

ITC XVIII- Lipid-Lipid interactions

Heerklotz H. and Epand R. M. (2001) The enthalpy of acyl chain packing and the apparent water-accessible apolar surface area of phospholipids. *Biophys J* **80**, 271-279.

Abstract: The energetics of phospholipid aggregation depend on the apparent water-accessible apolar surface area (ASA_{ap}), ordering effects of the chains, and headgroup interactions. We quantify the enthalpy and entropy of these interactions separately. For that purpose, the thermodynamics of micelle formation of lysophosphatidylcholines (LPCs, chains C10, C12, C14, and C16) and diacylphosphatidylcholines (DAPCs, chains C5, C6) and C7) are studied using isothermal titration calorimetry. The critical micelle concentration (CMC) values are 90, 15, and 1.9 mM (C5-C7-DAPC) and 6.8, 0.71, 0.045, and 0.005 mM (LPCs). The group contributions per methylene of $\Delta\Delta G(0) = -3.1$ kJ/mol and $\Delta\Delta CP = -57$ J/(mol. K) for LPCs agree with literature data on hydrocarbons and amphiphiles. An apparent deviation of DAPCs (-2.5 kJ/mol, 45 J/(mol. K)) is due to an intramolecular interaction between the two chains, burying 20% of the surface. The chain/chain interaction enthalpies in a micelle core are by approximately -2 kJ/(mol) per methylene group more favorable than in bulk hydrocarbons. We conclude that the impact of the chain conformation and packing on the interaction enthalpy is very pronounced. It serves to explain a variety of effects reported on membrane binding. Interactions within the water-accessible region show considerable ΔH , but almost no $\Delta G(0)$. The heat capacity changes suggest about three methylene groups (ASA_{ap} approximately 100 Å²) per LPC remain exposed to water in a micelle (DAPC: 2 CH₂/70 Å²).

Li Z., Mintzer E., and Bittman R. (2004) The critical micelle concentrations of lysophosphatidic acid and sphingosylphosphorylcholine. *Chem Phys Lipids* **130**, 197-201.

Abstract: The critical micelle concentrations (CMC) of lysophosphatidic acid (LPA) and sphingosylphosphorylcholine (SPC) were measured by isothermal titration calorimetry. The CMC of LPA decreases with salt concentration and acyl chain length. In water at 25 degrees C, the CMC values of 1-acyl-2-lyso-sn-glycero-3-phosphatidic acid are 1.850, 0.540, 0.082, and 0.346 mM, respectively, when the acyl group is myristoyl, palmitoyl, stearoyl, and oleoyl. The CMC of SPC in 10 mM sodium phosphate buffer, pH 7.4, at 25 degrees C was 0.158 mM, and did not change with an increase in salt concentration.

Mel'nikov S. M., Seijen ten Hoorn J. W., and Eijkelenboom A. P. (2004) Effect of phytosterols and phytostanols on the solubilization of cholesterol by dietary mixed micelles: an in vitro study. *Chem Phys Lipids* **127**, 121-141.

Abstract: The effect of a plant sterol, beta-sitosterol (SI), and a plant stanol, sitostanol (SS), on the solubilization of cholesterol (CH) by model dietary mixed micelles was examined under in vitro conditions with the use of gas chromatography, isothermal titration calorimetry, NMR spectroscopy and cryogenic transmission electron microscopy techniques. Free SI and SS were shown to reduce the concentration of CH in dietary mixed micelles via a dynamic competition mechanism. CH, SI and SS affect the microstructure of lipid vesicles and influence the process of amphiphilic self-assembly of nutrients in the gut with the formation of dietary mixed micelles in a similar manner. Therefore, substitution of CH by phytosterols and phytostanols in the diet does not lead to the notable changes in the mechanism of dietary mixed micelle formation and does not affect the process of the intestinal transport of nutrients and drugs via the micellar diffusion mechanism. Our experimental findings demonstrate that the introduction of plant sterols and plant stanols into the diet is clearly beneficial for the reduction of the intestinal uptake of cholesterol. Due to the limited capacity of dietary mixed micelles to embody hydrophobic sterol/stanol molecules, the micellar concentration of cholesterol is reduced and hence, its transport towards the intestinal brush border membrane decreases.

Paleos C.M., and Tsiourvas D.(2006) Interaction between complementary liposomes: a process leading to multicompartment systems formation. *J Mol Recognit.* **19**, 60-7.

Abstract: Interaction of complementary liposomes induces a series of processes, involving reorganization of their membrane lipids, which lead to the formation of large aggregates. In several cases these aggregates exhibit multicompartment structures and only primitively mimic, in some aspects at least, the multicompartmental features of cells. Similar multicompartment structures were repeatedly obtained following the interaction of a diversity of complementary liposomal pairs. Thus, a working hypothesis is

proposed, according to which, molecular recognition of liposomes induces the formation of multicompartiment structures. Copyright 2005 John Wiley & Sons, Ltd.

Tsamaloukas A. D., Szadkowska H., Slotte P. J., and Heerklotz H. H. (2005) Interactions of cholesterol with lipid membranes and cyclodextrin characterized by calorimetry. *Biophys J*. (epublication)

Abstract: Interactions of cholesterol (cho) with different lipids are commonly believed to play a key role in the formation of functional domains in membranes. We introduce a novel approach to characterize cholesterol-lipid interactions by isothermal titration calorimetry. Cholesterol is solubilized in the aqueous phase by reversible complexation with methyl-beta-cyclodextrin (cyd). Uptake of cholesterol into the membrane is measured upon a series of injections of lipid vesicles into a cyd/cho solution. As an independent assay, cholesterol release from membranes is measured upon titrating lipid/cho mixed vesicles into a cyd solution. The most consistent fit to the data is obtained with a mole fraction (rather than mole ratio) partition coefficient and considering a cholesterol:cyd stoichiometry of 1:2. The results are discussed in terms of contributions from (i) the transfer of cholesterol from cyd into a hypothetical, ideally mixed membrane and (ii) from non-ideal interactions with POPC. The latter are exothermic but opposed by a strong loss in entropy, in agreement with cholesterol-induced acyl chain ordering and membrane condensation. They are accompanied by a positive heat capacity change which cannot be interpreted in terms of the hydrophobic effect, suggesting that additive-induced chain ordering itself increases the heat capacity. The new assays have a great potential for a better understanding of cholesterol-lipid interactions and yield suggestions how to optimize cholesterol extraction from membranes.

Tsamaloukas A., Szadkowska H., and Heerklotz H. (2006) Thermodynamic comparison of the interactions of cholesterol with unsaturated phospholipid and sphingomyelins. *Biophys J* **90**, 4479-4487.

Abstract: A comparative analysis of the interaction of cholesterol (Chol) with palmitoyl-oleoyl-phosphatidylcholine (POPC) and sphingomyelins (SM) was performed in largely homogeneous, fluid-phase membranes at 50 degrees C. To this end, three independent assays for isothermal titration calorimetry were applied to POPC/SM/Chol mixtures. Cholesterol is solubilized by randomly methylated-beta-cyclodextrin and the uptake of Chol into (or release from) large unilamellar vesicles is measured. The affinity of Chol to a POPC/SM (1:1) membrane with 30 mol % Chol is approximately two times higher than to POPC alone; extrapolation to pure SM yields an affinity ratio of R(K) approximately 5. Bringing Chol in contact with SM is highly exothermic (-7 kJ/mol for POPC/SM (1:1), and -13 kJ/mol extrapolated to pure SM, both compared to POPC). No pronounced differences were observed between egg, bovine brain, and palmitoyl SM. With decreasing Chol content, R(K) increases and ΔH becomes more exothermic, suggesting a trend toward superlattice formation. That SM/Chol-interactions are enthalpically favorable implies that the preference of Chol for SM increases upon cooling and can induce domain formation below a certain temperature. The enthalpy gain is partially compensated by a loss in entropy in accordance with the concept of Chol-induced chain ordering, which improves intermolecular interactions (van der Waals, H-bond) but reduces conformational and motional freedom. The ability of cyclodextrin to extract sphingomyelin from membranes is twofold-weaker than for POPC.

Wenk M. R. and Seelig J. (1998) Proton induced vesicle fusion and the isothermal L α ->HII phase transition of lipid bilayers: a ³¹P-NMR and titration calorimetry study. *Biochim Biophys Acta* **1372**, 227-236.

Abstract: The proton-induced isothermal fusion of unilamellar lipid vesicles (Duzgunes et al., *Biochemistry* 24 (1985) 3091-3098) is compared with the lamellar (L α)->hexagonal (HII) phase transition of multilamellar lipid dispersions. Both lipid systems are composed of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (POPE) and oleic acid (OA) at a 7:3 molar ratio. Using solid-state phosphorus-31 nuclear magnetic resonance (³¹P-NMR) it is demonstrated that the multilamellar lipid dispersions are in the bilayer state at physiological pH and undergo a L α ->HII phase transition between pH 6.3 and 5.7. This phase transition can also be induced at constant pH by increasing the temperature. The midpoint of the temperature-induced L α ->HII transition is $T_H=56$ degrees C (at pH 7.4) and the corresponding transition enthalpy is $\Delta H=0.7 \pm 0.1$ kcal/mol as determined with differential scanning calorimetry. Both the proton-induced and the temperature-induced phase transition can be completely inhibited by addition of 30 mol% of 1-palmitoyl-2-hydroxy-sn-glycero-3-phosphocholine (LPC). In a second set of experiments unilamellar vesicles are prepared either by sonication or by extrusion through polycarbonate filters at pH 7.4 and are titrated into buffer at pH 5.7. The proton-induced fusion of the lipid vesicles is monitored with isothermal titration calorimetry, light scattering and fluorescence

spectroscopy. The fusion reaction is characterized by an endothermic enthalpy of $\Delta H = 0.5 \pm 0.2$ kcal/mol (at 28 degrees C). The fusion enthalpy is independent of the vesicle diameter and is only slightly reduced by an increase in temperature to 50 degrees C. Vesicle fusion is accompanied by an increase in light scattering, indicating the formation of larger lipid structures. The transition from unilamellar vesicles to fused lipid structures occurs in the same narrow pH range of 6.3-5.7 as observed for the L α ->HII transition of multilamellar dispersions. Vesicle fusion can be inhibited with 30% LPC. The virtually identical set of parameters found for the L α ->HII phase transition and the vesicle fusion reaction suggests that vesicle fusion also entails a L α ->HII phase transition.