Differential lipid affinity of xenobiotics and natural compounds

Heinrich Sandermann Jr.*

GSF-National Research Centre for Environment and Health, Institute of Biochemical Plant Pathology, D-85764 Neuherberg, Germany

Received 14 July 2003; revised 13 September 2003; accepted 19 September 2003

First published online 16 October 2003

Edited by Guido Tettamanti

Abstract Octanol-1/water partitioning currently provides the most widely used model system to simulate both phospholipid target lipids and triglyceride storage lipids. A differentiation between the two lipid classes is now achieved by making use of a water-induced lipid phase separation. Coefficients ($K_{TG/PC}$) for partitioning between trioleoylglycerol (TG) and phosphatidylcholine (PC) were determined for 20 xenobiotics and two biological lipids. $K_{TG/PC}$ values are related to K_{OW} through the relationship, log $K_{TG/PC}$ = 0.33 log K_{OW} − 1.078. The present results will allow better predictions on whether drugs and xenobiotics are bioaccumulated, degraded or reach toxicity-related sites. In addition, applications to natural lipophilic compounds and disease-related proteins are discussed.

Key words: Lipid affinity; Phosphatidylcholine; Triacylglycerol; Octanol-1/water partitioning coefficient; Xenobiotic

1. Introduction

The importance of the lipophilicity of chemicals for passive membrane permeation and narcotic activity was recognized more than 100 years ago (Meyer–Overton rule, reviewed [1,2]). Olive oil (triglyceride)/water [1–3], biomembrane/water [1,2], phospholipid/water [1,2,4] and octanol-1/water [1,3,5,6] partitioning coefficients have been used to characterize lipophilicity. Octanol-1/water partitioning coefficients (K_{OW}) became the most widely used lipophilicity parameter that has been employed in the systematic analysis of quantitative structure–activity relationships (QSAR) [7–10]. Hundreds, if not thousands, of pharmacokinetic and physiological processes have in this way been positively correlated with lipophilicity. However, it has usually been ignored that the two major biological lipid classes (phospholipids, triglycerides) have opposing functions. Triglycerides predominate in adipose tissue and in fat droplets. Their main pharmacokinetic function is storage and bioaccumulation of lipophilic xenobiotics and drugs. In contrast, phospholipids predominate in biological membranes where many of the target proteins and metabolic enzymes for lipophilic chemicals are located. Often, there is an obligatory functional dependence on phospholipids [11]. The opposing functions of the two major lipid classes have been recognized in ecotoxicological studies where target lipids (phospholipids) and non-target lipids (triglycerides) have been distinguished [12,13]. Any prediction of the biological activity and fate of lipophilic drugs and xenobiotics, as well as natural compounds, clearly requires knowledge of their differential affinity for either phospholipid or triglyceride. Such data are presented here for the first time.

2. Materials and methods

2.1. Materials

Partitioning was carried out between trioleoylglycerol (TG; Sigma No. T-7140) and either egg yolk phosphatidylcholine (PC; Sigma No. P-4279) or 1-palmitoyl-2-oleoyl-PC (Sigma No. P-3017). Results with the two types of PC were closely similar. Defined solutions of TG were prepared gravimetrically, whereas PC solutions were quantitated by phosphorus determination. Substances to be partitioned were purchased in ^{14}C- or ^{3}H-labeled form. Purities of TG, PC and of the radioactive chemicals were checked by thin layer chromatography [14,15].

2.2. Partitioning procedure

The radioactive test substance (0.2–10 nmol, 20 000–100 000 dpm, in 5–10 μl ethanol, propanol-2 or *n*-hexane) was mixed in 1 ml polycarbonate ultracentrifugation tubes with 3800 nmol PC in 30 μl ethanol and 4170 nmol TG in 30 μl *n*-hexane. The mixture was frozen in liquid nitrogen and lyophilized. A 300 μl aliquot of 100 mM nitrogen-saturated potassium phosphate buffer of pH 6.6 and 600 μl nitrogen-saturated water were added. The tube was gassed with nitrogen, sealed with Parafilm and shaken with maximal power for 30 s on a Vortex mixer. This treatment resulted in a heavy emulsion. The sample was incubated under nitrogen for 7–15 h at 25°C in a shaking water bath and then ultracentrifuged for 20 h at 20°C (Beckman rotor 25, 25 000 rpm). This resulted in separation of a TG-rich lipid layer at the top and a PC-rich lipid layer at the bottom of the tube. The tube was frozen in liquid nitrogen and dissected manually with a small saw into five slices whose radioactivity was determined using a detergent-containing scintillation fluid. A tube slice containing the TG layer was followed by three control slices containing water phase and the bottom tube slice containing the PC layer. External standardization of counting rates was checked by internal standardization using toluene standards of known radioactivity. Each substance was examined in 4–12 independent experiments; the relative standard deviation was ± 10–15%. In control experiments the amounts of TG or PC were varied between 0.5- and 2-fold and $K_{TG/PC}$ values were found to remain the same. In many cases, the amount of test substance was increased up to 50 nmol, again without significant effect on $K_{TG/PC}$.

3. Results

3.1. Basis of differential lipid partitioning

Preliminary experiments to determine coefficients ($K_{TG/PC}$) for partitioning of lipophilic chemicals between TG and PC have been described [14], but the physical basis of the procedure remained undefined. The phase behavior of TG and PC has now been determined in the absence and presence of

*Fax: (49)-89-3187 3383.
E-mail address: sandermann@gsf.de (H. Sandermann Jr.).

Abbreviations: TG, trioleoylglycerol; PC, phosphatidylcholine; K_{OW}, octanol-1/water partitioning coefficient

0014-5793/03/$22.00
doi:10.1016/S0014-5793(03)01143-8

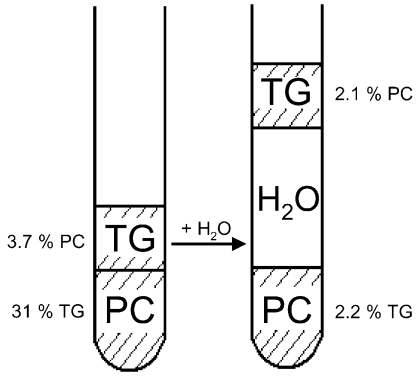

Fig. 1. Phase separation of egg PC and TG. Absence of water (tube depicted on the left): The tube received 215 μmol 1-palmitoyl-2-oleoyl-PC and 189 μmol TG that were lyophilized together from *n*-hexane solutions. [^{14}C]1-Palmitoyl-2-oleoyl-PC or [^{14}C]TG markers (~0.5 nmol, 70 000 dpm) had been added. After lyophilization, the dried lipids were thoroughly dispersed on a Vortex mixer and incubated under nitrogen in a shaking water bath at 25°C (12 h). Ultracentrifugation of the dried lipid mixtures in a Beckman 50Ti rotor at 33 000 rpm resulted in an upper oil phase and a semi-solid bottom phase. The associated radioactivities were determined so that the indicated mol% mutual miscibilities could be calculated. Four independent experiments with closely similar results (S.D., ±8%) were performed. Presence of water (tube depicted on the right): The standard TG/PC-partitioning procedure described in Section 2 was performed with inclusion of the same two ^{14}C-lipid markers. Mol% mutual miscibility is shown (n = 4, S.D., ±11%).

water. As depicted in Fig. 1, a phase separation of TG and PC occurs upon addition of aqueous buffer. Only one of the four parameters of Fig. 1, association of TG with PC in the presence of water, has previously been determined in the literature

Table 1
Experimental $K_{TG/PC}$ values

Chemical	$K_{TG/PC}$ value
1. DDE	9.8
2. DDT	9.3
3. DEHP	9.3
4. PCB-Cl$_5$	5.9
5. DDD	5.0
6. Methoxychlor	3.8
7. Aldrin	3.5
8. HCB	3.3
9. Dieldrin	2.8
10. Lindane	2.7
11. Biphenyl	2.5
12. Parathion	1.9
13. Kelthane	1.4
14. Dichlobenil	0.59
15. Benzylalcohol	0.58
16. 2,4,6-Trichlorophenol	0.48
17. Carbaryl	0.32
18. 2-Nitrophenol	0.28
19. 4-Nitrophenol	0.14
20. Phenol	0.105

The standard partitioning procedure of Section 2 was employed. The dpm ratios between the TG- and PC-rich lipid phases were converted to molar fraction ratios by taking the mutual lipid contamination into account. The values were not corrected for dissociation of functional groups. The common designations of xenobiotics are used; the corresponding chemical designations have been listed [45]. The least lipophilic chemical was phenol with $K_{TG/PC}$ = 0.105. Xenobiotics with still lower lipophilicity such as 2,4-D, 2,4,5-T, DDA, MEHP and phthalic acid showed distinct lipid associations, in particular to PC, but the elevated aqueous radioactivities prevented a reliable determination of $K_{TG/PC}$ values.

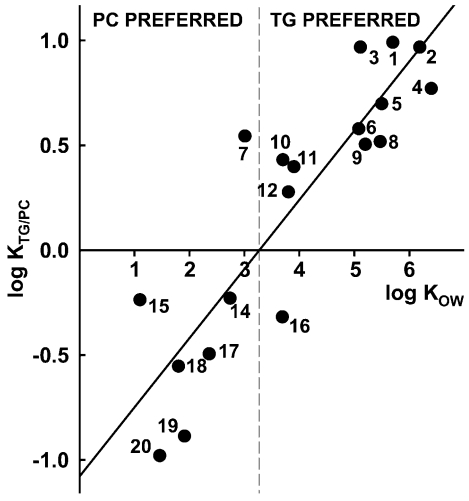

Fig. 2. Relationship between log $K_{TG/PC}$ (values derived from Table 1) and log K_{OW}. The latter values were adopted from [19] that states that errors in reported data are identified and 'best' values are reported. Xenobiotics were numbered according to Table 1. Xenobiotic No. 13 of Table 1 was not plotted because no log K_{OW} value could be found. The regression line conforms to the equation, log $K_{TG/PC}$ = 0.33 log K_{OW} − 1.078. The correlation coefficient was 0.77. The log K_{OW} regions of preferential partitioning into PC or TG are labelled.

[16,17]. The association of PC with TG may in part be due to the well-known monolayer coverage of TG droplets [17,18]. The importance of the phase separation process for the differential partitioning between TG and PC was further shown by the failure of the two lipophilic chemicals, cholesterol ($K_{TG/PC}$, 0.12) and oleoyl-cholesterol ($K_{TG/PC}$, 25), to equilibrate through the water phase. These substances were dried with either PC or TG and were then dispersed in aqueous buffer containing a dispersion of the second lipid. After incubation and ultracentrifugation, radioactivity of the TG and PC layers was determined. In all cases, the test substances remained completely associated with the lipid initially employed. The standardized protocol of Section 2 was used in these and all subsequent experiments.

3.2. Partitioning values of xenobiotics

The $K_{TG/PC}$ values of 20 radioactively labelled xenobiotics were determined, as summarized in Table 1. A linear relationship between log $K_{TG/PC}$ and published values of log K_{OW} [19] appeared to exist (Fig. 2). Chemicals with log K_{OW} below 3.3 had a higher affinity for PC, those with log K_{OW} above 3.3 partitioned preferentially into TG. The noticeable scatter of the data (correlation coefficient, 0.77) may be due to the relatively high standard deviation (±10–15%) of the partitioning procedure, and to the recently discussed unreliability of reported K_{OW} values [20]. The slope of the regression line was only 0.33 and was thus significantly lower than the slope of 1.0 that has been documented in numerous studies of narcosis and QSAR studies [1–10]. Slope values near 1.0 are expected from the Meyer–Overton rule due to the dominating 'hydrophobic effect' that arises from interactions with water [21]. In contrast, partitioning between TG and PC appears to involve more subtle free energy increments that result from differences between anisotropic phospholipid bilayers and isotropic triglyceride droplets. It has previously been determined for long-chain alcohols that the transfer free energy increment

per methylene group was only −40 cal/mol in the TG/PC-partitioning system [15], compared to some −800 cal/mol for partitioning systems involving a water phase [21]. Log K_{OW} values above ∼5 are difficult to determine experimentally [7,8], and TG/PC partitioning may be used for substances of high lipophilicity.

4. Discussion

Log K_{OW} has been well documented to be linearly related to bioaccumulation of DDT and other persistent xenobiotics [3,7–10], as well as to rate and equilibrium constants of numerous physiological and pharmacokinetic processes that are influenced by xenobiotics and drugs [1,2,5–10]. Typically, numerous lipid-related steps exist, such as membrane permeation, binding to membranes and serum lipoproteins, binding to membrane-localized receptors and enzymes, bioaccumulation in adipose tissue and lipid droplets. Multiple partitioning steps between target versus non-target lipids thus occur, but could so far not be simulated. This limitation is overcome by the present partitioning assay involving PC and TG. At the same time, the widespread use of K_{OW} receives new support because $K_{TG/PC}$ values can be calculated from existing K_{OW} values on the basis of the relationship given in Fig. 2. Below a transition point near log K_{OW} 3.3, octanol-1 acted as a model for PC, above log K_{OW} 3.3, as a model for TG.

Bioaccumulating xenobiotics are known to reach ppb or ppm levels in adipose lipids as well as the lipid droplets of human milk [22,23]. On the basis of lipid content, human brain contains much lower levels of these xenobiotics [22,24]. These observations may be due to the presence of only ∼0.6% triglycerides in brain lipids, compared, for example, to ∼48% triglycerides in liver lipids [25]. $K_{TG/PC}$ values may also be applied to analyze the functional role of natural compounds. For example, cholesterol and oleoyl-cholesterol are both practically water-insoluble but on the basis of their log $K_{TG/PC}$ values of −0.92 and 1.40, respectively, are predicted (cf. Fig. 2) to have greatly different biological functions. In fact, cholesterol, but not its esters, is specifically associated with biomembranes and the outer layer of soluble lipoproteins, whereas cholesterol esters occur as characteristic interior components of soluble lipoproteins [26]. With regard to other natural compounds, the partitioning of cannabinoid drug analogues into mouse brain has been correlated with their $K_{TG/PC}$ values [27,28]. The lipid-related pharmacokinetics of lipophilic food constituents that activate nuclear receptors has been reviewed [29]. The uptake and differential lipid partitioning of carotenoids has been studied in particular detail, including the use of TG/PC emulsions [30,31].

The self-assembly of blood lipoproteins from TG, cholesterol esters, cholesterol and phospholipids and the apoproteins has been well studied [32–35]. Phospholipids are known to play a chaperone-like role in the membrane incorporation of integral membrane proteins [36], although some membrane proteins, such as the nicotinic acetylcholine receptor [37], require the presence of sterols in addition to phospholipids. In loose analogy to the soluble lipoprotein complexes of blood, cellular TG droplets are known to be associated with characteristic proteins [38]. More recently, certain disease-related proteins, such as the hepatitis C virus capsid protein [39], α-synuclein [40], a cancer-related placental protein [41] and caveolins [42], have been found to differentially interact with cellular TG droplets or biomembranes. Differential lipid/protein interactions may also be involved in the formation, aggregation and clearance of β-amyloid peptides involved in Alzheimer's disease. The presence of the apolipoprotein E4 allele or lowered cholesterol levels are known to lead to higher, respectively lower, disease incidence [43,44].

In conclusion, differential interactions with TG- or PC-rich lipid phases may be important not only for drugs and xenobiotics, but also for lipophilic natural compounds and proteins. $K_{TG/PC}$ values may be applied in analogy to the theoretical plate numbers used to describe chromatographic processes: multiple partitioning steps between TG- and PL-rich phases decide whether lipophilic chemicals and proteins will be bioaccumulated, degraded or reach toxicity-related target sites.

Acknowledgements: Experimental contributions by Ulrike Kaiser and Drs. Elisabeth Willwacher and Dierk Scheel are gratefully acknowledged. Financial support came from the Deutsche Forschungsgemeinschaft.

References

[1] Seeman, P. (1972) Pharmacol. Rev. 24, 583–655.
[2] Miller, K.W. (1985) Int. Rev. Neurobiol. 27, 1–61.
[3] Chiou, C.T. (1985) Environ. Sci. Technol. 19, 57–62.
[4] Vaes, W.H., Ramos, E.U., Verhaar, H.J. and Hermens, J.L. (1998) Environ. Toxicol. Chem. 17, 1380–1384.
[5] Franks, N.P. and Lieb, W.R. (1986) Arch. Toxicol. Suppl. 9, 27–37.
[6] Sandberg, W.S., Dubois, B.W. and Miller, K.W. (1999) Curr. Topics Membr. 48, 311–350.
[7] Leo, A.J. (1991) Methods Enzymol. 202, 544–591.
[8] Schwarzenbach, R.P., Gschwend, P.M. and Imboden, D.M. (1993) Environmental Organic Chemistry, pp. 124–156, Wiley, New York.
[9] Hansch, C. and Leo, A. (1995) Exploring QSAR. Fundamentals and Applications in Chemistry and Biology, American Chemical Society, Washington, DC.
[10] Pliska, V., Testa, B. and v.d. Waterbeemd, H. (Eds.) (1996) Lipophilicity in Drug Action and Toxicology, VCH Publishers, Weinheim.
[11] Sandermann, H., Duncan, T.M., McIntyre, J.O. and Fleischer, S. (1993) in: Protein–Lipid Interactions (Watts, A. Ed.), pp. 67–86, Elsevier, Amsterdam.
[12] v. Wezel, A.P., Punte, S.S. and Opperhuizen, A. (1995) Environ. Toxicol. Chem. 14, 1579–1585.
[13] Escher, B.I. and Hermens, J.L. (2002) Environ. Sci. Technol. 36, 4201–4217.
[14] Sandermann, H. (1979) Chemosphere 8, 499–508.
[15] Sandermann, H., Addona, G.H. and Miller, K.W. (1997) Biochim. Biophys. Acta 1346, 158–162.
[16] Small, D.M. (1997) Methods Enzymol. 286, 153–167.
[17] Miller, K.W. and Small, D.M. (1987) in: New Comprehensive Biochemistry, Vol. 14, Plasma Lipoproteins (Gotto, Jr., A.M. Ed.), pp. 1–75, Elsevier, Amsterdam.
[18] Tauchi-Sato, K., Ozeki, S., Houjou, T., Taguchi, R. and Fujimoto, T. (2002) J. Biol. Chem. 277, 44507.
[19] Mackay, D., Shiu, W.Y. and Ma, K.C. (1992–97) Illustrated Handbook of Physical-Chemical Properties and Environmental Fate for Organic Chemicals, Vols. 1–5, Lewis, Boca Raton, FL.
[20] Renner, R. (2002) Environ. Sci. Technol. 36, 411A–413A.
[21] Tanford, C. (1980) The Hydrophobic Effect, Formation of Micelles and Biological Membranes, 2nd edn., Wiley, New York.
[22] Hayes, W.J. (1975) in: Toxicology of Pesticides, pp. 339–362, Williams and Wilkins, Baltimore, MD.
[23] Jensen, A.A. and Slorach, S.A. (1991) Chemical Contaminants in Human Milk, CRC Press, Boca Raton, FL.
[24] Dewailly, E., Mulvad, G., Pedersen, H.S., Ayotte, P., Demers, A., Weber, J.-P. and Hansen, J.C. (1999) Environ. Health Perspect. 107, 823–828.

[25] Davison, A.N. (1970) in: Comprehensive Biochemistry, Vol. 18, Lipid Metabolism, pp. 293–329, Elsevier, Amsterdam.
[26] Yeagle, P.L. (Ed.) (1988) Biology of Cholesterol, CRC Press, Boca Raton, FL.
[27] Binder, M., Witteler, F.-J., Schmidt, B., Franke, I., Bohnenberger, E. and Sandermann, H. (1984) in: The Cannabinoids: Chemical, Pharmacologic, and Therapeutic Aspects (Agurell, S., Dewey, W.L. and Willette, R.E., Eds.), pp. 709–727, Academic Press, Orlando, FL.
[28] Franke, I., Grothey, A., Koch, S., Witteler, F.-J., Binder, M. and Sandermann, H. (1985) Biochem. Pharmacol. 34, 3983–3986.
[29] Chawla, A., Repa, J.J., Evans, R.M. and Mangelsdorf, D.J. (2001) Science 294, 1866–1870.
[30] Borel, P., Grolier, P., Armand, M., Partier, A., Lafont, H., Lairon, D. and Azais-Braesco, V. (1996) J. Lipid Res. 37, 250–261.
[31] Tyssandier, V., Lyan, B. and Borel, P. (2001) Biochim. Biophys. Acta 1533, 285–292.
[32] Atkinson, D. and Small, D.M. (1986) Annu. Rev. Biophys. Biophys. Chem. 15, 403–456.
[33] Derksen, A. and Small, D.M. (1989) Biochemistry 28, 900–906.
[34] Saito, H., Minamida, T., Arimoto, I., Handa, T. and Miyajima, K. (1996) J. Biol. Chem. 271, 15515–15520.
[35] Saito, H., Tanaka, M., Okamura, E., Kimura, T., Nakahara, M. and Handa, T. (2001) Langmuir 17, 2528–2532.
[36] Bogdanov, M. and Dowhan, W. (1999) J. Biol. Chem. 274, 36827–36830.
[37] Addona, G.H., Sandermann, H.Jr., Kloczewiak, M.A. and Miller, K.W. (2003) Biochim. Biophys. Acta 1609, 177–182.
[38] Murphy, D.J. and Vance, J. (1998) Trends Biochem. Sci. 24, 109–115.
[39] Dubuisson, J., Penin, F. and Moradpour, D. (2002) Trends Cell Biol. 12, 517–523.
[40] Cole, N.B., Murphy, D.D., Grider, T., Rueter, S., Brasaemle, D. and Nussbaum, R.L. (2002) J. Biol. Chem. 277, 6344–6352.
[41] Than, N.G., Sumegi, B., Bellyei, S., Berki, T., Szekeres, G., Janaky, T., Szigeti, A., Bohn, H. and Than, G.N. (2003) Eur. J. Biochem. 270, 1176–1188.
[42] van Meer, G. (2001) J. Cell Biol. 152, F29–F34.
[43] Herz, J. and Bock, H.H. (2002) Annu. Rev. Biochem. 71, 405–434.
[44] Selkoe, D.J. and Podlisny, M.B. (2002) Annu. Rev. Genomics Hum. Genet. 3, 67–99.
[45] Tomlin, C. (Ed.) (1995) The Pesticide Manual, Crop Protection Publications, British Crop Protection Council, Farnham.