Monitoring Lipid Membrane Translocation of Sodium Dodecyl Sulfate by Isothermal Titration Calorimetry

Supporting Information

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1 Experimental Procedures

1.1 Materials

POPC was purchased from Avanti Polar Lipids (Alabaster, U.S.A.) and SDS from Sigma–Aldrich (Steinheim, Germany). All other chemicals were obtained from Merck (Darmstadt, Germany). All experiments were performed in 10 mM phosphate buffer, 154 mM NaCl, pH 7.4.

1.2 Vesicle Preparation

POPC dissolved in chloroform at 20 mg/mL was dried in a rotary evaporator and subsequently under high vacuum overnight. For the preparation of pure lipid vesicles, dry lipid films were suspended in buffer by vortex mixing for 5 min, yielding large multilamellar vesicles. Bilayers preloaded with SDS in both leaflets were made in the same manner by suspending lipid films in buffer containing the detergent at the desired final concentration of 2 mM. LUVs were prepared by 35 extrusion steps through two stacked polycarbonate filters with a pore diameter of 100 nm using a LiposoFast extruder (Avestin, Ottawa, Canada). The vesicle size was narrowly distributed around 100 nm, as checked by dynamic light scattering on an N4 Plus particle sizer (Beckman Coulter, Fullerton, U.S.A.) equipped with a 10-nW helium/neon laser with a wavelength of 632.8 nm at a scattering angle of 90°. Under the conditions used here, the presence of SDS during vesicle formation had no effect on the size of the liposomes.

1.3 Isothermal Titration Calorimetry

High-sensitivity isothermal titration calorimetry1 was performed on a VP-ITC (MicroCal, Northampton, U.S.A.) after vacuum degassing of the samples. Uptake experiments consisted of injecting 25 10-µL aliquots of 20 mM POPC into the 1.4-mL calorimeter cell containing 100 µM SDS. In release assays, 25 10-µL aliquots of 10 mM POPC preloaded with 2 mM SDS were titrated into pure buffer. The time spacings between consecutive injections were always chosen long enough to allow for return of the ITC signal to baseline level and strongly depended on temperature. Baseline subtraction and peak integration were accomplished using Origin 5.0 as described by the manufacturer (MicroCal Software, Northampton, U.S.A.). Reaction heats measured in both uptake and release experiments were normalized with respect to the molar amount of lipid injected. The first injection was always excluded from evaluation because it usually suffers from sample loss during the mounting of the syringe and the equilibration preceding the actual titration. All experiments were carried out at least in triplicate and were highly reproducible.

2 Alternative Partitioning Model

2.1 Theory

A frequently used and mathematically straightforward partitioning model² taking into account nonideal interactions in the membrane phase is based on a mole ratio partition coefficient defined as

\[ \tilde{K}_{b/aq}^{D} \equiv \frac{R_{b}^{D}}{c_{b}^{D}} = \frac{c_{D}^{b}}{c_{D}^{aq}} \]

where \( R^b_D \equiv c^b_D/c_L \) is the detergent/lipid mole ratio in the membrane. Substituting \( c^aq_D = c_D - c^b_D \), we obtain

\[
\tilde{K}^{b/aq}_D = \frac{c^b_D}{(c_D - c^b_D)c_L} \tag{2}
\]

which gives the concentration of detergent in the membrane as

\[
c^b_D = \frac{\tilde{K}^{b/aq}_D c_L}{1 + \tilde{K}^{b/aq}_D c_L} \tag{3}
\]

Transbilayer distribution and electrostatic effects are accounted for in the same way as outlined in the main text for the model based on ideal mixing.

### 2.2 Results and Discussion

Evaluation of uptake and release experiments performed in the temperature range from 25 to 65 °C in terms of nonideal mixing in the membrane according to eq 1 returns the results compiled in Table S 1. These values are in reasonable accord with those reported previously\(^3\) for somewhat different conditions (10 mM Tris buffer, 100 mM NaCl, pH 7.4). Here, the standard molar Gibbs free energy change reads

\[
\Delta G^{b/i,0}_D = -RT \ln \left( \tilde{K}^{b/i}_D c_W \right) \tag{4}
\]

The conversion rule\(^4\) between the mole fraction partition coefficient, \( K^{b/i}_D \), and the mole ratio partition coefficient, \( \tilde{K}^{b/i}_D \), is given by

\[
\tilde{K}^{b/i}_D = \frac{K^{b/i}_D}{c_W} \frac{1}{1 - X^b_D} \tag{5}
\]

Thus, \( \tilde{K}^{b/i}_D \to K^{b/i}_D/(55.5 \text{ M}) \) for \( X^b_D \to 0 \) but must be greater than this limiting value at finite detergent concentrations to compensate for the unfavorable nonideal contribution inherent in this model. For the present case, comparison of Tables 1 and S 1 reveals that \( \tilde{K}^{b/i}_D \approx K^{b/i}_D/(48 \text{ M}) \). The differences in the other parameters, particularly in \( \gamma \), between the two partitioning models are well within the experimental error limits and, hence, are not significant. Likewise, the temperature dependence of \( K^{b/i}_D \) (evaluation not shown) is as consistent as that of \( \tilde{K}^{b/i}_D \) in Figure 3b, indicating that, at low detergent concentrations, both models are equally well suited for describing SDS partitioning into and translocation across POPC bilayers.

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