Colloid Polym Sci (1995) 273:96–100

ORIGINAL CONTRIBUTION

K. Yamauchi
K. Togawa
T. Moriya
M. Kinoshita

Novel properties of molecular assemblies of isoprenoid surfactants

Received: 15 March 1994
Accepted 22 June 1994

Prof. K. Yamauchi (✉) · K. Togawa
T. Moriya · M. Kinoshita
Dept. of Bioapplied Chemistry
Faculty of Engineering
Osaka City University
Sumiyoshi-ku, Osaka 558, Japan

Abstract Unlike micelles of straight hydrocarbon chain-surfactants, isoprenoid surfactants, $CH_3[CH(CH_3)CH_2CH_2CH_2]_3CH(CH_3)CH_2-R$ ($R = CH_2N^+(CH_3)_3\ Br^-$, $CH_2OPO_3H^-\ Na^+$, $CH_2OSO_3^-\ Na^+$, $CO_2^-\ Na^+$), gave large globular and cellular assemblies in water which could be observed directly by transmission electron microscopy; critical micelle concentration of $0.3 \sim 1.4 \times 10^{-3}$ M at 20 °C, aggregation number of $2 \sim 15 \times 10^4$, and diameter of 200–2000 Å. A basic structure of the assemblies was a thin layer with a thickness (about 30 Å) which was close to the molecular length of the surfactants. The assemblies were decomposed during gel column chromatography; viz., they were not as stable as the liposomes of lecithins. The morphology was discussed in conjunction with a steric effect of the isoprenoid chain.

Key words: Molecular assembly – archaebacterium – isoprenoid surfactant – transmission electron microscopy (TEM)

Introduction

Archaebacteria grow in extreme habitats such as hot springs, salt lakes and acidic aqueous spots [1–4]. The bacterial lipids are always characterized by long polyisoprenoid chains. It has been shown that the liposomal membranes made of such lipids are much more thermostable than those of ordinary lipids which have straight hydrocarbon chains. Their membranes do not fuse readily with each other, resulting in formation of a highly stable aqueous suspension of vesicles [5–8]. It seemed that these features owe chiefly to the branched hydrophobic chains with methyl groups.

In this paper, we describe molecular assemblies prepared from a single species of surfactants (Ia–d; Fig. 1) bearing a isoprenoid chain [9]. The surfactants were designed in the hope that they may inherit the novel property of the archaebacterial lipids.

Experimental

The isoprenoid surfactants (Ia-d) were prepared previously from phytol; all of them were epimeric mixtures, and the viscous oil did not solidify above −20 °C [9]. Straight chain-surfactants such as cetyltrimethylammonium bromide (CTAB) were commercially available. Gel permeation chromatography was carried out on modified dextran (Sephadex G-25). Ultrasonication was preformed by using a probe-type ultrasonic disintegrator (Ohtake Works Co., model 5201). Phase transition was studied by means of a Rigaku 8240 and a Microcal MC-2 scanning calorimeters. Other instruments used were a Shimazu ST-1

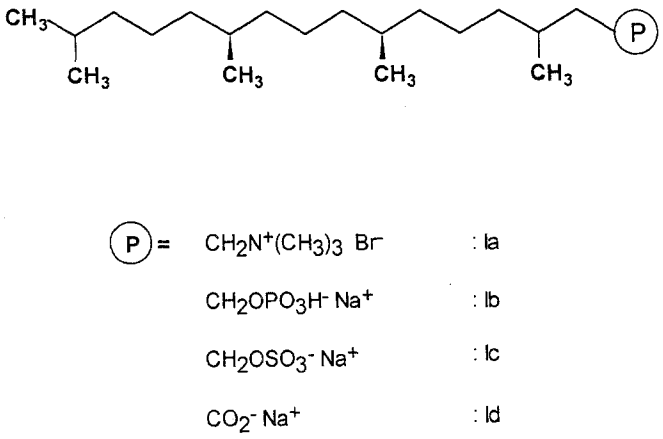

Fig. 1

surface tension balance, a Hitachi H-7000 transmission electron microscope, and the Ohtsuka DLS-700 light-scattering spectrometer which was controlled by a microprocessor, NEC-9801.

Preparation of molecular assemblies

The mixture of a surfactant and twice-distilled water (3 ml/about 15 mg of surfactant) was either vortexed at 20–25 °C for 30 min or sonicated at about 20 °C (ambient temperature) and 35 W for 20 min. The sonication was followed by centrifugation at 2000 g for 15 min. Both methods gave clear suspensions of the molecular assemblies which were indistinguishable in physicochemical properties, including CMC and morphology. The results were reproducible for at least 1 week after preparation of the suspensions. The sonication method was adopted in the present study to obtain homogeneous suspensions.

Measurement of critical micelle concentration (CMC)

The methodology was based on Whilhelmy's method. About 10^{-2} M aqueous solutions of the phytanyl surfactants were prepared as stock solutions. Each of them was diluted with water to various apparent concentrations and sonicated at 20–25 °C for 5 ~ 20 min. After the weight was readjusted by addition of water, surface tension was measured at 20 ± 1 °C by means of the balance equipped with the flat glass tip, which was dipped for at least 10 min in the aqueous solution. The CMC was taken as the concentration which showed a well-defined refraction in the plots of the surface tensions vs. log[surfactant]; experimental error, ± 3%. Typical results are shown in Fig. 2. The CMC values were not affected by sonication conditions (temperature and time).

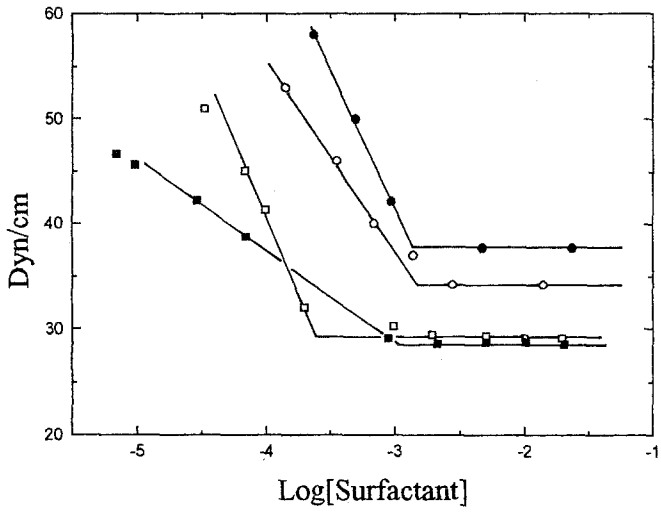

Fig. 2 Surface tension of the aqueous suspensions of Ia-d as a function of log [surfactant] at 20 ± 1 °C. Ia, ○; Ib, ●; Ic, □; Id, ■

Transmission electron microscopy (TEM)

A half drop of the sonicated surfactant-suspension prepared above was laid on the collodion film (on a copper grid) which was coated with a thin carbon (about 10 nm thickness). A half drop of 1% (w/v) aqueous phosphotungstic acid/sodium hydroxide (pH 7) or aqueous uranium acetate was then added to the suspension on the grid, and staining was allowed to proceed for 2–3 min at room temperature. Excess liquid on the grid was removed with the tip of an absorbing paper to give the specimen which was mounted to the electron microscope in order to observe the image of the molecular assemblies at a magnification of $1 \sim 2 \times 10^4$. The picture was usually enlarged by a factor of 5 ~ 10 (Fig. 3).

Light scattering

The sonicated surfactant-suspensions obtained above were filtered through a cellulose acetate membrane (Corning #21033 − 13; pore size, 0.45 μm), and scattering intensity was measured at 25 ± 0.5 °C using a standard DLS-cell to give the size-distribution of the particles. Typical results are shown in Fig. 4. The size from the scattering data, the morphology from TEM, and the molecular volume of the surfactants, which was calculated to be about 1500 Å^3 (= cross-sectional area of about 50 Å^2 × the molecular length of 30 Å; c.f., Langmuir membrane studies of isoprenoid lipids have revealed about 40 ~ 50 Å^2/the isoprenoid chain at a limiting compression [5]), were used to estimate an aggregation number of the assemblies.

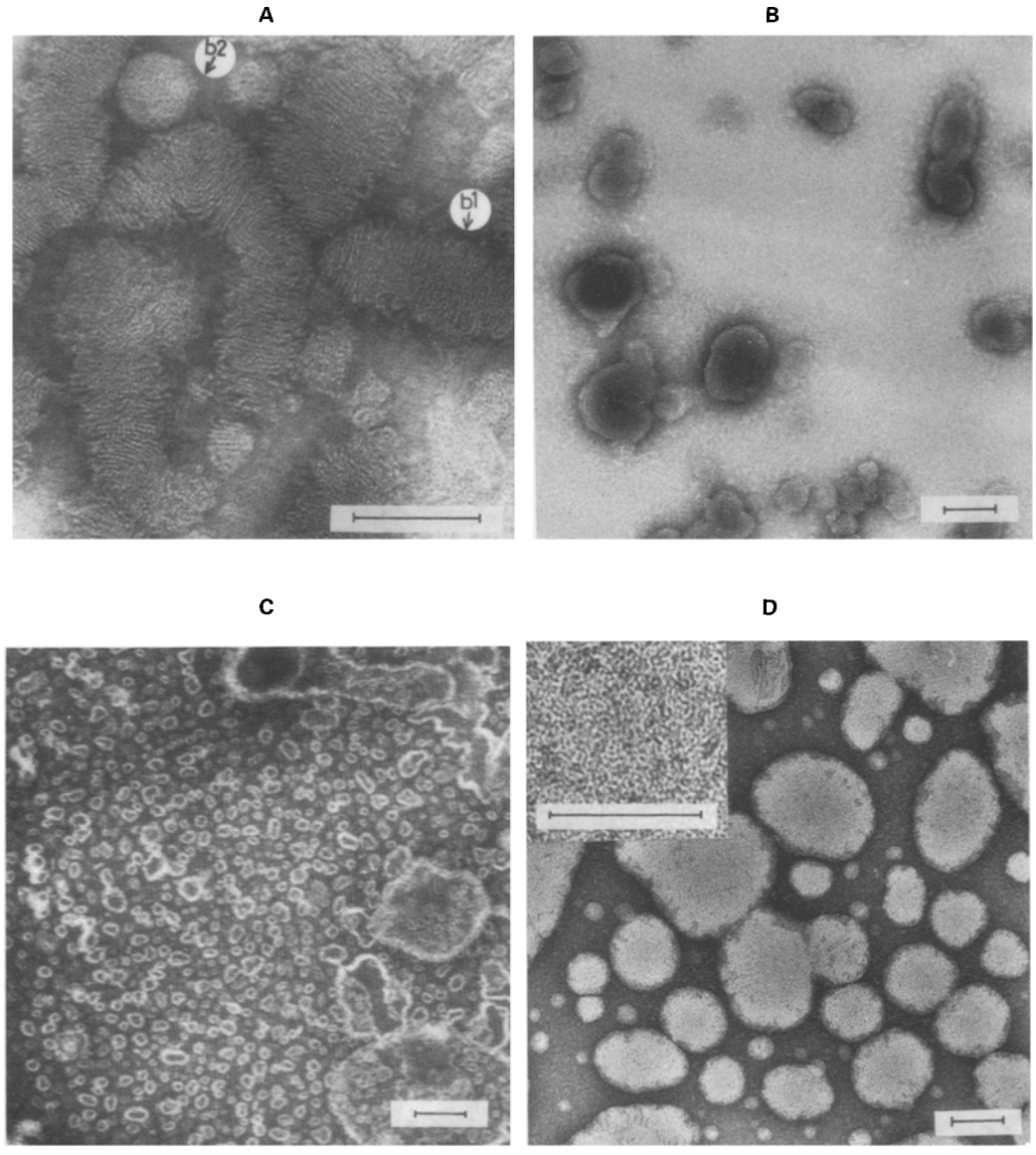

Fig. 3 Transmission electron micrographs; negative staining with phosphotungstic acid/sodium hydroxide (pH 7). The bar is 1000 Å. (A) Ia, (B) Ib, (C) Ic, (D) Id

Results and discussion

Despite the large CMC (Fig. 2; Ia: 1.4; Ib: 1.3; Ic; 0.3; Id: 1.6 10^{-3} M) the isoprenoid amphiphiles (1–2 mg/ml of water) upon sonication at ambient temperature gave rise to large globular and cellular assemblies as seen in TEM pictures (Fig. 3A–D). It may be estimated from the size of the assemblies in TEM or light scattering and a molecular volume of Ia–d ($1200 \sim 1500$ Å^3) that an aggregation number of the assemblies was approximately as $2 \sim 15 \times 10^4$ molecules. In contrast, the "micelles" of straight chain-surfactants such as n-$C_{16}H_{33}N^+(Me)_3$ Br^- (CTAB) and n-$C_{16}H_{33}SO_4^-$ Na^+ have never exhibited and, indeed, did not display any structures in TEM, presumably because of a transient existence of the micelles equilibrating rapidly with free surfactant molecules in a bulk aqueous phase [10].

The morphology of the assemblies was varied with the polar groups. The compound, Ia, gave a neat stack of the discus-like plates (Fig. 3A). Each plate has a thickness of $30 \sim 40$ Å and a diameter of 200–1000 Å. The stacking of the disks from various angles, is shown in the TEM picture, Fig. 5. The compound, Ib, furnished multilamellar concentric globules as seen in Fig. 3B. The thickness of each layer was of about 35 Å. The cellular assemblies from

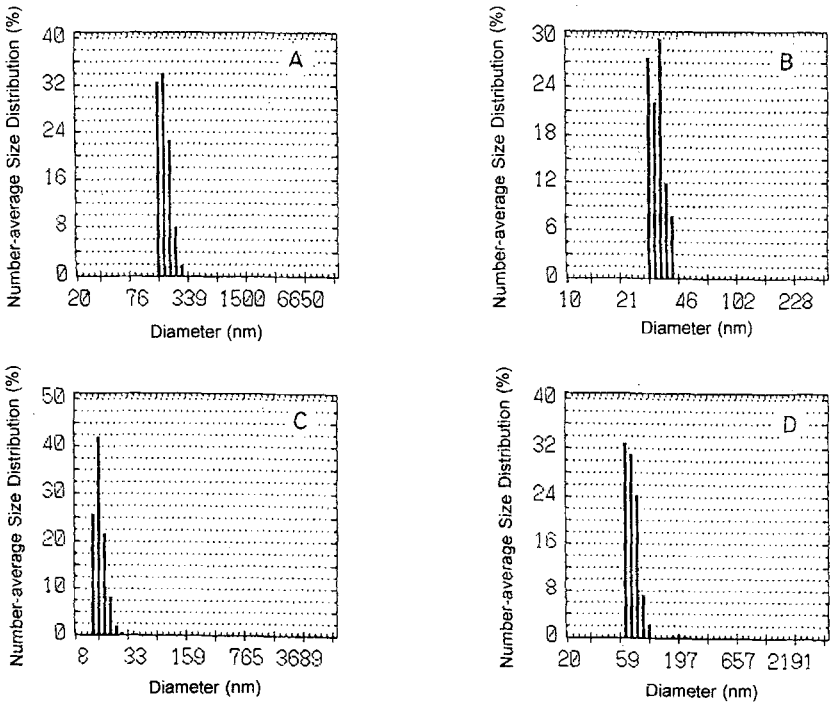

Fig. 4 The number average size distribution (%) of the diameters measured by light scattering. (A) Ia; (B) Ib; (C) Ic; (D) Id. Assay temperature, 20 °C

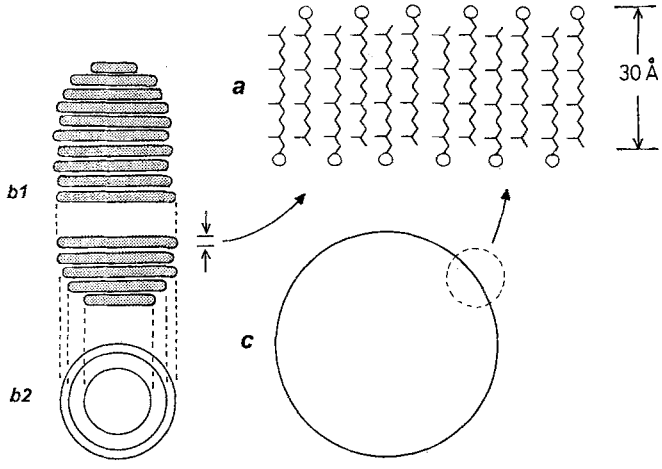

Fig. 5 Schematic representation of the molecular assemblies of the isoprenoid surfactants. The surfactants interdigitate to form a plate (*a*) as the primary assembly, which gives globules (*b1* = side-view; *b2* = top-view) or bubbles-in-water (*c*) as the secondary structure. See Fig. 3A for *b1* and *b2*, and Fig. 3C for *c*

Ic may be regarded as "soap bubbles-in-water" (Fig. 3C). Most of them (> 70%) are 200–500 Å in diameter. The wall is about 40 Å in thickness. The layer thickness of these assemblies would suggest that the surfactant molecules were packed by interdigitating each other to locate their polar heads on both sides (Fig. 5a); c.f., the molecules have a length of about 30 Å (= 2.5 Å of one C–C–C zig-zag × 8 + about 10 Å of a polar hydrated head) in water [11]. The assemblies from Id were unusual. Such a complex and porous structure has often arisen from amphiphiles bearing small polar heads, such as with a $-CO_2^-$ moiety [12, 13].

The direct observation by TEM implies that the molecular assemblies from Ia–d were stationary at least for a period (about 2 min) of negative staining in the preparation of the specimens. The assemblies were thus more definite in structure than micelles. It might be ascribed to a steric hindrance against association/dissociation of the bulky isoprenoid surfactants to/from the assemblies, resulting in a slow equilibrium with the free molecules in a bulk aqueous phase. A stabilizing effect of the phytanyl chain has also been observed in the membranes of archaebacterial lipids [5–8]. The assemblies, however, were disintegrated during gel-permeation chromatography. They could not retain glucose and 5(6)-carboxyfluorescein, either; viz., these water-soluble probes, if there were any in the assemblies, were released during gel chromatographic separation. None of the aqueous suspensions of these assemblies underwent any gel-to-liquid crystalline phase transition, at least not above 3 °C. Molecular assemblies from most archaebacterial lipids also have not exhibit phase transition above − 10 °C [5, 14]. A detailed mechanism for the formation of abnormal assemblies would be obtained from a kinetic study, and an investigation in this direction has been under consideration.

Acknowledgement A part of the investigation was supported financially by the Ministry of Education, Japan.

References

1. Kates M (1978) Prog Chem Fats Lipids 15:301–342
2. Langworthy TA (1985) In: Woese CR, Wolfe RS (eds) The Bacteria. The Treaties on Structure and Function, Academic Press, Orlando, FL, pp 459–497
3. De Rosa M, Gambacorta A, Gliozzi A (1986) Microbiol Rev 50:70–80
4. Koga Y, Nishihara M, Morii H, Akagawa-Matsushita M (1993) Microbiol Rev 57:164–182
5. Yamauchi K, Kinoshita M (1993) Prog Polym Sci 18:763–804
6. Yamauchi K, Sakamoto Y, Moriya A, Yamada K, Hosokawa T, Higuchi T, Kinoshita M (1990) J Am Chem Soc 112:3188–3191
7. Yamauchi K, Doi K, Kinoshita M, Kii F, Fukuda H (1992) Biochim Biophys Acta 1110:171–177
8. Yamauchi K, Doi K, Yoshida Y, Kinoshita M (1993) Biochim Biophys Acta 1146:178–182
9. Yamauchi K, Yoshida Y, Moriya T, Togawa K, Kinoshita M (1994) Biochim Biophys Acta 1193:41–47
10. Fendler JH, Fendler EJ (1975) Catalysis in Micellar and Macromolecular Systems, Academic Press, New York, pp 23–33
11. Hauser H (1985) Chimia 39:252–264
12. Luzzati V, Gulik A (1986) System Appl Microbiol 7:262–265
13. Israelachvill JN, Marcelja S, Horn RG (1980) Quart Rev Biophys 13:121–200
14. Stewart LC, Kates M, Ekiel IH, Smith ICP (1990) Chem Phys Lipids 54:115–129