI YAMAMOTO†

Department of Civil, Architectural and Environmental Engineering, The University of Texas at Austin, 1 University Station C1786, Austin, Texas, 78712-0273, and Department of Mathematical and Natural Sciences, The University of Tokushima, 1-1 Minamijosanjima-cho, Tokushima, Japan, 770-8502

The thermodynamics of partitioning of selected endocrine disruptors between water and synthetic membrane vesicles were investigated. For most of the chemicals investigated, partitioning is dominated by the enthalpy change for unsaturated lipid membrane vesicles and by the entropy contribution for saturated lipid membrane vesicles. The contribution of the entropy terms in determining the free-energy change becomes more important compared with the enthalpy terms with increased branching of *p*-substituted phenols. These results suggest that the thermal energy required for and the entropy gain associated with the creation of the cavity in the lipid bilayer is of critical importance in differentiating the process from 1-octanol/water partitioning. In addition, partitioning thermodynamics are significantly influenced by cholesterol content in the lipid membranes. Results of the present study and those in the literature suggest that partitioning processes significantly depend on the physical state of the lipid membranes and log K_{OW}-based quantitative structure−activity relationships should be carefully applied for predicting bioconcentration by considering lipid compositions.

Introduction

It has been generally acknowledged that hydrophobic organic pollutants accumulate in lipid compartments of the body. For this reason, the bioconcentration of xenobiotic chemicals has been estimated from equilibrium partition coefficients between water and surrogate organic phases, such as 1-octanol/water partition coefficient (K_{OW}) (e.g., *1*−*3*). Although K_{OW} has been shown to be a good parameter for the estimation of biopartitioning especially for nonpolar organic chemicals, many researchers have indicated that the 1-octanol/water partition coefficient has only limited potential for estimating bioconcentration for a wide range of organic chemicals in phytoplanktons (*4*) and in fish (*5*). Moreover, recent studies have shown that K_{OW} for diverse estrogenic chemicals does not correlate well with the partition coefficient between water and lipid membrane vesicles (K_{lipw}), theoretically better surrogates for biological membranes (*6*,*7*).

Most of the quantitative structure−activity relationships (QSARs) between K_{OW} and bioconcentration factors (or K_{lipw}) rely on the assumption that the free-energy change of a solute transfer from water to 1-octanol is linearly related to that from water to biological lipid phases (*1*). However, Opperhuizen et al. (*5*) showed that the partitioning of chlorinated benzenes between water and fish is thermodynamically different from that between water and 1-octanol. Whereas the former is characterized by a positive enthalpy change and is driven by the entropy change, the latter is driven by a large negative enthalpy change. Similarly, Woodrow and Dorsey (*8*) reported that the free-energy of solute transfer is dominated by the entropy change for surfactant micelle/water partitioning, although the enthalpy change (ΔH) is slightly negative. However, van Wezel and Opperhuizen (*9*) showed that ΔH of partitioning between water and fish storage lipids for chlorobenzenes was negative and thus the partitioning process is driven by the enthalpy change, as is the case with K_{OW}. Because storage lipids do not form highly organized bilayers or micelles, it may be deduced that the higher organization of lipid molecules in biological membranes differentiates its partitioning thermodynamics from that in the water/lipid membranes system.

In spite of the large variation in body lipid components, bioconcentration factors (BCFs) in aquatic animals are typically normalized by the total lipid content (e.g., refs *10*, *11*). There have been a few studies that relate bioaccumulation with lipid composition (*12*, *13*). Ewald and Larsson (*13*) observed that lipid-normalized BCF is significantly lower in fish species containing a high phospholipid content using 2,2′,4,4′-tetrachlorobiphenyl as a model compound, indicating that the compound is more soluble in non-membrane lipids. However, there are large gaps between measured lipid-normalized BCFs even when bioconcentration is dominated by chemical equilibrium between water and biological organic phases of an organism. Fish have various types of fatty acids in their membranes. In addition, fatty acid composition varies significantly from one species to another as well as from one organ to another within an individual (*14*). Biopartitioning is also affected by temperature (e.g., refs *15*, *16*) and diet (e.g., ref *17*). Thus, a better characterization of the roles of fatty acid components in these membranes is needed for development of more reliable QSARs.

Consequently, we evaluated the enthalpy change (ΔH) and entropy change (ΔS) of partitioning between water and lipid membrane vesicles formed from two model phospholipids, dipalmitoylphosphatidylcholine (DPPC, C16:0, 16:0) and palmitoyloleoylphosphatidylcholine (POPC, C18:1, 16:0), which serve as a representative saturated and unsaturated lipid, respectively. Sixteen structurally diverse endocrine disrupting chemicals (EDCs) were investigated for evaluating the effects of saturation in the lipid tails. The effects of lipid components were further evaluated using additional liposomes comprised of dimyristoylphosphatidylcholine (DMPC), distearylphosphatidylcholine (DSPC), and dioleoylphosphatidylcholine (DOPC) for 17β-estradiol, diethylstilbestrol, bisphenol A, and *p*-*n*-nonylphenol. Finally, the effects of cholesterol, a common membrane strengthening agent, on the partitioning thermodynamics in saturated and unsaturated liposomes were investigated by varying cholesterol content.

* Corresponding author phone: 1-512-471-4604; e-mail: liljestrand@mail.utexas.edu.
† The University of Tokushima.
‡ Present address: Department of Environmental Toxicology (UTOX), Swiss Federal Institute of Aquatic Science and Technology(EAWAG), CH-8600 Dübendorf, Switzerland.

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Materials and Methods

Chemicals. Sixteen moderately hydrophobic EDCs were selected to investigate the thermodynamics of partitioning between water and synthetic lipid membrane vesicles, including natural hormones (17β-estradiol, estrone), synthetic estrogens (ethynylestradiol, diethylstilbestrol, mesohexestrol, and dienestrol), break-down products of alkylphenol ethoxylates (4-*n*-nonylphenol, 4-*tert*-octylphenol, 4-*sec*-butylphenol, and 4-*tert*-amylphenol) and industrial estrogenic compounds (bisphenol A, benzyl-4-hydroxybenzoate, butyl-4-hydroxybenzoate, 4-phenylphenol, dibutylphthalate, benzylbutylphthalate). Estrone (99%), 17α-ethynylestradiol (98%), diethylstilbestrol (99%), mesohexestrol (98%), dienestrol (98%) were purchased from Sigma Chemical Co. (St. Louis, MO). 17β-Estradiol (97%), 4-*tert*-octylphenol (97%), 4-*sec*-butylphenol (98%), 4-*tert*-amylphenol (99%), bisphenol A (97%), benzyl-4-hydroxybenzoate (99%), and benzylbutylphthalate (98%) were obtained from Aldrich Chemical Co. (Milwaukee, WI). 4-Phenylphenol (98%), dibutylphthalate (98%), and butyl-4-hydroxybenzoate (99%) were purchased from Fluka Chemical Co. (Milwaukee, WI). The 4-*n*-nonylphenol (99%) was purchased from Sigma-Aldrich Co. (St. Louis, MO). Dipalmytoylphosphatidylcholine (DPPC, C16:0, 16:0) and palmytoyloleoylphsphatidylcholine (POPC, C18:1, 16:0) were chosen as model saturated and unsaturated lipid membrane phases due to their abundance in biological membranes. Dimyristoylphosphatidylcholine (DMPC, C12:0, 12:0), distearylphosphatidylcholine (DSPC, C18:0, 18:0), and dioleoylphosphatidylcholine (DOPC, C18:1, 18:1) were also chosen for further investigation for 17β-estradiol, diethylstilbestrol, bisphenol A, and nonylphenol. DPPC and DOPC liposomes with varying cholesterol contents were used to evaluate the effects of cholesterol in lipid membranes on the thermodynamics. Chloroform solutions of model lipid components, DOPC, POPC, DMPC, DPPC, and DSPC were purchased from Avanti Polar Lipids (Albaster, AL). Cholesterol was purchased from Aldrich Chemical Co. (Milwaukee, WI).

Preparation of Liposome Suspensions. Large unilamella vesicle suspensions of selected lipid components were prepared using the thin film hydration technique (*18*) followed by rapid extrusion processes (*19*), as described previously (*7*). The chloroform solution of the lipid was evaporated under a gentle nitrogen stream and the thin residue film was dissolved in dilution water (buffered at pH 7.0 and ionic strength of 0.02 M, using KH_2PO_4, NaOH, and NaCl) to make the liposome suspension. The suspension was further extruded through a polycarbonate membrane several times to reduce vesicle polydiversity.

Determination of K_{lipw} Using Equilibrium Dialysis Technique. Partition coefficients of the selected chemicals were determined by the equilibrium dialysis technique described in the literature (*7*, *20*). Two 2 mL amber vials were separated by a dialysis membrane (Por7 MWCO 10 000, purchased from Spectrum Scientific, Rancho Dominguez, CA). EDCs were dissolved directly in water and the aqueous solution was filtered through glass membrane. Initial aqueous concentration of each chemical did not exceed half aqueous solubility. The solution containing the EDC was used to fill one cell and lipid vesicle suspension (concentration of lipid, *m*) was used to fill the other. Equilibrium partition coefficients were determined by analyzing the remaining aqueous EDC concentrations in the vial without lipid membrane vesicles in the sample reactors (C_w, mg/L) and both sides of the reference reactors (C_{ref}, mg/L) that initially contained buffer solution instead of lipid suspensions. Chemical analyses were performed after 14 day incubation (28 day incubation at 11 °C). Preliminary studies showed that apparent equilibrium was obtained after 14 days (28 days at 11 °C) using a custom-made tumbling device. The aqueous concentrations of the selected chemicals were measured using a Waters 2690 high-performance liquid chromatography system equipped with a Waters 996 photodiode array detector (Milford, MA). The partition coefficient (K_{lipw}) was calculated using

$$K_{lipw}\,(\mathrm{L/kg-lipid}) = \frac{C_{ref} - C_w}{C_w m} \tag{1}$$

Determination of Thermodynamics of a Solute Transfer. For the evaluation of partitioning thermodynamics, K_{lipw} values were obtained in a temperature-controlled room at 11, 22, 30, and 37 °C. Measured K_{lipw} values were converted to mole fraction partition coefficients ($\log K^{m}_{lipw}$) using molar density of water ($V_{water} = 55.5$ mol/L) and each lipid ($V_{lip,\,DPPC} = 1.36$ mol/kg and $V_{lip,\,POPC} = 1.32$ mol/kg) for the calculation of the enthalpy change (ΔH) and the entropy change (ΔS) of solute transfer from water to lipid membrane vesicles using the van't Hoff relationship:

$$\log K^{m}_{lipw} = \log K_{lipw} + \log\left(\frac{V_{water}}{V_{lip}}\right) \tag{2}$$

$$\log K^{m}_{lipw} = -\frac{\Delta H}{2.303R}\frac{1}{T} + \frac{\Delta S}{2.303R} \tag{3}$$

where R is gas constant (8.314 J/mol-K) and T is temperature (K).

Results and Discussion

Effects of Membrane Compositions. Table 1 shows K_{lipw} values between water and POPC vesicles ($K_{lipw,POPC}$) and between water and DPPC vesicles ($K_{lipw,DPPC}$) at 11, 22, 30, and 37 °C. For most of the EDCs investigated, $K_{lipw,POPC}$ decreases with increasing temperature whereas $K_{lipw,DPPC}$ increases with increasing temperature. The enthalpy change (ΔH) and entropy contribution ($T\Delta S$) at 22 °C in the partitioning process calculated using eq 3 are shown in Table 2 with r^2 values of the linear regressions. As can be seen from Table 2, solute transfer from water to POPC liposomes is generally driven by thermal energy gain (i.e., negative ΔH), whereas solute transfer from water to DPPC liposomes is driven by increased entropy (i.e., positive $T\Delta S$) for most EDCs. Entropy term dominates in determining $K_{lipw,\,POPC}$ for dibutylphthalate and benzylbutylphthalate and it is difficult to say that enthalpy term dominates in this process for many other EDCs due to relatively large standard errors. However, their entropy contribution in partitioning between water and DPPC liposomes is significantly greater than that between water and POPC liposomes for all EDCs except for 4-*tert*-octylphenol. There are negligible changes in enthalpy and entropy for 4-*tert*-octylphenol between the two model saturated and unsaturated lipids. Thus, partitioning into saturated liposomes requires more thermal energy, and the overall process is more likely to be driven by the entropy change compared with that into the unsaturated analogues. The mole fraction of 4-*tert*-octylphenol in lipid phases was very large (0.1∼0.4 in our experimental conditions), whereas this was below 0.1 for 4-*n*-nonylphenol and 0.01 for all other EDCs. At these relatively high concentrations, the infinite dilution assumption in the liposome phases may not be applicable, and alteration of the membrane fluidity is possible as sorbed non-ionic surfactants in lipid membranes may increase membrane fluidity (*21*).

Excluding 4-*tert*-octylphenol, the results are generally consistent with those reported in the literature, which show that partitioning into gel phase liposomes is typically driven by entropy change (*22*,*23*) whereas partitioning into liquid-crystalline phase liposomes is driven by enthalpy change

TABLE 1. Liposome−Water Partition Coefficients for 16 EDCs Obtained at Four Different Temperatures Using Palmitoyloleoylphosphatidylcholine (POPC) and Dipalmitoylphosphatidylcholine (DPPC) Liposomes[a]

	K_{lipw} ($\times 10^3$ L/kg liposomes)							
	POPC liposomes				DPPC liposomes			
chemical	11°C	22 °C	30 °C	37 °C	11 °C	22 °C	30 °C	37 °C
estrone	6.58 (0.76)	5.65 (1.03)	4.15 (1.17)	1.22 (0.21)	0.69 (0.24)	0.70 (0.14)	0.55 (0.14)	0.44 (0.07)
17β-estradiol	2.13 (0.25)	1.63 (0.67)	1.43	1.41	0.15 (0.04)	0.17 (0.04)	0.21	0.25
ethynylestradiol	5.95 (1.74)	5.12 (0.09)	3.96 (0.26)	2.76 (0.37)	0.45 (0.05)	0.34 (0.12)	0.52 (0.02)	0.82 (0.01)
diethylstilbestrol	77.1	57.9 (3.6)	55.4 (10.6)	35.3 (3.8)	8.42 (0.73)	14.9 (3.3)	14.4 (1.7)	24.6 (2.9)
meso-Hexestrol	36.8	30.4 (10.2)	25.1	10.5 (1.6)	4.37 (0.49)	4.96 (0.97)	5.74 (1.32)	5.54 (0.58)
dienestrol	201	117	94.4 (5.5)	64.5 (25.9)	18.5 (2.3)	11.3 (0.9)	19.3	17.8 (0.3)
4-*n*-nonylphenol	966 (319)	664 (35)	442 (114)	304 (130)	512 (143)	617 (199)	543 (75)	885 (406)
4-*tert*-octylphenol	630 (40)	275 (55)	219	145 (75)	482 (20)	409	256 (119)	132 (16)
4-*tert*-amylphenol	3.73 (0.05)	3.81 (0.18)	3.46 (0.22)	2.18 (0.36)	0.35 (0.04)	0.43 (0.06)	0.66 (0.09)	1.20 (0.79)
4-*sec*-Butylphenol	2.73 (0.22)	1.95 (0.23)	1.96 (0.13)	1.30 (0.10)	0.22 (0.03)	0.18 (0.02)	0.29 (0.02)	0.42 (0.02)
4-phenylphenol	7.48 (0.87)	5.41(0.16)	3.87	2.25 (0.52)	0.64 (0.01)	0.53 (0.01)	0.66 (0.01)	1.17 (0.04)
bisphenol A	12.0	7.59	6.78 (0.18)	3.17 (0.32)	1.10 (0.02)	0.79 (0.06)	1.38 (0.16)	1.67 (0.08)
dibutylphthalate	3.96 (0.33)	6.11 (0.28)	4.28 (0.17)	3.18 (2.26)	0.43 (0.03)	0.46 (0.04)	0.86 (0.07)	1.19 (0.38)
benzylbutylphthalate	12.8 (0.9)	18.3	12.1 (4.3)	15.1 (4.8)	2.01 (0.16)	4.19 (0.11)	4.80 (2.37)	4.56 (0.15)
butyl-4-hydroxybenzoate	3.73 (0.09)	3.08 (0.04)	3.09 (0.11)	1.20 (0.30)	0.36 (0.05)	0.39 (0.03)	0.39 (0.03)	1.03 (0.11)
benzyl-4-hydroxybenzoate	8.29 (0.74)	6.96 (0.04)	6.32	3.21 (0.07)	0.70 (0.04)	0.60 (0.02)	0.66 (0.05)	1.20 (0.05)

[a] Values in parentheses are standard deviation of triplicate analyses. Average values are used when duplicates are used without standard deviation.

TABLE 2. Enthalpies (ΔH) and Entropies (ΔS) of Partitioning between Water and Synthetic Membrane Vesicles

	$K_{lipw,\ POPC}$			$K_{lipw,\ DPPC}$		
chemicals	ΔH (kJ/mol)	$T\Delta S$[a] (kJ/mol)	r^2	ΔH (kJ/mol)	$T\Delta S$[a] (kJ/mol)	r^2
estrone	−42.6 (18.8)	−12.9 (18.6)	0.72	−12.5 (4.8)	12.4 (4.7)	0.78
17β-estradiol	−12.2 (2.3)	15.3 (2.3)	0.93	14.4 (2.0)	36.3 (2.0)	0.96
ethynylestradiol	−21.0 (4.8)	8.9 (4.8)	0.91	16.4 (12.5)	40.7 (12.4)	0.46
diethylstilbestrol	−19.7 (5.3)	16.4 (5.3)	0.87	27.0 (6.8)	59.4 (6.7)	0.89
meso-hexestrol	−31.7 (12.5)	2.5 (12.4)	0.76	7.5 (2.0)	37.5 (2.0)	0.88
dienestrol	−30.9 (2.4)	7.1 (2.4)	0.99	2.0 (11.6)	34.9 (11.5)	0.02
4-*n*-nonylphenol	−32.5 (3.2)	9.4 (3.2)	0.98	12.2 (7.4)	53.9 (7.4)	0.57
4-*tert*-octylphenol	−40.0 (4.5)	0.3 (4.5)	0.98	−35.3 (10.1)	4.9 (10.0)	0.86
4-*tert*-amylphenol	−13.8 (7.6)	15.3 (7.6)	0.62	33.8 (8.4)	58.3 (8.4)	0.89
4-*sec*-butylphenol	−18.6 (5.0)	9.3 (5.0)	0.87	18.7 (10.5)	41.3 (10.4)	0.61
4-phenylphenol	−32.5 (5.9)	−2.5 (5.9)	0.94	15.1 (11.6)	40.2 (11.5)	0.46
bisphenol A	−28.9 (5.6)	2.2 (5.5)	0.93	13.7 (11.5)	40.0 (11.4)	0.41
dibutylphthalate	−6.5 (11.7)	23.3 (11.6)	0.13	30.2 (8.3)	55.0 (8.2)	0.87
benzylbutylphthalate	1.4 (8.6)	34.0 (8.5)	0.01	23.7 (8.6)	52.8 (8.6)	0.79
butyl-4-hydroxybenzoate	−26.8 (14.1)	1.8 (14.0)	0.64	23.8 (15.6)	47.9 (15.4)	0.54
benzyl-4-hydroxybenzoate	−23.6 (9.6)	7.1 (9.5)	0.75	12.3 (11.4)	37.6 (11.3)	0.37

[a] Entropy contribution ($T\Delta S$) to the free energy change (ΔG) calculated at 22 °C. Values in parentheses are standard errors of the regression.

(*23*−*26*). Partitioning into mixed lipids such as egg-phosphatidylcholine has been reported to be driven by either enthalpy change (*27*) or entropy change (*28*).

Experimental partition coefficients at four temperatures (five for DMPC liposomes) using five different types of liposomes are shown in Figure 1 for the selected EDCs, 17β-estradiol, bisphenol A, diethylstilbestrol, and 4-*n*-nonylphenol. For these selected model lipid membranes, the thermodynamics of partitioning strongly depends on lipid saturation. Partition coefficients for 4-*n*-nonylphenol are not significantly different with respect to lipid composition (Figure 1d). For the selected chemicals, the partitioning process is dominated by the enthalpy gain for unsaturated DOPC liposomes, whereas it is dominated by the entropy change for saturated DSPC liposomes, except for bisphenol A. In addition, the partition coefficient between water and DMPC liposomes generally increases with increasing temperature below the main transition temperature ($T_m \cong 23$ °C; *29*), but decreases with increasing temperature above T_m, except for 4-*n*-nonylphenol. Thus, the physical state of the lipid membrane is critical in determining the partitioning behavior.

Partitioning of moderately hydrophobic organic chemicals between water and synthetic membrane vesicles is conceptually divided into two sequential processes, evaporating a solute from water and dissolving it to the organic phase. Dissolution in each solution can be further divided into two processes including the creation of a cavity for a solute and the formation of a new interaction between the solute and solvent molecules (*30*, *31*). The logarithm of the partition coefficient between water and an organic phase is related to the free energy of solute transfer from water to the organic phase ($\Delta G_{w\rightarrow org}$), which can be estimated from the theoretical free-energy changes including cavity creation and the free

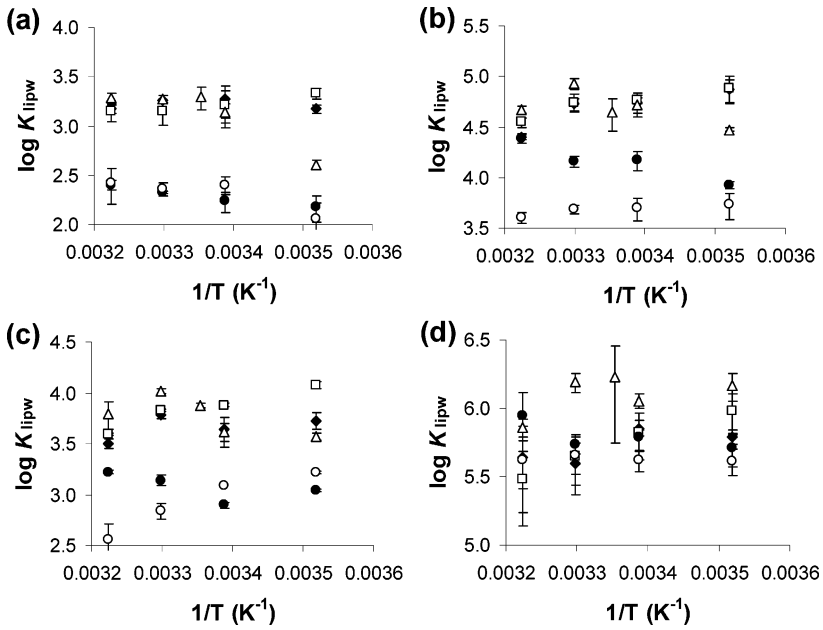

FIGURE 1. Effects of temperature on partitioning between water and various synthetic lipid membrane vesicles for (a) 17β-estradiol, (b) diethylstilbestrol, (c) bisphenol A, and (d) 4-*n*-nonylphenol. Error bars denote standard deviations. (♦: DOPC, □: POPC, △: DMPC, ●: DPPC, ○: DSPC).

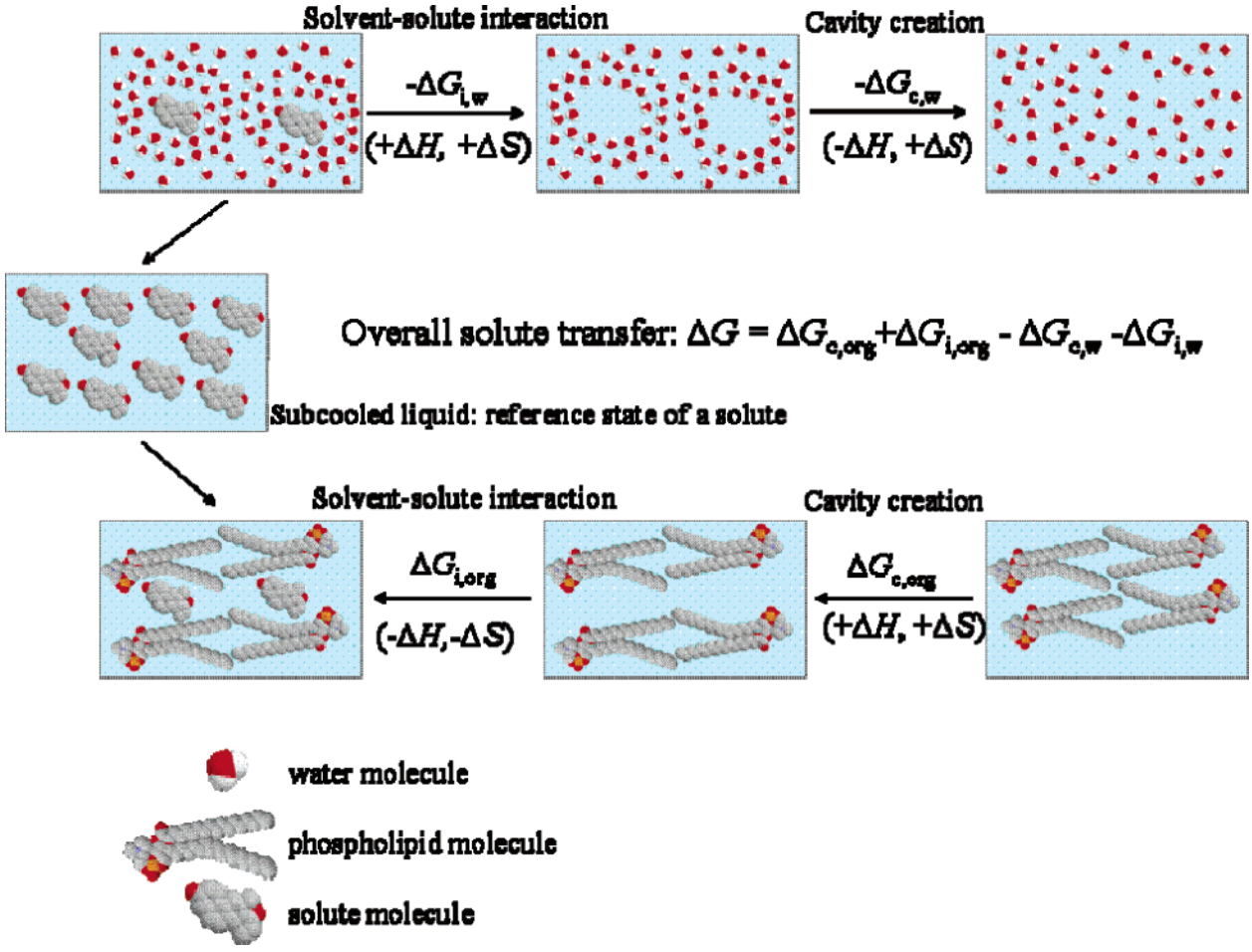

FIGURE 2. Thermodynamic cycle for solute transfer.

energy of interaction between solute and solvent, as follows:

$$\Delta G_{w\rightarrow org} = \Delta G_{s,org} - \Delta G_{s,w} = (\Delta G_{c,org} + \Delta G_{i,org}) - (\Delta G_{c,w} + \Delta G_{i,w}) = (\Delta G_{c,org} - \Delta G_{c,w}) + (\Delta G_{i,org} - \Delta G_{i,w}) \quad (4)$$

where subscript c denotes energy required for creating a cavity for a solute, i denotes interaction energy, and org and w represent the organic phase and water, respectively. As depicted in Figure 2, thermodynamic constants are affected differently by each process. Loss of a solute from water could

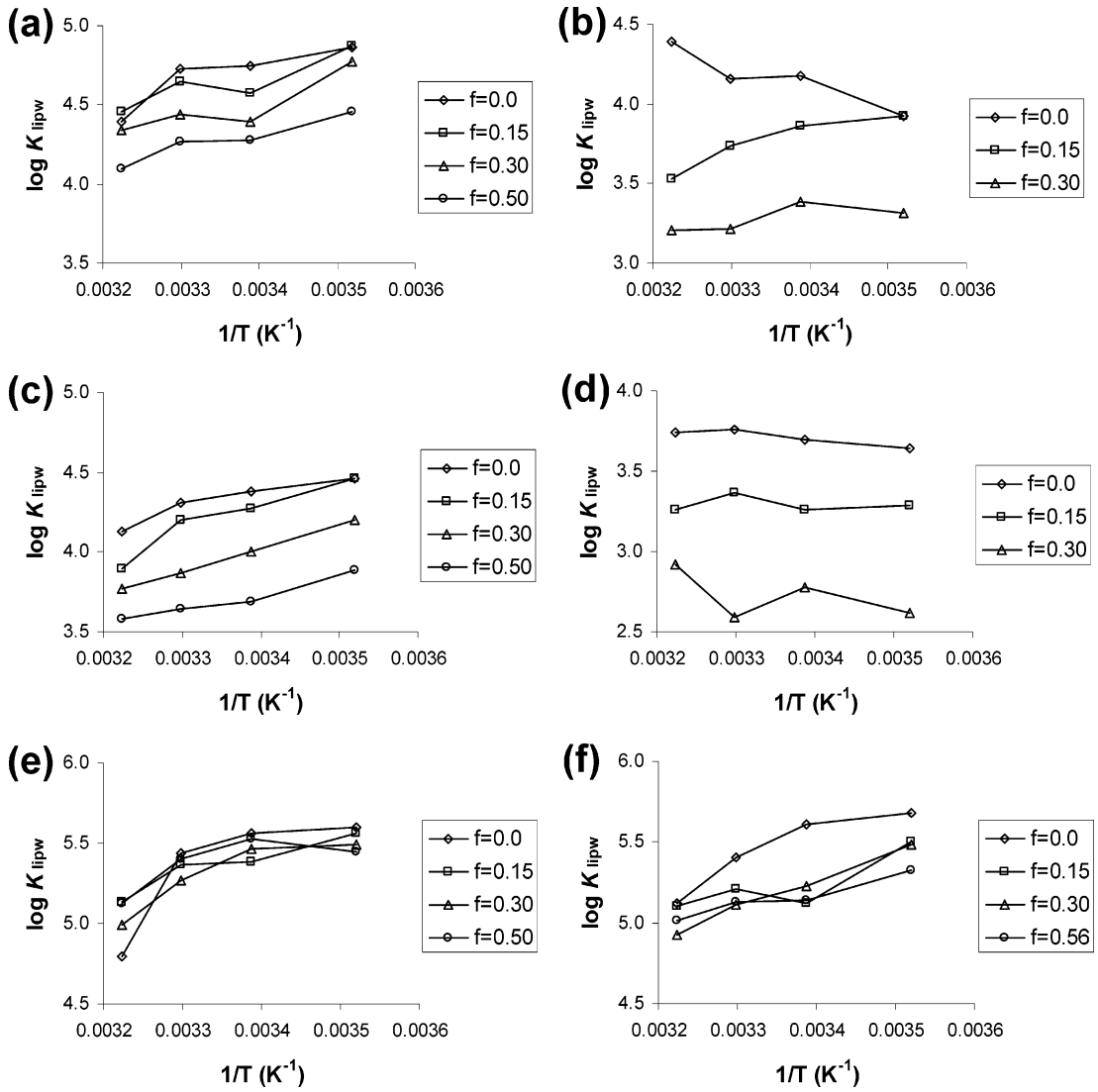

FIGURE 3. Effects of cholesterol on partitioning thermodynamics between water and membrane vesicles for (a) diethylstilbestrol using dioleoylphosphatidylcholine (DOPC) liposomes, (b) diethylstilbestrol using dipalmitoylphosphatidylcholine (DPPC) liposomes, (c) meso-hexestrol using DOPC liposomes, (d) meso-hexestrol using DPPC liposomes, (e) 4-*tert*-octylphenol using DOPC liposomes, and (f) 4-*tert*-octylphenol using DPPC liposomes. *f* denotes mole fraction of cholesterol in the membranes.

be accompanied by a significant increase in entropy because water molecules near a hydrophobic solute are strictly organized (*32*). Negative ΔH is expected by filling a cavity in water. Creation of a cavity in the lipid bilayers requires heat energy because of their highly organized structure and this will be compensated by thermal energy gain due to newly formed van der Waals interaction. The average distance between lipid components would be increased by introducing a sufficiently large molecule in the bilayers. This would result in positive ΔH and positive ΔS values. Noticeable differences in partitioning thermodynamics in Table 2 between DPPC and POPC liposomes should be attributed to $\Delta G_{c,org}$ because all processes in aqueous phase are the same and it can be assumed that thermal energy gain due to the van der Waals interaction in one liposomes are not significantly different from the other. This also distinguishes partitioning into biological membranes from that into 1-octanol or natural organic matter that is dominated by the solute's activity in aqueous solution (*33*).

In addition, evaluation of thermodynamic constants for three moderately hydrophobic *p*-substituted phenols (4-*tert*-amylphenol, 4-*sec*-butylphenol, and 4-phenylphenol) provides an insight on the "branching effect". Partitioning enthalpy and entropy values follow the order 4-*tert*-amylphenol > 4-*sec*-butylphenol > 4-phenylphenol for partitioning into both liposomes (Table 2), although this trend is not statistically significant. The enthalpy contribution decreases and the entropy contribution increases in the partitioning process with increased branching in the *p*-substitution of these phenols. Although we cannot exclude the effects of branching on the dissolution processes in water, the significance of $\Delta G_{c,org}$ may explain these effects on K_{lipw}.

Effects of Cholesterol in the Membrane. Figure 3 shows changes in log K_{lipw} with cholesterol content and temperature for diethylstilbestrol, meso-hexestrol, and 4-*tert*-octylphenol. The partition coefficient decreases much more for diethylstilbestrol and meso-hexestrol with increasing cholesterol content in the lipid membranes. In contrast, only a slight decrease in the partition coefficient is observed for 4-*tert*-octylphenol at room temperature (*6*). The partition coefficients for diethylstilbestrol and meso-hexestrol between water and DOPC liposomes increased with decreasing temperature regardless of the amount of cholesterol in the membrane (Figure 3a and c). The slopes and the intercepts of these regressions are not statistically different, although

TABLE 3. Trends in ΔH and ΔS for Chemicals between Water and Different Organic Phases[a]

organic phases		chemicals	ΔH	ΔS	reference
lipid bilayer membranes	1-octanol	CBs	−	+	*5*
		benzocaine	−	+	*22*
		CBs	−	n.d.	*38*
		CBs, PAHs, PCNs, PCBs	−	n.d.	*39*
		mefloquine/quinine	−/+	−/+	*40*
		dipyridamole	+	+	*41*
	fish storage lipids	CBs	-−	+	*9*
	surfactant micelles (SDS)	various water pollutants	−/+	+	*8*
	DPPC (16:0, 16:0)	benzocaine	+	+	*22*
		CBs	+ ($<T_m$) − ($>T_m$)	+	*23*
		dipyridamole	+	+	*41*
		EDCs	+	+	this study
	DPPC with cholesterol	EDCs	+	+	this study
	DSPC (18:0, 18:0)	EDCs		+	this study
	DMPC (12:0, 12:0)	benzocaine	− ($>T_m$)	-	*22*
		small nonelectrolytes	+	+	*30*
		mefloquine/quinine	− ($>T_m$) + ($<T_m$)	- ($>T_m$) + ($<T_m$)	*40*
		dipyridamole	+	+	*41*
		EDCs	− ($>T_m$) + ($<T_m$)	+	this study
	POPC (18:1, 16:0)	pharmaceuticals	−	+	*25*
		pharmaceuticals	−	±	*24*
		EDCs	−	±	this study
	DOPC (18:1, 18:1)	EDCs	−	±	this study
	DOPC with cholesterol	EDCs	−	±	this study
	EPC (mixture)	phosphonium homologues	+	+	*28*
		phenothiazine drugs	−	+	*27*
living organisms	phytoplankton	CBs	+	+	*36*
	fish	CBs	+	+	*5*

[a] CBs=chlorobenzenes, DMPC=dimyristoylphosphatidylcholine, DOPC=dioleoylphosphatidylcholine, DPPC=dipalmytoylphosphatidylcholine, DSPC=distearylphosphatidylcholine, EDCs=endocrine disrupting chemicals, EPC=egg-yolk phosphatidylcholine, PAHs=polyaromatic hydrocarbons, PCBs=polychlorinated biphenyls, PCNs=polychlorinated naphthalenes, POPC=palmytotyloleoylphosphatidylcholine, SDS=sodium dodecylsulfate, T_m=main transition temperature.

log K_{lipw} values are consistently lower with increasing amount of cholesterol in the DOPC membranes over the temperature range investigated.

In contrast, cholesterol has a much different effects on the partitioning between water and saturated liposomes, DPPC, than that observed using unsaturated liposomes. Partitioning of diethylstilbestrol into DPPC liposomes is endothermic as observed for other EDCs into DPPC. However, adding a small amount of cholesterol (15% by mole fraction) into DPPC dramatically changed the partitioning thermodynamics for diethylstilbestrol to an exothermic process strongly driven by enthalpy change (Figure 3b). The noticeable difference between the two synthetic estrogens may be due to their chemical structures (cf. Supporting Information). Diethylstilbestrol has a conjugated double bond between two aromatic rings, making it rigid and flat, whereas meso-hexestrol does not. Rotations around the single bonds even allow stacking of the aromatic rings in meso-hexestrol. The small amount of cholesterol may increase membrane fluidity of the gel phase liposome by separating phospholipids molecules, a process that does not happen in unsaturated liposomes which have cavities for cholesterol (*34*). The effects of low cholesterol content in saturated lipid membranes are not apparent for meso-hexestrol, possibly as a result of its size requirements relative to diethylstilbestrol, because this meso-hexestrol can freely rotate around the central C−C single bond. Adding more cholesterol into the membrane gradually decreases fluidity, and the thermodynamic trends become consistent with that for the pure DPPC liposomes. At high fractions of cholesterol in the phospholipids, K_{lipw} values for diethylstilbestrol and meso-hexestrol decrease by almost 2 orders of magnitude, which may be due to competition with cholesterol (*7*, *35*).

Effects of cholesterol on the partitioning of 4-*tert*-octylphenol are negligibly small compared with synthetic estrogens even though their hydrophobicities are not significantly different. This may be related to the location of partitioning or due to an alteration of the membrane fluidity at high concentration of 4-*tert*-octylphenol in the membrane as indicated previously.

Implication for Bioconcentration Assessment. Table 3 shows typical trends in the thermodynamic constants of partitioning between water and different organic phases for some environmental contaminants and pharmaceuticals. Although the literature values and results in this study are somewhat chemical-specific, different organic phases can be categorized into two groups in terms of partitioning thermodynamics, highly ordered membrane phases (i.e., gel phase membrane lipids and surfactant micelles) and less ordered organic phases including bulk organic solvents, non-membrane lipids, and membrane lipids above the main transition temperature. The driving forces for partitioning into saturated lipid membranes can be differentiated from those for more fluidic surrogate phases. The existence of saturated lipid components below their main transition temperature partly explains the endothermic nature of biopartitioning in fish (*5*) and phytoplankton (*36*), although they are simplified models for real plasma membranes. However, partitioning into lipids with saturated tails does not fully support the observed temperature dependency in guppies (*5*), and thus there may be many other factors affecting the overall bioconcentration in fish at the organism level, even if bioconcentration is dominated by equilibrium partitioning processes. Fish contain large amount of poly unsaturated fatty acids in their plasma membrane and triacylglycerols as storage lipids, which do not form organized

bilayers (*14*). Van Wezel and Opperhuizen (*9*) reported that partitioning into storage lipids is driven by enthalpy change. Thus, there should be several compensating effects for fish bioconcentration to be exothermic. For example, existence of sterols and membrane proteins can make the plasma membrane more rigid which could cause the overall biopartitioning to be driven by entropy change. Environmental adaptation of lipid compositions (e.g., refs 15, 16) would also affect measured partition coefficient at different temperature.

Although bioconcentration in fish and other aquatic organisms is a comprehensive process including absorption, distribution, metabolism, and excretion, disequilibrium between water and lipid tissues is the most significant driving force of many transport processes such as gill membrane permeation. Quantitative structure−activity relationships based on log K_{OW} for predicting bioconcentration would be more useful for species containing more storage lipids, because it correlates better with the partition coefficient between water and storage lipids than that between water and membrane lipids (*37*). However, there are significant differences in fatty acids composition among different species and phospholipids are dominant in some species (*13*, *14*). Further investigation is needed for evaluating species-to-species variation in relation with lipid compositions as well as physiological processes such as distribution and metabolic degradation.

Supporting Information Available

Structures of the two synthetic estrogens. This material is available free of charge via the internet at http://pubs.acs.org.

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