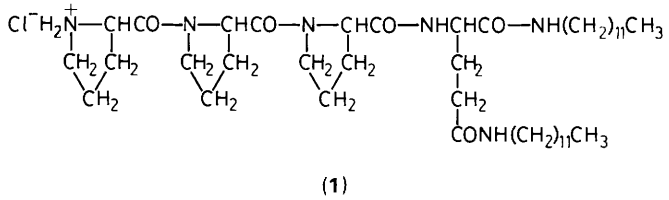
Synthetic Peptidic Amphiphile: Reduction in Length of a Helical Bilayer Assembly due to Interaction with a Metal Cation

Toshimi Shimizu,* Mariko Mori, Hiroyuki Minamikawa, and Masakatsu Hato

Research Institute for Polymers and Textiles, 1-1-4 Higashi, Tsukuba, Ibaraki 305, Japan

In the presence of Ba^{2+}, a remarkable reduction in the length of helical bilayer assemblies, through interaction between the metal cation and peptide-carbonyl groups, has been observed.

Helical superstructures have been found to be formed from chiral amphiphiles such as glutamate-based lipids,[1—3] aspartate-based lipids,[4] and diacetylenic lecithins.[5—6] Such helices generally appear as intermediates on incubation, and eventually they are converted into rod-like structures over a long time period. However, there are few studies on the artificial control of the dimensions of bilayer assemblies.[6] We have reported the formation of helical superstructures from synthetic chiral peptidic amphiphiles.[7—8] In studying the characteristics of those helices, we noticed that the morpholo-



(1)

Table 1. Length of helical superstructures as a function of cation concentration and incubation time.[a]

Salt	Molar ratio salt : (1)	Incubation time/h	Averaged length/μm	Number of selected helices
	0	3	19.4	51
No salt	0	24	64.7	45
	0	264	63.8	37
	1.0	24	11.7	100
KCl	5.0	24	8.9	79
	10.0	24	3.7	75
	1.0	312	6.3	67
$BaCl_2$	5.0	312	2.8	87
	10.0	312	3.0	76

[a] Concentration of (1): 1 mg ml^{-1}.

gies were strongly affected by inorganic or organic additives in aqueous dispersion. In this communication we describe the marked reduction in the length of the helical assemblies in the presence of alkaline or alkaline earth metal cations.

Peptidic amphiphile (1) forming a bilayer assembly was synthesized as reported elsewhere.[7] Observation of the morphological change in the assembly was accomplished as follows: amphiphile (1) (1 mg) was dispersed in deionized distilled water (1.2×10^{-3} M; 1 ml) containing a certain amount of metal chloride [$BaCl_2$, $CaCl_2$, $MgCl_2$, NaCl, KCl, NH_4Cl, and $FeCl_3$; molar ratio salt: (1) 0, 0.5, 1.0, 5.0, and 10.0]. The pH of the aqueous media ranged from 5.9 to 6.5. The slightly turbid aqueous dispersion was then sonicated for 3 min at 40 °C (above the phase transition temperature). At this time, only very small globular vesicles (diameter <1 μm) were present, showing rapid Brownian motion. Next, the dispersion was incubated at 18—20 °C over a 3—3.5 h time period. The droplet was then observed using dark-field optical microscopy.

When no salt was added, fine fibrous assemblies were observed on incubation (30 min) and gradually transformed into well known right-handed helical superstructures (length 50—100 μm)[1—4] (Figure 1a). In the presence of $BaCl_2$, $CaCl_2$, $MgCl_2$, and KCl, the length of such a superstructure was suppressed. Especially, remarkable reduction in the length was observed when $BaCl_2$ coexisted with the amphiphile. Figure 1b shows well defined shorter right-handed helices (length 5—10 μm) produced by the addition of an equimolar amount of $BaCl_2$. On the other hand, NaCl, NH_4Cl, and $FeCl_3$ have shown no suppressive effect on the length of the assemblies.

Thirty to a hundred pieces of helical assemblies were selected on the optical micrograph and traced on paper. Table 1 summarizes the lengths of the helices in the presence of $BaCl_2$ and KCl, which were determined by an area-curve meter (X-PLAN 360, Ushikata Co., Ltd.), clearly indicating that the presence of KCl or $BaCl_2$ reduces the lengths of helices, which are strongly dependent on the concentration of

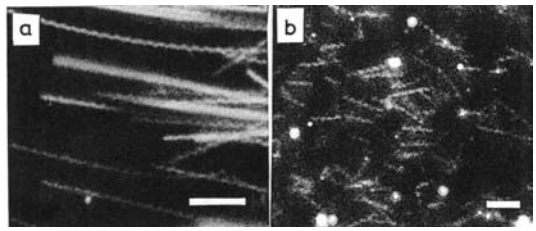


Figure 1. Enlarged dark-field optical micrographs of amphiphile (1) in aqueous dispersion (1 mg ml^{-1}). Aging conditions: 1 day at 18—20 °C. Scale: 5 μm. (a) No salt coexisted. (b) Equimolar $BaCl_2$ was added before aging.

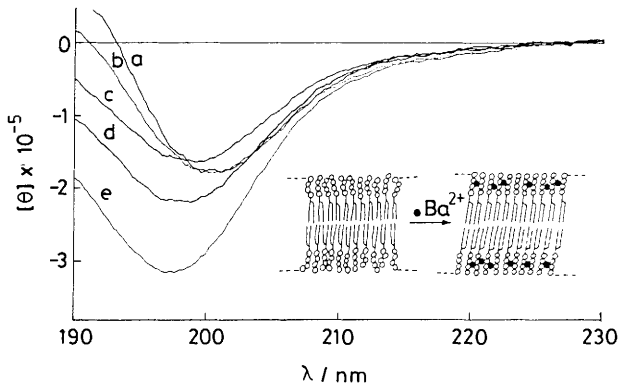


Figure 2. CD spectra of amphiphile (1) in aqueous solution containing $BaCl_2$ or KCl at 25 °C. Molar ratios of the salt to the peptidic amphiphile (PA) (1): (a) no salt, (b) [KCl]/[PA] = 0.5, (c) [KCl]/[PA] = 1.0, (d) [$BaCl_2$]/[PA] = 0.5, and (e) [$BaCl_2$]/[PA] = 1.0. Aging conditions: 2 days at 18—20 °C. Concentration of PA: 1.27×10^{-4} M.

the salt. $BaCl_2$ is more suppressive than KCl, and the helices were of fairly uniform length. It should be noted here that no remarkable changes in length, width, or morphology of the helices were induced by the addition of $BaCl_2$ during the course of incubation. This observation suggests that the salt affects the initial assembling process from globular vesicles to fine fibres.

It is predicted that the reduction in the critical aggregate concentration (CAC) of (1) will be too small to affect the length at concentrations of the salts below 10^{-2} M. Actually, the CAC values of (1) decreased somewhat from 1.2×10^{-5} M to 5×10^{-6} M with added $BaCl_2$ or NH_4Cl up to 10^{-1} M. Also, the protonated state of the amino head group of (1) will be little changed over the pH range of the aqueous media (pH 5.9—6.5). Therefore, it could be suggested that the length is not determined by end group or coulombic effects alone.

Circular dichrosim (CD) is a useful probe to assess the regularity of arrangement of the amide chromophores in bilayer assemblies.[8] Figure 3 shows the CD spectra of assemblies of (1) in aqueous solution, with $BaCl_2$, over 2 days incubation at 18—20 °C. The cation-free assembly of (1) gave a negative Cotton band at 201 nm. As the amount of $BaCl_2$ increased, the CD intensity around 200 nm increased, accompanied by a small blue-shift of the peak minimum. This enhancement of the CD intensity is caused by the regular arrangement of the amide chromophores due to the lowering of the membrane fluidity.[8] Significantly, a similar CD

enhancement could be obtained by cooling the cation-free aqueous dispersion to 18 °C. The spectral change induced by KCl was smaller than that induced by $BaCl_2$. This difference may originate from strong binding of Ba^{2+} to oxygen atoms in the peptide-carbonyl groups.[9—11]

CD data suggest that complexation of the assemblies with Ba^{2+} may be responsible for the more suppressive effect on the length. The approximate order of suppression by the cations ($Ba^{2+} \gg Ca^{2+} \sim Mg^{2+} \sim K^{+} > Na^{+} \sim NH_4^{+} \sim Fe^{3+}$) is consistent with the selectivity in cation-binding by synthetic oligopeptides.[10,11] While it is not possible to give a definite interpretation at this stage, some possibilities are presented. When more Ba^{2+} is added, nucleation may be promoted by the assembling of the molecules through complexation. Since the initial self-assembling process is more rapid, much more nuclei seem to result in shorter helices. A similar situation occurs on addition of water to a solution of diacetylenic lecithin.[6] Electron microscopy studies, to obtain further information on the initial assembling process, are in progress.

Received, 16th May 1989; Com. 9/02060H

References

1 N. Nakashima, S. Asakuma, J.-M. Kim, and T. Kunitake, *Chem. Lett.*, 1984, 1709.
2 K. Yamada, H. Ihara, T. Ide, T. Fukumoto, and C. Hirayama, *Chem. Lett.*, 1984, 1713.
3 N. Nakashima, S. Asakuma, and T. Kunitake, *J. Am. Chem. Soc.*, 1985, **107**, 509.
4 H. Ihara, T. Fukumoto, C. Hirayama, and K. Yamada, *Polym. Commun.*, 1986, **27**, 282.
5 P. Yager, J. P. Sheridan, and W. L. Peticolas, *Biochim. Biophys. Acta*, 1982, **693**, 485.
6 J. H. Georger, A. Singh, R. R. Price, J. M. Schnur. P. Yager, and P. E. Schoen, *J. Am. Chem. Soc.*, 1987, **109**, 6169.
7 T. Shimizu and M. Hato, *Thin Solid Films*, 1989, **178**, in the press.
8 T. Shimizu, M. Mori, H. Minamikawa, and M. Hato, *Chem. Lett.*, 1989, 1341.
9 Y. A. Ovchinnikov, V. T. Ivanov, and A. M. Shkrob, in 'Membrane Active Complexones,' B.B.A. Library, Elsevier, New York, 1974.
10 V. Madison, C. M. Deber, and E. R. Blout, *J. Am. Chem. Soc.*, 1977, **99**, 4788.
11 T. Shimizu, Y. Tanaka, and K. Tsuda, *Bull. Chem. Soc. Jpn.*, 1985, **58**, 3436.