Self-Replicating Micelles: Aqueous Micelles and Enzymatically Driven Reactions in Reverse Micelles§

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Abstract: In this paper we present a further development of the self-replication system that was originally described for reverse micelles (Bachmann, P. A.; Walde, P.; Luisi, P. L.; Lang, J. *J. Am. Chem. Soc.* **1990**, *112*, 8200-8201). The definition of the term "self-replication" requires that the population growth of the structure is due to a reaction which takes place within the geometrical boundary of the structure itself. Here it is shown that it is possible to design both reverse and aqueous micelles that are able to self-replicate due to a reaction occurring within the micelle structure. The surfactant used was sodium octanoate, which forms micelles in water in the presence of 1-octanol as cosurfactant. Oxidation of the cosurfactant by permanganate ion converts it to molecules of the corresponding salt, which spontaneously assemble into new micelles. The reaction goes to completion, producing a 43% increase in the micelle concentration, as judged by time-resolved fluorescence quenching. Octanoate in the presence of octanol also forms reverse micelles in 85:15 (v/v) isooctane/1-octanol, and the same oxidation reaction brings about an increase in the micelle concentration by a factor of 9. The reverse micellar system sodium octanoate/octylamine is capable of hosting lipases, which are restricted to the water pool. The hydrolysis of trioctanoyl glycerol, a substrate for these enzymes, which is only soluble in the organic phase, takes place at the micellar interface and increases the micelle concentration by a factor of 4. The implication of these data for chemical autopoiesis (a theory that attempts to define the living in terms of chemical self-organization and self-production) is discussed.

Introduction

In a recent communication[1] we have described a reverse micellar system that can self-replicate. The principle is the following: the reverse micelle hosts a reaction which leads to production of the same surfactant of which the micelle is constituted, the freshly produced surfactant spontaneously redistributing to form a larger number of micelles, i.e., an increase in the micelle concentration. The appropriate definition of "self-replication" (as opposed to a trivial chemist-aided reaction) is thus the following: it is a population increase of a geometrically closed structure, the growth being due to a reaction which takes place within the boundary of the structure itself and which is made possible by the intrinsic properties of the structure (in the case we have described[1] it is the surface-active properties of the micelle which makes a reaction between a strongly hydrophilic component (LiOH) and a lipophilic, membrane-bound component (octyl octanoate) possible, the reaction taking place at the micellar interface).

In contrast to other self-replicating structures proposed in the literature, which are based on template reactions,[2,3] the emphasis in our work is on the self-reproduction of geometrically closed chemical entities, i.e., structures possessing a chemically clearly defined boundary ("bounded structures").

The reason why bounded structures are important to us is tied in with the theory of autopoiesis. This term has been introduced by Maturana and Varela to define the essence of the living,[4,5] i.e., to discriminate between living and nonliving. The presence of a boundary, which operationally defines the inside from the outside, is essential to the functioning of an autopoietic system (as, for example, a living cell), as is the existence of a network of interconnected reactions that take place within the bounded structure. The reaction network should lead to self-generation, via the production of the boundary components which should then spontaneously assemble into the bounded structure itself.

The objective of our work is to explore whether, and to what extent, autopoietic systems can be constructed out of simple supramolecular structures, such as micelles and liposomes. The aim of the present paper is to extend the self-replication scheme originally designed for reverse micelles and to show that it can be generalized to other micellar systems. In particular, we will show that an oxidation reaction (the oxidation of 1-octanol to the corresponding acid by permanganate ion) can form the basis for micelle self-replication in both aqueous and reverse micelles. We will also show that the self-replication of reverse micelles can be based on an enzymatically driven reaction, the principle here being that each autopoietic unit contains the catalyst for its own replication. All these data should allow us to draw some general conclusions about the self-replication of micellar systems and implications for autopoiesis.

Materials and Methods

Reagents. 1-Pyrenesulfonic acid sodium salt (PSA) was from Molecular Probes; pyrene, methylviologen chloride (MV), and 1-octanol from Aldrich; and $K_3Fe(CN)_6$ from Merck. All were of high-purity grade. All other chemicals were of the highest purity available from Fluka.

Tetradecylpyridinium chloride was obtained by exchanging the bromide ions of tetradecylpyridinium bromide (obtained by reacting pyridine with bromo-1-tetradecane) for chloride ions on an ion-exchange resin (Merck III) and was purified by threefold crystallization from ethyl acetate. Tris(bipyridyl)ruthenium chloride (Ru(bipy)) was a gift from Dr. Ziesel (University Louis Pasteur, Strasbourg, France).

Octyl octanoate was synthesized by heating an excess of octanoic acid with 1-octanol in concentrated sulfuric acid. The ester product was washed with water and purified by distillation. *N*-Octyloctanamide was synthesized by aminolysis of octylamine and methyl octanoate and was twice crystallized from methanol/water 1:1 (v/v). The purity (>95%) of both compounds was confirmed by FTIR.

Lipase from *Chromobacterium viscosum* (type XII) was from Sigma, and the lipase from *Humicola lanuginosa* (Lipolase) was a gift from Novo Nordisk Ferment AG (Switzerland).

Quasi-Elastic Light Scattering. Quasi-elastic light scattering experiments were carried out with a Malvern 4700 PS/MW spectrometer and an argon ion laser (Coherent, Innova Model 200-10, λ_0 = 488 nm). Hydrodynamic radii of the octanoate reverse micelles were determined from a cumulant analysis of the intensity autocorrelation function with a reproducibility of ±5%.

Time-Resolved Fluorescence Quenching. Fluorescence measurements were carried out at the Institut Charles Sadron in Strasbourg, France.

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§ Dedicated to the memory of Professor P. Pino.
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(1) Bachmann, P. A.; Walde, P.; Luisi, P. L.; Lang, J. *J. Am. Chem. Soc.* **1990**, *112*, 8200-8201.
(2) von Kiedrowski, G. *Angew. Chem.* **1986**, *10*, 932-934.
(3) Tjivikua, T.; Ballester, P.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1990**, *112*, 1249-1250.
(4) Varela, F. G.; Maturana, H. R.; Uribe, R. *BioSystems* **1974**, *5*, 187-196.
(5) Fleischaker, G. R. *BioSystems* **1988**, *22*, 37-40.

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The fluorescence decay curves of the solubilized fluorescent probe and of the probe and quencher were obtained by the single photon counting technique and analyzed according to a nonlinear weighted least-squares procedure.[6]

For comparison with the light-scattering data, the hydrodynamic radius can be calculated as the sum of the water pool radius and the length of the extended surfactant molecule, which is 10.4 Å for octanoate.[7]

FTIR Spectroscopy. All Fourier transform infrared spectra were recorded on a Nicolet 5SXC FTIR spectrometer, using a CaF_2 cell from Textronica AG with a fixed path length of 0.01 cm. Thirty-six scans were taken of each sample at a resolution of 4 cm^{-1}.

UV Spectroscopy. All UV spectra were recorded with a Kontron Uvikon 810 spectrophotometer using quartz cells from Hellma with fixed path lengths of either 0.1 cm or 1 cm.

Preparation of the Reaction Mixtures and Quantification of the Reactions

System IIA and IIB: Oxidation of Octanol in Aqueous and Reverse Micelles. Aqueous micelles were prepared by mixing 1-octanol (final concentration 0.48 M), sodium octanoate (final concentration 1.2 M), and water. The reaction was started by adding sodium permanganate (final concentration 0.64 M) solubilized by stirring. Reverse micelles were prepared by mixing and then vortexing aqueous solutions of sodium permanganate (initial concentration 3.6 M) and sodium octanoate (final concentration 50 mM) and 85:15 (v/v) isooctane/1-octanol. The reaction mixtures were stirred at room temperature and samples were withdrawn at time intervals; the concentration of permanganate was determined spectrophotometrically, assuming $\epsilon_{546} = 2300\ M^{-1}\ cm^{-1}$.[8]

MnO_2 was removed by centrifugation, and, since some water is also lost at this step, the final water content in the reverse micelles was determined by Karl-Fischer titration.[9]

After the reaction was complete, the sodium octanoate concentration was determined by infrared spectroscopy using a calibration curve of concentration versus absorbance at 1570 cm^{-1} with sodium octanoate in either water or in the reverse micellar solution as applicable. For the aqueous system, the spectrum was recorded after diluting 50 μL of the aqueous solution with 1 mL of pentanol, whereas no dilution was necessary for the reverse micellar system.

System III: Enzyme-Catalyzed Ester Hydrolysis in Reverse Micelles. Stock aqueous solutions (2 mg/mL) of *H. lanuginosa* and *C. viscosum* lipases were prepared in 0.1 M borate buffer pH 9.0 and 0.1 M Tris-HCl buffer pH 7.0, respectively. The solvent system was prepared by adding an appropriate amount of buffer (~0.8% v/v) to 50 mM sodium octanoate in 85:15 (v/v) isooctane/octylamine, and the reagents were solubilized by vortexing and sonification until a clear solution was obtained. Sufficient enzyme stock solution, 1% (v/v), to give the desired w_0 value (w_0 = 20; w_0 = [water]/[surfactant]) and enzyme concentration (20 μM overall concentration) was added, and the mixture was shaked and vortexed for about 2 min. The reaction was started by adding trioctanoyl glycerol with a microsyringe (typically 100 mM). The reaction mixture was kept at room temperature without stirring, and samples were withdrawn at time intervals and analyzed by FTIR spectroscopy as follows.

As the ester is hydrolyzed, the intensity of the C=O (st) band around 1750 cm^{-1} decreases, while at the same time the intensity of the C=O (st) band of the octanoate around 1560 cm^{-1} increases. In the case of ester aminolysis the intensity of the C=O (st) band around 1750 cm^{-1} again decreases, while the intensities of the amide C=O (st) around 1560 cm^{-1}, the amide N—C=O (st) around 1650 cm^{-1}, and the amide N—H (st) around 3300 and 3350 cm^{-1} all increase.

The sodium octanoate band at 1560 cm^{-1} is superimposed on the amide band of the same wavenumber, making a octanoate concentration determination impossible without deconvolution. To determine the extent of hydrolysis and aminolysis, two concentration versus absorbance calibrations were necessary: one for the ester at 1750 cm^{-1} in the presence of known amounts of trioctanoyl glycerol in the organic solvent, the other for the *N*-octyloctanamide at 1650 cm^{-1} with known amounts of the amide in the reverse micellar solution, taking into account the effect of the reverse micelles on the spectrum of the amide. In order to calculate the intensity of the band at 1650 cm^{-1}, it was assumed that the bending mode of the water (around 1643 cm^{-1}) does not change significantly during the reaction.[10] With these two calibrations giving the decrease in trioctanoyl glycerol and the increase in the amide concentrations as a function of time, the increase in octanoate concentration was calculated using eq 1.

$$[\text{octanoate}]_t = 3\{[\text{trioctanoyl glycerol}]_0 - [\text{trioctanoyl glycerol}]_t\} - [\textit{N}\text{-octyloctanamide}]_t \quad (1)$$

Table I. Oxidation of Octanol in Aqueous and Reverse Micelles

	starting conditions	final conditions
System IIA:	Aqueous Micelles	
[sodium octanoate][a] (M)	1.2	1.68
[1-octanol] (M)	0.48	0
[$NaMnO_4$][b] (M)	0.64	0
[M][c] (mM)	14	20
N_M[d]	86[f]	68[e]
System IIB:	Reverse Micelles[g]	
[sodium octanoate][a] (mM)	49.98	99.4 (81.15)
[1-octanol] (M)	0.95	0.9 (0.92)
w_o[h]	30 (20)	13.7 (10.5)
[$NaMnO_4$][b] (mM)	97.2 (64.8)	0 (0)
[M][c] (mM)	0.132 (0.357)	1.24 (1.73)
N_M[d]	380 (140)	80 (47)
R_w[i] (Å)	43.33 (27.14)	19.85 (15.22)
R_H[j] (Å)	69.03 (42.7)	43.7 (21.7)

[a] As determined by FTIR; uncertainty ±2%. [b] As determined spectrophotometrically by UV absorption. [c] Micelle concentration ([M]) as determined by fluorescence quenching; uncertainty ±8%. [d] Number of octanoate molecules per micelle, as calculated from [M].[6] [e] Taking a cmc of 0.34 M.[27] [f] Assuming that the high concentration of octanol reduces the cmc to zero. [g] In parentheses are the values for the system with starting w_0 20, for the same octanoate and octanol concentration. [h] As determined by Karl-Fischer titration.[9] [i] Radius of the water pool as calculated from [M].[6] [j] Hydrodynamic radius as determined by quasi-elastic light scattering;[28] uncertainty ±20%; $R_H = R_w + 10.4$ Å.

Results and Discussion

System IIA: 1-Octanol Oxidation in Aqueous Micelles. Octanoate was used as a surfactant as it forms micelles in water that can host 1-octanol,[11] which is itself insoluble. Since permanganate ion is localized either in the water pool or, because it is anionic, as a cosurfactant at the micellar interface, oxidation takes place at the latter site. The alcohol is slowly converted into the cognate acid, which is, of course, the surfactant of which the micelle is composed. The resultant growth and replication of the micellar system, as well as the chemical reaction, are illustrated in Figure 1.

Note that the oxidation reaction generates 1 equiv of NaOH which may accelerate the kinetics of the reaction, due to the partial ionization of the alcohol. Furthermore the high ionic strength introduced by the permanganate ion reduces the surface potential and thereby also enhances the oxidation rate.[12]

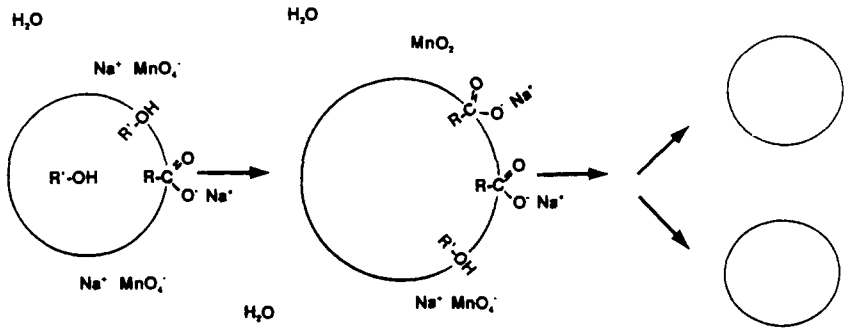
Figure 2 shows the time course of the reaction, as followed by visible absorbance change at 546 nm (alternatively, the concentration of octanoate formed can be followed by FTIR at 1570 cm^{-1}). Also shown is a control experiment in which the oxidation of octanol was carried out in an aqueous suspension containing 0.64 M permanganate. The faster reaction rate observed in the presence of micelles is an example of "micellar catalysis" and can be ascribed to the solubilization of octanol in the hydrophobic domain of the octanoate micelles; i.e., in this case the reaction takes place in a quite different microenvironment.[13]

Under the experimental conditions described, all of the initial 1-octanol was oxidized after 20 h, leading to an increase in the octanoate concentration from 1.20 M to 1.68 M.

In order to measure the corresponding increase in micelle concentration directly, the technique of time-resolved fluorescence quenching was employed. Pyrene was used as a fluorescent probe with tetradecylpyridinium chloride as a quencher, a system that

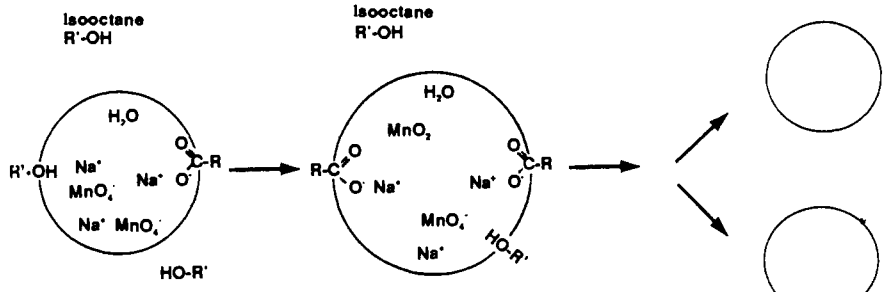
(6) Lang, J.; Jada, A.; Malliaris, A. *J. Phys. Chem.* **1988**, *92*, 1946–1953.
(7) Small, D. M. *Handbook of Lipid Research*; Plenum Press: New York, 1986; Vol. 4, p 251.
(8) Wünsch, G. *Optische Ananlysemethoden zur Bestimmung anorganischer Stoffe*; de Gruyter Sammlung: Göschen, 1976; p 170.
(9) Fischer, K. *Angew. Chem.* **1935**, *48*, 394.
(10) Walde, P.; Luisi, P. L. *Biochemistry* **1989**, *28*, 3353–3360.
(11) Ekwall, P. *Advances in Liquid Crystals*; Brown, H. G., Ed.; Academic Press: New York, 1975; Vol. 1, pp 1–142.
(12) We thank one of the reviewers for mentioning these two points.
(13) In this two-phase system, the reaction most probably takes place at the water–alcohol phase boundary. As shown in Figure 2, the rate in this case depends on the interfacial area.

System IIA: 1-octanol oxidation in aqueous micelles



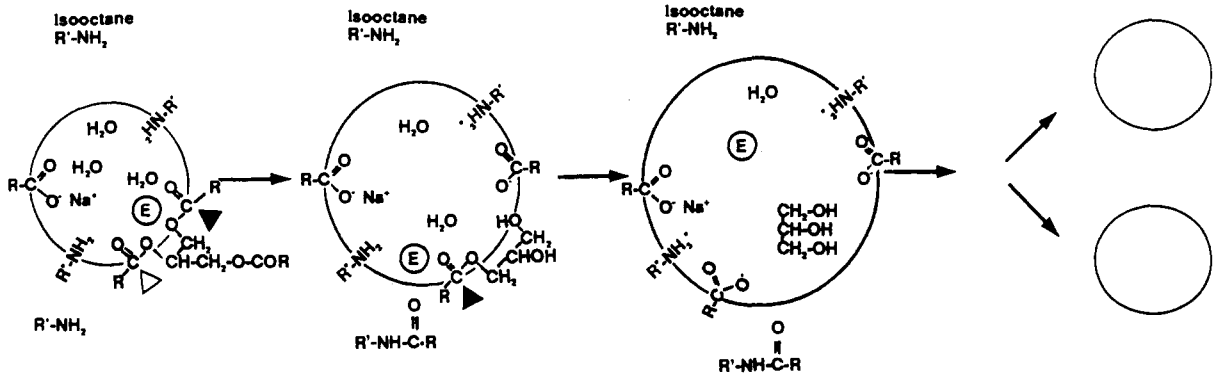
$$3\ CH_3\text{-}(CH_2)_7\text{-}OH + 4\ NaMnO_4 = 3\ CH_3\text{-}(CH_2)_6\text{-}COO^-\ Na^+ + 4MnO_2 + NaOH + 4\ H_2O$$

System IIB: 1-octanol oxidation in reverse micelles



$$3\ CH_3\text{-}(CH_2)_7\text{-}OH + 4\ NaMnO_4 = 3\ CH_3\text{-}(CH_2)_6\text{-}COO^-\ Na^+ + 4MnO_2 + NaOH + 4\ H_2O$$

System III: enzyme-catalyzed ester hydrolysis in reverse micelles



▲ **Hydrolysis:** TOG + 3 H_2O = Glycerol + 3 OA

△ **Aminolysis:** TOG + 3 OM = Glycerol + 3 N-OM

Figure 1. Schematic representation of the micellar reaction systems used: R = $-(CH_2)_6CH_3$, R' = $-(CH_2)_7CH_3$.

has been described previously.[14] Results are reported in Table I. Note that the micelle concentration increases from an initial value of 14 mM to 20 mM after 20 h.

System IIB: 1-Octanol Oxidation in Reverse Micelles. Sodium octanoate forms reverse micelles in isooctane in the presence of a cosurfactant. Reverse micelles with this surfactant in 85:15 (v:v) isooctane/1-octanol were prepared, the alcohol again being both a cosolvent and a cosurfactant.

The reaction utilized was again the oxidation of 1-octanol by permanganate and, as before, oxidation takes place at the micellar interface. In a control experiment, carried out by suspending the same amount of aqueous sodium permanganate in the 1-octanol/isooctane solvent mixture in the absence of sodium octanoate, there was no decrease of permanganate concentration within the time period studied (Figure 3). Therefore, once more, the oxidation of the alcohol is mediated by the micellar interface, and reverse micelles also display micellar catalysis.

Table I shows the results of the self-replication experiments. The micelle concentration was again determined by time-resolved fluorescence quenching using either Ru(bipy) and $K_3Fe(CN)_6$ or PSA and sodium iodide as probe and quencher, depending on the micellar size. The amount of freshly produced surfactant is approximately equal to the initial amount of octanoate, and, correspondingly, the final w_0 value is about 50% less than the initial value.[15] The micelle concentration increases very significantly,

(14) Malliaris, A.; Lang, J.; Zana, R. *J. Chem. Soc., Faraday Trans. 1* **1986**, *82*, 109–118.

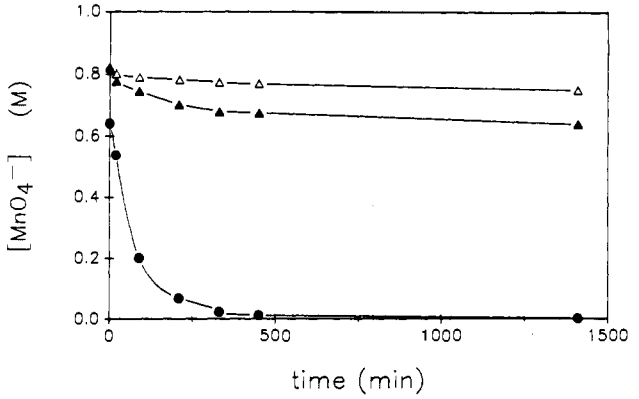


Figure 2. The decrease of permanganate concentration with time for the permanganate oxidation of 1-octanol in sodium octanoate micelles, followed spectrophotometrically assuming ϵ_{546} = 2300 L mol^{-1} cm^{-1} at T = 25 °C. Starting concentrations are (●) 1.2 M sodium octanoate, 0.48 M 1-octanol, 0.64 M sodium permanganate; (△) control reaction, 6.2 cm^2 water–octanol interfacial area; and (▲) control reaction, 12.6 cm^2 water–octanol interfacial area. This interfacial area is given by the diameter of the test tube. In agreement with the literature,[25] it was found that the permanganate concentration decreases with a rate which is first order with respect to permanganate; the overall rate constant, as estimated from half-time determinations at different permanganate concentrations, is 2.2 × 10^{-4} s^{-1}.

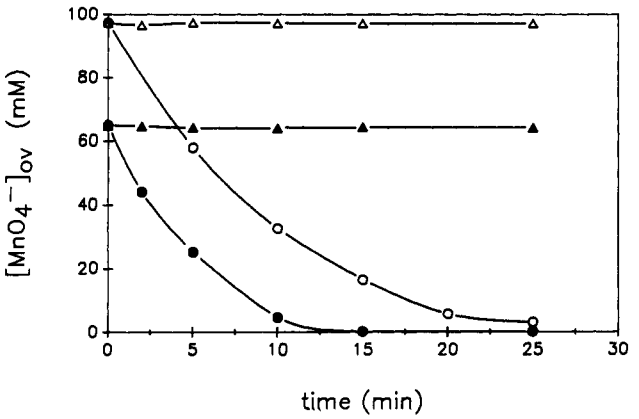


Figure 3. The decrease of permanganate concentration with time for the permanganate oxidation of 1-octanol in sodium octanoate reverse micelles, followed spectrophotometrically, assuming ϵ_{546} = 2300 L mol^{-1} cm^{-1} at T = 25 °C. Starting conditions are (●) 50 mM sodium octanoate, 64.8 mM sodium permanganate, w_0 20, isooctane/1-octanol 85:15 (v/v); (○) 50 mM sodium octanoate, 97.2 mM sodium permanganate, w_0 30, isooctane/1-octanol 85:15 (v/v); (▲) and (△) corresponding control reactions. By analogy to system IIA, the decrease in permanganate concentration is first order, the overall rate constants being 3.08 × 10^{-3} s^{-1} (w_0 20) and 1.85 × 10^{-3} s^{-1} (w_0 30), respectively.

and there is a corresponding decrease in micellar size as measured by quasi-elastic light scattering.

The most significant result in these experiments was the enormous increase in the number of the reverse micelles, which was at first sight surprising, in view of the modest increase in the number of the aqueous micelles in the corresponding experiment (Table I). This is partly determined by the geometrical constraints of the reverse micellar system. In fact, a calculation of the theoretically expected final micelle concentration based on the experimentally determined surfactant concentration at equilibrium[16] gives 1.04 mM for the micelle concentration and a decrease of w_0 from 30 to 15. The somewhat larger experimental value can probably be ascribed to changes in ionic strength, (i.e., the increase in hydroxide concentration during the reaction (see Figure 1), and the corresponding decrease of w_0 produces an increase in the local concentration of salt in the reverse micelles) which in turn induces a decrease in the average size of the micelles.[6] This secondary effect may further accelerate the rate of replication.

The limiting factor in the aqueous system is the concentration of octanol (final concentration 0.48 M), which determines the maximum quantity of octanoate and, hence, new micelles that can be produced. A greater extent of self-replication can be achieved in the reverse micellar system because of the high solubility of octanol in isooctane and smaller initial octanoate concentration (due to the much lower cmc in the organic solvent, compared with water).

Another important difference between the two systems is that, in contrast to the aqueous micellar system IIA, the concentration of octanoate produced in the reverse micellar system indicates that oxidation is incomplete even though all the permanganate is reduced (Table II). In other words, the amount of octanoate produced is lower than expected, as calculated from the equation given in Figure 1. This suggests that competitive oxidation reactions occur which are much more important in reverse micelles than in aqueous solution.[17]

Table II. Enzyme-Catalyzed Ester Hydrolysis in Reverse Micelles

	starting conditions[a]	final conditions		
		H.l. lip.	*C.v.* lip.	base
[octanoate] (mM)	50	156.9	234.5	99.1
w_0[b]	20	5.8	3.4	9.7
g_0[b]	0	0.6	0.4	1
[trioctanoyl glycerol][c] (mM)	100	0	0	0
[N-octyloctanamide][c] (mM)	0	193.1	115.5	250.9
[M][d] (mM)	1.52 (1.61)	1.91	5.86	0.45
N_M[e]	33 (31)	82	40	220
R_w[f] (Å)	16.7 (16.4)	16.9	11.3	27.8
R_H[g] (Å)	43.4 (45.4)	40.7	34.3	56.9

[a] In parentheses are the starting conditions for the system without enzyme (referring to the final conditions of the last column). [b] w_o = [H_2O]/[octanoate]; g_o = [glycerol]/[octanoate]. [c] As determined by FTIR; uncertainty ±2%. [d] Micelle concentration ([M]) as determined by fluorescence quenching; uncertainty ±8%. [e] Number of octanoate molecules per micelle, as calculated from [M].[6] [f] Radius of the water pool as calculated from N_M with simple geometrical considerations: $R_w = \{3N_M(V_{H_2O}w_0 - V_{glycerol}g_0)/4\pi\}^{1/3}$ ($V_{glycerol}$ = 121 Å^3, V_{H_2O} = 29.9 Å^3). [g] Hydrodynamic radius as determined by quasi-elastic light scattering;[28] uncertainty ±20%; $R_H = R_w$ + 10.4 Å.

System III: Enzyme-Catalyzed Ester Hydrolysis in Reverse Micelles. The reaction investigated was the lipase-catalyzed hydrolysis of trioctanoyl glycerol, the surfactant being again octanoate and the solvent 85:15 (v/v) isooctane/octylamine.[18] Octylamine was used in place of octanol as cosolvent and cosurfactant, because it was not possible to perform the reaction in the presence of excess octanol as the enzyme catalyzes the back-synthesis of trioctanoyl glycerol. The presence of octylamine, however, introduces a complication as it reacts with trioctanoyl glycerol, forming *N*-octyloctanamide.

Two different lipases were used: that from *C. viscosum*, which has been used previously for ester hydrolysis in reverse micelles,[19] and that from *H. lanuginosa* because it is known to be stable in the presence of anionic surfactants.[20] As these enzymes are not soluble in the organic phase but only in the water pool and trioctanoyl glycerol is only soluble in the organic phase, hydrolysis

(15) Although the reaction produces 4 equiv of water for 3 equiv of octanoate, this is not enough to keep a constant w_0 30, as 30 equiv water would be required per freshly produced octanoate molecule.

(16) An increase in the surfactant concentration by a factor of n leads theoretically to a increase in the micelle concentration of a factor of n^3 and to a decrease in w_0 of a factor $1/n$, as has been shown previously.[24]

(17) One possible side reaction is the decomposition of permanganate catalyzed by dispersed MnO_2.[25] The high water pool concentration of permanganate (3.6 M) and initially produced MnO_2 favors this reaction. The disappearance of permanganate can therefore be ascribed to a side reaction which is more important in reverse micelles because of local concentration effects. We have also observed (data not shown) that the higher the local permanganate concentration, the lower the percentage yield of octanoate.

(18) Using the method described by Bowcott and Schulman,[26] we have demonstrated that 8% (70 mM) of the total octylamine is localized at the micellar interface.

(19) Flechter, P. D. I.; Robinson, B. H.; Feedman, R. B.; Oldfield, C. *J. Chem. Soc., Faraday Trans. 1* **1985**, *81*, 2667–2679.

(20) Novo product information.

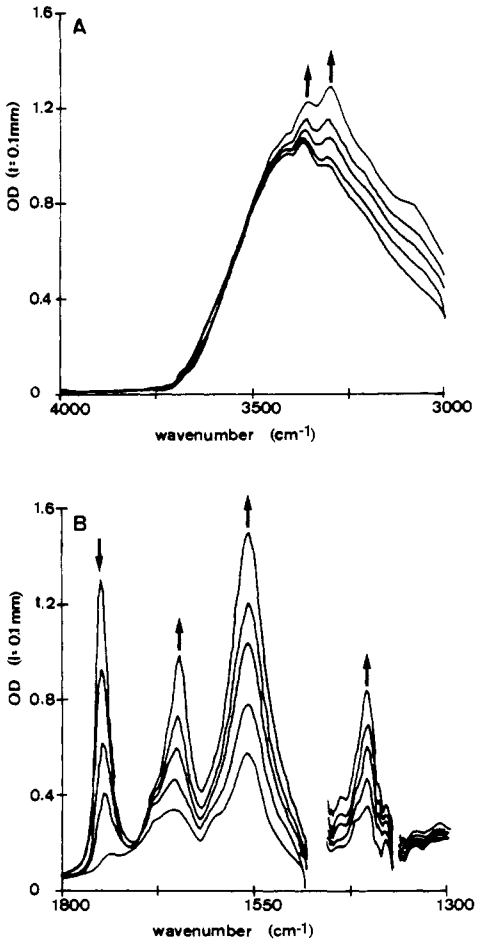


Figure 4. The enzyme-catalyzed hydrolysis of trioctanoyl glycerol in sodium octanoate reverse micelles: the FTIR spectrum, as function of time, between 4000 and 3000 cm^{-1} (A); 1800 and 1300 cm^{-1} (B). Sodium octanoate (50 mM) in isooctane/octylamine 85:15 (v/v), w_0 20, [trioctanoyl glycerol]$_0$ = 100 mM, 20 μg/mL of lipase from *C. viscosum*. Path length = 0.1 mm, CaF_2 cell. Incubation times: 3, 6, 9, 12, and 24 h.

takes place at the micellar interface and trioctanoyl glycerol is distributed between the bulk organic solvent and the micellar interface[21] (see Figure 1).

The first step in the main reaction can be outlined as follows:

trioctanoyl glycerol + $H_2O \rightleftarrows$ 1,2-dioctanoyl glycerol + octanoate (2)

1,2-Dioctanoyl glycerol is also susceptible to enzymatic hydrolysis, and the reaction can proceed further, eventually yielding three molecules of octanoate and one of glycerol, glycerol being preferentially localized in the water pool. The side reaction proceeds according to the following equation:

trioctanoyl glycerol + octylamine $\rightleftarrows$ 1,2-dioctanoyl glycerol + *N*-octyloctanamide (3)

In absence of lipase, both hydrolysis which is base-catalyzed (the base being produced by the reaction of octylamine with water) and aminolysis reactions are observed in the reverse micellar systems.

The time course of the reaction was followed by FTIR spectroscopy, as is shown in Figure 4A,B for a typical experiment.

(21) The infrared spectrum of trioctanoyl glycerol in the carbonyl stretching region between 1800 and 1500 cm^{-1} is affected by the presence of reverse micelles, indicating that at least some of the trioctanoyl glycerol molecules are present at the micellar interface. The carbonyl stretching region between 1800 and 1500 cm^{-1} of the infrared spectrum of *N*-octyloctanamide is also affected by the presence of reverse micelles, indicating surface activity, i.e., activity as a new surfactant.

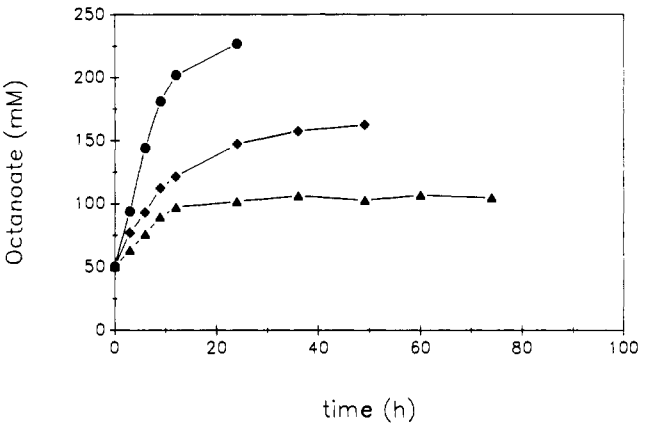


Figure 5. The increase of octanoate concentration for lipase-catalyzed and base-catalyzed hydrolysis reactions in sodium octanoate reverse micelles. Starting concentrations were 50 mM sodium octanoate, 100 mM trioctanoyl glycerol, w_0 20, isooctane/octylamine 85:15 (v/v), 20 μg/mL of lipase from *H. lanuginosa*, 20 μg/mL of lipase from *C. viscosum*: (●) lipase from *C. viscosum*, (◆) lipase from *H. lanuginosa*, (▲) base-catalyzed hydrolysis.

Figure 5 shows the increase of octanoate as function of time for the two lipase-catalyzed reactions and for the base-catalyzed reaction. Note that the use of *C. viscosum* lipase results in a shorter reaction time and in higher quantities of newly produced octanoate (Table II). Note also that the time course of the base-catalyzed hydrolysis in reverse micelles in the absence of the enzyme is much slower, resulting in a lower yield of octanoate.

The changes in micelle concentration, measured by time-resolved fluorescence quenching (using Ru(bipy) and MV as probe and quencher), and the changes in the micellar radius for the enzyme- and base-catalyzed reactions, calculated from both the fluorescence measurement and from the results of quasi-elastic light scattering, are shown in Table II, together with the product distribution. In both cases, there was a 100% consumption of trioctanoyl glycerol. Thus, the greatly increased yields in micelle concentration with the *C. viscosum* lipase were due to kinetic effects; i.e., the competitive reaction was less significant, as is shown by the differences in the concentration of *N*-octyloctanamide at equilibrium. The reaction was also carried out with a 1:1 mixture of the two enzymes (data not shown) with the expected result of an algebraic average of the data found with the single enzymes. In the nonenzymatic reaction there was a significant decrease of the micelle concentration.

Concluding Remarks

We have shown that it is possible to devise micellar systems of various kinds that possess the property of self-replication. Aqueous micelles have the advantage of greater biological relevance (for example, they may have been the first prebiotic structures that were able to self-replicate), although, at the moment, the reverse micelle appears to be the system which can more readily be manipulated to produce a high yield of "daughter" micelles.

In most of the cases illustrated here, the fidelity of replication is not perfect; during replication the reverse micelles become smaller, and in the aqueous system that uses permanganate oxidation, the concentration of octanol is depleted. Enzymatically driven reverse micellar self-replication suffers from a similar problem, as the number of catalyst molecules per micelle becomes smaller during replication. Varela[4] has considered the implications of this problem for the case of self-replication induced by a catalyst which is itself not able to replicate in a computer simulation of an autopoietic system based on a polymerization reaction. In principle, the problem could be avoided by the exogenous addition of the substance that is being depleted during replication (e.g., water, octanol, or enzyme). The corresponding complication of the experimental setup was not pursued in this paper. The present work is more concerned with the basic principle of micellar self-replication and, in particular, with the realization of exper-

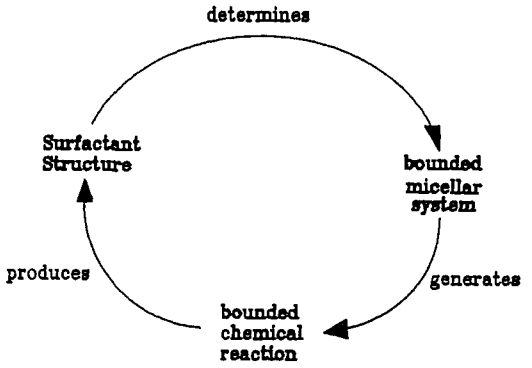


Figure 6. The circularity of "self" in autopoietic self-production, a reworking of a more general figure presented by Fleischaker.[22] Input of reactants (energy) takes place at the level of the bounded chemical reaction.

imental systems which conform to the definition of autopoiesis. The situation which we have been able to reach here is represented schematically in Figure 6, which is based on an illustration by Fleischaker.[22] There is a cyclic relationship between the production of the surfactant and micelle population growth; the micellar structure makes possible the reactions which create the surfactants, which in turn spontaneously assemble into the micelle, which then further supports the chemical reaction, and so on. This cycle seems indeed to simulate the "circularity of the self", which is one of the features of the autopoietic unit.[4,22]

The self-replicating systems, based on template reactions which have been developed by von Kiedrowski[2] and Rebek and collaborators,[3] have the virtue of simulating DNA replication (or analogues thereof), which the present systems do not. On the other hand, as suggested by Ringsdorf,[23] supramolecular polymeric structures, such as micelles and liposomes, allow extensive variation in structural organization and consequently a higher functional complexity. For example, these systems can act as host to guest molecules (including enzymes) situated either inside the micelle, and/or at the micellar interface, and this structural flexibility might prove useful in devising models for cell function, including cellular evolution. Recognizing the possible prebiotic relevance of liposomes, we are currently trying to extend the principles of self-replication to enzyme-containing liposomes.

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(22) Fleischaker, G. R. *Origins Life Evol. Biosphere* **1990**, *20*, 127–137.
(23) Ringsdorf, H.; Schlarb, B.; Venzmer, J. *Angew. Chem.* **1988**, *100*, 117–162.
(24) Luisi, P. L.; Varela, F. *Origins Life Evol. Biosphere* **1989**, *19*, 633–634.
(25) Zhao, L.; Davis, J. G.; Young, V. Y. *Langmuir* **1990**, *6*, 168–172.
(26) Bowcott, J. E.; Schulman, J. H. *Z. Elektrochem.* **1955**, *59*, 283–290.
(27) Fendler, J. H.; Fendler, E. J. *Catalysis in Micellar and Macromolecular Systems*; Academic Press: New York, 1975; p 26.
(28) The data shown in Tables I and II give the initial decay obtained from a second-order cumulant analysis. In all cases the values for the hydrodynamic radius are higher than the those determined by fluorescence quenching although the qualitative trends are in agreement. An analysis of the quasi-elastic light scattering measurements using a Laplace inversion method indicates that the samples are polydisperse, making it impossible to give exact quantitative values. The different weighting of the data obtained by quasi-elastic light scattering with respect to the fluorescence quenching gives different quantitative values in the case of polydisperse samples.