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In Situ Modification of Plain Liposomes with Lipidated Coiled Coil Forming Peptides Induces Membrane Fusion

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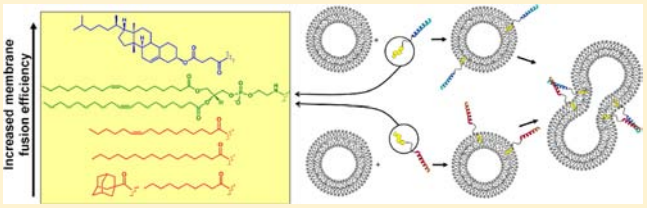
Supporting Information

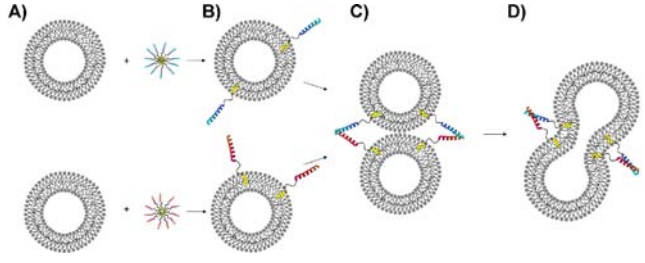
ABSTRACT: Complementary coiled coil forming lipidated peptides embedded in liposomal membranes are able to induce rapid, controlled, and targeted membrane fusion. Traditionally, such fusogenic liposomes are prepared by mixing lipids and lipidated peptides in organic solvent (e.g., chloroform). Here we prepared fusogenic liposomes in situ, i.e., by addition of a lipidated peptide solution to plain liposomes. As the lipid anchor is vital for the correct insertion of lipidated peptides into liposomal membranes, a small library of lipidated coiled coil forming peptides was designed in which the lipid structure was varied. The fusogenicity was screened using lipid and content mixing assays showing that cholesterol modified coiled coil peptides induced the most efficient fusion of membranes. Importantly, both lipid and content mixing experiments demonstrated that the in situ modification of plain liposomes with the cholesterol modified peptides yielded highly fusogenic liposomes. This work shows that existing membranes can be activated with lipidated coiled coil forming peptides, which might lead to highly potent applications such as the fusion of liposomes with cells.



INTRODUCTION

The advancement of supramolecular chemistry in recent decades has supplied scientists with new strategies to design functional materials.[1−4] However, the most stunning examples of controlled self-assembly of well-defined architectures are found in nature. In these living systems, especially proteins are able to carry out a wealth of processes through their precisely arranged structure. In particular, the well-defined secondary, tertiary, and quaternary structures present in proteins allow for the specific recognition of DNA,[5] RNA,[6] carbohydrates,[7] and other proteins.[8] As it is a nontrivial task to manage interactions between complete proteins, chemists have turned to peptide amphiphiles.[9−16] By coupling of a hydrophobic element to a peptide, aggregation in aqueous solution is induced, and this often results in aggregates in which the peptide moieties display a well-defined secondary structure.[17−20] These peptide amphiphiles can be used to mimic naturally occurring, protein driven processes.[21−23] One example of such a highly specific, regulated process that is based on protein−protein recognition and can be mimicked by peptide amphiphiles is membrane fusion.[24] This process is defined by the merging of opposing membranes into one, which results in content transfer. Proteins located in opposite membranes form a coiled coil motif, thereby forcing the membranes into proximity. Subsequently, membrane fusion can occur. In living systems this process is vital because it aids, for example, the transport of proteins between intracellular compartments and the controlled release of neurotransmitters. We have designed a synthetic supramolecular system that is based on a pair of complementary lipidated peptides and is capable of inducing rapid membrane fusion, i.e., lipid and content mixing, between liposomes.[25,26] In this contribution we aim to induce membrane fusion of plain liposomes through addition of aqueous solutions of peptide amphiphiles to preformed plain liposomes (Scheme 1). This is in stark contrast to the conventional way to prepare fusogenic liposomes, which is based on the mixing of fusogens (e.g., peptides,[27] carbohydrates,[28] glycopeptides,[29,30] DNA conjugates,[31−33] boronic acid and inositol[34]) and lipids in organic solvent prior to liposome formation. Importantly, this strategy

Scheme 1. Schematic Representation of Coiled Coil Driven Membrane Fusion[a]



[a]Fusogenic liposomes are prepared by in situ modification of plain liposomes with solutions of the peptide amphiphiles (A). Insertion of the lipidated peptides in the liposomal membranes (B) and subsequent coiled coil formation of the complementary peptides (C) lead to membrane fusion (D).

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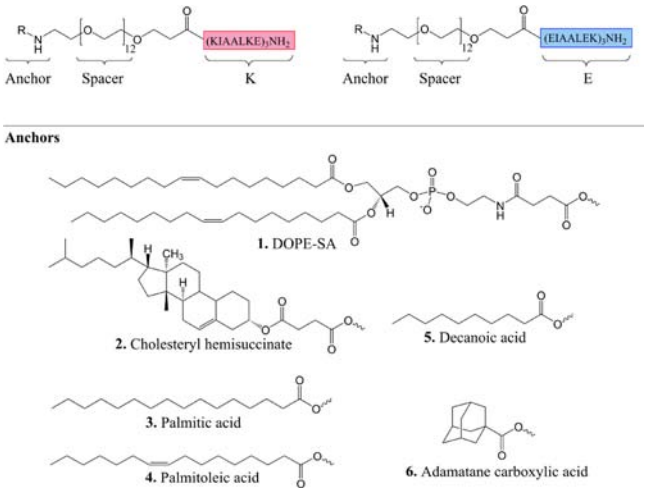
opens up various new applications for our model system, such as the activation of cell membranes in order to induce fusion of liposomes with cells.

We anticipated that the membrane anchor is the key component of the lipidated peptides, as it determines their aggregation behavior and insertion into lipid bilayers. Our strategy was therefore to synthesize lipidated peptides with a variety of membrane anchors (Scheme 2) in order to find a set

Scheme 2. Chemical Structures of the Lipidated Peptides and Various Membrane Anchors Used in This Study



of optimized lipidated coiled coil forming peptides that could (1) spontaneously insert into plain liposomes and (2) trigger membrane fusion once embedded into the liposomal membranes. Furthermore, different hydrophobic anchors have been used in the field of membrane fusion model systems, such as single or double cholesterol moieties,[31,33] stearic acid,[34] phospholipids,[29,30] peptides,[27] and lipid phosphoramidite.[32] However, no studies have been conducted examining various anchors for their effect on the membrane fusion process. Therefore, we examined the influence of the different membrane anchors on membrane fusion, through lipid and content mixing assays. For this screening experiment the conventional preparation route was used. The data then unequivocally show whether a set of lipidated peptides that was already incorporated into liposomal membranes could induce fusion.

In our membrane fusion model system, the general structure of the peptide amphiphiles is lipid−spacer−peptide (Scheme 2). We use a heterodimeric coiled coil forming peptide pair denoted E $(EIAALEK)_3$ and K $(KIAALKE)_3$[35] as the recognition units. Successful in situ modification of liposomes by these lipidated peptides is beneficial for several reasons. First and foremost, efficient insertion of peptides into plain liposomes that are subsequently available for coiled coil formation would be a strong indication that the lipidated peptides could also be easily added to natural membranes such as cell membranes. As the coiled coil motif acts as molecular Velcro, these peptides could then be used as a handle to which the complementary peptide can be attached. When the complementary peptide is anchored into a liposome, this might even lead to fusion of liposomes with cells. Furthermore, efficient encapsulation of biomolecules of interest such as DNA into liposomes typically requires high lipid concentrations.[36] If lipids and peptides are mixed before liposome formation, large amounts of lipidated peptide are required. In situ modification of the liposomes with encapsulated DNA would then be a viable and molecule efficient option.

EXPERIMENTAL SECTION

Synthesis of the Lipidated Peptides. Peptides E and K were synthesized using standard solid phase Fmoc chemistry on a Sieber amide resin. HCTU and DIPEA were used as the activator and base, respectively. Fmoc-PEG_{12}-COOH was subsequently coupled using the same reagents. For the conjugation of lipids **2**−**6** the acid form was activated with DIC and HOBT. Coupling of DOPE was preceded by coupling succinic anhydride to the N-terminus of the pegylated peptides. Cleavage of the peptide amphiphiles from the resin was performed by adding a mixture containing TFA/TIS/water (95:2.5:2.5). Precipitation of the crude products in cold diethyl ether and subsequent drying under reduced pressure yielded crude powders. All compounds were purified using reversed phase HPLC. The mass was confirmed by LCMS, and the purity was always above 95%.

Preparation of Fusogenic Liposomes. The liposomes used throughout this study were typically composed of a ternary mixture of DOPC/DOPE/CH (50:25:25 mol %) and 1 mol % of lipidated peptide, at a total concentration of 0.1 mM. In the traditional preparation of fusogenic liposomes (used for the screening experiments), lipidated peptides and lipids were mixed in a 1:1 mixture of chloroform and methanol. Subsequently, the solvent was evaporated, buffer was added, and the samples were sonicated for 1 min at 55 °C. The in situ modification of liposomes was performed by preparing plain liposomes as described above. Subsequently, aqueous solutions of lipidated peptides (final concentration of 1 μM, or 1 mol %) were added to the plain liposomes and left to incubate for 15 min at room temperature. DLS measurements showed that the liposomes were ∼100 nm in diameter in all instances.

RESULTS AND DISCUSSION

Upon mixing of two batches of liposomes that bear complementary peptides E and K, heterodimeric coiled coil formation brings opposite liposomes into proximity, resulting in an increase in particle size, which was investigated by measuring the optical densities at 400 nm. Surprisingly, only the cholesterol, DOPE, and palmitic acid anchors induced aggregation of the liposomes (Figure 1). These data were confirmed by DLS measurements, which showed particle sizes over 1 μm (after 30 min) when the cholesterol anchored

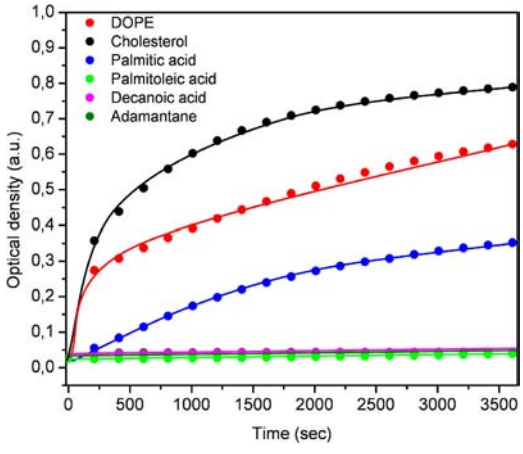


Figure 1. Optical densities (scattering of 400 nm light) were measured upon combining batches of E- and K-decorated liposomes (0.25 mM liposomes with 1% lipopeptide).

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peptides were incorporated in the liposomal membranes (see Supporting Information Figure S1). It is plausible that the other anchors, which are less hydrophobic, are not embedded into the liposomal membranes firmly enough to hold the liposomes together once coiled coil formation has occurred.[31]

To support this hypothesis, the hydrophobicity of the lipidated K peptides was determined using RP-HPLC. As the solvent gradient was set to 10−90% actenotrile in H_2O, longer retention times correspond to a more hydrophobic character. As expected, the DOPE peptides yielded the longest retention time, followed by cholesterol, palmitic acid, palmitoleic acid, decanoic acid, and adamantane carboxylic acid (See Supporting Information Figure S2). The three most hydrophobic lipidated peptides induced aggregation of liposomes, whereas the more hydrophilic lipidated peptides do not. This might indicate that the membrane anchor needs to be of sufficient hydrophobicity to be able to induce aggregation and subsequent membrane fusion (vide infra). Most likely the less hydrophobic lipopeptides are rather weakly embedded in the liposomal membranes and are forced out of these membranes upon coiled coil formation.

To assess the efficiency with which the six sets of peptides induce fusion between liposomes, a standard lipid mixing assay was conducted.[26,37] The liposomes decorated with peptide K also contained 0.5 mol % DOPE-NBD (donor) and 0.5 mol % DOPE-LR (acceptor). Upon fusion of these liposomes with nonfluorescent liposomes bearing the complementary E peptide, an increase in the average distance between the donor and acceptor dyes will ensue, resulting in an increased donor emission. Consistent with the optical density measurements we observed that both the cholesterol and DOPE anchor induced rapid and efficient fusion, whereas the other anchors only produced moderate to low levels of fusion (Figure 2).

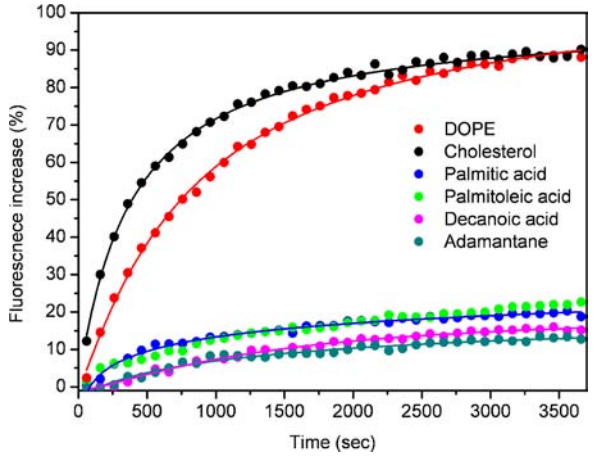

Figure 2. Lipid mixing between E- and K-decorated liposomes as indicated by an increase in NBD emission. Total lipid concentrations were 0.1 mM, with 1% lipopeptide in PBS.

Furthermore, control experiments in which one of the peptides was omitted, or when identical peptides were present on the separate batches of vesicles, showed negligible fusion rates (see Supporting Information Figures S3 and S4). This shows that this is a targeted fusion process and only occurs when both the complementary peptides E and K are present at the surface of liposomes to form heterodimeric coiled coils.

This lipid mixing assay only detects one round of fusion, as additional fusion events between already fused liposomes do not increase the average distance between the donor and acceptor dyes any further. Therefore, the similar fluorescence increase observed for cholesterol and DOPE anchored peptides does not necessarily reflect equal amounts of lipid mixing. Multiple rounds of fusion can be probed using the lipid mixing assay by adding multiple nonfluorescent liposomes to a single fluorescent liposome. This increases the chance that fusion events will result in an increase of the distance between the donor and acceptor dyes. When the liposome ratio was changed to 1:5 (fluorescent/nonfluorescent), it was observed that the cholesterol anchor induces fusion events more efficiently compared to the DOPE anchor (see Supporting Information Figure S5).

While lipid mixing is the initial step in the fusion process, full fusion requires mixing of the aqueous compartments of the liposomes. This process can be monitored by encapsulating sulforhodamine at a self-quenching concentration (20 mM) into one batch of liposomes. Upon mixing with liposomes that do not contain a fluorescent probe in their aqueous interior, content mixing results in relief of self-quenching of sulforhodamine and thus gives rise to an increase in fluorescence emission. Consistent with the lipid mixing data, the cholesterol anchor gave the most efficient content mixing, followed by DOPE, whereas all other anchors induced only negligible levels of content mixing (Figure 3). Also, omitting one of the peptides

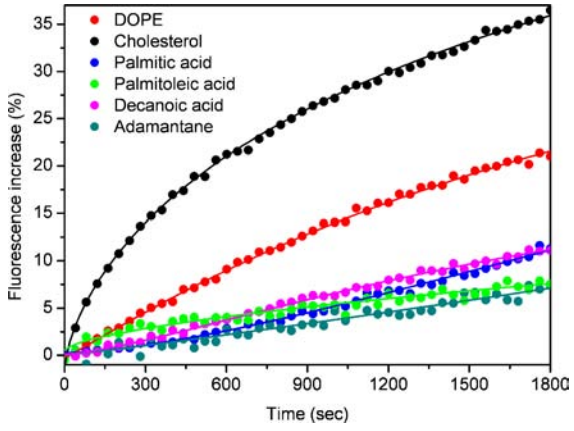

Figure 3. Content mixing between E- and K-decorated liposomes as indicated by an increase in sulforhodamine emission. Total lipid concentrations were 0.1 mM, with 1% lipopeptide in HEPES.

resulted in a low fluorescence increase (see Supporting Information Figure S4), confirming that coiled coil formation between the lipopeptides E and K is required in order to induce full membrane fusion. Finally, a leakage test was performed, in which both E- and K-decorated liposomes were loaded with sulforhodamine. If leakage occurs, this would result in an increase in rhodamine fluorescence; however, this was not observed (see Supporting Information Figure S6).

Although the membrane anchor of the fusogenic peptides is not directly involved in the binding the liposomes, this study demonstrates through lipid and content mixing assays that the membrane anchor has a surprisingly large effect on lipid and content mixing rates. As discussed above, the hydrophobicity of the anchor is likely to play an important role. In order to be able to formulate further hypotheses concerning the different results obtained with the various anchors, we examined the effect that the membrane anchor has on the secondary and quaternary structures of the peptides by conducting circular

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dichroism (CD) measurements. Upon the combination of E- and K-decorated liposomes, coiled coil formation between peptides E and K takes place, which can be measured by examining the ratio of the ellipticity minima at around 222/208 nm. A ratio of <1 is considered to indicate single helices, whereas a ratio of ≥1 is evidence for interacting helices. However, the use of 222/208 ratios is not uncontroversial, as other possible factors such as scattering might influence this ratio. Nonetheless, ratios of >1 were observed for cholesterol and DOPE peptides, while the other lipidated peptides show ratios of ∼1 (see Supporting Information Figure S7 and Table S1). This indicates that coiled coils were formed between peptides E and K for all the different lipidated peptide pairs. As lipid and content mixing data showed that only cholesterol and DOPE bearing peptides induced membrane fusion, the CD data bolster the hypothesis that the less hydrophobic anchors are removed from the liposomal membrane upon coiled coil formation. However, further studies are needed to confirm this hypothesized phenomenon. Furthermore, CD data of separate E- and K-decorated liposomes (Figure 4) reveal that the anchor

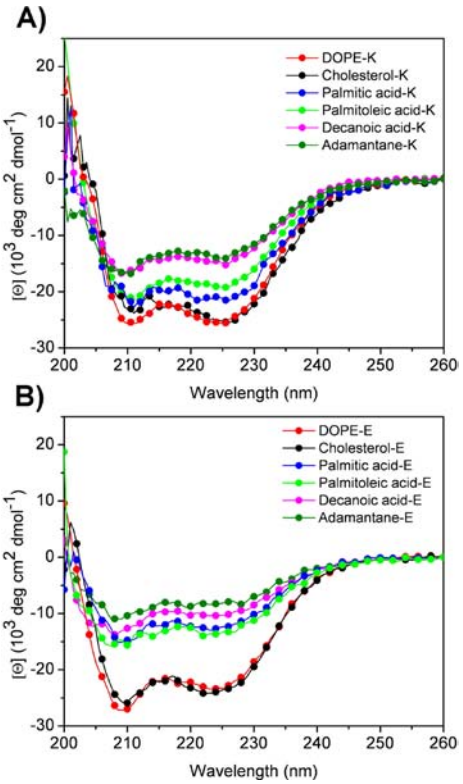

Figure 4. CD data of (A) K-decorated liposomes and (B) E-decorated liposomes. Total lipid concentration was 0.25 mM and 3 mol % lipidated peptide, in PBS.

has a large influence on the helical content of the peptide segments (see Supporting Information Table S2). When peptides E and K were conjugated to cholesterol and DOPE, the peptides were more helical, compared to less hydrophobic anchors and free E and K peptides in solution (i.e., without a membrane anchor). Furthermore, when the K peptide was anchored in liposomal membranes through a cholesterol or DOPE anchor, the ellipticity ratios of ∼1 indicate that homocoiling of the K peptides might occur. It is possible that this homocoiling results in a locally elevated concentration of peptide strands which might be necessary to initiate fusion events as it is very likely that several coiled coils need to be formed to initiate a fusion event. Part of the natural membrane fusion machinery is formed by SNARE proteins, and it has been argued that several SNARE complexes are needed for full membrane fusion to occur.[38−40] As the coiled coil motif employed here is much smaller than the natural occurring SNARE complex, it is plausible that aggregates of several E/K coiled coils are required to spark liposome fusion.

We were interested in the in situ modification of plain liposomes with these lipidated peptides. This enables the activation of membranes with coiled coil forming peptides that could lead to future applications such as the fusion of liposomes with cells. To examine whether in situ modification of plain liposomes could indeed induce membrane fusion, plain liposomes were prepared via hydration of a lipid film. Next, a solution of the lipidated peptide (1 mol % with respect to the lipids) in PBS was added to the plain liposomes and left to incubate for 15 min at room temperature. Subsequently, in situ modified liposomes (E or K) were mixed with conventionally prepared liposomes (K or E, respectively, Figure 5). Also,

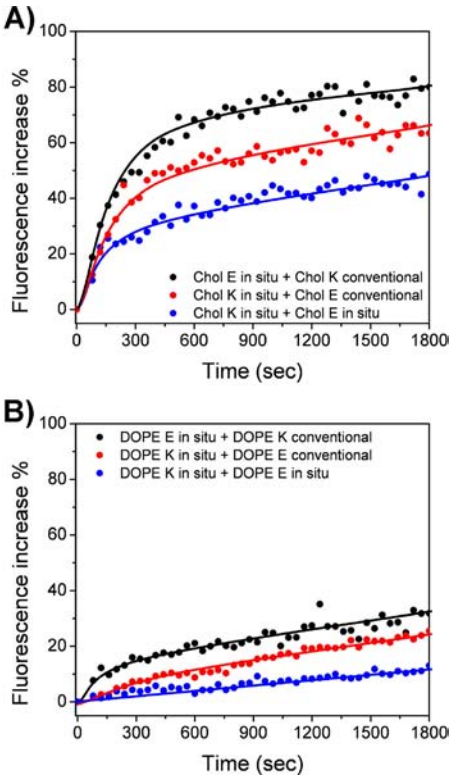

Figure 5. Fluorescence graphs indicating lipid mixing kinetics between in situ modified liposomes and complementary liposomes (either in situ or conventionally modified). The lipidated peptides were added (1 μM final concentration) to preformed liposomes (0.1 mM): (A) addition of cholesterol-PEG-E (CPE) and/or cholesterol-PEG-K (CPK) to preformed liposomes and (B) addition of (DOPE-PEG-E) LPE and/or (DOPE-PEG-K) LPK to preformed liposomes.

separate batches of liposomes that were both in situ modified with lipidated peptides E and K were combined. The fusogenity of these systems were first studied with the lipid mixing assay described earlier. As only the cholesterol and DOPE modified peptides showed efficient membrane fusion, these lipidated peptides are tested here.

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Figure 5 shows that addition of cholesterol peptides to plain liposomes yields highly fusogenic liposomes, comparable to the traditional liposome preparation method. In contrast, the in situ modification of plain liposomes with the DOPE peptides resulted in much lower lipid mixing rates. Additionally, in situ modification with the E peptides is more favorable compared to the K peptides (Figure 5A). This was shown by adding liposomes that were modified in situ with lipidated E or K to traditionally prepared fusogenic liposomes, bearing the complementary peptide. This phenomenon could be due to the observation that the K peptide interacts more with liposomal membranes then the E peptide.[41] This might inhibit the proper insertion of the cholesterol moiety from inserting into the lipid membrane.

To prove that the fusion events are due to incorporation of the lipidated peptides in the liposomal membranes, we performed an additional content mixing experiment. A solution of lipidated peptides was added to sulforhodamine loaded liposomes, and any nonbound peptide was removed by size exclusion chromatography. Both the DOPE and cholesterol modified peptides induced content mixing after in situ modification of plain liposomes with the E peptides. However, the cholesterol modified peptides induced significantly more content mixing after in situ modification of plain liposomes with the E peptides (Figure 6). This is consistent with the lipid mixing data, and it is further evidence that the cholesterol anchor inserts efficiently in the liposomal membranes.

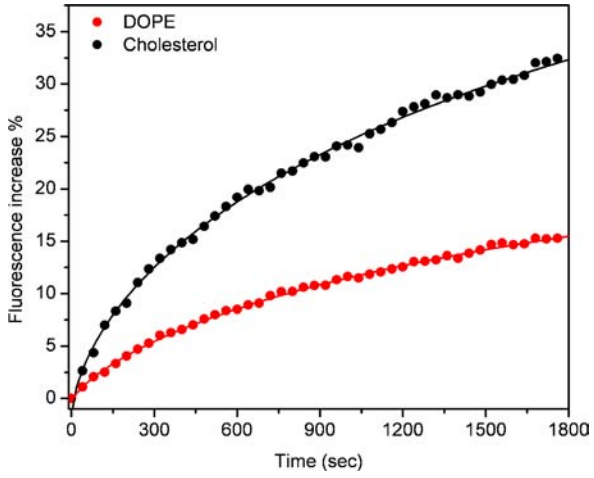

Figure 6. Fluorescence graphs indicating the rate of content mixing between conventionally prepared cholesterol-PEG-K or DOPE-PEG-K liposomes and liposomes to which cholesterol-PEG-E and DOPE-PEG-E were added in situ. Final lipopeptide concentrations were 1 μM, and liposome concentrations were 0.1 mM. 20 mM sulforhodamine B was encapsulated in E-decorated liposomes.

CONCLUSIONS

We have shown that the in situ modification of plain liposomes with peptide amphiphiles cholesterol-PEG-E and cholesterol-PEG-K yields highly fusogenic liposomes. The process of membrane fusion is targeted and occurs with efficient lipid and content mixing without leakage. This is a strong indication that both these lipidated peptides spontaneously enter lipid membranes. Consequently, we should now be able to decorate biological membranes such as cell membranes with these peptide amphiphiles. This opens up the opportunity to form coiled coils at the surface of biological membranes and even induce fusion of liposomes with cells. Furthermore, we found that the hydrophobicity of the membrane anchor is vital for inducing membrane fusion; short single alkyl chains are not sufficient to hold the liposomes in close proximity upon coiled coil formation, whereas phospholipid modified peptides do not readily insert into preformed membranes. Also, the anchor appears to have a large influence on the secondary structure of the peptides at the surface of liposomes; i.e., higher helicity values were obtained for peptide amphiphiles with more hydrophobic membrane anchors. Finally, the cholesterol and DOPE anchored peptide amphiphiles show more homocoiling compared to the other lipidated peptides. This aggregation of peptides at the surface of liposomes might aid fusion efficiency, as it is likely that multiple coiled coil motifs are needed, a phenomenon that is also observed in SNARE induced membrane fusion.

The addition of a set of complementary cholesterol anchored coiled coil forming peptides to separate batches of plain liposomes yielded highly fusogenic liposomes that fused in a targeted and controlled manner. With this fusion machinery in hand, we are now able to activate pre-existing membranes, which might be used as nanoreactors with controlled mixing of the components, to dock a wide variety of molecular constructs to (natural) membranes, and even to induce fusion between liposomes and cells.

ASSOCIATED CONTENT

Supporting Information

Dynamic light scattering and circular dichroism data of the lipidated peptides at liposomal surfaces, control experiments for lipid and content mixing assays, and LC diagrams of lipidated K peptides. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Lehn, J. M. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 1304−1319.
(2) Elemans, J.; Rowan, A. E.; Nolte, R. J. M. *J. Mater. Chem.* **2003**, *13*, 2661−2670.
(3) Kwak, M.; Herrmann, A. *Angew. Chem., Int. Ed.* **2010**, *49*, 8574−8587.
(4) Voskuhl, J.; Ravoo, B. J. *Chem. Soc. Rev.* **2009**, *38*, 495−505.
(5) Landschulz, W. H.; Johnson, P. F.; McKnight, S. L. *Science* **1988**, *240*, 1759−1764.
(6) Lund, J. M.; Alexopoulou, L.; Sato, A.; Karow, M.; Adams, N. C.; Gale, N. W.; Iwasaki, A.; Flavell, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 5598−5603.
(7) Sacchettini, J. C.; Baum, L. G.; Brewer, C. F. *Biochemistry* **2001**, *40*, 3009−3015.
(8) Jones, S.; Thornton, J. M. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 13−20.

(9) Versluis, F.; Marsden, H. R.; Kros, A. *Chem. Soc. Rev.* **2010**, *39*, 3434−3444.
(10) Hartgerink, J. D.; Beniash, E.; Stupp, S. I. *Science* **2001**, *294*, 1684−1688.
(11) Yu, Y. C.; Tirrell, M.; Fields, G. B. *J. Am. Chem. Soc.* **1998**, *120*, 9979−9987.
(12) Kokkoli, E.; Mardilovich, A.; Wedekind, A.; Rexeisen, E. L.; Garg, A.; Craig, J. A. *Soft Matter* **2006**, *2*, 1015−1024.
(13) Paramonov, S. E.; Jun, H. W.; Hartgerink, J. D. *J. Am. Chem. Soc.* **2006**, *128*, 7291−7298.
(14) Cui, H.; Muraoka, T.; Cheetham, A. G.; Stupp, S. I. *Nano Lett.* **2009**, *9*, 945−951.
(15) Muraoka, T.; Koh, C. Y.; Cui, H. G.; Stupp, S. I. *Angew. Chem., Int. Ed.* **2009**, *48*, 5946−5949.
(16) Boato, F.; Thomas, R. M.; Ghasparian, A.; Freund-Renard, A.; Moehle, K.; Robinson, J. A. *Angew. Chem., Int. Ed.* **2007**, *46*, 9015−9018.
(17) Cavalli, S.; Albericio, F.; Kros, A. *Chem. Soc. Rev.* **2010**, *39*, 241−263.
(18) Yuwono, V. M.; Hartgerink, J. D. *Langmuir* **2007**, *23*, 5033−5038.
(19) Fields, G. B.; Lauer, J. L.; Dori, Y.; Forns, P.; Yu, Y. C.; Tirrell, M. *Biopolymers* **1998**, *47*, 143−151.
(20) Behanna, H. A.; Donners, J.; Gordon, A. C.; Stupp, S. I. *J. Am. Chem. Soc.* **2005**, *127*, 1193−1200.
(21) Boyle, A. L.; Woolfson, D. N. *Chem. Soc. Rev.* **2011**, *40*, 4295−4306.
(22) Silva, G. A.; Czeisler, C.; Niece, K. L.; Beniash, E.; Harrington, D. A.; Kessler, J. A.; Stupp, S. I. *Science* **2004**, *303*, 1352−1355.
(23) Tysseling-Mattiace, V. M.; Sahni, V.; Niece, K. L.; Birch, D.; Czeisler, C.; Fehlings, M. G.; Stupp, S. I.; Kessler, J. A. *J. Neurosci.* **2008**, *28*, 3814−3823.
(24) Jahn, R.; Lang, T.; Sudhof, T. C. *Cell* **2003**, *112*, 519−533.
(25) Marsden, H. R.; Elbers, N. A.; Bomans, P. H. H.; Sommerdijk, N.; Kros, A. *Angew. Chem., Int. Ed.* **2009**, *48*, 2330−2333.
(26) Marsden, H. R.; Tomatsu, I.; Kros, A. *Chem. Soc. Rev.* **2011**, *40*, 1572−1585.
(27) Meyenberg, K.; Lygina, A. S.; van den Bogaart, G.; Jahn, R.; Diederichsen, U. *Chem. Commun.* **2011**, *47*, 9405−9407.
(28) Kashiwada, A.; Tsuboi, M.; Takamura, N.; Brandenburg, E.; Matsuda, K.; Koksch, B. *Chem.—Eur. J.* **2011**, *17*, 6179−6186.
(29) Gong, Y.; Ma, M.; Luo, Y.; Bong, D. *J. Am. Chem. Soc.* **2008**, *130*, 6196−6205.
(30) Gong, Y.; Luo, Y. M.; Bong, D. *J. Am. Chem. Soc.* **2006**, *128*, 14430−14431.
(31) Stengel, G.; Simonsson, L.; Campbell, R. A.; Hook, F. *J. Phys. Chem. B* **2008**, *112*, 8264−8274.
(32) Chan, Y. H. M.; van Lengerich, B.; Boxer, S. G. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 979−984.
(33) Stengel, G.; Zahn, R.; Hook, F. *J. Am. Chem. Soc.* **2007**, *129*, 9584−9585.
(34) Kashiwada, A.; Tsuboi, M.; Matsuda, K. *Chem. Commun.* **2009**, 695−697.
(35) Litowski, J. R.; Hodges, R. S. *J. Biol. Chem.* **2002**, *277*, 37272−37279.
(36) Fillion, P.; Desjardins, A.; Sayasith, K.; Lagace, J. *Biochim. Biophys. Acta, Biomembr.* **2001**, *1515*, 44−54.
(37) Struck, D. K.; Hoekstra, D.; Pagano, R. E. *Biochemistry* **1981**, *20*, 4093−4099.
(38) Hua, Y. Y.; Scheller, R. H. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 8065−8070.
(39) Shi, L.; Shen, Q. T.; Kiel, A.; Wang, J.; Wang, H. W.; Melia, T. J.; Rothman, J. E.; Pincet, F. *Science* **2012**, *335*, 1355−1359.
(40) Mohrmann, R.; de Wit, H.; Verhage, M.; Neher, E.; Sorensen, J. B. *Science* **2010**, *330*, 502−505.
(41) In lipid and content mixing assays the mixing of K decorated liposomes with plain liposomes consistently yields higher fluorescence increase than the mixing of E decorated liposomes with plain liposomes. It is likely that this observation is caused by interactions between the K peptide and lipid membranes.