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Biomimetic Generation and Remodeling of Phospholipid Membranes by Dynamic Imine Chemistry

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Abstract

Biomimetic liposomes have a wide array of applications in several areas, ranging from medicinal chemistry to synthetic biology. Due to their biocompatibility and biological relevance, there is particular interest in the formation of synthetic phospholipid vesicles and the development of methods to tune their properties in a controlled manner. However, while true biological membranes are capable of responding to environmental stimuli by enzymatically remodeling their composition, synthetic liposomes are typically static once formed. Herein we report the chemoselective reaction of the natural amine-containing lysosphingomyelin with a series of long-chain aldehydes to form imines. This transformation results in the formation of phospholipid liposomes that are in dynamic equilibrium with the aldehyde–amine form. The reversibility of the imine linkage is exploited in the synthesis of vesicles that are capable of responding to external stimuli such as temperature or the addition of small molecules.

Phospholipid membranes are essential features of every living organism, as they define boundaries and provide needed compartmentalization for executing metabolic reactions.¹ The synthesis of phospholipids in cells is carried out by membrane-bound acyltransferases which link a single-chain lysophospholipid and an activated fatty acid.² In addition to their ability to synthesize phospholipids from single-chain precursors, organisms can tune the properties of their lipid membranes by replacing the aliphatic chain of their components through the action of phospholipases, transacylases, and acyltransferases (Figure 1A).³ Inspired by these transformations, several nonenzymatic systems have been created, allowing the *de novo* formation of artificial phospholipid membranes.^{4–7} Remodeling of *in situ* generated membranes can be achieved by leveraging the ability of *N*-methylated cysteine-modified lysolipids to undergo reversible native chemical ligation (RNCL) with thioesters. This process resembles the activity of natural transacylases and is able to replace both headgroups and acyl tails in an efficient manner.⁸ Despite being a step toward the facile remodeling of synthetic membranes, there are several drawbacks to this technique. First, since the method is based on transacylase activity, the addition of an entirely new lysolipid is

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Supporting Information

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Detailed procedures, spectral data, Scheme S1, Tables S1–S2, and Figures S1–S7 (PDF)

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required to replace one of the aliphatic tails, leading to byproducts of high complexity and size. In addition, the ratio of the starting versus remodeled lipids in the equilibrium can only be controlled by adding a high excess of one of the lysolipids. This feature is due to the similar stability of the different amides formed in the process. Therefore, a system able to replace only the acyl chains of the phospholipid, mimicking phospholipase/acyltransferase activity, would be a significant advance in the biomimicry of membranes. Furthermore, the ability to drive the equilibria based on the stability of the different phospholipids would enable applying the principles of dynamic combinatorial chemistry (DCC) to phospholipid membranes.

To meet these demands, we required a reversible transformation whose product stability could be readily tuned. One of the most important reversible reactions in organic chemistry is the formation of imines from amines and aldehydes, leading to a dynamic system that can be tuned by the careful choice of both reaction partners, as well as other parameters such as temperature or pH.^{9,10} Reversible imine formation is a cornerstone of several fields including organocatalysis,^{11,12} supramolecular chemistry,^{13,14} and DCC.^{15,16} Despite the potential of this reaction to generate robust and biocompatible membranes under physiological conditions, there are limited examples of imine-based vesicles. Moreover, their composing amphiphiles lack resemblance of natural phospholipids.^{17,18} Herein we demonstrate that a natural amine-containing lysophospholipid, lysosphingomyelin, reacts chemoselectively with long-chain aldehydes to form imines, driving the *de novo* generation of dynamic biomimetic phospholipid liposomes (Figure 1B). We also show how the reversibility of the imine can be used to engineer vesicles capable of responding to external stimuli.

Initially, we combined lysosphingomyelin (**1**) and the long-chain aliphatic oleylaldehyde (**2a**), whose reaction would give rise to **3a**, a mimic of the naturally occurring N-oleoyl-D-erythro-sphingosylphosphorylcholine (Figure 1B).¹⁹ Although the equilibrium of imine formation in water is usually shifted toward the starting materials,²⁰ it has been shown that the formation of amphiphiles capable of self-assembling can lead to the stabilization of the imine, presumably by shielding the electrophilic carbon of the imine from water molecules.^{21–25} The reaction between **1** and **2a** was carried out in 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) aqueous buffer at pH 7.5 under millimolar concentrations. Immediately after adding both reaction partners to the buffer, we observed the presence of oil droplets by microscopy. Ten minutes after the mixing, tubules and vesicles began to appear on the surface of the droplets (Figure 2A) until the oil droplets were fully consumed and only liposomes remained in solution (Figure 2B, Figure S3C). The formed vesicles were characterized by transmission electron microscopy (TEM) (Figure 2C, Figure S4). When the resulting mixture was analyzed by LC-MS and evaporative light-scattering detection (ELSD), negligible quantities of the proposed product were detected (Figure S5A). We reasoned this was likely due to hydrolysis of the imine during the elution. To avoid such decomposition and to reproducibly capture the different species in the solution, the imine equilibrium was frozen by the addition of an excess of NaBH₄,¹⁸ which reduced the proposed iminophospholipid **3a** to the corresponding stable aminophospholipid **4a** (Figure 2D). Remarkably, the imine formation exhibits a high chemoselectivity toward

the lysosphingomyelin. When the reaction was carried out in the presence of other biologically relevant amines (adenine, glucosamine, and lysine), the main product detected was **4a** (Figure S1). This effect is most likely due to the hydrophobic effect, which leads to the retention of **2a** in the bilayer favoring the reaction of **1** over the other amines.²⁶

We next explored the ability of other aldehydes to form imines and vesicles upon reaction with lysosphingomyelin **1** (Figure 3). Adding short-chain aldehydes (**2b,c**) to **1** did not result in the formation of liposomes, and after reduction of the mixtures, no amines were detected (Figure 3C). This behavior can be explained by the inability of the putative imines **3b,c** to assemble into bilayers (Figure S3). Without the formation of liposomes, the imines would lack the necessary protection from hydrolysis. Interestingly, aromatic long-chained aldehydes such as 4-*n*-dodecyloxybenzaldehyde (**2d**) also led to the formation of liposomes (Figure S3D). To obtain hydrolysis-resistant iminophospholipids, 4-*n*-octyloxysalicylaldehyde (**2e**), which provides extra stabilization through intramolecular hydrogen bonding between the imine and *ortho* hydroxy group,^{27,28} was added to the lysosphingomyelin **1**. Combining **1** and **2e** resulted in the formation of vesicles under the standard reaction conditions (Figure S3E). Moreover, the equilibrium is completely shifted to the imine (Figure 3C). Remarkably, this imine can be detected by LC-MS without the need for reduction (Figure S5E), proving the ability of hydrogen bonding to significantly stabilize imine formation in water, even at slightly acidic pH.

Having demonstrated the generation of phospholipid vesicles by imine formation from various aldehydes, we next studied how the dynamic covalent system would respond to external stimuli (Figure 3D). A classic phenomenon observed in living organisms is the ability of cells to change their membrane composition in response to external temperature.²⁹ Intriguingly, we found that a solution of 2 equiv of oleylaldehyde **2a** and 1 equiv of benzyl aldehyde **2d** with lysosphingomyelin **1** responded to temperature changes by shifting the composition of the species in the equilibrium (Figure 3D). When the reaction was carried out at 37 °C for 16 h, the major product obtained after reduction was the benzylamine **4d**. Lowering the temperature resulted in a higher proportion of oleyl-based aminophospholipid **4a**, which became the main component of the mixture when stirred at 23 °C (Figure 3D). This response to external conditions can be explained by kinetic versus thermodynamic control.³⁰ At low temperature, when hydrolysis is slow, the product is controlled by the rate of formation of imines leading to oleylimine **3a**, due the higher electrophilicity of aldehyde **2a**.³¹ However, at increased temperatures, hydrolysis has to be taken into account, favoring the formation of the more thermodynamically stable conjugated aromaticimine.³²

Once the dynamic nature of the system and its capacity to distinguish between aldehydes had been proven, we decided to test whether addition of salicylaldehyde derivatives could induce the remodeling of one of the tails of the preformed sphingomyelin analog **3a** (Figure 3E). As previously shown, salicylaldehyde **2e** forms a very stable imine bond with **1**, so we envisioned that **2e** should be able to take the place of the oleylaldehyde **2a**, affording sphingolipid **3e**. As we expected, the addition of **2e** to a solution of **3a** in HEPES, followed by chemical reduction, led to the formation of **4e** (Figure 3E, top). Exchanging a lipid tail of a phospholipid bears close resemblance to the Land's cycle of membrane remodeling in cells, where one acyl chain is removed and a new one reattached enzymatically.³

The successful remodeling of the iminophospholipids prompted us to investigate potential applications. We found that the addition of a salicylaldehyde without an aliphatic substituent (2f) to 3a also resulted in imine exchange (Figure 3E, bottom). Since salicylaldehyde 2f can displace the aliphatic chain of iminophospholipid 3a, it should be possible to permeabilize or disassemble aliphatic imine-constituted liposomes by adding a short-chain salicylaldehyde that can replace the long aliphatic tail, thus yielding an amphiphile that is unable to form membranes. We hypothesized that remodeling could be used to trigger the release of cargo encapsulated within the liposomes. We therefore studied the encapsulation and release of fluorophores using iminophospholipid liposomes (Figure 4). A solution of 1 in HEPES buffer was added to a mixture of a Cy5 dye and 2a in HEPES buffered solution (Figure 4B). After stirring the reaction for 16 h and removing the nonencapsulated fluorophore, we observed the presence of dye-loaded vesicles (Figure 4C). The addition of salicylaldehyde 2f and tris(2-carboxyethylphosphine) hydrochloride (TCEP-HCl), a known fluorescence quencher of Cy5 derivatives,³³ followed by 10 h of stirring resulted in the disappearance of fluorescence along with the formation of new oil droplets (Figure 4D). In contrast, the addition of TCEP-HCl alone to a solution of oleylimine-3a-based vesicles containing the Cy5 dye resulted in negligible quenching of the encapsulated dye (Figure S7), demonstrating that the phosphine is unable to reach the confined Cy5 derivative unless membrane integrity is compromised by tail exchange. These experiments show how dynamic iminophospholipid vesicles can be modified to undergo drastic changes in the aggregation state of the supramolecular assemblies in response to a small molecule trigger.

In summary, we have shown that imine-based phospholipid analogs are capable of spontaneously self-assembling into bilayers. We have also established the relationship between the ability of the resulting iminophospholipids to form vesicles and the stability of a given imine bond. This approach constitutes the first example of dynamic combinatorial chemistry in phospholipid membranes, which mimics the Land's cycle mechanism for membrane remodeling. Finally, iminophospholipid liposomes can respond to external stimuli, such as temperature and the addition of chemicals, which allows the release of cargo encapsulated within. We foresee future applications of dynamic phospholipid vesicles on the study of transient lipid membrane protein interactions, the development of vesicle carriers, and the depletion of lipids in proteoliposomes for the crystallization of membrane proteins.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

- (1). Alberts B; Johnson A; Lewis J; Raff M; Roberts K; Walter P Mol. Biol. Cell, 4th ed.; Garland Science: New York, 2002.
- (2). Yamashita A; Sugiura T; Waku KJ Biochem 1997, 122, 1.
- (3). Lands WEM J. Biol. Chem 1958, 231, 883. [PubMed: 13539023]
- (4). Devaraj NK J. Org. Chem 2017, 82, 5997. [PubMed: 28467841]
- (5). Brea RJ; Bhattacharya A; Devaraj NK Synlett 2017, 28, 108.
- (6). Brea RJ; Cole CM; Devaraj NK Angew. Chem., Int. Ed 2014, 53, 14102.
- (7). Budin I; Devaraj NK J. Am. Chem. Soc 2012, 134, 751. [PubMed: 22239722]
- (8). Brea RJ; Rudd AK; Devaraj NK Proc. Natl. Acad. Sci. U. S. A 2016, 113, 8589. [PubMed: 27439858]
- (9). Belowich ME; Stoddart JF Chem. Soc. Rev 2012, 41, 2003. [PubMed: 22310886]
- (10). Giuseppone N; Lehn J-M Chem. - Eur. J 2006, 12, 1715. [PubMed: 16402400]
- (11). Murphy JJ; Bastida D; Paria S; Fagnoni M; Melchiorre P Nature 2016, 532, 218. [PubMed: 27075098]
- (12). Morales S; Guijarro FG; García Ruano JL; Cid MB J. Am. Chem. Soc 2014, 136, 1082. [PubMed: 24359453]
- (13). Ratjen L; Vantomme G; Lehn J-M Chem. - Eur. J 2015, 21, 10070. [PubMed: 26061683]
- (14). Meyer CD; Joiner CS; Stoddart JF Chem. Soc. Rev 2007, 36, 1705. [PubMed: 18213980]
- (15). Li J; Nowak P; Otto SJ Am. Chem. Soc 2013, 135, 9222.
- (16). Nowak P; Saggiomo V; Salehian F; Colomb-Delsuc M; Han Y; Otto S Angew. Chem., Int. Ed 2015, 54, 4192.
- (17). Li W; McManus D; Liu H; Casiraghi C; Webb SJ Phys. Chem. Chem. Phys 2017, 19, 17036. [PubMed: 28642943]
- (18). Minkenberg CB; Li F; van Rijn P; Florusse L; Boekhoven J; Stuart MCA; Koper GJM; Eelkema R; van Esch JH Angew. Chem., Int. Ed 2011, 50, 3421.
- (19). Aljohani AJ; Munguba GC; Guerra Y; Lee RK; Bhattacharya SK Mol. Vis 2013, 19, 1966. [PubMed: 24068864]
- (20). Godoy-Alcántar C; Yatsimirsky AK; Lehn J-MJ Phys. Org. Chem 2005, 18, 979.
- (21). Wu X; Chen X-X; Zhang M; Li Z; Gale PA; Jiang Y-B Chem. Commun 2016, 52, 6981.
- (22). Sheng L; Kurihara K Chem. Commun 2016, 52, 7786.
- (23). Takakura K; Yamamoto T; Kurihara K; Toyota T; Ohnuma K; Sugawara T Chem. Commun 2014, 50, 2190.
- (24). Janeliunas D; van Rijn P; Boekhoven J; Minkenberg CB; van Esch JH; Eelkema R Angew. Chem., Int. Ed 2013, 52, 1998.
- (25). Minkenberg CB; Florusse L; Eelkema R; Koper GJM; van Esch JH J. Am. Chem. Soc 2009, 131, 11274. [PubMed: 19627081]
- (26). Kronberg B Curr. Opin. Colloid Interface Sci 2016, 22, 14.
- (27). Tomás-Gamasa M; Serdjukow S; Su M; Müller M; Carell T Angew. Chem., Int. Ed 2015, 54, 796.
- (28). Crueiras J; Rios A; Riveiros E; Richard JP J. Am. Chem. Soc 2009, 131, 15815. [PubMed: 19807092]
- (29). de Mendoza D; Cronan JE Trends Biochem. Sci 1983, 8, 49.
- (30). Kulchat S; Chaur MN; Lehn J-M Chem. - Eur. J 2017, 23, 11108. [PubMed: 28628248]
- (31). Appel R; Mayr HJ Am. Chem. Soc 2011, 133, 8240.
- (32). Layer RW Chem. Rev 1963, 63, 489.
- (33). Vaughan JC; Dempsey GT; Sun E; Zhuang XJ Am. Chem. Soc 2013, 135, 1197.

Figure 2.

Generation of vesicles driven by imine formation. Fluorescence microscopy images of a mixture of **1** (1 mM), **2a** (1 mM), and Nile red (1 μ M) in HEPES buffer pH 7.5 after 10 min (A) and 14 h (B). Scale bars denote 5 μ m. (C) TEM images of the vesicles composed of **3a**. Scale bar denotes 250 nm. (D) HPLC/ELSD traces of the reaction between **1** and **2a** after reduction with NaBH₄, showing the formation of amine-containing sphingolipid **4a**.

Figure 3.

Reactivity of different aldehydes toward iminophospholipid formation and evidence for the dynamic nature of the system. (A) Schematic representation of the dynamic imine formation and subsequent reduction. (B) Structures of the aldehydes used herein. (C) Relative amount of iminophospholipids after combining different aldehydes with **1** [measured after reduction of the mixture with NaBH₄ and normalized using 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) as internal standard]. (D) Ratio of formation of iminophospholipids **3a** and **3d** as a function of the temperature (measured as the relative yield of **4d** versus **4a**

after reduction of the mixture). (E) HPLC/ELSD traces after the addition of 1 equiv of **2e** (top) and **2f** (bottom) to a 1 mM solution of 3a in HEPES solution, followed by reduction.

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Figure 4.

Encapsulation and release of a fluorescent dye. (A) Schematic representation showing the vesicle assembly from precursors **1** (dark blue) and **2a** (light blue) and concurrent encapsulation of a Cy5 dye (green) followed by remodeling and quenching of the fluorophore. (B) Fluorescence microscopy image of **1** and **2a** in the presence of free dye immediately after mixing. (C) Fluorescence microscopy image of iminophospholipid **3a** vesicles containing the encapsulated fluorophore, after dialysis. (D) Fluorescence microscopy image of the release and quenching of the Cy5 dye after treatment of vesicles from panel C with **2f** (orange) and quencher TCEP·HCl (red). Insets represent images captured using phase-contrast microscopy, showing oil droplets before vesicle formation (B) and after the remodeling with **2f** (D), while vesicles are present after imine formation (C). Scale bars denote 5 μm .