Organic & Biomolecular Chemistry

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Cite this: *Org. Biomol. Chem.*, 2012, **10**, 260

www.rsc.org/obc

PAPER

Flexible oligocholate foldamers as membrane transporters and their guest-dependent transport mechanism†

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Received 10th August 2011, Accepted 29th September 2011
DOI: 10.1039/c1ob06364b

Dimeric, trimeric, and tetrameric oligocholates with flexible 4-aminobutyroyl spacers caused the efflux of hydrophilic molecules such as carboxyfluorescein (CF) and glucose from POPC/POPG liposomes. Transport was greatly suppressed across higher-melting DPPC membranes. Lipid-mixing assays and dynamic light scattering (DLS) indicated that the liposomes were intact during the transport. Kinetic analysis supported the involvement of monomeric species in the rate-limiting step of CF transport, consistent with a carrier-based mechanism. Glucose transport, on the other hand, displayed a highly unusual zero-order dependence on the oligocholate concentration at low loading of the transporter. Different selectivity was observed in the oligocholate transporters depending on the guest involved.

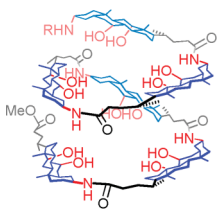
Introduction

Proteins and nucleic acids carry out molecular recognition, catalysis, transport, informational transfer, and numerous other tasks vital to cells. In recent years, many chemists have become interested in their synthetic analogues (*i.e.*, foldamers) with ordered and yet tunable conformations.[1] Part of the motivation comes from the fact that the conformation of a molecule can impact its size, shape, distribution of functional groups, and, in turn, physical and chemical properties.

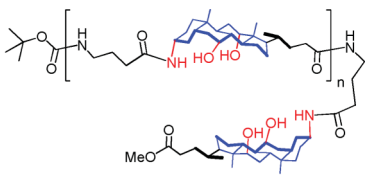
Although new folding motifs[2] and building blocks[3] continue to emerge in the literature, chemists recently focused increasingly on the applications of foldamers, creating novel antimicrobial agents,[4] protein surface-binders and inhibitors,[5] vesicles and organogellators,[6] and biomimetic enantioselective catalysts[7] from these conformationally controllable materials.

Because the conformation of a molecule is often dependent on its interactions with other molecules, understanding how a molecule behaves in different environments is important to its applications. The lipid membrane is a unique environment due to its amphiphilicity, liquid crystalline nature, and nanodimension in the lipid normal. Membrane proteins represent an extremely important class of biofoldamers and are responsible for a wide variety of cellular functions. Although membrane proteins are coded by ~30% of genes in a genome[8] and account for nearly 50% of all drug targets,[9] the majority of synthetic foldamers fold in solution and in the solid date.[1]

We have prepared foldamers by connecting cholate groups head-to-tail by amide bonds.[10] These facially amphiphilic oligomers typically fold in nonpolar solvents containing a few percent of a polar solvent. Folding creates a helix, whose interior is filled disproportionally with the polar solvent that solvates the introverted hydrophilic groups. Such an arrangement is beneficial to both the entrapped polar solvent and the hydrophilic groups of the cholates. Having a hydrophilic internal cavity and a hydrophobic exterior, the folded helix seems perfect for translocating hydrophilic molecules across a nonpolar medium.



Parent Oligocholate



Flexible Oligocholate

1, n = 1; **2**, n = 2; **3**, n = 3

Recently, we discovered that oligocholate-based foldamers,[11] baskets,[12] and macrocycles[13] were effective transporters in lipid membranes. Chemists have long been interested in transporting ions and molecules across lipid bilayers using synthetic carriers, channels, and pores.[14] Our research was inspired by other cholate-derived transporters reported in the literature.[15] Herein, we report the transport of hydrophilic molecules by flexible oligocholates **1–3**. Previously, the 4-aminobutyroyl-spaced oligocholates were found to fold better than the parent foldamers in the solution. Whereas the rigid, parent oligocholates need at least 5 cholate groups to fold cooperatively,[16] the flexible ones could do so with 3–4 cholates, in similar or more competitive solvents.[17] In the current work, the spacers once again turned out critical to the transport. Another finding was that, although the structure of the transporter was important to its performance, the transport efficiency did not correlate directly to its foldability in solution.

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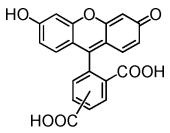
† This article is part of an Organic & Biomolecular Chemistry web theme issue on Foldamer Chemistry.

Results and discussion

Transport of CF

To study the transport across lipid bilayers, we employed the well-established CF leakage assay in liposomal research.[18] Briefly, the water-soluble fluorescent dye was trapped inside large unilamellar vesicles (LUVs) at a concentration (50 mM) that caused significant self-quenching. The extravesicular CF was removed by gel filtration. Leakage was monitored by the increase of the CF emission upon injection of the oligocholates in DMSO. The efflux was followed for 120 min, at which the LUVs were lysed by the addition of Triton X-100 to release all the CF.



CF

Fig. 1 shows the leakage profiles of CF induced by the three oligocholates. All three compounds were quite effective at triggering the release. Leakage generally increased with an increasing concentration of the oligocholates. At the same concentration, the shorter oligomers (Fig. 1a,b) were noticeably more capable of transporting the dye than the longer one (Fig. 1c). Based on the slopes of the curves, leakage in all three cases started out fast and then slowed down—this was quite normal because the driving force for the leakage became smaller as the intravesicular concentration of CF decreased over time.

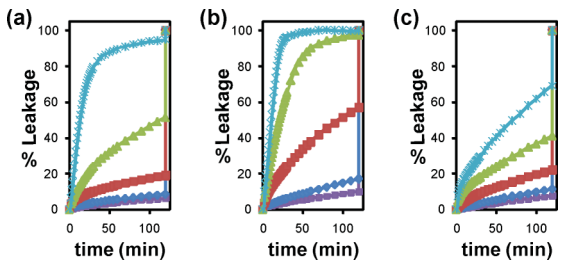
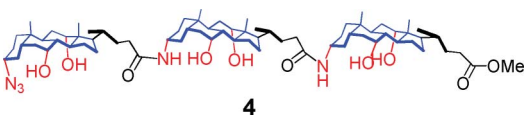


Fig. 1 Percent leakage of CF from POPC/POPG LUVs upon the addition of (a) **1**, (b) **2**, and (c) **3**. The concentrations of the oligocholates added were 0.0174, 0.035, 0.073, 0.15, and 0.29 μM from bottom to top. [phospholipids] = 2.9 μM. The liposomes were lysed at 120 min upon the addition of 1% Triton X-100.

Fig. 2 plots the CF leakage at 120 min against the concentrations of the oligocholates. In addition to the flexible oligomers **1**–**3**, we included the data for rigid trimer **4** (×) for comparison. Overall, the flexible, 4-aminobutyroyl-spaced oligocholates were far better transporters than the rigid trimer, regardless of the number of the cholates in the structure. In fact, compound **4** was completely inactive—the 5–10% leakage during the course of the assay was the same as the background value.



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Clearly, how the cholates arrange with one another was important to the transport and the 4-aminobutyroyl spacers were critical. The flexible oligocholates are known to fold better than

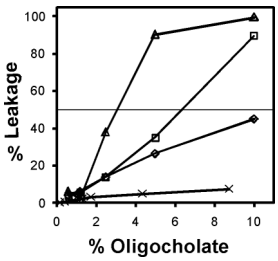

Fig. 2 Percent leakage of CF at 120 min from POPC/POPG LUVs as a function of [oligocholate]/[phospholipids] for **1** (□), **2** (△), **3** (◇), and **4** (×). [phospholipids] = 2.9 μM.

the rigid ones in solution.[17] Among the fleixble oligomers (**1**–**3**), however, neither the number of cholates nor the foldability of the oligomers directly correlated to the leakage. The most foldable tetramer (**3**), for example, was by far the worst among the three and the best transporter was the trimer (**2**). It was also clear that foldability in solution was not a prerequisite for the transport, as dimer **1** was unable to fold in solution[17] but remained effective as a transporter.

It is important to verify that the LUVs were intact during the transport. Detergents, for example, can destroy liposomes to release the dye. This is the reason why Triton X-100 was added at the end of the leakage assay (to calibrate the 100% leakage point).[18] One way to confirm the membrane's integrity was through the lipid-mixing assay.[19] In this experiment, one batch of LUVs containing 1 mol % NBD- and rhodamine-functionalized lipids was mixed with another batch of unlabeled LUVs. If the oligocholate acts as a detergent to destroy the liposomes or cause fusion, the labels would be diluted, resulting in smaller fluorescence resonance energy transfer (FRET) from NBD to rhodamine. In our hands, <10% fusion was observed upon the addition of the flexible oligocholates (Fig. 3a). Hence, no fusion or disintegration of membranes occurred under the experimental condition.

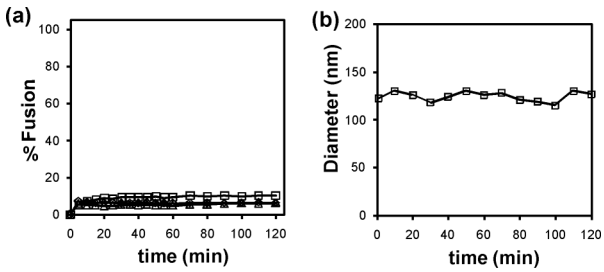

Fig. 3 (a) Percent fusion of POPC/POPG LUVs after the addition of **1** (□), **2** (△), and **3** (◇). [oligocholate] = 2.5 μM, [phospholipids] = 54 μM. (b) DLS diameter of the liposomes after the addition of **1**. [oligocholate] = 0.58 μM. [phospholipids] = 2.9 μM.

Different concentrations of the LUVs had to be used in the CF and lipid-mixing assays, as the methods have different sensitivity. To further confirm the integrity of the liposomes, we monitored the size of the LUVs by DLS, using the same concentration of LUVs as that in the CF leakage assay (*i.e.*, [phospholipids] = 2.9 μM). Again, even upon the addition of 0.58 μM of the oligocholate (twice of the highest concentration in the leakage assay), the liposomes stayed constant in size over the entire period of 120 min (Fig. 3b). The

experiment corroborated with the lipid-mixing assay and ruled out disintegration, aggregation, or fusion of the liposomes.

The initial leakage rate of CF (v_0) could be obtained by polynomial curve-fitting of the leakage curves in Fig. 1. It is useful to examine v_0 because the concentration gradient of CF across the membrane started out the same but changed to different degrees as time went by. As shown in Fig. 4, v_0 for all three transporters was linearly related to their concentration. The first-order relationship was consistent with a carrier mechanism that involved monomeric species.[20] Alternatively, in some pore-based transport, if the formation of a self-assembled pore is exergonic, the concentration of the pore would be directly proportional to the concentration of the monomer present. Transport, in such a case, could also display first-order kinetics.[21]

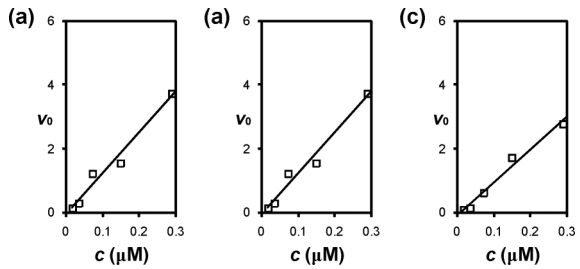

Fig. 4 Initial leakage rate v_0 (percent leakage per min) of CF from POPC/POPG LUVs as a function of oligocholate concentration for (a) **1**, (b) **2**, and (c) **3**. The initial leakage rates were obtained by polynomial curve-fitting to the leakage profiles in Fig. 1.

Thickness, hydrophobicity, and fluidity of lipid membranes have different effects on different transport mechanisms. To further probe the transport mechanism, we studied the CF leakage from DPPC (dipalmitoyl-phosphatidylcholine) LUVs. The saturated lipid has a gel–liquid crystalline transition temperature of 41 °C. It is known that carrier-based ion transporters such as nonactin and valinomycin become essentially inactive in DPPC membranes at room temperature but channel-forming gramicidin is not affected by the high melting lipid.[22] Experimentally, all three oligocholates were observed to lose most of their activity (Fig. 5). Quite interestingly, although the transport was slow, the tricholate **2** (△) remained the most effective among the three.

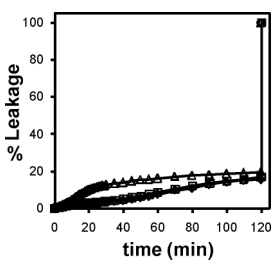

Fig. 5 Percent leakage of CF from DPPC LUVs upon the addition **1** (□), **2** (△), and **3** (◇). [oligocholate] = 0.58 μM. [phospholipids] = 2.9 μM. The liposomes were lysed at 120 min upon addition of 1% Triton X-100.

Even though none of the above tests by itself could pin down the transport mechanism, the data all together was most consistent with a carrier-based mechanism. These oligocholates conceivably could aggregate within the membranes to form "hydrophilic crevices" in the membranes (see next section for further discussion). It is difficult, nonetheless, to imagine that a molecule as wide as 1 nm could squeeze through such crevices. Also, because pores need to overcome substantial external pressure to stay open,[23] most synthetic nanopore-forming agents tend to be quite rigid.[14f,14h] The flexible oligocholates, therefore, may have difficulty forming and keeping open nanosized pores.

If the oligocholates indeed operate as carriers, why was the trimer most effective among the three (Fig. 2)? In order for a carrier to shuttle CF from one side of the membrane to the other, it needs to bind the guest and diffuse across the bilayer. For a preorganized transporter, binding may not be an issue and diffusion is frequently the rate-limiting step for the transport. For a flexible oligocholate, however, binding could only occur when the transporter wraps around the guest. Depending on its state prior to binding,[24] this step could become rate-limiting and certainly is critical to the transport process.

In the solution, dimer **1** does not have enough solvophobic driving force to fold.[17] Even though it does not need to adopt a perfectly folded conformation to transport, **1** needs to turn its hydrophilic groups inward and hydrophobic ones outward to shield the CF from the lipid hydrocarbon. Its poor folding, thus, could affect its transport. Another possible reason for the poor transportability of the dimer is related to its size. A cholate is about 1.4 nm from head-to-tail and 0.6–07 nm wide. CF is triangular in shape, *ca.* 1.0 nm on the side. Geometrically, it seems challenging for **1** to encompass the guest completely. In contrast, as shown by the molecular models (Fig. 6), the trimer (**2**) is nearly perfect for encapsulating CF, with multiple hydrogen bonds formed between the peripheral oxygens on the guest and the inwardly facing polar groups of the oligocholate.

Fig. 6 Photograph of the CPK molecular models of CF protected by trimer **2**.

Tetramer **3** was the best folder in solution[17] but the worst transporter among the three. Folding, therefore, could not be the most important factor in its performance. There are at least two possible reasons for its low activity. (a) Its larger size may make it slow to diffuse across the membranes. If three cholate groups are enough to shield CF (Fig. 6), having another cholate may not be helpful, as the extra hydrophilic groups on the fourth cholate cannot be easily satisfied in hydrogen-bonding. In general, if the hydrophilic groups of a molecule are not properly shielded from the lipid hydrocarbon, this molecule will have difficulty moving across the membrane—the same should apply to a guest or a transporter-guest complex. (b) The oligocholates very likely exist in multiple states after they come in contact with the lipid membranes.[24] If a large fraction of the oligocholates is aggregated, dissociation will be more difficult as the number of the cholates increases. Not only the hydrogen bonding interactions between

the cholate hydrophilic faces become stronger with a longer chain length, chain entanglement is also expected to be more severe. If the oligocholates transport CF as monomeric carriers, the concentration of the carrier is anticipated to decrease as the chain gets longer.

Transport of glucose

Changing the size of the guests is useful for probing the transport mechanism. In comparison to CF, glucose is much smaller in size, although both have their hydrophilic groups on the periphery. For the glucose leakage assay, LUVs were first prepared in the presence of 300 mM of glucose.[25] After the removal of extravesicular glucose, hexokinase, glucose-6-phosphate dehydrogenase, NADP, and ATP were added to liposomal solution. Without any transporting agents, the glucose will stay inside the LUVs and remain intact. The enzymes are generally too large to permeate into the LUVs by the transporters. In case any leakage occurs, the escaped glucose will be converted by the enzymes to glucose-6-phosphate while NADP reduced to NADPH. Because of the fast enzymatic kinetics, the formation of NADPH at 340 nm normally correlates directly with the rate of glucose efflux.[26]

As shown by Fig. 7, the glucose leakage profiles were drastically different from those for CF (Fig. 1). Instead of a fast-to-slow transition, the leakage rate, as judged by the slopes of the leakage curves, stayed constant in the majority of experiments. In addition, the leakage in all three cases was fairly constant at low concentrations of the oligocholates and jumped up until a certain (higher) concentration was reached, also unlike the CF transport.

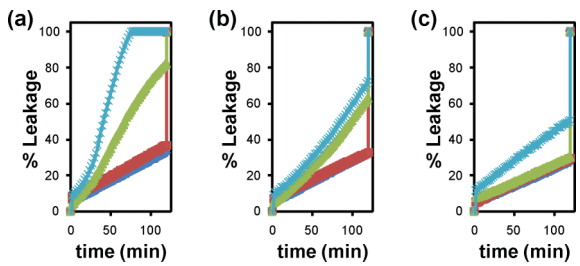

Fig. 7 Percent leakage of glucose from POPC/POPG LUVs upon the addition of (a) **1**, (b) **2**, and (c) **3**. The concentrations of the oligocholates added were 1.3, 2.7, 5.4, and 10.7 μM from bottom to top. [phospholipids] = 107 μM. The concentration of glucose was 300 mM within the LUVs. The liposomes were lysed at 120 min upon addition of 1% Triton X-100.

The linear leakage profiles in Fig. 7 indicate that the transport was independent of the glucose concentration under the experimental conditions. Linearity was observed in some cases even when 60–70% of the glucose escaped or only 30–40% of initial glucose was left in the LUVs (Fig. 7b). The behavior was quite unusual and distinctively different from what was observed in either the pore-forming[11b,13] or carrier-based oligocholates.[11a,12]

In the case of the tetramer (**3**), the leakage rate stayed exactly the same until the oligocholate reached 10.7 μM or 10 mol % of the phospholipids (Fig. 7c). Thus, v_0 had a zero-order dependence on the concentration of **3** until its concentration reached 10 mol % in the membrane—we assume that all oligocholates added went into the membranes because the oligocholates were essentially insoluble in water. The zero-order dependence was also observed for **1** and **2**, at lower concentrations (Fig. 7a,b).

The leakage induced by these flexible oligocholates overall was small in comparison to that induced by other cholate-based transporters. For example, it took <0.5 mol % of a pore-forming tricholate macrocycle in POPC/POPG membranes to leak 50% of the entrapped glucose in 60 min.[13] The same required about 2 mol % of a carrier-based tricholate basket.[12] In contrast, doing so with twice as much time required ~4 mol % of flexible tricholate **2** (estimated from Fig. 8a, △).

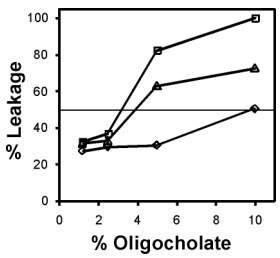

Fig. 8 Percent leakage of glucose at 120 min from POPC/POPG LUVs as a function of oligocholate concentration for **1** (□), **2** (△), **3** (◇). [phospholipids] = 107 μM.

It is difficult to directly compare the leakage of CF and glucose because the concentrations of both the guest and the liposome were different in the two assays. However, if we use the molar percentage of the oligocholate in the membrane to cause 50% leakage at 120 min (*i.e.*, EC_{50}) for comparison, the value (obtained by interpolation) for **2** was $EC_{50} \approx 3$ mol % for CF (Fig. 2) and 4 mol % for glucose (Fig. 8). To the extent that these values can be compared, the tricholate seemed to transport CF more easily than glucose.

Both the leakage profiles (Fig. 7) and the sluggishness of the glucose transport were unusual, especially given the relative ease for the CF transport. Our tentative proposal for the transport mechanism is that the guest escaped the LUVs by squeezing its way out of small "crevices" formed by the aggregated oligocholates. The reason we suggest "crevices" is that, when the oligocholates aggregate in the hydrophobic region of the membrane, they have to satisfy the hydrogen-bonding needs of the hydroxyl and amide groups. They also prefer to form these hydrogen bonds by involving as little water as possible because putting water molecules inside a hydrophobic membrane is energetically unfavorable. For these reasons, the oligocholate aggregates in the membranes are expected to be quite compact, with minimal exposure of the hydrophilic groups to the lipid hydrocarbon. When there is a large concentration gradient across the membrane (*i.e.*, 300 mM), glucose wants to escape from the LUVs for entropically reasons. Although the hydrophilic faces of the oligocholates are hydrogen-bonded in the membrane, glucose conceivably can insert itself in between the aggregated hydrophilic faces (*i.e.*, into the "crevices") by forming new hydrogen bonds with the cholates. As the glucose form and break hydrogen bonds with the (aggregated) oligocholates, the molecule essentially is squeezing its way out of the membrane, taking advantage of the facial amphiphilicity of the oligocholates.

The proposed mechanism for glucose leakage is consistent with the lack of activity of **4**, which has the same number of

cholate groups as **2**. Our previous work shows that the flexible oligocholates in general aggregate better than the rigid parent oligocholates because the flexible spacers in between the cholates help the awkwardly shaped facial amphiphiles pack more tightly.[27] In fact, flexible spacers were found to help the aggregation in both aqueous medium (when the oligocholates aggregate through the hydrophobic interactions of the hydrophobic faces)[27] and nonpolar environments (when the oligocholates aggregate through the hydrogen-bonding interactions of the hydrophilic faces).[28] If rigid trimer **4** cannot pack tightly to form stable aggregates in lipid membranes, it would prefer to lie at the lipid-water interface, with the hydrophilic faces toward water—this could be the reason for the inactivity of **4**.

The proposed mechanism, although tentative, can explain the order of transport efficiency (**1** > **2** > **3**, Fig. 8). A longer oligocholate has more cholates groups involved in the aggregation. As glucose tries to squeeze its way out, the oligocholates need to make adjustment to let the guest pass through. The longer the chain length, the more difficult the adjustment would be. This is because, not only a longer oligocholate can form more hydrogen-bonds during aggregation, there is also more entanglement of the chain in the aggregates.

The proposed mechanism is also consistent with the unusual linear leakage profiles in Fig. 7. As glucose pushes its way out of the membranes, it inserts itself in between the aggregated oligocholates. Such insertion is likely to be difficult—which is consistent with the sluggishness of the transport. If formation and breaking of the hydrogen bonds between glucose and the oligocholates are rate-limiting, constant leakage rates (*i.e.*, linear leakage profiles) would be expected. The "jump" in the leakage rate at high oligocholate loading is anticipated as well, because the overall hydrophobicity of the membrane will decrease when the membrane contains a large number of amphiphilic oligocholates, making it easier for the glucose to migrate through.

Why did the CF rely on the carrier mechanism to escape but glucose did not? A possible explanation is that the larger size and smaller number of hydrophilic groups of CF make it difficult to squeeze through the aggregated oligocholates. Glucose, on the other hand, being smaller but having more hydrophilic groups, may not be protected well by a monomeric oligocholate while all the hydrogen-bonding groups of the transporter and the guest are satisfied simultaneously. Exposure of the polar groups to the lipid hydrocarbon would make glucose transport difficult. Another reason, which is even more likely, is that multiple mechanisms might be operating and the dominant mechanism could change, depending on the guest and the concentration of the oligocholate. Dimer **1**, for example, started to display a slight downward curvature in the leakage profile at 5 and 10 mol % loading in the membrane (Fig. 7a). It is thus possible that carrier-based or other leakage mechanisms were also operative for the dimer, at least at higher concentrations.

Conclusions

The flexible oligocholates (**1–3**) were able to transport both relatively large and small hydrophilic molecules across POPC/POPG membranes. The transport of CF had several signatures consistent with the carrier-based mechanism, including the linear relationship between the initial leakage rate (v_0) and the transporter concentration, the suppression of leakage in the higher-melting DPPC membranes, and the chain length-dependence of the transport efficiency.

The glucose leakage displayed highly unusual kinetics, with the leakage rate essentially independent of the transporter concentration at low oligocholate loading. The proposed mechanism involves the guest pushing its way through the aggregated oligocholates. This process is considered sluggish and mainly dependent on the rates of formation and breaking of hydrogen bonds between glucose and the oligocholates, as well as the ability of the aggregates to adjust to the passing of the guest.

The most interesting discovery of this work is the vastly different leakage profiles for the different guests. The 4-aminobutoryl spacers were critical to both types of transport, as the rigid trimer (**4**) was completely inactive in both CF and glucose leakage. It is a relatively recent effort for chemists to emulate nature in constructing conformationally tunable molecules. Membrane proteins are a unique class of biofoldamers with diverse functions. The oligocholates, as an interesting class of membrane protein mimics, may enable new applications in drug delivery and controlled release. The zero-order dependence on the transporter concentration, in particular, could be highly desirable in a delivery application due to the constant release rate.

Experimental

General

The syntheses of **1–3**[17] and **4**[16] were reported previously. All reagents and solvents were of ACS-certified grade or higher, and were used as received from commercial suppliers. Millipore water was used to prepare buffers and the liposomes. UV-vis spectra were recorded on a Cary 50 Bio UV-visible spectrophotometer. Fluorescence spectra were recorded on a Varian Cary Eclipse Fluorescence spectrophotometer. Dynamic light scattering (DLS) was performed on a PD2000DLS^{PLUS} detector.

Preparation of the LUVs

CF-containing LUVs were prepared according to a slightly modified literature procedure.[29] A chloroform solution of POPC (25 mg mL^{-1}, 198 μL) and POPG (50 mg mL^{-1}, 10.0 μL) was placed in a 10 mL test tube and dried under a stream of nitrogen. The residue was dried further under high vacuum overnight. A solution of CF-HEPES buffer (0.5 mL, 50 mM CF, 10 mM HEPES, 10 mM NaCl, pH = 7.4) was added. Rehydration of the lipids was allowed to continue for 30 min with occasional vortexing. The opaque dispersion was subjected to ten freeze-thaw cycles. The resulting mixture was extruded twenty-nine times through a polycarbonate filter (diameter = 19 mm, pore size = 100 nm) at room temperature using an Avanti Mini-Extruder. A portion (0.1 mL) of the liposome solution was passed through a column of Sephadex G-50 using HEPES buffer (10 mM HEPES, 107 mM NaCl, pH = 7.4) as the eluent to remove the extravesicular CF. The liposome fractions were combined and diluted to 10.0 mL with the HEPES buffer. The concentration of phospholipids in the stock solution was 0.14 mM.

CF leakage assay

For fluorescence measurements, aliquots of the above LUV solution (40 μL) were diluted with the HEPES buffer (1.96 mL,

10 mM HEPES, 107 mM NaCl, pH = 7.4) in separate cuvettes, resulting in a lipid concentration of 2.9 μM in each cuvette. Aliquots of the appropriate oligocholate in DMSO were added to different cuvettes *via* a microsyringe. The amount of DMSO introduced to each sample was ≤20 μL. The change of emission intensity at 520 nm (λ_{ex} = 492 nm) was monitored over time. After 2 h, 40 μL of Triton X-100 (1% v/v) was added, disrupting the vesicles and releasing the remaining CF (100% release). The percent leakage was defined as % leakage = $(F_t - F_0)/(F_{max} - F_0) \times 100$, in which F_0 and F_t are the initial and intermediate emission intensity, respectively, and F_{max} was taken as the fluorescence intensity after lysis of the LUVs by Triton X-100.

Lipid-mixing assay

Unlabeled POPC/POPG LUVs were prepared with a mixture of POPC (25 mg mL^{-1}, 198 μL) and POPG (50 mg mL^{-1}, 10 μL) using HEPES buffer (10 mM HEPES, 107 mM NaCl, pH = 7.4), following the procedure described above. Gel filtration was not needed in this experiment. Labeled POPC/POPG LUVs containing 1 mol % of NBD–DPPE and Rh–DPPE were prepared in the same manner. The labeled and the unlabeled LUVs were mixed in 1 : 4. An aliquot of the mixed LUVs (15 μL) was placed in a cuvette and diluted with the HEPES buffer to 2.0 mL. The concentration of lipids was 54 μM in the final mixture. The change of NBD fluorescence (λ_{ex} = 450 nm and λ_{em} = 530 nm) was measured upon injection of the oligocholate solution (0.5 mM in DMSO, 10 μL). An increase of NBD emission indicates dilution of membrane bound probes caused by membrane fusion. The percentage of fusion was determined using equation % Fusion = $(F_t - F_0)/(F_{max} - F_0) \times 100\%$, in which F_t is the emission intensity of NBD during the assay, F_0 the initial intensity, and F_{max} the maximum intensity (measured for LUVs containing 0.2 mol % each of NBD–DPPE and Rh–DPPE).

DLS study

An aliquot of **1** in DMSO (5.0 μL, 0.10 mM) was added to 2.0 mL of POPC/POPG LUVs ([total lipids] = 2.9 μM) in a quartz cuvette. After the sample was gently shaken by hand for 10 s, DLS measurements were taken. Intensity data from each sample were collected in three replicates and analyzed by the Precision Deconvolve software.

Glucose leakage assay

Glucose-loaded LUVs were prepared according to a slightly modified literature procedure with 300 mM of D-(+)-glucose in 50 mM Tris buffer (0.5 mL, pH = 7.5).[30] The concentration of phospholipids in the stock solution was 0.86 mM. Glucose released from the liposomes was measured enzymatically by a slightly modified literature procedure.[25] Aliquots of the above LUV solution (250 μL), Tris buffer (750 μL, 50 mM Tris, pH = 7.5, 145 mM NaCl, 3.5 mM $MgCl_2$, and 0.15 mM $CaCl_2$), the enzyme solution (500 μL, 10 units/mL of hexokinase/glucose-6-phosphoate dehydrogenase and 2 mM ATP dissolved in the above Tris buffer), and NADP solution (500 μL, 1 mM dissolved in the above Tris buffer) were placed in a series of cuvettes. The concentration of phospholipids in each cuvette was 107 μM. Aliquots of the oligocholate solution in DMSO were added to different cuvettes *via* a microsyringe. The amount of DMSO introduced to each sample was ≤20 μL. The absorbance of NADPH at 340 nm was monitored. To measure the nonspecific glucose leakage from the liposomes, the sample was prepared in an identical fashion and DMSO instead of the oligocholate solution was added. After 2 h, the liposomes were lysed by the addition of 100 μL of Triton X-100 (1% v/v) and the absorbance at 340 nm (A_{max}) was used to calculate the percent leakage [= $(A_t - A_0)/(A_{max} - A_0) \times 100$]. A_0 and A_t are the initial and intermediate absorbance, respectively.

Acknowledgements

We thank NSF (DMR-1005515) for supporting the research.

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