Autopoietic Self-Reproduction of Fatty Acid Vesicles

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Abstract: Conditions are described under which vesicles formed by caprylic acid and oleic acid in water are able to undergo autopoietic self-reproduction—namely an increase of their population number due to a reaction which takes place within the spherical boundary of the vesicles themselves. This is achieved by letting a certain amount of the neat water-insoluble caprylic or oleic anhydride hydrolyze at alkaline pH: the initial increase of the concentration of the released acid/carboxylate is extremely slow (several days to reach the conditions for spontaneous vesicle formation), but afterwards, the presence of vesicles brings about a rapid second phase leading to more and more vesicles being formed in an overall autocatalytic process. The catalytic power of the caprylic acid and oleic acid vesicles toward the hydrolysis of the corresponding anhydride is documented in a set of independent experiments. In these experiments, the hydrolysis was carried out in the presence of vesicles at a pH corresponding approximately to the p*K* of the acid in the vesicles. The process of autopoietic self-reproduction of caprylic acid and oleic acid vesicles is studied as a function of temperature: by increasing temperature (up to 70 °C), the exponential time progress of vesicle formation tends to become steeper while the long initial slow phase is significantly shortened. The caprylic acid and oleic acid vesicles are characterized by electron microscopy and by determining their internal volume. The question whether and to what extent these vesicles form a classic chemical equilibrium system—in which namely the free surfactant is in equilibrium with the aggregates—is also investigated.

Introduction

During the last few years we have focused attention on the autopoietic self-reproduction[1] of micelles,[2−5] seen as the simplest bounded structures (i.e., provided with a physically well-defined boundary). The term autopoietic indicates that the self-reproduction process is due to a reaction which takes place within a spherically closed boundary, a situation which, according to the proponents of the theory of autopoiesis,[6−8] defines a prerequisite structural condition for minimal life.

The biological relevance of this kind of work would be greater if vesicles instead of micelles were used. In fact, vesicles (or liposomes) have the bilayer structure of biological membranes and actually they have been proposed as possible precellular systems.[9,10] It is also interesting to mention that, according to some authors, fatty acids might well have been the material for primordial cell walls.[9,11]

This paper deals with vesicles from fatty acids, in particular from oleic acid and caprylic acid. As shown by Hargreaves and Deamer[12] as well as by other researchers,[13−15] fatty acids are able to form vesicles in a particular pH region. This is the pH region at which the pH equals approximately the p*K* of the acid in the bilayer: in this way, the equimolar protonated form and the ionized form of the acid form a cylindrical dimer structure stabilized by hydrogen bonding which is able to build the vesicle.[14,16]

The aim of this paper is to describe conditions under which fatty acid (caprylic and oleic) vesicles are able to self-reproduce and, preliminary to that, to characterize these vesicles.

Materials and Methods

Reagents. Caprylic acid (>99.5%), sodium caprylate (ca. 99%), sodium oleate (>99%), arsenazo III, *N,N*-bis(2-hydroxyethyl)glycine (Bicine), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), potassium hexacyanoferrate, sodium cholate, and isooctane (for UV spectroscopy) were from Fluka, Switzerland. Caprylic anhydride (ca. 99%), oleic anhydride (ca. 99%), pinacyanol chloride, and tris-(hydroxymethyl)aminomethane (Tris) were from Sigma. 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) was from Avanti Polar Lipids. Sepharose 4B was from Pharmacia, Sweden.

Preparation of Vesicles. Vesicles of caprylic acid or oleic acid were prepared in two different ways, either by titrating with HCl an alkaline micellar solution of the corresponding salt or by dispersing a dry lipid film in buffer solution of defined concentration and pH.

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(1) We use in this paper the nomenclature recently proposed by one of the authors (P.L.L.) at the 1993 NATO workshop on Self-Production of Supramolecular Structures (Maratea, Italy, September 12−16, 1993). According to this proposal, the term *self-replication* is limited to linear structures whose replication is based on the template chemistry of nucleic acids; the term *self-reproduction* is a more general one, valid for all other structures (including micelles vesicles, and liposomes); and the term *autopoietic self-reproduction* is for the particular case in which reactions leading to self-reproduction take place within the spherical boundary of the reproductive unit (e.g.. within the boundary of a micelle or a vesicle).

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Typical preparations will be described in the following for oleic acid vesicles. The procedure for caprylic acid vesicles was essentially the same, except that the concentration and pH had to be adapted accordingly.

(a) Titration with HCl. Sodium oleate (0.24 mmol) was dissolved in 3 mL of 50 mM Tris/HCl buffer (pH 9), resulting in a transparent micellar solution of ca. pH 10.9. The pH was then readjusted with 1 M HCl to pH 9, leading to the formation of a turbid suspension containing spontaneously formed oleic acid vesicles with a total concentration of oleic acid and oleate ([oleic acid/oleate]) of 80 mM.

(b) Lipid film dispersion. Sodium oleate (0.24 mmol) was first dissolved in 3 mL of methanol. This methanolic solution was then added to a 100 mL round-bottom flask, and the methanol was removed under reduced pressure with a rotatory evaporator. The lipid film formed was dried over night at high vacuum and then dispersed under vortexing in buffer by adding 3 mL of 200 mM Tris/HCl (pH 9). The pH of the vesicle suspension was measured and if necessary readjusted to pH 9 by adding HCl.

(c) "Extrusions". In certain cases, vesicle dispersions were repeatedly pressed through two stacked polycarbonate filters with pores of defined pore diameters by using "The Extruder" from Lipex Biomembranes Inc., Vancouver, Canada.[17] A total of 10 extrusions were made for each filter type.

Light Microscopy. Vesicle dispersions were analyzed for the presence of giant vesicles by using an Axioplan light microscope from Zeiss, to which a Sony XC-75CE video camera system with screen and printer were connected.

Electron Microscopy. Electron micrographs of fatty acid vesicles were taken by using the freeze-fracture method as described before.[5,18] The size of the vesicles was determined from the electron micrographs by taking into account nonequatorial fracturing.[19] The mean vesicle size and the size distribution were obtained by analyzing 20 different micrographs of the same vesicle preparation.

Cmc Determinations. Cmc[20] determinations of sodium caprylate and sodium oleate have been carried out at a constant temperature of 25 °C in two different ways, either conductometrically with a PR9501 instrument from Philips or spectrophotometrically with the help of pinacyanol chloride.[21,22] In the latter case, 5 μL of a 1.21 mM solution of pinacyanol chloride in methanol was added to 3 mL of the aqueous fatty acid salt solution and the absorbance was measured at 610 nm with an Uvikon 810 spectrophotometer from Kontron, Switzerland.

Internal Volume Determination. The internal aqueous volume of oleic acid vesicles has been determined as described in the literature,[23] using potassium hexacyanoferrate or arsenazo III as water-soluble dye markers. The principle of the method is briefly outlined. If the vesicles are prepared in the presence of a water-soluble dye molecule, some of these dye molecules will be entrapped in the interior aqueous space of the vesicles. Non-entrapped dye molecules are then separated from the high molecular weight vesicles by size exclusion chromatography (sepharose 4B). The coelution of the water-soluble dye molecules with the vesicle fraction is proof for the presence of an aqueous interior, and the internal aqueous vesicle volume (in microliters per micromole of lipid) can be determined by assuming an equal distribution of the dye molecules between the vesicles interior and the external solution during the preparation.

Determination of the Concentrations of Caprylic Acid/Caprylate and Oleic Acid/Oleate. The total concentration of protonated and deprotonated fatty acid in the vesicle suspensions was determined by FTIR spectroscopy using a Nicolet 5SXC FTIR spectrophotometer and a 0.02 cm CaF_2 cell. The procedure was the following: To 1 mL of the vesicle suspension were added 1 mL of 1 M HCl and 1.5 mL of isooctane. The mixture was vortexed for 2 min and then stored at room temperature for 2 h. The FTIR spectrum of the upper isooctane phase was then recorded, and the concentration of the fatty acid was determined by analyzing the peak intensity at 1715 cm^{-1}, using molar extinction coefficients of 879 M^{-1} cm^{-1} for caprylic acid and 855 M^{-1} cm^{-1} for oleic acid.

Anhydride Hydrolysis Catalyzed by Fatty Acid Vesicles. (a) Caprylic Anhydride. To 10 mL of a suspension of caprylic acid vesicles ([caprylic acid/caprylate] > 120 mM, prepared in 0.5 M HEPES buffer (pH 6.8)) in a flat-bottom test tube of 2.5 cm diameter was added 149 μL of caprylic anhydride (0.5 mmol), and the reaction mixture was kept under slight magnetic stirring at 40 °C. The added anhydride forms a water immiscible oil phase on top of the aqueous solution. From time to time, 50 μL of the aqueous phase was withdrawn, diluted with 1 mL of 1 M HCl, and analyzed by FTIR spectroscopy for caprylic acid/caprylate concentration as described above. In control measurements, caprylate solutions were used at pH 6.8 and at a concentration below the cac.[20]

(b) Oleic Anhydride. To 10 mL of a suspension of oleic acid vesicles (prepared in 0.2 M Bicine buffer (pH 8.5)) in a flat-bottom test tube of 2.5 cm diameter was added 150 μL of oleic anhydride (0.25 mmol) saturated with 8 μL of oleic acid (0.025 mmol),[24] and the reaction mixture was kept under slight magnetic stirring either at 25 or at 40 °C. From time to time, 50 μL of the aqueous phase was withdrawn and analyzed by FTIR spectroscopy for oleic acid/oleate concentration as described above. In control measurements, the hydrolysis was studied by using plain buffer solution.

Solubilization of Oleic Anhydride by Oleic Acid Vesicles. The uptake of oleic anhydride by oleic acid vesicles during the course of the oleic anhydride hydrolysis has been determined by withdrawing from the vesicle phase 50 μL samples. To each sample were added 1 mL of 1 M HCl and 1.5 mL of isooctane, and the mixture was vortexed for 2 min. After equilibration for 2 h at room temperature, the FTIR spectrum of the isooctane phase was measured and the concentration of oleic anhydride was determined at 1825 cm^{-1} using a molar extinction coefficient of 418 M^{-1} cm^{-1}.

Anhydride Hydrolysis Leading to Vesicle Self-Reproduction. (a) Caprylic Anhydride. Caprylic anhydride (0.746 mL, 2.5 mmol) was added onto 10 mL of an aqueous solution containing 265 mM NaOH and 100 mM NaCl. The reaction mixture was kept under slight magnetic stirring at a fixed temperature (oil bath), and the pH of the aqueous phase was monitored continuously with a pH electrode. From time to time, 50 μL of the aqueous phase was withdrawn for the determination of the concentration of caprylic acid/caprylate (see above).

(b) Oleic Anhydride. The experiments with oleic anhydride were carried out in a similar way under the following initial conditions: 0.153 mL of oleic anhydride was added onto 10 mL of 35 mM NaOH, containing 100 mM NaCl.

Results and Discussion

Characterization of the Vesicles. As already shown in the literature, caprylic acid vesicles[5,12] and oleic acid vesicles[12-15,23] form spontaneously when the pH of the water solution is properly adjusted. In this case, a rather broad distribution of sizes is obtained, as shown in the case of oleic acid in the electron micrograph of Figure 1A. Also giant vesicles, with diameters up to a few micrometers, are formed (see Figure 1B): these structures are now under investigation and we will report on them at a later stage. Figures 1C,D shows electron micrographs of vesicles obtained by extrusion through 400 and 100 nm, respectively, polycarbonate filters. In this case, the size and the size distribution are as expected much smaller.

The internal volume of spontaneously formed oleic acid vesicles (80 mM) is in the range of 1–1.6 μL/μmol of lipid, as

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(24) When oleic acid is not added to oleic anhydride at the beginning of the experiment, a slight decrease in oleic acid/oleate concentration (ca. 2.5 mM) is generally observed at the initial stage of the anhydride hydrolysis. This decrease is due to an initial transfer of oleic acid from the vesicles into the anhydride phase. In order to eliminate this effect, 0.025 mmol of oleic acid was initially added to 10 mL of the reaction mixture (this had the effect of saturating the anhydride with the acid).

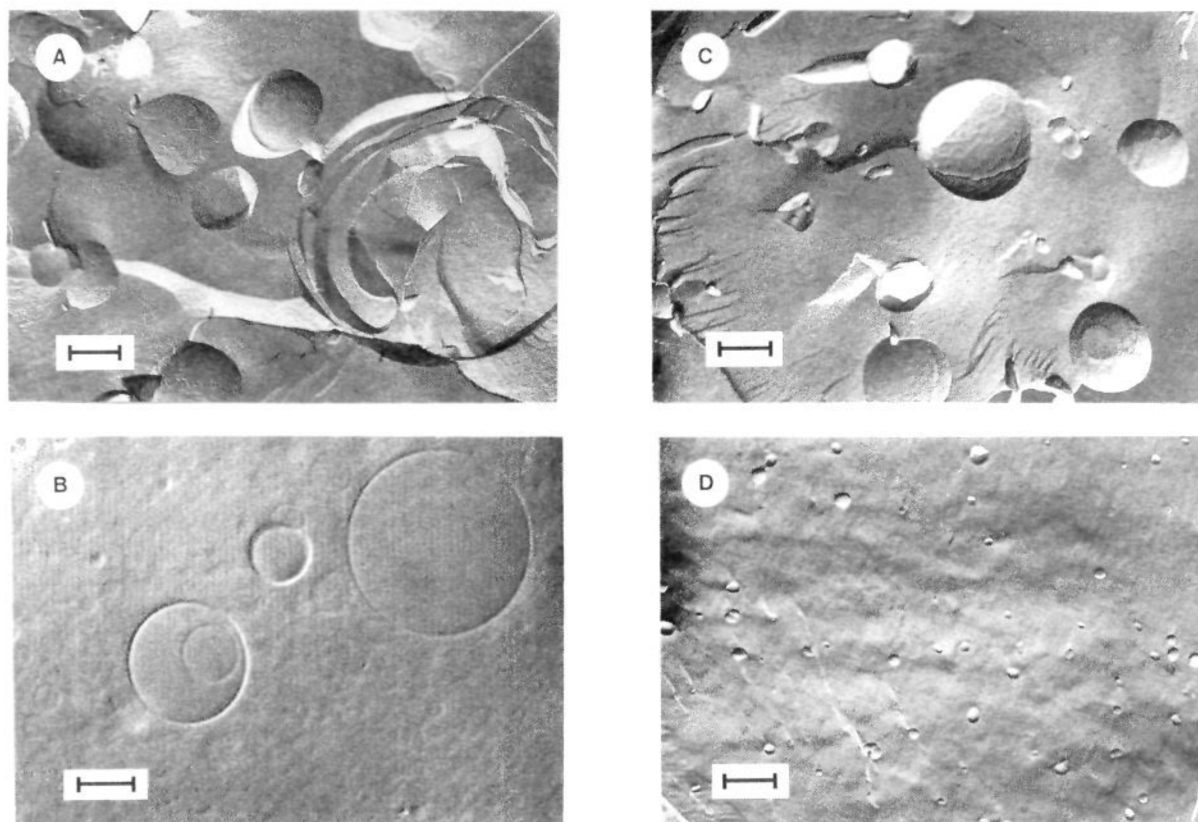

Figure 1. Microscopic analysis of vesicles prepared from oleic acid (20 mM) at pH 8.5 (0.2 M Bicine buffer). Spontaneously formed vesicles, as analyzed by freeze fracture electron microscopy (A) and light microscopy (B). (C,D) Electron micrographs of spontaneously formed vesicles which have been extruded through polycarbonate filters with pore sizes of 400 nm (C) and 100 nm (D). The lengths of the bars in A, C, and D correspond to 200 nm; the length of the bar in B corresponds to 10 μm.

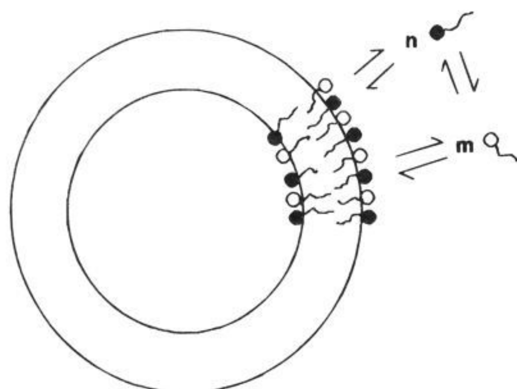

Figure 2. Schematic representation of the equilibrium between the vesicles and free, nonassociated fatty acid molecules. (Molecules with filled head groups symbolize the protonated acid; molecules with empty head groups stand for the deprotonated molecules. n and m stand for different populations of monomeric protonated and deprotonated acids.)

determined by hexacyanoferrate or arsenazo III as the marker dye. This value is about 3 times higher than the internal volume determined for oleic acid vesicles which have been extruded through 100 nm polycarbonate filters.[23] A higher value for the spontaneously formed vesicles confirms—at least qualitatively—the observation that the mean size of spontaneously formed vesicles is larger than that of the extruded vesicles.

Vesicles as Equilibrium Systems. One important question related to the vesicle systems used in the present work concerns the equilibrium between the fatty acid/salt molecules in the vesicles and the free, nonaggregated fatty acid/salt molecules, as represented in Figure 2.

Kinetic investigations[25,26] show that the rate of dissociation of fatty acid/salt molecules from (phospholipid) vesicles containing fatty acids is relatively fast, which leads us to the suggestion that our fatty acid vesicles are chemical equilibrium systems. In order to verify that this is the case, we have carried out simple dilution experiments. By starting with 400 mM caprylic acid/caprylate vesicles (0.1 M HEPES buffer, pH 7) and diluting with 0.1 M HEPES buffer (pH 7), we observe a decrease in turbidity and pH with increasing dilution until, at 120−150 mM, the pH reaches a minimum of 6.2. Further dilution with pH 7 buffer leads to a continuous increase in pH, approaching the value of 7. These pH changes can be understood as a direct consequence of the dissociation of caprylic acid molecules from the vesicles and the consequent increase of acidity of the water solution (due to the lower pK of the fatty acid in bulk water than in the bilayer). At 120−150 mM, all the vesicles disappear.[27] These data can be readily interpreted in terms of the influence of dilution on the equilibrium depicted in Figure 2: dilution shifts the equilibrium toward the monomers, and due to the lower pK of the monomer in comparison with the aggregated acid, the pH decreases.

Further proof that fatty acid vesicles are rapid chemical equilibrium systems stems from the observations made with gel permeation chromatography. After applying spontaneously formed caprylic acid vesicles (400 mM, prepared in 0.1 M HEPES buffer (pH 7)) onto a Sepharose 4B gel filtration

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(27) These values, as expected, are close to one-half of the cmc values for sodium caprylate micelles in water: between 270 mM (as determined conductometrically) and 320 mM (as determined with pinacyanol chloride), in good agreement with values reported in the literature.[21] For sodium oleate, the cmc value for micelles is found to be in the range 0.7 mM (as determined conductometrically) to 1.4 mM (pinacyanol chloride), again in good agreement with already reported values.[15,22]

column, all the vesicles are destroyed during the passage through the column due to the dilution with elution buffer (0.1 M HEPES (pH 7)). As it is well-known, this is not the case, for example, for POPC liposomes and other classic liposomes,[28] which are not destroyed by dilution due to their extremely low cac's.

In the case of oleic acid vesicles, the situation is in principle the same as for caprylic acid vesicles. The only difference lies in the much lower cac values due to the longer hydrophobic chain in the molecule. The cac for oleic acid/oleate at pH 8.5–9 is around 0.4–0.7 mM. This relatively low value allows experiments which involve moderate dilutions, such as gel filtration, to be carried out, and we have used this technique to determine the entrapped volume of oleic acid vesicles (see above).

Chemical Properties and Catalysis. Let us consider now the chemical properties which are directly relevant to self-reproduction experiments. In particular, let us consider the hydrolysis of caprylic anhydride catalyzed by caprylic acid vesicles and the hydrolysis of oleic anhydride catalyzed by oleic acid vesicles. Catalysis operated by micelles is well established,[29] but catalysis by vesicles (liposomes), although a few examples are reported in the literature,[29g,30] is a much less known phenomenon.

Figure 3 shows the hydrolysis of caprylic anhydride with and without preformed caprylic acid vesicles. Since the anhydride is water insoluble, it has been added as a neat oil to the aqueous vesicle suspension and the concentration of the acid product has been measured in the overall water solution by FTIR spectroscopy. The catalytic effect of the aggregates is significant: whereas the initial rate of anhydride hydrolysis at pH 6.8 at caprylate concentrations below the cac is rather low (see Figure 3), it is considerably increased in the presence of vesicles (at a fatty acid concentration above the cac). For example, at an initial concentration of 260 mM caprylic acid/caprylate, about 25 mM anhydride is hydrolyzed within 450 min, yielding additional 50 mM caprylic acid/caprylate.

The catalytic effect of oleic acid vesicles in the hydrolysis of oleic anhydride is similar: the presence of oleic acid vesicles clearly catalyzes the hydrolysis of oleic anhydride, and the higher the vesicle concentration, the greater the effect (Figure 4A).

If one follows the hydrolysis of oleic anhydride not only at the early stage but until completion of the reaction, the following is observed (Figure 4B): If initially no oleic acid vesicles are present, the reaction starts with a low rate until after about 4–5 h (at 40 °C) the rate of hydrolysis increases autocatalytically due to the formation of vesicles (cac < 1 mM). After about 15 h, all anhydride molecules are hydrolyzed. In the presence of vesicles, the initial rate of hydrolysis is larger, leading to a faster conversion of the anhydride molecules: the equilibrium is reached after already 5 h for initially 20 mM oleic acid/oleate vesicles (Figure 4B). If the same reactions are carried out at 25 °C, the reaction again is autocatalytic but the time needed for completion is about 3 times longer (data not shown).

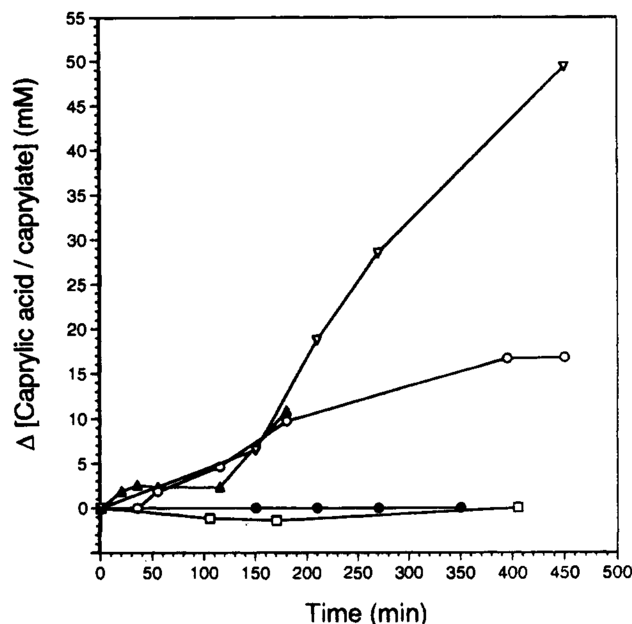

Figure 3. Hydrolysis of caprylic anhydride catalyzed by spontaneously formed caprylic acid vesicles at 40 °C. A vesicle suspension (10 mL in 0.5 M HEPES buffer (pH 6.8)) was overlaid with 0.5 mmol of neat caprylic anhydride. The increase of the concentration of caprylic acid/caprylate is plotted as a function of reaction time. Initial concentration of caprylic acid/caprylate: 0 mM (●), 90 mM (□), 125 mM (○), 180 mM (▲), 260 mM (▽).

Since the anhydride molecules are water insoluble, the hydrolysis reaction takes place either at the interface of the supernatant anhydride phase or at the surface of the vesicles. The amount of oleic anhydride solubilized by oleic acid vesicles during the course of the reaction has been determined by FTIR spectroscopy as described in the experimental part. The results are shown in Figure 4B. A maximum of 7 mM oleic anhydride solubilized by the oleic acid vesicles (initial oleic acid/oleate concentration of 20 mM) is reached after about 3 h.

Autopoietic Self-Reproduction of Vesicles. The oleic acid vesicle catalyzed hydrolysis of oleic anhydride as described above (Figure 4) leads to an increase of the surfactant molecules and as a consequence to a growth of vesicles—both size and number. This can be shown by analyzing electron micrographs at the beginning and at the end of the reaction. Let us consider a typical experiment, as shown in Figure 5, in which 25 mM of neat oleic anhydride is added to a 20 mM oleic acid/oleate suspension of 100 nm extruded vesicles. (In this way, the concentration of the surfactant increases by a factor of 3.5.) The statistical analysis of a large number of electron micrographs shows that the mean diameter of the vesicles increases from 41 nm ($t = 0$) to 52 nm ($t = 48$ h). This slight increase is due to the fact that a small amount of large vesicles appears after the self-reproduction reaction. If the analysis is restricted to the main fractions alone (diameter ≤ 120 nm), then the mean diameter goes from 40 to 43 nm. More generally, the fact that the reaction mixture at the end of the reaction contains increased amounts of smaller as well as of larger vesicles compared to the situation at the beginning of the reaction suggests that during the time course of the reaction two different processes occur, namely growth and multiplication of the vesicles.

The autopoietic self-reproduction of vesicles can be carried out with a different configuration, namely without preformed vesicles. The system is thus simply a biphasic system initially

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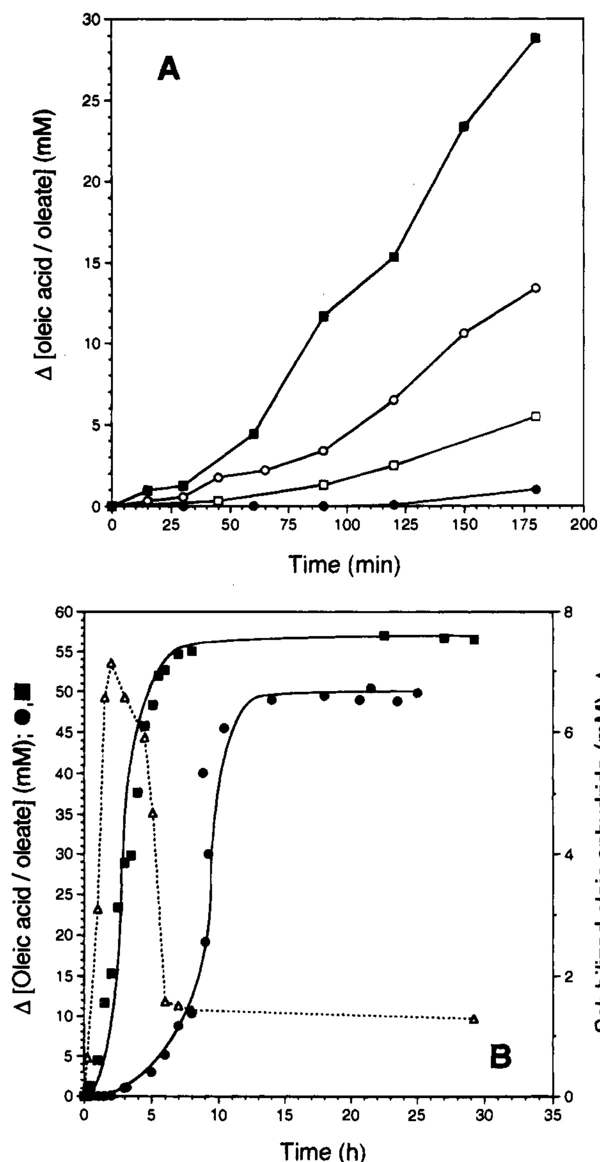

Figure 4. Hydrolysis of oleic anhydride catalyzed by spontaneously formed oleic acid vesicles at 40 °C during the first 3 h (A) and during a long observation time (B). A vesicle suspension (10 mL in 0.2 M Bicine buffer (pH 8.5)) was overlaid with 0.25 mmol of oleic anhydride and 0.025 mmol of oleic acid. The increase of the concentration of oleic acid/oleate is plotted as a function of reaction time. Initial concentration of oleic acid/oleate: 0 mM (●), 5 mM (□), 10 mM (○), 20 mM (■). For an initial oleic acid/oleate concentration of 20 mM, the concentration of oleic anhydride (△) present in the vesicles during the reaction is also plotted (B).

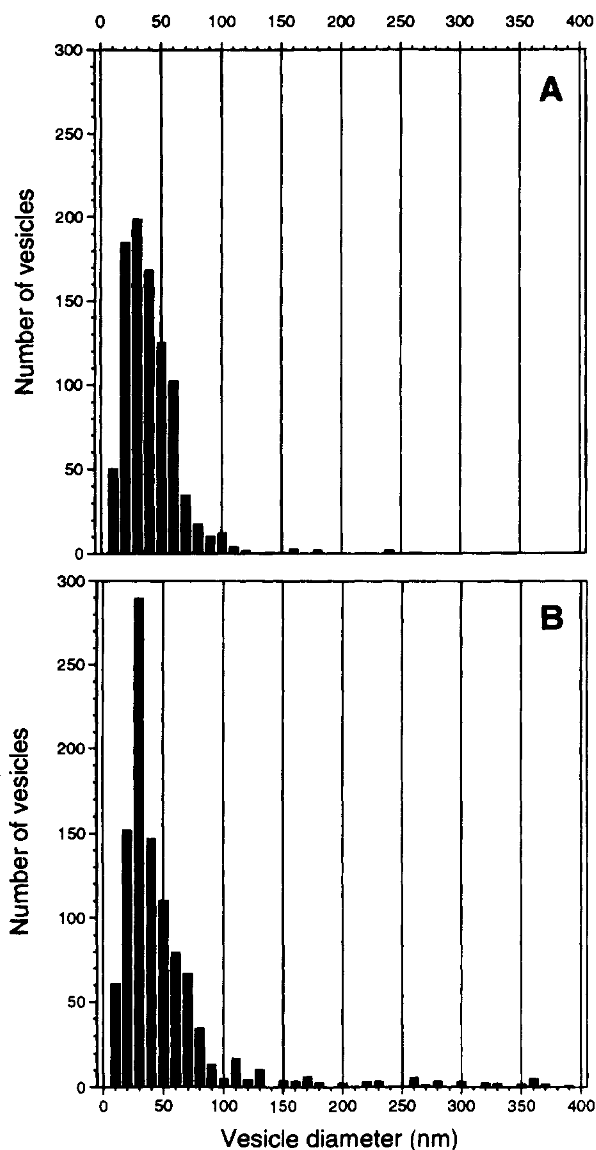

Figure 5. Autopoietic self-reproduction of oleic acid vesicles. 100 nm extruded oleic acid vesicles (20 mM oleic acid/oleate, 0.2 M Bicine (pH 8.5)) catalyzed the hydrolysis of 25 mM oleic anhydride at 25 °C. Analysis of the mean size and size distribution of the vesicles at the beginning (A) and at the end (B) of the reaction.

composed of two immiscible phases, an upper anhydride phase and a lower unbuffered aqueous solution of high pH. The anhydride molecules are hydrolyzed at the interface with the alkaline solution, leading to the release of fatty acids into the aqueous phase, which causes a decrease in pH. The reaction is initially extremely slow, until micelles and/or vesicles spontaneously form. At this point, there is a larger increase of interfacial area and the rate of hydrolysis increases due to the solubilization of the anhydride molecules within the domain of the micelles and/or vesicles. This process ultimately leads to an autocatalytic increase in the concentration of micelles and/or vesicles. As discussed in the previous experiments already, this process is autocatalytic in the sense that the more aggregates are produced, the higher the rate of formation of more aggregates becomes.

Let us now look in more detail at one typical reaction of this type. (Figure 6A). In starting with a biphasic system containing 10 mL of 0.265 M NaOH, 0.1 M NaCl (ca. pH 13), and 746 μL of caprylic anhydride (2.5 mmol) at 40 °C under slight stirring, the anhydride molecules are rather slowly hydrolyzed during the first 15−16 days until 230 mM total caprylic acid/caprylate is present in the aqueous phase. During this time, the pH drops from initially ca. 13 to 9.5−10.5 (Figure 6A). Within the next 24 h, the pH further drops, the aqueous phase becomes turbid, and the rate of anhydride hydrolysis considerably increases in the concentration region of 240 mM (formation of micelles, pH 9.4) and 260 mM (formation of vesicles, ca. pH 7). The fact that micelles are formed as intermediate structures has been proven by using pinacyanol chloride[21,22] (see the experimental part). The transformation of caprylate micelles into vesicles at 260 mM is obvious from the appearance of turbidity as well as from the electron micrographs of the corresponding samples.

The same reaction with caprylic anhydride has been studied at different temperatures by following the change in pH as a function of reaction time. The influence of temperature is very significant: the higher the reaction temperature, the shorter the lag phase (Figure 6B).

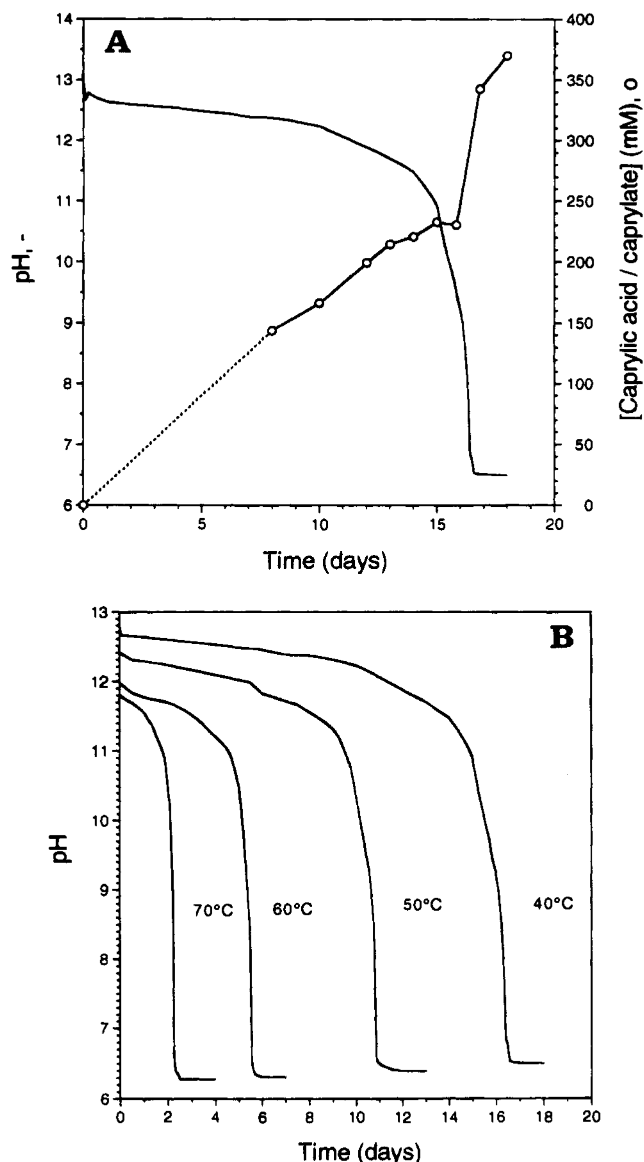

Figure 6. Hydrolysis of caprylic anhydride leading to the formation of self-reproducing caprylic acid vesicles. A mixture of 10 mL of 0.265 M NaOH, 0.1 M NaCl, and 2.5 mmol of caprylic anhydride was incubated at a fixed temperature under slight stirring. (A) Change in pH and caprylic acid/caprylate concentration in the aqueous phase as a function of reaction time at 40 °C. (B) Variation of the reaction temperature.

Also the hydrolysis of oleic anhydride has been studied at different temperatures by using 10 mL of 0.035 M NaOH, 0.1 M NaCl (ca. pH 12), and 153 μL of oleic anhydride (0.25 mmol). In this case, oleic acid vesicles appear in the solution as soon as the pH reaches a value of 9.5.

In all cases, the process is strongly autocatalytic, leading to a quantitative hydrolysis of the anhydride and to an autopoietic self-reproduction of vesicles. Notice again the simplicity of the reaction—hydrolysis of an anhydride. The reaction yields surfactants which self-assemble into autocatalytically active aggregates which are then able to increase their number.

Concluding Remarks

We have shown that vesicles can increase their population number owing to an autocatalytic process taking place within their own spherical boundary. The boundary determines the reaction in two very significant ways: it allows the binding of a water-insoluble substrate, which is thus energetically drawn into the hydrophobic bilayer; and it is able to display efficient catalysis. These observations may have a biological meaning at large, indicating that the compartmentation in biological systems is more than a simple protection shell. Note also the simplicity of the system described here: all one needs to have an autopoietic self-reproductive system is an hydrophobic molecule dispersed in a slightly alkaline water solution. This observation permits one to suggest—as we have done in our previous paper dealing with the self-reproduction of aqueous caprylate micelles[5]—that these kind of systems may have been one of the first self-reproductive shells in prebiotic times.

A series of questions remain open, first of all the very mechanism of vesicle multiplication. Although some electron micrographs suggest the existence of "budding" vesicles, the most likely way of vesicle multiplication is a continuous statistical process and not a discontinuous one, so that the analogy with self-reproduction in cellular systems is probably minor. The determination of this shell reproduction permits however a step forward toward the construction of a minimal cell model. In fact, due to the process described here, we were able to design systems[31,32] characterized by "core-and-shell self-reproduction". In these systems, inside the vesicles, enzymatic replication of RNA takes place simultaneously with the shell reproduction of the oleic acid vesicles.

The main gist of this paper has been the autopoietic self-reproduction, but in order to describe this process, it has been necessary to characterize the vesicular systems. This characterization has permitted some observations which are of general interest for the field of supramolecular chemistry of surfactant aggregates. One is the catalytic effect of the vesicles—a phenomenon which is not well documented in the literature yet. It is not easy to give an interpretation of this effect. One may say that the agent of the hydrolysis (the OH^-) becomes more nucleophilic in the bilayer; however, due to the negative charged carboxylates in the bilayer, one might also expect a negative catalysis such as those obtained in negatively charged micelles.[29a,b,e] Most probably, the catalytic effect is to be ascribed to the huge increase in surface area which follows the formation of aggregates in water. We are now investigating this point with a theoretical approach—preliminary calculations indicate that, when in the experiments of Figure 6 the oil droplet of the water insoluble anhydride is converted into vesicles, the interfacial area increases by more than 4−5 orders of magnitude!

Finally, it is interesting to observe that the caprylic acid and oleic acid vesicles behave as classic chemical equilibrium systems. This brings about many experimental difficulties in handling such systems, particularly when the cac is large (as in the case of caprylic acid/caprylate): by diluting during chromatography, for example, the vesicles may disappear. This suggests the following observation which may be of importance in the field at large: a system characterized by a very small cac—as for example POPC—offers the advantage of a greater "stability"; however, in this case, the permeability to substrate molecules (for example, AMP, ADP, or ATP[32]) is much lower—which is a great disadvantage if one wishes to use vesicles or liposomes as microreactors or cell models.

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