

Transbilayer Complementarity of Phospholipids in Cholesterol-Rich Membranes

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(Supporting Information)

As discussed in the text, the interchange of monomers of **A** and **B** among **AA**, **BB** and **AB** is governed by an equilibrium constant, K , which characterizes their mixing behavior (eqs 1 and 2).



$$K = \frac{[\mathbf{AB}]^2}{[\mathbf{AA}][\mathbf{BB}]} \quad (2)$$

Because **A'A'** and **AA**, **B'B'** and **BB**, and **A'B'** and **AB**, overlapped in the HPLC traces, dimer ratios $[\mathbf{AB}]/[\mathbf{BB}]$, $[\mathbf{AB}]/[\mathbf{AA}]$, and $([\mathbf{AB}]+[\mathbf{A'B'}])/[\mathbf{BB}]$ were used to calculate K values when **A'A'**, **B'B'** and **A'B'** were employed as the templates, respectively, as outlined below:

Experiments using 25 mol% A'A'. Liposomes were prepared using 25% A'A', 25% AA and 50% BB, and also 25% A'A', 25% BB and 50% AB. In both cases, the molar ratio of A/B equals 1/2. Since essentially all of the phospholipids are in the dimer form at the end of the exchange reaction (quantitative TLC indicates that <0.6% of the thiol monomer is present, see Experimental section), the mass balance in these systems can be expressed according to equation 3. Here, [AA], [AB] and [BB] refer to the dimer concentrations that are present when equilibrium is reached, and [A]₀/[B]₀ is the molar ratio of exchangeable monomer units that are present in the form of dimers, which is constant.

$$\frac{2[\text{AA}] + [\text{AB}]}{2[\text{BB}] + [\text{AB}]} = \frac{[\text{A}]_0}{[\text{B}]_0} = \frac{1}{2} \quad (3)$$

Equation 4 is a rearranged form of eq 3, that expresses [AA] in terms of [BB] and [AB]. Substitution of eq 4 into eq 2 then affords *K*, which can be calculated from the experimentally measured dimer ratio [AB]/[BB], according to eq 5.

$$[\text{AA}] = \frac{[\text{BB}]}{2} - \frac{[\text{AB}]}{4} \quad (4)$$

$$K = \frac{[\text{AB}]^2}{[\text{AA}][\text{BB}]} = \frac{[\text{AB}]^2}{\left(\frac{[\text{BB}]}{2} - \frac{[\text{AB}]}{4}\right)[\text{BB}]} = \frac{4\frac{[\text{AB}]}{[\text{BB}]}}{2\frac{[\text{BB}]}{[\text{AB}]} - 1} \quad (5)$$

The standard deviation of *K* can then be calculated based on the standard error transfer formula according to eqs. 6 and 7. Thus, if *x*₁, *x*₂, ... *x*_{*n*} are experimentally measured values, and *y* is calculated from these values using the formula, *y* = *f*(*x*₁, *x*₂, ... *x*_{*n*}), then the standard deviation of *y* is given by eq 7.

$$y = f(x_1, x_2, \dots, x_n) \quad (6)$$

$$dy = \frac{\partial f}{\partial x_1} dx_1 + \frac{\partial f}{\partial x_2} dx_2 + \dots + \frac{\partial f}{\partial x_n} dx_n \quad (7)$$

Here, *y*=*K* and *f*(*x*) = (4*x*)/(2/*x* - 1), where *x* = [AB]/[BB]. Equation 8, which is obtained by substituting these parameters into eq 7, represents the standard deviation of *K*.

$$dK = \frac{\partial K}{\partial \frac{[AB]}{[BB]}} d \frac{[AB]}{[BB]} = \left(\frac{16 \frac{[AB]}{[BB]} - 4 \left(\frac{[AB]}{[BB]} \right)^2}{\left(2 - \frac{[AB]}{[BB]} \right)^2} \right) d \frac{[AB]}{[BB]} \quad (8)$$

Experiments using 25 mol% B'B'. Using an approach that is identical to that described above, eqs 9, 10 and 11 are applicable when B'B' is used as the template:

$$[BB] = \frac{[AA]}{2} - \frac{[AB]}{4} \quad (9)$$

$$K = \frac{[AB]^2}{[AA][BB]} = \frac{[AB]^2}{[AA] \left(\frac{[AA]}{2} - \frac{[AB]}{4} \right)} = \frac{4 \frac{[AB]}{[AA]}}{2 \frac{[AA]}{[AB]} - 1} \quad (10)$$

$$dK = \frac{\partial K}{\partial \frac{[AB]}{[AA]}} d \frac{[AB]}{[AA]} = \left(\frac{16 \frac{[AB]}{[AA]} - 4 \left(\frac{[AB]}{[AA]} \right)^2}{\left(2 - \frac{[AB]}{[AA]} \right)^2} \right) d \frac{[AB]}{[AA]} \quad (11)$$

Experiments using 25 mol% A'B'. Liposomes were prepared using 25% A'B', 37.5% AA and 37.5% BB, and also 25% A'B' and 75% AB. In both cases, the molar ratio, r_1 , of template A'B' to the sum of the exchangeable dimers is 1/3, as shown in eq 12. This ratio simply reflects the mass balance in the system, and is constant provided that the thiol monomer content is negligible, which has been found to be the case (see Experimental section). In eq 12, $[AA]_0$, $[BB]_0$ and $[AB]_0$ refer to the starting concentrations of the exchangeable dimers, and $[AA]$, $[BB]$ and $[AB]$ are the final equilibrium concentrations.

$$r_1 = \frac{[A'B']}{[AB]_0} = \frac{[A'B']}{[AA]_0 + [BB]_0} = \frac{[A'B']}{[AA] + [AB] + [BB]} = \frac{1}{3} \quad (12)$$

Since the concentration of exchangeable A and B are identical in these systems, and since the reaction coefficients of AA and BB are same (eq 1), the concentrations of AA and BB are always same throughout the course of the reaction, i.e., $[AA] = [BB]$. In other words, eq 12 can also be expressed as eq 13.

$$r_1 = \frac{[A'B']}{[AB] + 2[BB]} = \frac{1}{3} \quad (13)$$

From the HPLC analysis, one can readily measure the concentration of **BB** that is present, as well as the *sum* of **AB** and **A'B'**. Thus, if we define r_2 by eq 14, this parameter can be determined by HPLC analysis.

$$r_2 = \frac{[AB] + [A'B']}{[BB]} \quad (14)$$

If we now combine eqs 13 and 14 and eliminate the **A'B'** term, one may obtain eq 15.

$$\frac{[AB]}{[BB]} = \frac{r_2 - 2r_1}{1 + r_1} \quad (15)$$

Additionally, since $r_1 = 1/3$, eq 15 can then be expressed as eq 16,

$$\frac{[AB]}{[BB]} = \frac{3r_2 - 2}{4} \quad (16)$$

Finally, if one now combines eqs 2 and 16, where $[AA] = [BB]$, the equilibrium constant that governs the exchange reaction can now be expressed in a form that can be determined by r_2 (eq 17).

$$K = \left(\frac{[AB]}{[BB]} \right)^2 = \left(\frac{3r_2 - 2}{4} \right)^2 \quad (17)$$

By using standard error transfer formulas (eqs 6 and 7) on eq 17, the standard deviation of K is given by eq 18,

$$dK = \frac{\partial K}{\partial r_2} dr_2 = \frac{3(3r_2 - 2)}{8} dr_2 \quad (18)$$