CHEMISTRY LETTERS, pp. 1385-1388, 1987.

Selective Destruction of the Synthetic Vesicles by the Reductive Cleavage of Disulfide Linkage

Suk-Kyu CHANG,* Duck-Hee KIM, Nam-Yee KIM, and Iwhan CHO*†

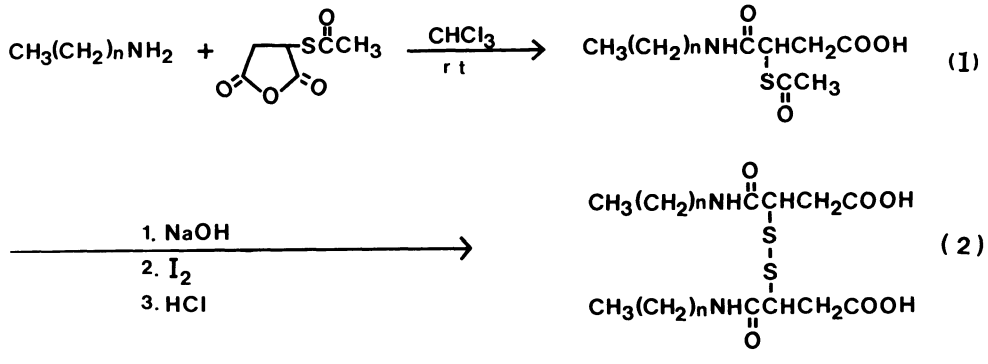
Department of Chemistry, Chung-Ang University, Seoul 151, Korea

†Department of Chemistry, Korea Advanced Institute of Science and Technology, Seoul 131, Korea

A series of dialkyl disulfide surfactants were prepared by the reaction of different aliphatic amines with S-acetylmercaptosuccinic anhydride followed by the oxidation with iodine. Selective destruction of the vesicles formed by the above surfactants were successfully performed by the reductive cleavage of dialkyl disulfide to its monoalkyl thiol surfactants with various mercaptans.

Recently there has been considerable interest in constructing synthetic vesicle systems which are stable but can be chemically switched-on(vesicle formation) and -off (vesicle destruction) under mild conditions, particularly in the biological environments.[1] However, there are only a few reports on this subject.[3]

One of the most widespread and thoroughly elucidated chemical functions in many significant biochemical systems is the thiol-disulfide redox couple as is represented by the cysteine-cystine couple of insuline. And many attempts have been made to mimic this exquisite biochemical switch for the manipulation of many interesting events such as red-ox crown ethers[4] and reversibly polymerized-depoly merized vesicles.[5] We here report a new type of amphiphiles which can be interconverted readily between micelle and vesicle states by thiol-disulfide redox couple, which appears very promising as a novel device for the synthetic drug carrier.[6]



a: n=11 b: n=13 c: n=15 d: n=17

S-Acetyl-protected thiol-containing surfactants (1a-d) were prepared as follows: Long chain aliphatic amines were first reacted with S-acetylmercaptosuccinic anhydride[7] and hydrolyzed. The products were then oxidized to their corresponding disulfide-bridged dialkyl amphiphiles (2a-d) with iodine in aqueous NaOH solution.[8] In the NMR spectra of disulfide amphiphiles taken in $CDCl_3$, a singlet around 2.4 ppm attributable to the resonance of S-acetyl protons of 1a-d disappeared completely and all the other resonance peaks were somewhat broadened.

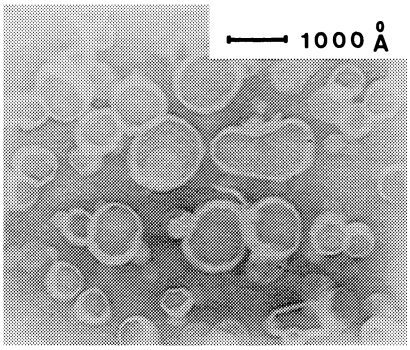


Fig. 1. Electron micrograph of vesicles derived from 2b in Tris buffer(pH 7.9).

These amphiphiles have strong tendency to disperse in water and gave a clear to slightly turbid solutions upon sonication in Tris buffer (pH 7.9). The solution morphology of these aqueous aggregates were examined by electron microscopy and [^{14}C] sucrose entrapment experiments. An electron micrograph of C_{14}-S-S-C_{14} disulfide vesicles is shown in Fig. 1.[9] As can be seen from the Figure well-defined vesicles of closed bilayer structure were prominent with approximate diameter of up to 900 Å and bilayer thickness of around 50 Å. The closedness of the present vesicles was also ascertained by [^{14}C] sucrose entrapment experiments.[10] Gel filtration chromatograph of the vesicle solution containing [^{14}C] sucrose through Sephadex G-50-80 column at 4°C were carried out and the results are summarized in Table 1.

To elucidate the morphological change the thermal transition behavior of thus obtained vesicles were investigated by DSC[11] and turbidity measurements,[12] and the results are also included in Table 1. While C_{14}-S-S-C_{14}, C_{16}-S-S-C_{16}, and C_{18}-S-S-C_{18} exhibited a sharp transition, characteristic of an organized bilayer assembly, C_{12}-S-S-C_{12} did not exhibit any notable transition behavior down to 4°C under the present experimental conditions. Temperature-dependent turbidity measurements (absorbance at 400 nm) also revealed phase transitions.

Table 1. Properties of Vesicles Formed by Dialkyl Disulfide Amphiphiles

Amphiphlies	Entrapment of [^{14}C] sucrose / %	Phase transition temperature / °C		EM
		DSC	Turbidity	
2a, C_{12}-S-S-C_{12}	-[a]	-[a]	5	-[a]
2b, C_{14}-S-S-C_{14}	0.03	24.5	25	vesicle
2c, C_{16}-S-S-C_{16}	0.10	42	41	vesicle
2d, C_{18}-S-S-C_{18}	0.59	56	56.5	vesicle

a) The values could not be determined because of the unstable nature of the vesicle.

Table 2. Changes in Fluorescence Intensity of 6-Carboxyfluorescencein upon Treatment with Various Reducing Agents

Reducing agent	Changes in intensity/10^3% h^{-1} a)		
	0.01 mol dm^{-3}	0.1 mol dm^{-3}	1 mol dm^{-3}
Ethanol	-	3.3	5.0
Dithiothreitol	15.4	17	-
2-Mercaptoethanol	-	18	100
Octylmercaptan	-	450	790

a) The values were estimated based on the values measured after complete destruction of the 6-CF-entrapped vesicles by boiling the solution at 100°C for 5 min.

In order to find certain applications we attempted to selectively destroy (switch-off) the present vesicles by converting dialkyl amphiphlies to its monoalkyl surfactants via reductive cleavage of disulfide to thiol functions.[13] Preliminary experiments aimed to determine the reductive efficiency of the various reducing agents upon the destruction of the present vesicle system were carried out by employing 6-carboxyfluorescein (6-CF) as a probe. 6-CF (120 mM) was entrapped within the vesicles of 2d (10 mM) in Tris buffer (pH 8.1) following the standard procedure.[14] After gel filtration over Sephadex G-50-80 column (12 mm x 250 mm) with Tris buffer the separated vesicle solution was treated with various reducing agents and stored at 4°C which is well below the transition temperature of 2d. The destruction of the vesicles were monitored for a certain time period by measuring the changes in fluorescence intensity of 6-CF (excitation wavelength = 490 nm; emission wavelength = 520 nm).[14]

Surfactants containing dialkyl moiety are known to have greater tendency to form vesicles compared with monoalkyl analogs. When the presently investigated dialkyl amphiphiles are reduced to their monoalkyl thiol amphiphiles, they can no longer interact strongly enough to sustain the vesicular structure. Thus, the vesicles became destructed (switched-off) with concomitant release of the entrapped 6-CF.[15] To draw a clear line from the unwanted contribution of lyolysis[16] control experiments with same equivalent amount of ethanol were performed. the results are given in Table 2. As can be seen from the Table, all the tested triggering reagents brought much pronounced changes in fluorescence intensity, thus manifesting their efficient destabilizing power. Without any reducing agents, the changes in fluorescence intensity were nearly negligible up to 5 days. Interestingly, octylmercaptan is much more efficient compared with the dithiothreitol and 2-mercaptoethanol which are well known and frequently employed reducing agent for the disulfide linkages. This is probably due to the higher hydrophobicity of octylmercaptan, which makes it readily accessible to the disulfide linkage of the vesicle.[5] With $Na_2S_2O_4$, the changes were not reproducible but great enough to confirm the efficient destailization effect. Further studies concerning the employment of more selective and milder triggering

agents such as enzyme analogs for the application of the present disulfide vesicle systems as a drug carrier are under way in our laboratory.

The authors thank Dr. Kyung-Woo Lee, Dr. Jong-Kee Yeo, and Dr. Nam-Sok Choi of Lucky Central Research Institute for helping with the EM measurements. The helpful discussions of Dr. Chung-Soo Kim and Dr. Kwang-Choon Chung are also greatly acknowledged.

References

1) J. H. Fendler, Chem. Br., 20, 1098 (1984).
2) S. L. Regen, Polymer News, 10, 68 (1984).
3) Y. Okahata, S. Hachiya, K. Ariga, and T. Seki, J. Am. Chem. Soc., 108, 2863 (1986).
4) S. Shinkai, K. Inuzuka, O. Miyazaki, and O. Manabe, J. Am. Chem. Soc., 107, 3950 (1985).
5) N. Y. P. Samuel, M. Singh, K. Yamaguchi, and S. L. Regen, J. Am. Chem., 107, 42 (1985). One of the referees suggested that a controlled experiment with octanol should be performed for the purpose of comparison. The authors will work on it and publish the results in a future report.
6) Cystine derivative with fluorocarbon chains which has a closely related structure to the present amphiphiles was genuinely employed to construct "uncorked liposome" by Ringsdorf et al. However, no detailed discussion on the amphiphile itself was reported: R. Buschl, T. Folda, and H. Ringsdorf, Markromol. Chem. Suppl., 6, 245 (1984).
7) I. M. Klotz and R. E. Heiney, J. Am. Chem. Soc., 81, 3802 (1959).
8) All the compounds gave satisfactory IR and NMR spectral properties. 1a(92%), mp 78-79°C; 1b(64%), mp 89.5-92°C; 1c(76%), mp 92-94°C; 1d(95%), mp 94°C, Found: C, 65.1; H, 10.3; N, 3.1%. Calcd for $C_{24}H_{45}NO_4S$: C, 65.0; H, 10.2; N, 3.2%. 2a(70%), mp 76.5-78.5°C; 2b(72%), mp 89-91°C; 2c(91%), mp 90-92.5°C; 2d(88%), mp 87°C. Found: C, 65.1; H, 10.5; N, 3.4%. Calcd for $C_{44}H_{84}N_2O_6S_2$: C, 66.0; H, 10.6; N, 3.5%.
9) Samples for electron microscopy were prepared by mixing vesicle solutions (10 mM) with the staining agent of 2% aqueous ammonium molybdate solution.
10) S. L. Regen, A. Singh, G. Oehme, and M. Singh, J. Am. Chem. Soc., 104, 791 (1982).
11) T. Kunitake and Y. Okahata, J. Am. Chem. Soc., 102, 549 (1980).
12) K. Kano, A. Romero, B. Djermouni, H. J. Ache, and J. H. Fendler, J. Am. Chem. Soc., 101, 4030 (1979).
13) G. E. Means and R. E. Feeney, "Chemical Modification of Proteins", Holden-Day, Inc., San Francisco (1971) pp. 149-174.
14) R. A. Moss and G. O. Bizzigotti, J. Am. Chem. Soc., 103, 6512 (1981).
15) The solution properties of the closely related cysteine amphiphlies are given in the reference: P. Heitman, Europenan, J. Biochem., 3, 346 (1986).
16) C. D. Tran, P. L. Klahn, A. Romero, and J. H. Fendler, J. Am. Chem. Soc., 100, 1622 (1978).

(Received December 25, 1986)