J|A|C|S
COMMUNICATIONS

Published on Web 10/25/2006

The Effect of Receptor Clustering on Vesicle−Vesicle Adhesion

Robert J. Mart, Kwan Ping Liem, Xi Wang, and Simon J. Webb*

Manchester Interdisciplinary Biocentre and the School of Chemistry, University of Manchester, 131 Princess St, Manchester M1 7DN, U.K.

Received August 8, 2006; E-mail: s.webb@manchester.ac.uk

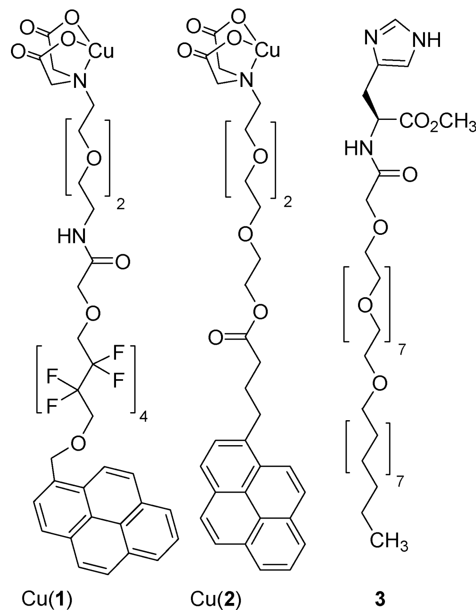
The lateral phase separation of cell membrane constituents into distinct regions called "lipid rafts" is central to important cellular functions like signal transduction, cell adhesion, and endocytosis.[1] These lipid rafts, phase separated domains composed of protein, glycosphingolipids, and cholesterol, are liquid ordered "islands" moving within a liquid disordered phospholipid bilayer matrix.[2] The localization of cell adhesion molecules (CAMs) within lipid rafts has recently been shown[3] to play a key role in cell adhesion and motility. Indeed the modification of adhesive properties caused by concentrating CAMs within lipid rafts has important medical consequences, affecting viral attachment to cells,[1] lymphocyte and axon chemotaxis,[4,5] and cancer cell metastasis.[3a]

We have developed a perfluoroalkyl-pyrene membrane anchor that enables synthetic lipids to phase separate within phospholipid bilayers in the solid ordered (s_o) or liquid ordered (l_o) phases, even at membrane concentrations as low as 1% mol/mol.[6a] This motif offers a unique opportunity to create lipid raft mimics; phase separated domains of synthetic receptors floating within a fluid l_o matrix. Given the key role of lipid rafts in cell adhesion, we aimed to create rafts of synthetic CAMs in the membranes of vesicles, then study how they affect the extent of vesicle adhesion. These simple mimics of cells would be crosslinked through relatively weak Cu(iminodiacetate)−histidine (Cu(IDA)−His) bonds.[7,8] This interaction, with K around 10^3 M^{-1}, is similar in strength to natural adhesive interactions, like selectin-sialyl Lewis X, which have individual strengths less than 10^4 M^{-1}.[9] Thus, we hope that receptor clustering will enhance these individually weak links as it does in nature.[10]

The creation of vesicle assemblies is of great current interest,[11] with both homogeneous vesicle assemblies[12] and assemblies of different types of vesicles[13] being created. To create the latter, which have more potential as tissue mimics and functional biomaterials, we synthesized a complementary pair of CAM mimics: a Cu(IDA)-capped lipid incorporating our perfluoroalkyl−pyrene motif, Cu(**1**), and its conjugate receptor, an L-histidine-capped lipid **3** (Chart 1). Cu(**2**) is a control compound that does not phase separate in vesicles of any composition, yet is known to mediate vesicle aggregation by poly L-histidine.[7] The pyrene groups in Cu(**1**) and Cu(**2**) have dual roles; the ratio of excimer to monomer emission intensity (E/M ratio) reflects the extent of lipid phase separation, and they also allow visualization of Cu(**1**) and Cu(**2**) containing vesicles by fluorescence microscopy.

Vesicles (0.8 μm diameter) containing 5% mol/mol of lipids H_2**1**, H_2**2**, or **3** (at 1 mM) in dimyristoyl phosphatidylcholine (DMPC) or DMPC mixed 50 % w/w with cholesterol (DMPC/chol) were formed by extrusion. Lipids H_2**1**, H_2**2**, or **3** were admixed into the appropriate phospholipids prior to vesicle formation; the affinity of the chelating headgroups in H_2**1** and H_2**2** for copper(II) is around 4×10^7 M^{-1},[7] so later addition of 1 equiv of copper(II) afforded synthetic receptors Cu(**1**) and Cu(**2**) quantitatively.

Chart 1. Fluorescent Lipids Cu(**1**) and Cu(**2**) with Conjugate Histidine-Capped Lipid **3**



Given that previous studies had established that lipids containing perfluoroalkyl groups formed domains in ordered bilayers,[6] we anticipated that Cu(**1**) would phase separate in DMPC/chol vesicles, which are in the l_o phase at 25 °C. However Cu(**1**) should not phase separate from vesicles composed solely of DMPC; since these are in the liquid disordered (l_d) phase at 25 °C, Cu(**1**) will be distributed evenly over the surface of the vesicles. As expected, fluorescence spectroscopy showed significant phase separation for [Cu(**1**)-DMPC/chol] vesicles, with $E/M = 0.5$, while only a small amount of excimer was observed in [Cu(**1**)-DMPC] vesicles, $E/M = 0.2$. Receptor Cu(**2**), at the same 5% mol/mol loading in DMPC/chol vesicles, did not form any measurable excimer.

The formation of vesicle aggregates can be inferred from increases in solution turbidity and directly observed using fluorescence microscopy. Job plots are particularly useful for detecting vesicle aggregation as the absorbance of a noninteracting mixture of vesicles can be predicted from the absorbances of the unmixed suspensions; positive deviations from the predicted absorbance indicate the formation of vesicle aggregates. Mixing our control vesicles without any phase separation, [Cu(**2**)-DMPC/chol], with [**3**-DMPC/chol] vesicles gave noninteracting mixtures, with no significant deviation from the expected absorbance and no aggregates observable by fluorescence microscopy. Given that previous work had shown that poly L-histidine can cross-link vesicles containing Cu(**2**),[7] this suggests that multivalent interactions with polymeric ligands are stronger than analogous multivalent interactions between vesicles. In contrast, a positive deviation in absorb-

10.1021/ja0657612 CCC: $33.50 © 2006 American Chemical Society

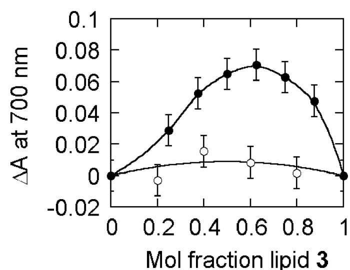


Figure 1. Job plots showing changes in turbidity observed for mixtures of 5% mol/mol **3** in DMPC/chol vesicles with vesicles containing 5% mol/mol Cu(**1**) in DMPC (○) and DMPC/chol (●).

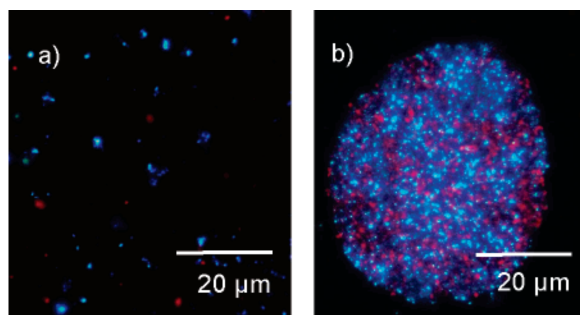


Figure 2. Fluorescence micrographs of vesicles containing 5% mol/mol lipid **3** in DMPC/chol (red) mixed with vesicles containing 5% mol/mol Cu(**1**) (blue) in (a) DMPC and (b) DMPC/chol.

ance of 11% was found when [Cu(**1**)-DMPC/chol] vesicles were mixed with [**3**-DMPC/chol] vesicles, implying the phase separation of Cu(**1**) was enabling the formation of vesicle aggregates (Figure 1). In line with this explanation, mixing [**3**-DMPC/chol] vesicles with [Cu(**1**)-DMPC] vesicles, which contain only weakly phase separated Cu(**1**), gave a much smaller (<2%) deviation in the absorbance at 700 nm.

To confirm the presence of vesicle aggregates, these three mixtures were analyzed by fluorescence microscopy. Since lipid **3** lacks a fluorophore, vesicles containing **3** were doped with 0.1% mol/mol of the red fluorescent membrane dye, rhodamine B DPPE, which contrasts with the blue fluorescence of Cu(**1**) and Cu(**2**). Fluorescence microscopy revealed that mixtures with little deviation in turbidity contained only isolated vesicles ([Cu(**2**)-DMPC/chol] + [**3**-DMPC/chol]) or small clusters of vesicles ([Cu(**1**)-DMPC] + [**3**-DMPC/chol]). In contrast, mixtures of [Cu(**1**)-DMPC/chol] and [**3**-DMPC/chol] vesicles showed large aggregates containing both types of vesicle (Figure 2).

These aggregates, with diameters between 20 and 80 μm, were fully formed within 2 min of mixing the two vesicle populations. The aggregates seemed stable for several days, and we observed no movement of the fluorescent synthetic lipids between the different vesicle populations.

The formation of these distinctive aggregates seems to depend upon the degree of phase separation of the Cu(IDA) lipid. If the structure of the membrane anchor in Cu(**1**) was changed to give a lipid, Cu(**2**), that no longer phase separated in the bilayer, then no adhesion was observed. If membrane composition was changed to diminish phase separation of Cu(**1**), then large vesicle aggregates did not form. Furthermore, if [**3**-DMPC/chol] vesicles were mixed with vesicles containing 5% mol/mol Cu(**1**) in distearoyl phosphatidylcholine (DSPC) (s_o phase at 25 °C), which have extensive phase separation of Cu(**1**) (*E*/*M* ratio of 0.8), then even larger aggregates were observed by fluorescence microscopy.[14]

To the best of our knowledge, this is the first time a biomimetic system has been used to show that the lateral distribution of adhesive agents within a membrane can directly affect the membrane's adhesive properties. It is revealing that partitioning receptors into adhesive clusters, acknowledged as a major factor in controlling the adhesive properties of cells, can be replicated and shown to enhance the adhesive properties of vesicles. This binding enhancement may stem from receptor preorganization, where part of the entropic penalty inherent in receptor clustering at a binding interface is prepaid though lipid phase separation.[15] Alternatively, the chelate effect may enhance vesicle−vesicle binding; after formation of the first crosslinking bond to phase-separated Cu(**1**), this tethering link between vesicles will facilitate additional intervesicular bonds to other Cu(**1**) receptors in the same phase separated patch.[16] We now hope to exploit this discovery to develop biocompatible tissue mimics that are structured on a submicrometer scale.

Acknowledgment. This work was supported by BBSRC Grant B19722. We would like to thank Dr. Jason Micklefield for use of a UV−vis spectrophotometer and the EPSRC National Mass Spectrometry Service Centre, Swansea, U.K. for HRMS.

Supporting Information Available: Syntheses and spectroscopic data for H_2**1**, H_2**2**, and **3**. Details of turbidimetric, fluorimetric, and microscopic analyses. Emission spectra of [H_2**1**-DMPC/chol] and [H_2**1**-DMPC] vesicles. Fluorescence micrographs of [**3**-DMPC/chol] vesicles with [Cu(**2**)-DMPC/chol] or [Cu(**1**)-DSPC] vesicles.

References

(1) Binder, W. H.; Barragan, V.; Menger, F. M. *Angew. Chem., Int. Ed.* **2003**, *42*, 5802−5827.
(2) (a) Pike, L. J. *Biochem. J.* **2004**, *378*, 281−292. (b) Vereb, G.; Szöllösi, J.; Matkó, J.; Nagy, P.; Farkas, T.; Vígh, L.; Mátyus, L.; Waldmann, T. A.; Damjanovich, S. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 8053−8058.
(3) (a) Huang, Q.; Shen, H.-M.; Shui, G.; Wenk, M. R.; Ong, C.-N. *Cancer Res.* **2006**, *66*, 5807−5815. (b) Causeret, M.; Taulet, N.; Comunale, F.; Favard, C.; Gauthier-Rouviere, C. *Mol. Biol. Cell* **2005**, *16*, 2168−2180.
(4) Pizzo, P.; Viola, A. *Microbes Infect.* **2004**, *6*, 686−692.
(5) Ibanez, C. F. *Neuron* **2004**, *42*, 3−5.
(6) (a) Webb, S. J.; Greenaway, K.; Bayati, M.; Trembleau, L. *Org. Biomol. Chem.* **2006**, *4*, 2399−2407. (b) Elbert, R.; Folda, T.; Ringsdorf, H. *J. Am. Chem. Soc.* **1984**, *106*, 7687−7692.
(7) Webb, S. J.; Trembleau, L.; Mart, R. J.; Wang, X. *Org. Biomol. Chem.* **2005**, *3*, 3615−3617.
(8) Kent, M. S.; Yim, H.; Sasaki, D. Y.; Satija, S.; Seo, Y.-S.; Majewski, J. *Langmuir* **2005**, *21*, 6815−6824.
(9) Jacob, G. S.; Kirmaier, C.; Abbas, S. Z.; Howard, S. C.; Steininger, C. N.; Welply, J. K.; Scudder, P. *Biochemistry* **1995**, *34*, 1210−1217.
(10) (a) Vitte, J.; Benoliel, A.-M.; Eymeric, P.; Bongrand, P.; Pierres, A. *Biophys. J.* **2004**, *86*, 4059−4074. (b) Chen, A.; Moy, V. T. *Biophys. J.* **2000**, *78*, 2814−2820. (c) Cohen, M.; Joester, D.; Geiger, B.; Addadi, L. *ChemBioChem* **2004**, *5*, 1393−1399.
(11) Paleos, C. M.; Tsiourvas, D. *Top. Curr. Chem.* **2003**, *227*, 1−29.
(12) (a) Tsogas, I.; Tsiourvas, D.; Nounesis, G.; Paleos, C. M. *Langmuir* **2005**, *21*, 5997−6001. (b) Menger, F. M.; Zhang, H. *J. Am. Chem. Soc.* **2006**, *128*, 1414−1415. (c) Kisak, E. T.; Kennedy, M. T.; Trommeshauser, D.; Zasadzinski, J. A. *Langmuir* **2000**, *16*, 2825−2831.
(13) (a) Marchi-Artzner, V.; Gulik-Krzywicki, T.; Guedeau-Boudeville, M.-A.; Gosse, C.; Sanderson, J. M.; Dedieu, J.-C.; Lehn, J.-M. *ChemPhysChem* **2001**, *2*, 367−376. (b) Menger, F. M.; Seredyuk, V. A. *J. Am. Chem. Soc.* **2003**, *125*, 11800−11801.
(14) However there was evidence of vesicle fusion in this case; vesicles up to 5 μm in diameter were observed. See the Supporting Information.
(15) (a) Marchi-Artzner, V.; Lorz, B.; Gosse, C.; Jullien, L.; Merkel, R.; Kessler, H.; Sackmann, E. *Langmuir* **2003**, *19*, 835−841.
(16) Doyle, E. L.; Hunter, C. A.; Phillips, H. C.; Webb, S. J.; Williams, N. H. *J. Am. Chem. Soc.* **2003**, *125*, 4593−4599.

JA0657612