

**KINETICS AND THERMODYNAMICS OF THE INTERACTION OF
AMPHIPHILES WITH SOFT LIPID INTERFACES**

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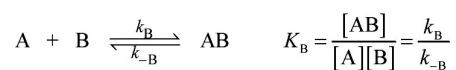
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The non-catalyzed association of amphiphilic molecules with soft lipid interfaces is the basic physical-chemical process that underlies the physiologically important maintenance of lipid homeostasis, compositional identity of cell membranes, exchange of lipids among circulating lipoproteins, and between circulating lipoproteins and cells. It is also an important process in the pharmacokinetics of amphiphilic drugs and other xenobiotics, in particular, in what concerns “tight” physiological barriers such as the blood-brain-barrier. Its complete description requires the quantitative definition of three processes: 1) the association of the amphiphile, coming from the aqueous phase, with the lipid surface (outer membrane leaflet in the case of membranes), characterized by the association rate constant, k_+ , 2) in the case of membranes, trans-membrane translocation of the amphiphile, characterized by the translocation (flip-flop) rate constant, k_f , and 3) the dissociation of the amphiphile from the lipid structure into the aqueous phase, characterized by the dissociation rate constant, k_- . Although this process has been studied in model membranes and lipoproteins, as well as in living cells by several laboratories for over thirty years, the three relevant kinetic rate constants are, to our knowledge, known only for the case of fatty acids and their derivatives [see Kleinfeld, A.M. **J. Membr. Biol.** 175 (2000) 79 and literature cited therein] and for a lysophosphatidylethanolamine derivative [Nichols, J.W. **Biochemistry** 24 (1985) 6390].

The principal difficulty in studying the detailed kinetics of amphiphile association with soft lipid interfaces has been the fact that the amphiphiles themselves aggregate in aqueous solution at very low concentrations with complex aggregation kinetics making their subsequent interaction with lipid interfaces not amenable to a straightforward and simple kinetic analysis. Thus, most reports have studied the first-order dissociation of an amphiphile associated with a lipid aggregate (membrane, lipoprotein, etc.) and have assumed the association process to be diffusion-limited or considerably faster than the dissociation step.

To overcome this difficulty we have recently developed a strategy [Vaz, W.L.C. and Melo, E. **J. Fluorescence** 11 (2001) 255 and Abreu, M.S.C. *et al.* **Biophys. J.** 84 (2003) 386] in which the amphiphile, A, of interest is first associated to a binding agent, B, whose concentration can be adjusted so that the concentration of free amphiphile in aqueous solution is well below its critical micellar concentration (CMC). Addition of the lipid interface (membranes, lipoproteins) of choice to this reaction mixture then results in a net transfer of amphiphile to

the lipidic structure from the binding agent through the aqueous interface. The whole process can then be defined as:



where L is the lipid interface (membrane vesicle, lipoprotein particle, etc.), K_B and K_L are equilibrium association constants, and k_i are the kinetic rate constants for the processes for which they are defined above. The strategy requires that the equilibrium and kinetic constants for the association of amphiphile with the binding agent be previously known, the best case being when the characteristic times for the association with B are negligibly small compared to the characteristic times for the association with L. However, analytical solutions are possible for all cases.

Using this methodology, with bovine serum albumin as the binding agent (B), we have studied the association of a fluorescent lipid-derived amphiphile, NBD-dimyristoyl-phosphatidylethanolamine (NBD-DMPE), with lipid bilayer membranes in the liquid-ordered and liquid-disordered phases [Abreu, M.S.C. *et al.* **Biophys. J.** 87 (2004) 353] and with several types of lipoproteins [Estronca, L.M.B.B. *et al.* **Biophys. J.** 88 (2005) 557]. For purposes of comparison, we have also studied the association of another lipid-derived fluorescent amphiphile, NBD-lyso-myristoyl-phosphatidylethanolamine (NBD-lysoMPE) with the same lipid bilayer membranes [Sampaio, J.L. *et al.* **Biophys. J.** (2005) in press]. The latter amphiphile could be used directly in these studies at concentrations below its CMC.

All equilibrium constants were obtained by equilibrium titrations as a function of temperature and all kinetic rate constants were also obtained as a function of temperature between 15 and 35°C. The rate constant for trans-bilayer translocation (flip-flop) of these amphiphiles in the membranes studied was also obtained over the same temperature range using an established procedure [McIntyre, J.C. and Sleight, R.G. **Biochemistry** 30 (1991) 11819]. This permitted us to evaluate the thermodynamics of every step (including activation steps) in the reaction mechanism. These parameters will be presented and discussed.

Contrary to the generally assumed and accepted mechanism, we find that the insertion of an amphiphile into a lipid bilayer membrane or a lipoprotein surface is not a diffusion-limited reaction but rather involves the diffusion-limited formation of an encounter complex (with the amphiphile at the surface of the lipid aggregate) followed by a much slower insertion process that we interpret to be dependent upon the existence, in the lipid interface, of a free volume large enough to accommodate the amphiphile.

It was also shown that amphiphile translocation across a bilayer membrane was dominated by the work of placing the polar head group of the molecule into the hydrophobic bilayer midplane and did not depend upon the size of the apolar portion of the amphiphile.