Hyperextended Amphiphiles. Bilayer Formation from Single-Tailed Compounds

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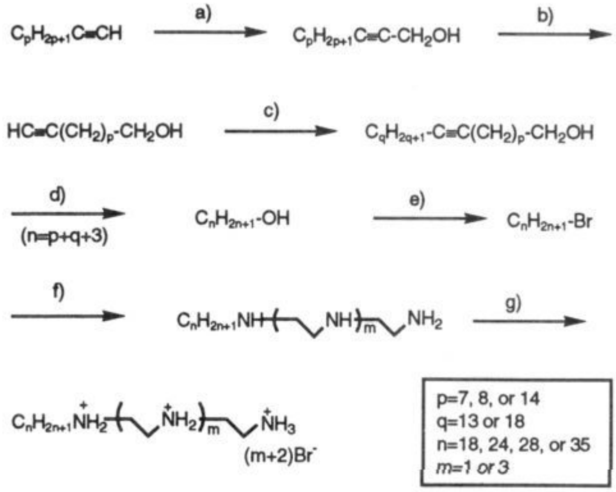
Bilayers are most often composed of amphiphiles that possess two aliphatic chains. Recent reports have shown, however, that bilayers are also formed from single-tailed amphiphiles when two different types, one cationic and one anionic, are mixed.[1-3] Presumably, ion-pairing between the components simulates the "double-tailed condition". We now report that bilayer structures can be prepared with a pure, unmixed amphiphile having but one aliphatic chain.[4] The results bear on important questions relating to the structural requirements for membrane formation.

As often happens, the investigation was originally motivated by simple curiosity. Thus, we wondered about the properties of "hyperextended" amphiphiles, *i.e.* amphiphiles with aliphatic chains up to 2–3 times longer than normally encountered. Since such extremely long chains would, no doubt, create a solubility problem in water, we endowed them with multiionic headgroups in hopes of maintaining a suitable hydrophilic/hydrophobic balance. The synthesis of amphiphiles with hyperextended chains (24, 28, or 35 carbons) and multiionic headgroups (3 or 5 cationic nitrogens) is given in Scheme I. A key step in the sequence, the so-called "zipper reaction" in step b, shifts a triple bond from one end of the chain to the other.[5] Note that Scheme I provides a new and useful entry into chain lengths in excess of 70. One morely has to convert $C_{35}H_{71}Br$ in Scheme I into a starting alkyne, $C_{35}H_{71}C{\equiv}CH$, and then repeat the sequence. The products of Scheme I will be designated by means of their chain length and number of cationic nitrogens (*e.g.* $C_{35}N_5$).[6]

Amphiphiles $C_{18}N_3$ and $C_{18}N_5$, synthesized as controls, formed micelles in water with critical micelle concentrations of 0.9×10^{-4} and 1.4×10^{-4} M, respectively, as determined by surface tension, conductivity, and ^{81}Br NMR. In contrast, all hyperextended amphiphiles aggregated at concentrations too low for observation by these three methods. We therefore relied on dynamic light scattering (DLS) and electron microscopy (SEM and STEM) for their characterization. DLS samples were prepared *via* bath sonication of 10 mM aqueous mixtures at 60–80 °C, dilution, and passage through 0.40- or 0.65-μm microfilters. SEM and STEM samples were prepared by spraying a mixture of 1 mM amphiphile and 2% uranyl acetate onto a Formvar-coated 200-mesh copper grid, vacuum-drying, and Cr-coating to a 1.5–2.0-nm thickness. This atomization method for high-resolution SEM and STEM examination of amphiphiles is new and will be described in detail elsewhere.[7]

Sizes and morphologies of aggregates made from hyperextended amphiphiles are given in Table I. Agreement is reasonably good

Scheme I[a]



[a] (a) *n*-BuLi, hexamethylphosphoramide (HMPA); paraformaldehyde, 78%; (b) ethylenediamine, NaH, 45–55 °C, 57–83%; (c) *n*-BuLi, HMPA; $C_qH_{2q+1}Br$, 42–57%; (d) H_2, 2.5–5.0 mol % Pd/C, EtOH, 60–92%; (e) 48% HBr, concentrated H_2SO_4, 90–110 °C, 56–63%; (f) excess $NH_2(CH_2CH_2NH)_mCH_2CH_2NH_2$, THF; NaOH, THF, 29–77%; (g) 48% HBr, THF, 56–93%. Exact yields depend on *n*.

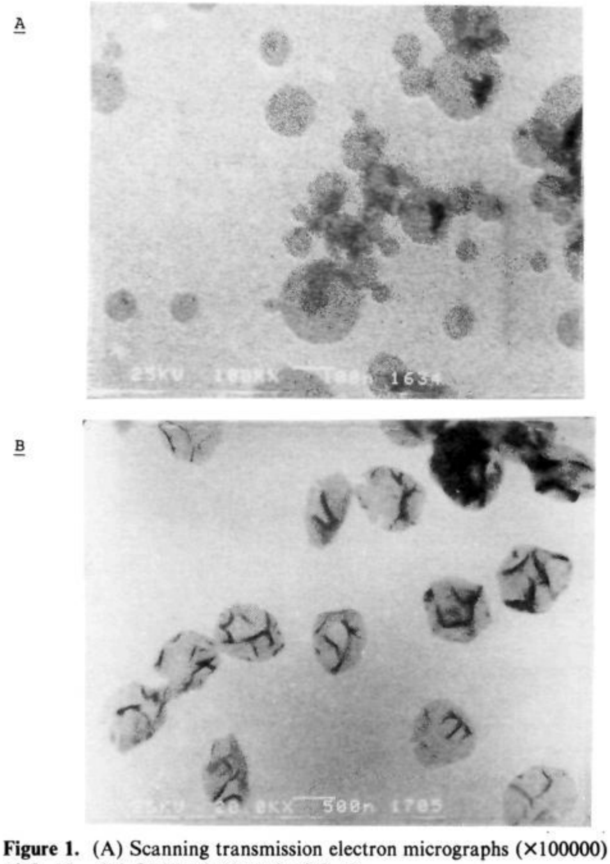


Figure 1. (A) Scanning transmission electron micrographs (×100000) of $C_{28}N_3$. (B) STEM (×20000) of $C_{24}N_3$.

between DLS data taken from solution systems and EM data taken from dry films.[8] As seen from the table, vesicular structures are formed from amphiphiles with chain lengths of 24, 28, and 35 carbons when the chains are attached to headgroups comprising three linearly arranged ammonium ions. The spherical shape of the aggregates (Figure 1A) and their large size (20–700 nm

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(1) Kaler, E. W.; Murthy, A. K.; Rodriquez, B. E.; Zasadzinski, J. A. *Science* **1989**, *245*, 1371.

(2) Fukuda, H.; Kawata, K.; Okuda, H.; Regen, S. L. *J. Am. Chem. Soc.* **1990**, *112*, 1635.

(3) Hargreaves, W. R.; Deamer, D. W. *Biochemistry* **1978**, *17*, 3759.

(4) Over a decade ago it was shown that single-tailed amphiphiles incorporating large rigid segments within the chain (*e.g.* biphenyl, azobenzene, etc.) can form vesicles. Kunitake, T.; Okahata, Y.; Shimomura, M.; Yasunami, S.; Takarabe, K. *J. Am. Chem. Soc.* **1981**, *103*, 5401.

(5) Abrams, S. R.; Nucciarone, D. D.; Steck, W. F. *Can. J. Chem.* **1983**, *61*, 1073. Brown, C. A.; Yamashita, A. *J. Am. Chem. Soc.* **1975**, *97*, 891.

(6) All free amines were solids characterized by IR, MS, ^{1}H and ^{13}C NMR, and elemental analysis. The corresponding hydrobromide salts were characterized by IR, MS, and elemental analysis.

(7) Apkarian, R. P.; Robinson, K. A.; Yamasaki, Y.; Menger, F. M. To be published in the *51st Annual Proceedings of the Microscope Society of America*.

(8) Caution in interpreting DLS data on small, highly charged particles is recommended since electrostatic repulsion can increase diffusivity.

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Table I. Dynamic Light Scattering (DLS) and Electron Microscopy (EM) Data on the Size and Morphology of Hyperextended Amphiphile Aggregates

	DLS	EM	
amphiphile	size (nm)	morphology[a]	size (nm)
$C_{24}N_3$	263	vesicular	500–700
$C_{24}N_5$	10	rodlike, globular	10[b]
$C_{28}N_3$	63	vesicular	20–150
$C_{28}N_5$	7	globular	15
$C_{35}N_3$	19, 65[c]	vesicular	20–110
$C_{35}N_5$	7, 25[c]	globular, vesicular	10–100

[a] Globules are defined as small spherical particles of uncertain structure manifesting no "bilayer staining" under SEM observation. [b] Aggregates showed small globules (10–20 nm in diameter) connected by thin filaments (10 nm in width). [c] The DLS software (using a NICOMP instrument equipped with a 3.5-Watt argon laser) imposed a bimodal distribution upon a polydisperse population ranging in size between the cited values.

compared to ca. 5 nm for micelles) testify to their vesicular structure. Note that the vesicles in the STEM picture of $C_{24}N_3$ (Figure 1B) are so large that they collapsed under the high-vacuum conditions of the experiment. Whether the hyperextended amphiphiles assemble into vesicles or some other structure (*e.g.* micelles or opaque "globules") depends critically upon the number of cationic nitrogens in the headgroup. As is apparent from Table I, amphiphiles with five cationic nitrogens are not as prone to produce vesicles. This observation runs counter to current dogma that micelle *vs.* vesicle formation is determined primarily by headgroup *vs.* chain cross sections.[9] Clearly, hydrophilic/hydrophobic balance is also a determining factor.

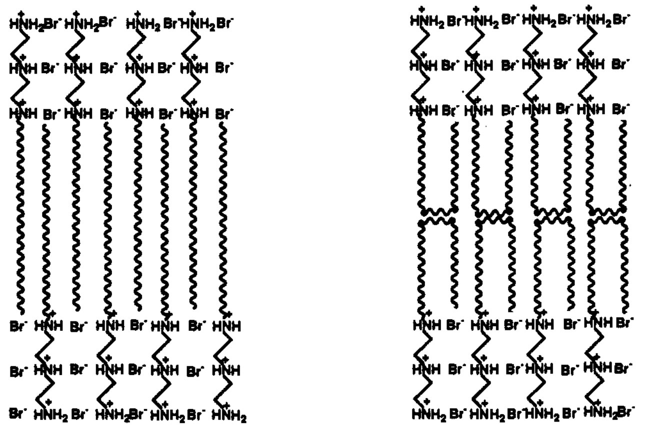
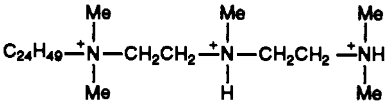


Figure 2. "Interdigitation" and "folding" models of bilayer formation with hyperextended amphiphiles.

Although we lack direct proof, it seems reasonable that the long chains either strongly interdigitate or, alternatively, fold in the center to create a "double-chained" entity (Figure 2). When the amphiphile was methylated or branched, as shown below, nonvesicular structures were observed. It appears as if vesicle formation is associated with tight ion-pairing, a feature that can be sterically disrupted by the indicated structural modifications.



$C_{24}H_{49}-\overset{Me}{\underset{Me}{\overset{+}{N}}}-CH_2CH_2-\overset{Me}{\underset{H}{\overset{+}{N}}}-CH_2CH_2-\overset{Me}{\underset{Me}{\overset{+}{N}H}}$

$C_{28}H_{57}\overset{+}{N}H(CH_2CH_2\overset{+}{N}H_3)_2$

In summary, we have shown that vesicular structures are indeed possible with single aliphatic chains provided that (a) the chains are hyperextended; (b) the headgroup is unsubstituted; and (c) the headgroup possesses a proper number of cationic nitrogens to counterbalance the chain hydrophobicity.[10]

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(9) Israelachvili, J. N.; Mitchell, D. J.; Ninham, B. W. *J. Chem. Soc., Faraday Trans. 2* **1976**, *72*, 1525.

(10) We call attention to a forthcoming paper in *Angew. Chem.* entitled "Can Single-Chain Perfluoroalkylated Amphiphiles Alone Form Vesicles and Other Organized Supramolecular Systems?" by M.-P. Krafft, F. Giulieri, and J. G. Riess.