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The pH-Sensitive Conversion of Molecular Aggregates of Rhamnolipid Biosurfactant

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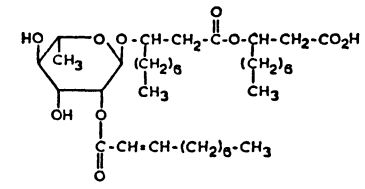
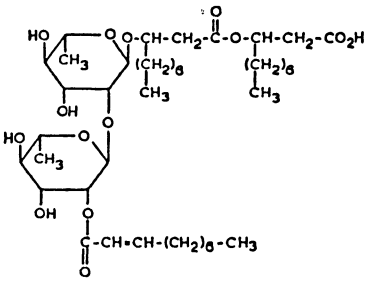
Rhamnolipid B and its precursor A, both microbial and membranous constituent, reversibly changed morphologies of their molecular aggregates from vesicles under acidic conditions, to lamella, lipid particles, and finally to micelles under weakly acidic conditions within a narrow pH range of about 5-7.

It is noteworthy that many kinds of surfactants and chemicals in addition to phospholipids[1-7] form vesicles in water. New vesicle-forming materials from biological origin have also been found,[8-11] and have been tried to apply their functions to superfine microcapsules, biomimetic biological membrane, and artificial cell constituents. There has been some studies on pH-responsive liposomes including preparation of SUV by pH adjustment,[12] drug-deliverable liposome containing the pH-sensitive palmitoyl homocysteine,[13] pH-dependent transition of the system of phosphatidylcholine and poly 2-ethylacrylate,[14] and pH-sensitive capsule membrane coated with dissociative bilayer-forming amphiphiles.[15,16] Hitherto, six kinds of rhamnolipids have been known as the growth stimulant for hydrocarbon-assimilating bacteria.[17-20]

Rhamnolipid A (2-O-α-decenoyl-α-L-rhamnopyranosyl-β-hydroxydecanoyl-β-hydroxydecanoic acid) and rhamnolipid B [2-O-(2-O-α-decenoyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranosyl-β-hydroxydecanoyl-β-hydroxydecanoic acid] belong to glycolipids, and were produced by Pseudomonas aeruginosa BOP 100 in the yield of A, 3.4 g and B, 10.2 g per liter of culture broth.[21] Rhamnolipid A was obtained as a syrupy substance which was $[\alpha]_D$ -44.2° and found: C, 65.72; H, 9.96; calcd.: C, 65.85; H, 9.79% for $C_{36}H_{64}O_{10}$. Rhamnolipid B was purified by recrystallization, which was $[\alpha]_D$ -58.3° and found: C, 62.74; H, 9.40; calcd.: C, 62.84; H, 9.22% for $C_{42}H_{74}O_{14}$. Aqueous suspensions of vesicles, lamella, and lipid particles were prepared as followed. Aliquots of



Rhamnolipid A



Rhamnolipid B

$CHCl_3$-MeOH(2:1) solutions of rhamnolipid A and B with and without the oil-soluble fluorescent dye of N-(5-fluoresceinthiocarbamoyl)dipalmitoyl-L-phosphatidyl-ethanolamine (mixed at 1/50 mol to lipid) were dried to thin films under a stream of nitrogen, and subsequently vacuum desicated for an hour. These lipid films were suspended in water or buffer solutions of pH 5.8-7.2 at the rhamnolipid concentrations of 0.5-20 mg/ml, and then vortexed for 20 min above the phase transition temperatures (Mettler TA 3000, elevated at the rate of 0.5 °C/min) of rhamnolipid A, 28.4; 31.6 °C (Fig. 1). Melting points of anhydrous forms are -3.2 for A and 84.7 °C for B. Kraft point of A, 31.8; B, 23.1 °C in micellar forms; phase transition temperature of A, main 28.4 and pretransition 18.2; B, 31.6 °C in vesicular forms; water, ca. 8 °C in micellar and vesicular forms. Resultant aqueous suspensions in the pH range of 4.3-5.8 were negatively stained with 1% aqueous phosphotungstic acid by the drop method.[22] Electron microscopy (Hitachi HU-12A) of rhamnolipid B demonstrate the formation of vesicles as shown in Fig. 2, which shows the presence of lipid rings of vesicles in the size of 50-100 nm (a), while their cord-like chains (b). Vesicles with the oil-soluble fluorescent dye within their palisade layers are observed using a fluorescent microscope by exciting at 410 nm as ring-like closed vesicles about 500 nm in diameter (Fig. 3). When vortex shaking in the M/15 phosphate buffers at the pH range of 6.0-6.5, lamellar structures are observed by a fluorescent microscope (Fig. 4), while lipid particles are observed as stains not like circular drops of mineral oils in the pH range of 6.2-6.6, based on their increased hydrophilic affinity (Fig. 5).

From the fact of the presence of three kinds of morphological patterns, unique effects of medium pHs may be rationalized by assuming conversion equilibria in the conformation of molecular aggregates as illustrated in Fig. 6. Thus, the micellar form [I] were anticipated and confirmed below. Critical micelle concentration (cmc) of sodium salts of rhamnolipid A and B were 6.22×10^{-5} and 1.50×10^{-4} mol dm^{-3}, respectively, as determined from the break points of the fluorescent intensity of 8-anilino-1-naphthalenesulphonate *vs.* concentration plots in PBS buffer at pH 7.35. Further, sodium salt of rhamnolipid B had the micellar weight of 6000 (average octamer) in the same PBS buffer by means of light scattering measurement (Shimadzu PG-21) at 25 °C and surface area per molecule at the air-water interface, 79.1 $Å^2$ at 30 °C from the surface tension measurement (Wilhelmy-type surface tensiometer, Shimadzu ST-1). On the other hand, it was not possible to detect each step (I-IV) of the pH-dependent aggregates by the pH titration (Hiranuma recording automatic titrator RAT-11), but the

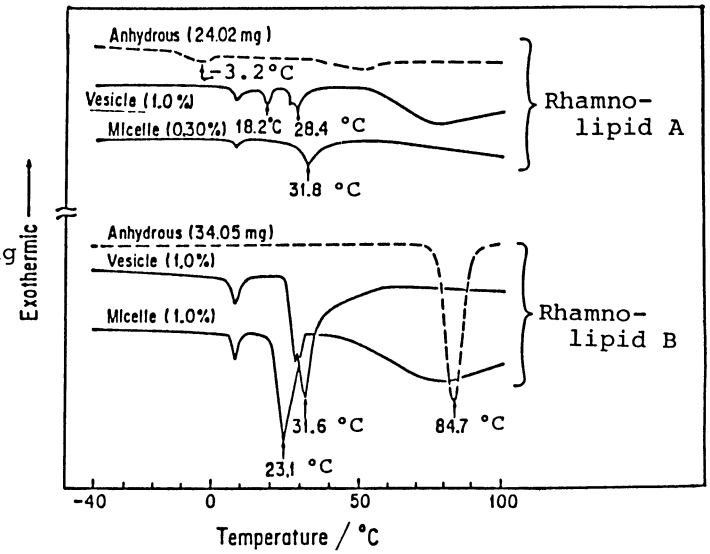


Fig. 1. DSC thermograms of rhamnolipid A and B.

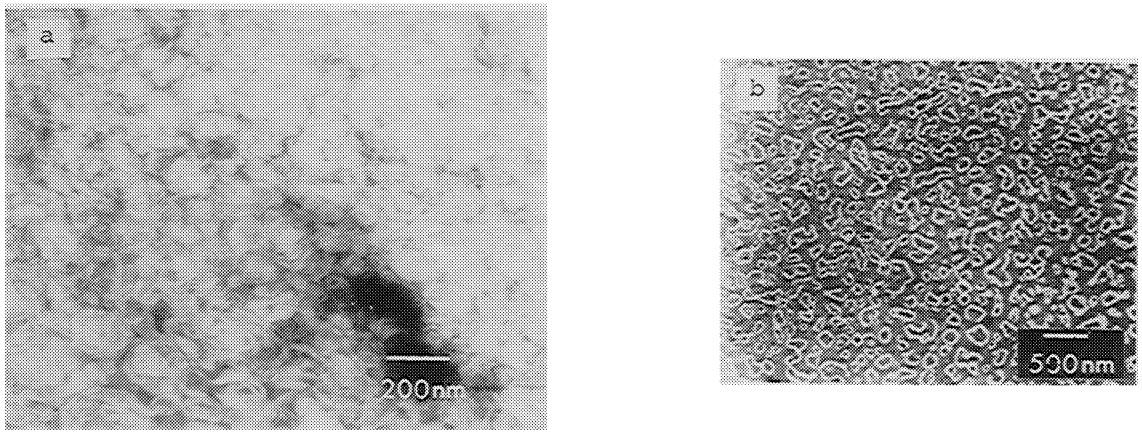


Fig. 2. Negative stain electron micrographs of vesicles [IV] of rhamnolipid B.

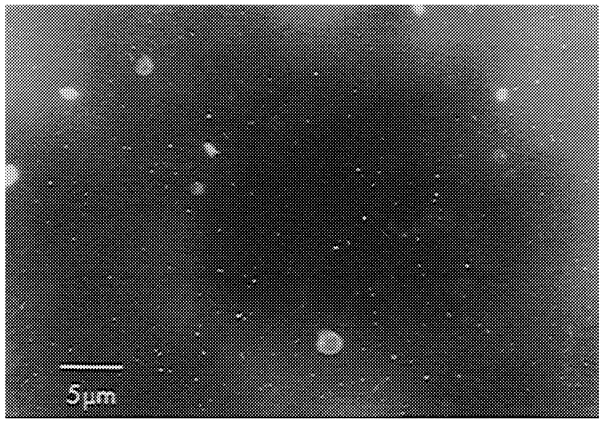


Fig. 3. Fluorescent micrograph of vesicles [IV] of rhamnolipid B.

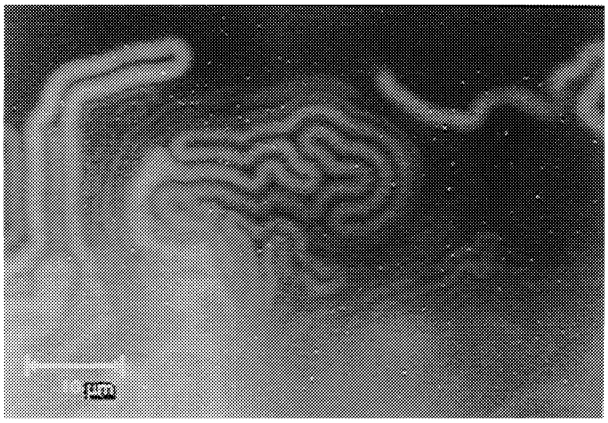


Fig. 4. Fluorescent micrograph of lamella from [III] of rhamnolipid B.

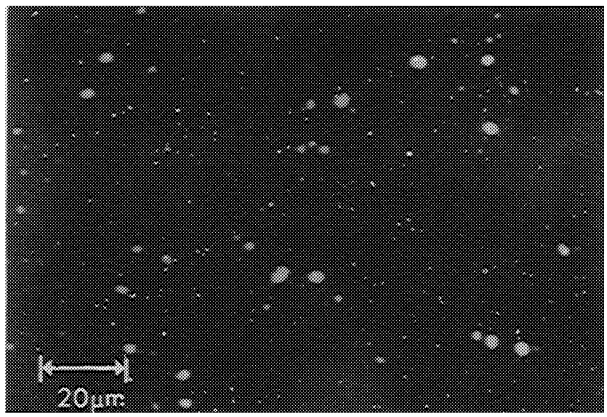


Fig. 5. Fluorescent micrograph of lipid particles [II] of rhamnolipid A.

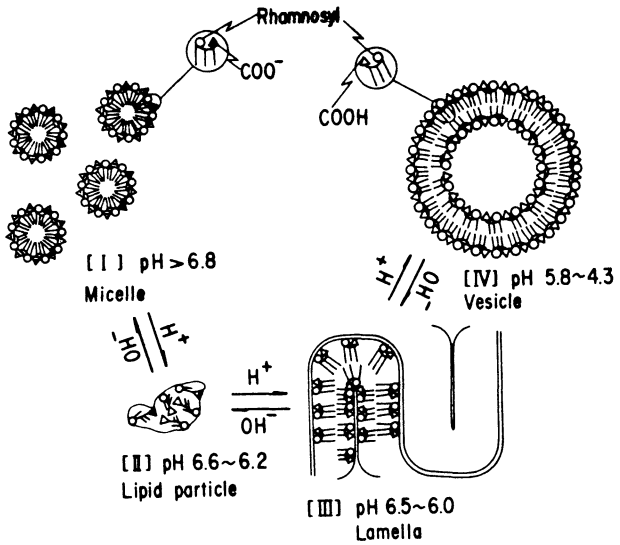


Fig. 6. pH-Sensitive conversion of molecular aggregates of rhamnolipids.

pKa values of rhamnolipid A and B were both 5.6 and were considerably consistent with the boundary zone of [IV] near [III], and end points, both pH 7.6 in [I].

In conclusion, it was found that rhamnolipids formed vesicles by themselves, and this finding may be comparable with the fact that some kinds of marine bacteria have biological membranes which predominantly consist of glycolipids instead of phospholipids.[23] The pH-dependent conversion of molecular aggregates of rhamnolipids would be associated with the biological functions inside and outside the bacterial membrane under weakly alkaline or neutral conditions in the hydrocarbon-assimilating bacterium. In the case of its acidic (bad) growing conditions, the bacterial cell membrane seems to be protected by rhamnolipids in the state of [III] and [IV].

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