Estimating the aqueous [FA] and the FA mole-fraction in the lipid bilayer

FA adsorption to lipid bilayers denotes partitioning between two immiscible phases, the aqueous phase and the membrane phase (1). The quantitative relation between the concentration of FA in the membrane phase (expressed as moles/volume, $[FA]_m$, or moles/area, $\{FA\}_m$), and the aqueous concentration $[FA]_a$ can be described using different frameworks (2). For the present analysis it is convenient to use both a bulk partition description:

$$[FA]_m = K_1 \cdot [FA]_a , \quad (1)$$

where K_1 is the (dimension-less) partition coefficient; and a surface adsorption model:

$$\{FA\}_m = K_2 \cdot [FA]_a , \quad (2)$$

where K_2 is the surface adsorption coefficient: $K_2 = K_1 \cdot d_0/2$, where d_0 is the bilayer thickness such that $\{FA\}_m$ denotes the amount adsorbed to each leaflet.

When a FA is added to the aqueous solution bathing a lipid bilayer, to a nominal concentration $[FA]_{nom}$, the FA will adsorb to the walls of the chamber and partition between the aqueous and membrane phases, where the latter includes not only the bilayer *per se* but also the bilayer-forming solution. The aqueous and membrane FA concentrations are estimated from Eq. 1 together with the conservation relation:

$$[FA]_{nom} \cdot V_{aq} = [FA]_a \cdot V_{aq} /(1 - F_T) + [FA]_m \cdot V_{lip} \quad (3)$$

where V_{aq} and V_{lip} denote the volumes of the aqueous and lipid solutions, respectively, and F_T denotes the fractional depletion due to adsorption to the Teflon chamber. We thus find that

$$[FA]_a = \frac{[FA]_{nom} \cdot V_{aq}}{\left(V_{aq} /(1 - F_T) + K_1 \cdot V_{lip}\right)} \quad (4)$$

and (using Eq. 2)

$$\{FA\}_m = \frac{[FA]_m \cdot d_0}{2} = \frac{K_1 \cdot [FA]_a \cdot d_0}{2} = \frac{K_1 \cdot [FA]_{nom} \cdot V_{aq} \cdot d_0}{\left(V_{aq} /(1 - F_T) + K_1 \cdot V_{lip}\right) \cdot 2} , \quad (5)$$

where Eqs 4 and 5 reduce to

$$[\mathrm{FA}]_a \approx \frac{[\mathrm{FA}]_{nom} \cdot V_{aq}}{K_1 \cdot V_{lip}} \quad (6)$$

and

$$\{\mathrm{FA}\}_m = \frac{[\mathrm{FA}]_m \cdot d_0}{2} \approx \frac{[\mathrm{FA}]_{nom} \cdot V_{aq} \cdot d_0}{V_{lip} \cdot 2} \quad (7)$$

when $K_1 \gg V_{aq}/(V_{lip} \cdot (1-F_T))$.

We measured the [FFA] in the Teflon chamber using ADIFAB. In the absence of lipid, only 29 ± 2% of the DHA and 25 ± 2% of the OA remains in the electrolyte; the rest is adsorbed to the Teflon.

Knowing F_T, K_1, d_0, V_{aq} and V_{lip}, it is now possible to estimate $[\mathrm{FA}]_a$, $\{\mathrm{FA}\}_m$ and the FA mole-fraction in the lipid bilayer, m_{FA}, in our experimental system. In our case, $F_T \approx 0.75$, $d_0 \approx 4$ nm, $V_{aq} = 5$ mL $V_{lip} \approx 2 - 4$ μL, and $K_1 = 2 \cdot 10^5$ (for DHA) and $2.5 \cdot 10^6$ (for OA); inserting into Eqs. 4 and 5 (or Eqs. 6 and 7) we find that $[\mathrm{DHA}]_a \approx [\mathrm{DHA}]_{nom} \cdot 1.2 \cdot 10^{-2}$ (for $V_{lip} = 2$ μL) and $\{\mathrm{DHA}\}_m \approx [\mathrm{DHA}]_{nom} \cdot 5 \cdot 10^{-4}$ cm (with [DHA] in moles/cm^3 and $\{\mathrm{DHA}\}_m$ in moles/cm^2); for OA, $[\mathrm{OA}]_a \approx [\mathrm{OA}]_{nom} \cdot 10^{-4}$ and $\{\mathrm{OA}\}_m \approx [\mathrm{OA}]_{nom} \cdot 5 \cdot 10^{-4}$ cm (note that $\{\mathrm{FA}\}_m/[\mathrm{FA}]_{nom}$ does not depend on K_1, cf. Eq. 7). For $[\mathrm{DHA}]_{nom} = 3$ μM, then $[\mathrm{DHA}]_a \approx 37$ nM and $\{\mathrm{DHA}\}_m \approx 1.5 \cdot 10^{-12}$ moles/cm^2 (or $9 \cdot 10^{11}$ molecules/cm^2) and $m_{DHA} \approx 6.3 \cdot 10^{-3}$ (assuming an area/lipid of 0.7 nm); for OA the corresponding values would be $[\mathrm{OA}]_a \approx 3$ nM and $\{\mathrm{OA}\}_m \approx 1.5 \cdot 10^{-12}$ moles/cm^2 (or $9 \cdot 10^{11}$ molecules/cm^2) and $m_{OA} \approx 6.3 \cdot 10^{-3}$. (Because most of the FA is in the hydrophobic phase, m_{FA} becomes independent of K_1.)

The above estimates are subject to uncertainty, but they are unlikely to be off by an order of magnitude. Indeed, when we attempted to measure the [FFA] in the experimental chamber in the

presence of a bilayer, and bilayer-forming solution, the concentrations were immeasurably small. We conclude that the FA concentrations in the electrolyte solutions in the present experiments are comparable (if higher) to those found in human serum (3), but less than those found in the cerebrospinal fluid after ischemic stroke (~1 μM (4)).

References

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