Selective phosphatidylethanolamine translocation across vesicle membranes using synthetic translocases

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Phospholipids are asymmetrically distributed between the outer and inner monolayers of biological plasma membranes. For example, most of the phosphatidylserine and phosphatidylethanolamine is sequestered in the inner monolayer of human erythrocytes, while the outer monolayer contains most of the phosphatidylcholine and sphingomyelin. Phospholipid translocation or ‘flip-flop’ across a bilayer membrane is inherently a slow process with a typical half-life of many hours. The asymmetric distribution is maintained by enzymes known generally as ‘translocases’ that facilitate translocation; however, there is little mechanistic understanding of how these enzymes work, or how translocation in general occurs on a molecular level. We are interested in designing synthetic translocases, which can be employed as pharmaceuticals or as chemical tools for biological membrane research. Recently, we reported that tren sulfonamide derivative 1 facilitates the translocation of the fluorescent probe PC-NBD across vesicle and cellular membranes. Compound 1 is thought to form a hydrogen-bonded complex with the phosphocholine headgroup at the surface of the membrane, effectively reducing the head-group polarity and lowering the barrier to diffusion across the lipophilic interior of the membrane. In this paper, we describe two second-generation tren derivatives (compounds 2 and 3) that selectively facilitate the translocation of PE-NBD, a fluorescent phospholipid probe that contains the phosphoethanolamine head-group.

Phospholipid translocation is monitored via the well-established 7-nitrobenz-2-oxa-1,3-diazol-4-yl (NBD)/dithionite quenching assay, which uses phospholipid probes that contain an NBD group in one of the acyl chains. The assay starts with surface-differentiated vesicles which are prepared with NBD-phospholipids in either the membrane outer monolayer (exo-labeled) or inner monolayer (endo-labeled). Upon treatment with sodium dithionite (Na2S2O4), the NBD fluorescence is quenched due to reduction of the NBD nitro group. Vesicle membranes are effectively impermeable to dithionite, therefore, only NBD-phospholipid located in the outer leaflet is chemically quenched. Exo-labeled vesicles, used to measure inward translocation (flip), are prepared by addition of a small aliquot of NBD-lipid (0.5 mol% of total phospholipid) in ethanol to a suspension of unlabelled 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) vesicles. The NBD-lipid readily inserts into the vesicle outer monolayer. Endo-labeled vesicles, used for outward translocation (flop) experiments, are produced by reducing the fluorescence intensity when a aliquot of NBD lipiddithionite mixture is added. In the presence of sulfonamide derivatives, the inward translocation of PE-NBD is decreased by about 55%, whereas the inward translocation of PC-NBD is increased by 110%.

The first attempt to design a phosphatidylethanolamine-selective translocase was with the sulfonamide crown ether. The benzo-18-crown-6 ring is well-known to associate strongly with ammonium cations in organic solvents and to select for phosphatidylethanolamine over phosphatidylcholine. As a starting point, first-generation sulfonamide 1 was evaluated for its ability to translocate PC-NBD and PE-NBD across pure POPC vesicle membranes (25 μM phospholipid). In the absence of synthetic translocase, the inward translocation of both probes is very slow with half-lives of more than several hours. In the presence of sulfonamide 1 (5 μM), inward translocation is greatly accelerated but by similar amounts (translocation half-lives of both PC-NBD and PE-NBD are reduced to ca. 6 min). In other words, sulfonamide 1 does not apparently differentiate between the two phospholipid headgroups.

The goal of this study was to find synthetic translocases that select for phosphatidylethanolamine over phosphatidylcholine. As a starting point, first-generation sulfonamide 1 was evaluated for its ability to translocate PC-NBD and PE-NBD across pure POPC vesicle membranes (25 μM phospholipid). In the absence of synthetic translocase, the inward translocation of both probes is very slow with half-lives of more than several hours. In the presence of sulfonamide 1 (5 μM), inward translocation is greatly accelerated but by similar amounts (translocation half-lives of both PC-NBD and PE-NBD are reduced to ca. 6 min). In other words, sulfonamide 1 does not apparently differentiate between the two phospholipid headgroups.
The extraordinary effectiveness of 3 to act as a phosphatidylethanolamine-like vesicle translocase is likely due to a combination of structural features. Not only are the sulfonamide hydrogens quite acidic due to the electron withdrawing effect of the trifluoromethyl groups, but the isopropyl chains located on the backbone enhance lipophilicity. The selectivity cannot be attributed to a binding preference for phosphatidylethanolamine over phosphatidylcholine by the neutral form of 3. In fact, the reverse is true. NMR titration experiments show that 3 binds to 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (POPE) in CDCl 3 at 25 °C with an association constant of 20 M⁻¹, whereas it binds to POPC with an association constant of 2500 M⁻¹. A plausible alternative explanation is that 3 exists as a zwitterionic species on the polar membrane surface due to proton transfer from one of the acidic trifluoromethylsulfonamide NH groups to the central tertiary amine. Molecular models suggest that this flexible zwitterionic version of 3 can adopt conformations that nicely complement the structure of the phosphatidylethanolamine head-group. Future studies will attempt to confirm this binding hypothesis.

In summary, the ten sulfonamides 2 and 3 selectively facilitate transbilayer migration of phosphatidylethanolamine over phosphatidylcholine. This differs from compound 1 which translocates both phospholipid head-groups equally. Compounds 2 and 3 may have potential uses in biomembrane research as synthetic phosphatidylethanolamine translocases.²⁻⁷

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Notes and references

† Compound 2 was prepared in 60% yield from tris(aminomethyl)amine and chlorosulfonylbenzo-18-crown-6; mp 168–172 °C; 1H NMR (CDCl 3, 300 MHz): δ 7.57 (d, 3H, 6.8 Hz), 7.50 (s, 3H), 6.99 (d, 3H, 7.2 Hz), 6.21 (s, br, 3H), 4.25 (m, 12H), 3.93 (m, 6H), 3.71 (m, 42H), 2.91 (s, br, 6H), 2.57 (s, br, 6H). 13C NMR (75MHz, CDCl 3); δ 151.9, 148.5, 131.5, 121.2, 112.1, 111.3, 70.6, 70.5, 70.4, 69.2, 69.1, 68.7, 68.5, 53.9, 40.9; HRMS calculated for C 54 H 85 N 4 O 24 S 3 1269.4716, found 1270.4793.

‡ As previously observed with sulfonamide 1, the crown derivative 2 (100 μM) is unable to facilitate inward translocation of PE-NBD across vesicle membranes composed of 1:1 POPC:PE (25 μM). This inhibitory effect is attributed to the presence of intermolecular hydrogen bonds between the POPE ammonium and neighboring phosphate head-groups. Sulfonamide 3 (8 μM), however, is still able to translocate PE-NBD effectively across 1:1 POPC:POPE vesicle membranes although the half-life is five times slower than in pure POPC vesicles.

§ The pKa of compound 3 is not yet known but the pKa of trifluoromethylsulfonamide in water is 6.3.¹¹