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Biochimica et Biophysica Acta 1559 (2002) 1–9

BIOCHIMICA ET BIOPHYSICA ACTA
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Self-assembled vesicles of monocarboxylic acids and alcohols: conditions for stability and for the encapsulation of biopolymers

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Received 22 June 2001; received in revised form 14 August 2001; accepted 14 August 2001

Abstract

We tested the ability of saturated *n*-monocarboxylic acids ranging from eight to 12 carbons in length to self-assemble into vesicles, and determined the minimal concentrations and chain lengths necessary to form stable bilayer membranes. Under defined conditions of pH and concentrations exceeding 150 mM, an unbranched monocarboxylic acid as short as eight carbons in length (*n*-octanoic acid) assembled into vesicular structures. Nonanoic acid (85 mM) formed stable vesicles at pH 7.0, the pK of the acid in bilayers, and was chosen for further testing. At pH 6 and below, the vesicles were unstable and the acid was present as droplets. At pH ranges of 8 and above clear solutions of micelles formed. However, addition of small amounts of an alcohol (nonanol) markedly stabilized the bilayers, and vesicles were present at significantly lower concentrations ($\sim$20 mM) at pH ranges up to 11. The formation of vesicles near the pK_a of the acids can be explained by the formation of stable $RCOO^-$...HOOCR hydrogen bond networks in the presence of both ionized and neutral acid functions. Similarly, the effects of alcohols at high pH suggests the formation of stable $RCOO^-$...HOR hydrogen bond networks when neutral RCOOH groups are absent. The vesicles provided a selective permeability barrier, as indicated by osmotic activity and ionic dye capture, and could encapsulate macromolecules such as DNA and a protein. When catalase was encapsulated in vesicles of decanoic acid and decanol, the enzyme was protected from degradation by protease, and could act as a catalyst for its substrate, hydrogen peroxide, which readily diffused across the membrane. We conclude that membranous vesicles produced by mixed short chain monocarboxylic acids and alcohols are useful models for testing the limits of stabilizing hydrophobic effects in membranes and for prebiotic membrane formation. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Self-assembly; Lipid vesicle; Encapsulation

1. Introduction

Phospholipids having two hydrocarbon chains are typical components of cell membranes, and the physical properties of such lipids permit self-assembly into fluid, lipid bilayers that represent the permeability barrier defining all cellular life. Gebicki and Hicks [1] and Hargreaves and Deamer [2] reported that single chain amphiphiles such as oleic acid can also form vesicular membranes under defined conditions of pH, ionic composition and concentration. This

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PII: S0005-2736(01)00400-X

property has been exploited as a way to prepare vesicles capable of growth [3] and encapsulation of a functional polymerase enzyme [4].

To test the minimal physical and chemical properties that can produce bilayer membranes in the form of vesicles, we investigated monocarboxylic acids and alcohols with chain lengths just sufficient to stabilize self-assembled structures. In the experiments reported here, we prepared membranous vesicles from such amphiphilic molecules and determined limits of stability with respect to chain length, concentration and pH in pure carboxylic acids and in carboxylic acid–alcohol mixtures. The results were used to estimate the contribution of hydrogen bonds in vesicle stabilization. We also established conditions under which osmotic solute gradients could be maintained and demonstrated that the vesicles could encapsulate functional macromolecules such as DNA and an enzyme. The vesicles can therefore serve as a model for prebiotic membranes.

2. Methods and materials

2.1. Preparation of vesicles

Vesicles of octanoic, nonanoic, and decanoic acid were prepared by adding aliquots of the acid to water to produce a range of concentrations. NaOH (1.0 M) was then added dropwise to the solution, vortexing between additions, until the acid was completely dissolved as micelles, typically near pH 11. HCl (1.0 M) was then added dropwise, vortexing and checking the pH after each addition until the pK_a of the acid was reached. At this point the solution appeared opalescent. If the chain length of the carboxylic acid was eight or more carbons and the solution was above a critical concentration, microscopic examination confirmed the presence of large multilamellar vesicles. This process was monitored by plotting absorbance of the solution at 480 nm as the titration progressed. If the pH was lowered further, droplets formed as the protonated acid became insoluble.

To prepare vesicles composed of carboxylic acid–alcohol mixtures, the acid was added to water and titrated with NaOH as described above to produce a micellar solution. Vesicle formation was then triggered by the addition of a small amount of an alcohol having the same chain length as the fatty acid at an alcohol to acid molar ratio of 1:10. Vesicles formed immediately upon vortexing and the solution was then adjusted to a lower pH appropriate for the experiment.

2.2. Microscopy

Vesicles were observed and photographed with both phase contrast and epifluorescence microscopy at 400× and 1000×. To increase contrast and resolution, the vesicles were stained with 1.0 μM rhodamine 6G.

2.3. Encapsulation of dye and macromolecules

Pyranine (1-hydroxypyrene-3,6,8-trisulfonic acid) is a relatively impermeant anionic fluorescent dye that can be used to estimate the internal volume of vesicles. Decanoic acid vesicles were prepared in the presence of 10 μM pyranine and then passed through a size exclusion column (Sephadex G-50) to separate the vesicles with entrapped dye from the unencapsulated dye in the surrounding solution. The amount of encapsulated pyranine was determined by raising the pH with NaOH to disperse the vesicles, then measuring the absorbance of the dye at 415 nm. Since the original concentration of dye was known, the encapsulated volume could be determined.

To encapsulate macromolecules, a dehydration/rehydration method was used [5] in which vesicles are mixed with macromolecules such as proteins or nucleic acids, then subjected to dehydration. Under these conditions the vesicles fuse and the macromolecules are captured between layers of the lipid. Upon rehydration, the vesicles that form encapsulate approximately half of the macromolecules that were originally present. In our experiments vesicles of decanoic acid (50 mM) were mixed with salmon testis DNA (Sigma) that had been sonicated to produce fragments under a thousand bases in length. The mass ratio of decanoic acid to DNA was 2:1, which was previously shown to be optimal for encapsulation. Aliquots of the mixture were allowed to dry on a microscope slide and then rehydrated by addition of water. After the vesicles had reformed, acridine orange was added to stain the DNA and the solution

was examined with fluorescence and phase contrast microscopy.

2.4. Osmotic activity measurements

Vesicle suspensions were placed in a spectrophotometer cuvette and absorbance was measured at 600 nm. Osmotically active solutes were then added to a concentration of 0.1 M. Osmotic flow of water out of the vesicles caused them to decrease in volume, thereby increasing light scattering, measured here as absorbance, to a maximum. This was followed by a slower decrease in absorbance if the solute crosses the membrane and the gradient is destroyed. The rate at which the vesicles return to their previous size and shape is a function of the bilayer permeability to a given solute [6]. Here we tested the permeability of fatty acid membranes to glycerol, KCl, and sucrose.

2.5. Encapsulation of catalase in fatty acid/alcohol vesicles

Decanoic acid/decanol vesicles were chosen to encapsulate a functional enzyme system. Catalase was chosen as a test enzyme because it is robust and its substrate – hydrogen peroxide – readily crossed the bilayer barriers.

Decanoic acid was added to 5.0 ml of water to a concentration of 40 mM and the pH was raised to 7.5 by the dropwise addition of NaOH, vortexing after each addition. At this pH the decanoic acid was present as micelles, and the catalase was stable. A stock solution of beef liver catalase (Sigma) in water (1 mg/ml) was prepared and 12.5 μl of the stock solution was added to the decanoic acid solution. The subsequent addition of 3.0 μl of decanol with vortex mixing initiated vesicle formation. HCl was then added to adjust the pH to 7.2, an optimum pH for enzyme function and membrane stability. The suspension of catalase and vesicles was equilibrated overnight at 25°C.

In order to eliminate unencapsulated enzyme activity, a 10 μl aliquot of a stock solution of *Streptomyces griseus* protease (Sigma, 10.0 mg/ml in water) was added to the catalase/vesicle solution and incubated for 6 h. One milliliter of the resulting solution was placed in a standard quartz cuvette (1.0 cm pathlength) and 10 μl of a 30% solution of hydrogen peroxide were added. Hydrogen peroxide has a UV absorbance peak at 240 nm, and the decrease in absorption at 240 nm was monitored as a measurement of enzyme activity. In control experiments, catalase was added after vesicles were formed so that the catalase remained outside the vesicles in the surrounding solution. Protease was again added, and after 6 h, the solution was tested for enzymatic activity.

3. Results

3.1. Vesicle formation and stability vary with pH

Carboxylic acids with chain lengths of eight or more carbons formed vesicles in pH ranges near the pK_a of their terminal carboxyl groups. Fig. 1 follows this process by plotting absorbance at 480 nm of a solution of 100 mM nonanoic acid as the pH was lowered by the addition of 1.0 M HCl. At higher pH the fatty acid was present in the form of micelles and did not scatter light. Near the pK_a of

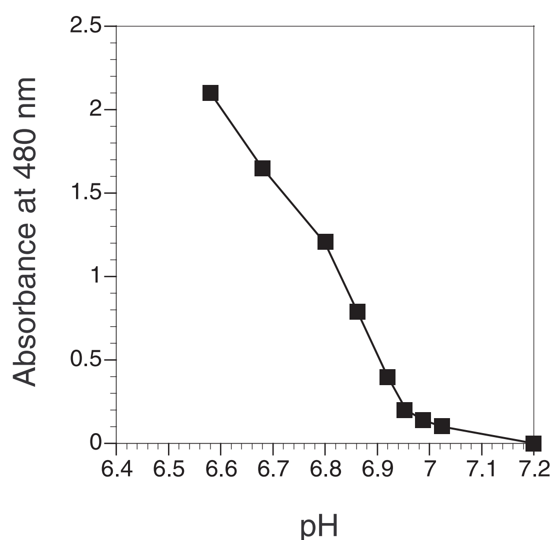


Fig. 1. Transitions of aqueous nonanoic acid dispersions from micellar to vesicle phases. Absorbance (480 nm) of 100 mM nonanoic acid was monitored as pH was varied over the range shown in the figure. The transition of nonanoic acid micelles to the vesicle phase near the pK of the acid (pH 6.8–7.0) is indicated by increased light scattering caused by the presence of vesicles. A second transition to acid droplets begins below pH 6.8 and is accompanied by further increase in light scattering.

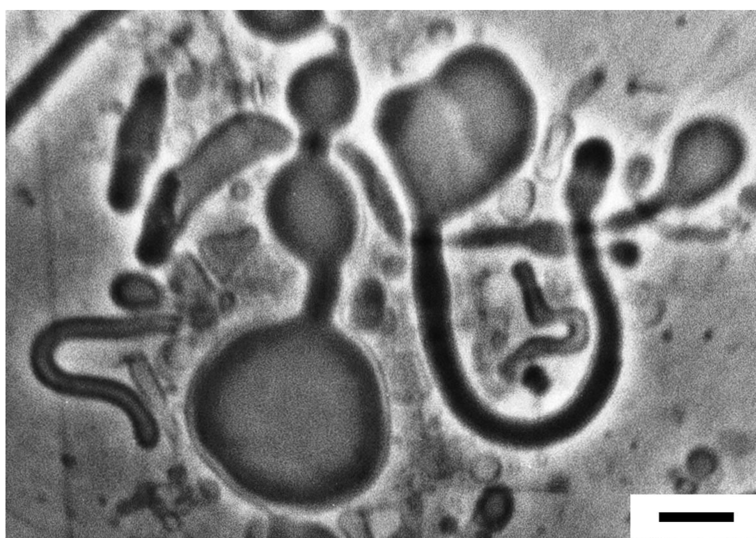


Fig. 2. Phase contrast image of vesicles formed by 100 mM nonanoic acid at pH 7.0. Bar shows 1.0 μm.

the acid (pH 6.9) the absorbance increased markedly, indicating the self-assembly of large multilamellar vesicles which could be visualized by phase and fluorescence microscopy (Fig. 2). Absorbance dramatically increased at pH ranges lower than the pK_a as the vesicles reverted to droplets of protonated acid.

3.2. Minimum chain lengths and concentrations necessary for bilayer vesicle formation

Straight chain fatty acids of eight, nine, and 10 carbons all formed vesicular membranes when dispersed at sufficiently high concentrations under conditions where the fatty acid was within half a pH unit of the pK_a value. No vesicles were observed with carboxylic acids having chain lengths shorter than eight carbons (data not shown). It was previously shown that fatty acids with chain lengths of 12 carbons or longer are present as solid phase crystals at 23°C, the temperature at which our experiments were carried out, but readily form vesicles when warmed to the melting point of the fatty acid [2].

The minimum concentration of monocarboxylic acid necessary to form vesicles in aqueous solution was found to be a function of chain length, with longer hydrocarbon chains permitting vesicle formation at lower concentrations. For example, the minimal concentrations for vesicle formation were 130 mM octanoic acid, 85 mM nonanoic acid and 40 mM decanoic acid. At lower concentrations, as the pH is lowered, the monocarboxylic acid changed directly from micelles to droplets and no vesicles were observed in the transition. Fig. 3 compares the ab-

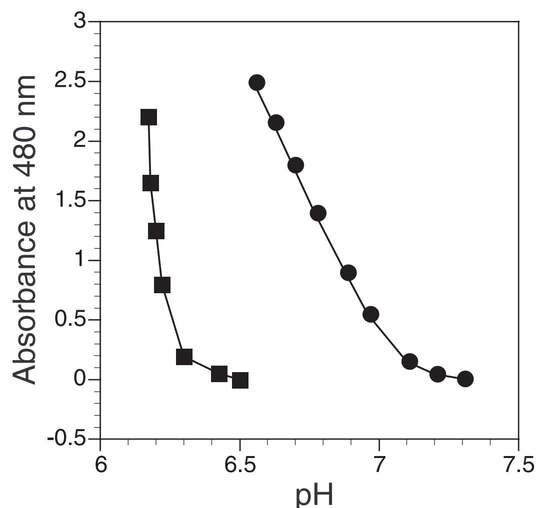


Fig. 3. Comparison of octanoic and nonanoic acid phase transitions with pH. The absorbance of 100 mM nonanoic acid (●) and 100 mM octanoic acid (■) was compared as pH was varied over the range shown. The concentration of 100 mM is below that required by octanoic acid for vesicle formation, and the steep slope of the curve corresponds to the transition from micelles directly to acid droplets. A concentration of 100 mM nonanoic acid is above that required for vesicle formation, and this is reflected in the less steep slope corresponding to the transition from micelles to vesicles to droplets as pH decreases.

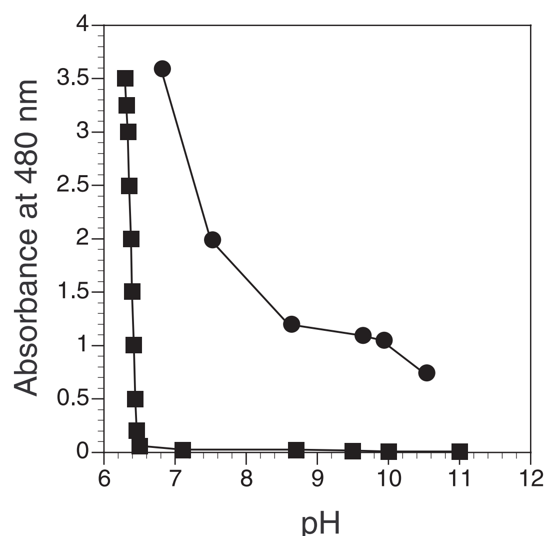


Fig. 4. Effect of alcohol admixtures on vesicle stability. Absorbance of 20 mM nonanoic acid was monitored as pH was varied over the range shown, in the absence (■) and presence (●) of 2.0 mM nonanol. The steep slope of the nonanoic acid solution corresponds to a transition directly from micelles to droplets. Nonanol reduces the nonanoic acid concentration required for vesicle formation to 20 mM, and stabilizes the vesicles over a range of 3 pH units.

sorbance of octanoic acid at 100 mM (below the critical vesicle concentration) with nonanoic acid at 100 mM (above the minimum concentration needed to form vesicles) as the pH is varied. The steepness of the octanoic acid curve is due to the transition directly from micelles to acid droplets, so that the vesicle phase never appears.

3.3. Size and morphology of observed vesicles

Vesicles of carboxylic acid with or without alcohol are large multilamellar vesicles ranging in size up to several microns in diameter. There is little correlation between composition and morphology visualized by microscopic observation. Vesicles may appear spherical or tubular due to deformation by small currents in the solution under the coverslip. Vesicles that have attached to the slide or coverslip are particularly vulnerable to deformation and assume a characteristic tubular form. At a pH significantly under the pK_a of the acid in vesicles, droplets are easily distinguished from vesicles by their appearance in phase micrographs.

3.4. Effect of alcohol on vesicle stability

The addition of alcohols of the same chain length as the monocarboxylic acid forming the bilayer membrane had a dramatic effect on vesicle stability, increasing the pH range of vesicle formation and markedly decreasing the concentration of fatty acid required. For example, an aqueous solution of nonanoic acid requires a concentration of at least 85 mM to form vesicles, but requires only 20 mM concentration of acid in the presence of 2 mM nonanol. Furthermore, the mixed phase vesicles are stable up to pH 11, well above those composed of the pure fatty acid. Fig. 4 compares the titration curves (absorbance at 480 nm) of a solution of 20 mM nonanoic acid with that of 20 mM nonanoic acid with 2 mM nonanol. The typical profile characteristic of carboxylic acid titration below the critical vesicle concentration is shown on the left. However, in the presence of the alcohol (right hand curve) a plateau of stable vesicle absorbance extends to above pH 10.

3.5. Encapsulated volume and osmotic permeability

Formation of vesicles in the presence of pyranine dye resulted in the capture of dye molecules within the vesicles. After size exclusion chromatography to separate the vesicles from unencapsulated dye, the

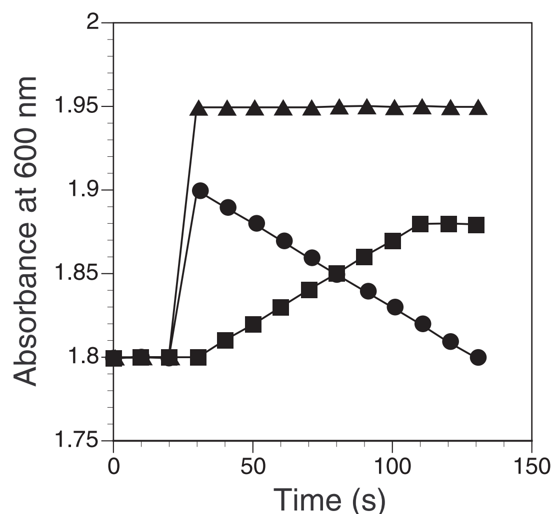
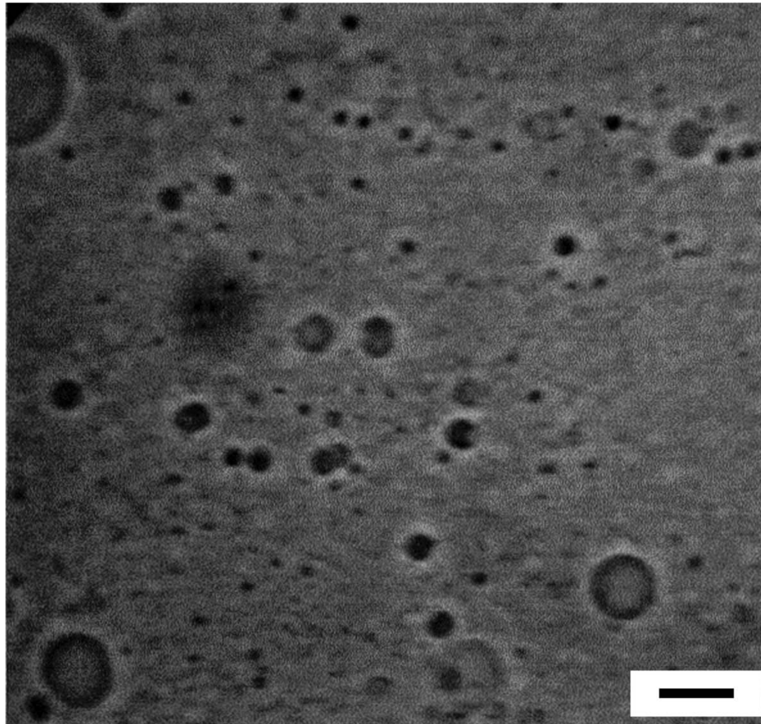


Fig. 5. Comparison of absorbance at 600 nm over time with the addition of three different solutes to a solution of nonanoic acid (120 mM). ●, permeable glycerol; ▲, impermeable KCl; ■, sucrose.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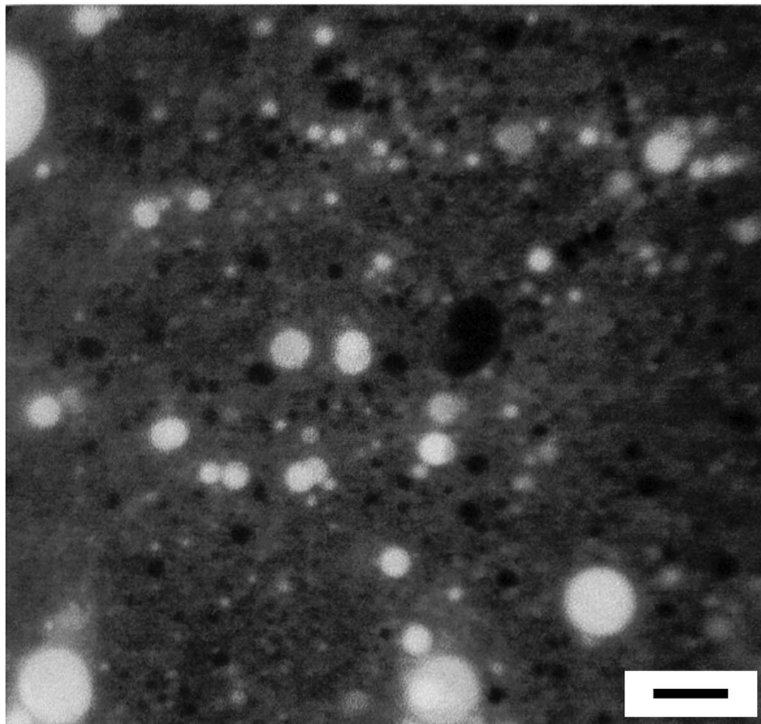
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vesicles containing dye were lysed and the amount of dye released was determined by absorbance spectroscopy to be in the range of 1–5% of the original total volume of the solution, depending on the composition of the mixture and the concentrations used.

Fig. 5 shows the results of permeability experiments in which osmotically active solutes (glycerol, sucrose and KCl) were added and absorbance changes measured over time, as described in Section 2. After addition of each solute, the vesicles responded by loss of water down an osmotic gradient. The decreased volume of the vesicles was reflected by increased light scattering measured as absorbance. In the case of glycerol addition, this was followed by a decrease in light scattering over a period of several minutes, indicating that glycerol permeated the membrane barrier of the vesicles. Sucrose and KCl were expected to be much less permeable, and in both cases only osmotic volume decrease was observed within the time course of the experiment. We conclude that the fatty acid vesicles respond osmotically to solutes, and that glycerol permeates at rates expected from previous research on phospholipid bilayers, while sucrose and KCl are relatively impermeant.

3.6. Encapsulation of macromolecules

Fig. 6A demonstrates that a macromolecule such as DNA can be encapsulated in decanoic acid vesicles by the dehydration/rehydration method. Following the encapsulation protocol described in Section 2, the resulting vesicles were visualized by phase and fluorescence microscopy. Vesicles with entrapped DNA appeared to be darker by phase contrast due to the difference in the index of refraction. Fig. 6B shows the same vesicles by epifluorescence microscopy. The encapsulated DNA showed strong fluorescence due to acridine orange staining, and the bright vesicles correspond to the phase-dark vesicles in Fig. 6A.

We were encouraged by this result to attempt encapsulation of a functional enzyme. Catalase was chosen due to the expected high permeability of bilayer membranes to its substrate, hydrogen peroxide, as illustrated in Fig. 7. Decanoic acid/decanol vesicles were selected to encapsulate the catalase because nonanoic and octanoic acid require a higher lipid concentration of lipid to form vesicles than does decanoic acid. The addition of decanol also dramatically lowered the concentration of lipid required, thereby reducing light scattering which would otherwise interfere with enzyme activity measurement.

Vesicles of decanoic acid/decanol were able to protect encapsulated catalase from degradation by protease, indicating that protease was unable to diffuse across the barrier. This permitted us to inhibit the activity of unencapsulated catalase by protease digestion. If catalase was added after vesicle formation the addition of protease was found to completely inhibit the enzyme activity after 6 h, while the encapsulated enzyme remained fully functional. Fig. 8 shows the degradation of hydrogen peroxide to oxygen and water observed as a decrease in absorbance over time. We conclude that fatty acid vesicles of decanoic acid/decanol are able to encapsulate an enzyme and protect it from degradation by a protease.

4. Discussion

The results reported here show that *n*-monocarboxylic acids ranging from eight to 10 carbons in length self-assemble into vesicles under defined conditions of pH and sufficiently high concentrations. The minimal chain length capable of forming vesicles is eight carbons in length (*n*-octanoic acid) at 150 mM, pH 6.9, the pK_a of the acid under these conditions. Nonanoic acid (>85 mM) formed stable vesicles at pH 7.0, and addition of small amounts of nonanol markedly stabilized the bilayers, so vesicles were present at significantly lower concentrations (~ 20 mM) at pH values up to 11. The vesicles provided a selective permeability barrier, as indicated by osmotic activity and ionic dye capture, and could

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Fig. 6. Encapsulation of DNA by fatty acid vesicles. (A) Phase contrast image of the vesicles with DNA, which appear dark due to the higher refractive index of the captured DNA. (B) The same field in which acridine orange fluorescence was excited by blue light of 400–450 nm wavelength. These phase dark vesicles are now fluorescent, confirming their DNA content. Scale bar = 1 μm.

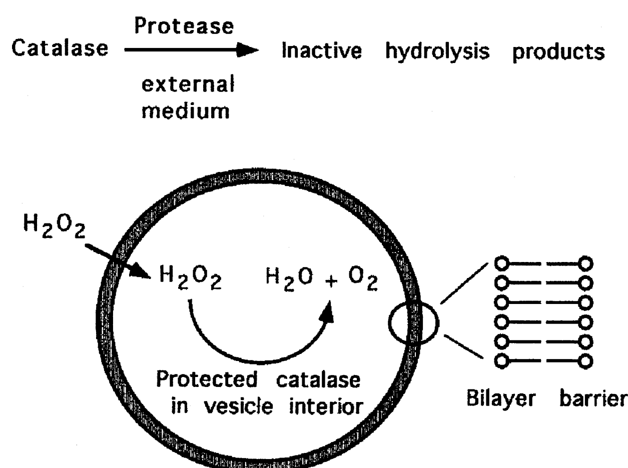
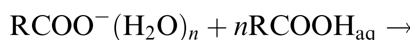


Fig. 7. Experimental scheme for encapsulating functional catalase. Following encapsulation, the subsequent addition of protease degrades the unencapsulated enzyme. Hydrogen peroxide, the substrate for catalase, readily diffuses across the membrane into the vesicle and is acted upon by the encapsulated enzyme.

encapsulate macromolecules such as DNA and catalase. In the case of catalase, the encapsulated enzyme was protected from degradation by protease, and retained enzymatic function. We conclude that membranous vesicles produced by mixed short chain monocarboxylic acids and alcohols are useful models for testing the limits of stabilizing hydrophobic effects in membranes.

4.1. *Role of hydrogen bonding in stabilizing bilayer vesicles*

An interesting observation in an earlier paper [2] and in the present work is that vesicles are produced in the pH range near the acid pK_a values. In this range the acid molecules are present as ionic $RCOO^-$ and the neutral form RCOOH. In this combination, each ionized $RCOO^-$ group can be stabilized by strong hydrogen bonds to the neutral molecules forming $RCOO^-...(RCOOH)_n$ hydrogen bonded aggregates. The stability of these bonds were measured recently in model gas phase $CH_3COO^-(CH_3COOH)_n(H_2O)_m$ clusters [7]. The results showed that the formation of the $RCOO^-...HCOOH$ hydrogen bond is greatly stabilizing. In fact, in the gas phase clusters, the strength of this bond is 29.3 kcal/mol, and the total stability of the hydrogen bonded aggregate $RCOO^-(RCOOH)_6$ is extrapolated from the cluster data as 85 kcal/mol. In comparison, the strength of the hydrogen bond to a water molecule $RCOO^-...H_2O$ is much weaker, 15.9 kcal/mol, and the stability of the hydrated ion assembly $RCOO^-(H_2O)_n$ is also much weaker, 72 kcal/mol. In general, the formation of the $RCOO^-(RCOOH)_n$ aggregates with solvent displacement in reaction 1 is thermodynamically favorable.



$$RCOO^-(H_2O)_n + nRCOOH_{aq} \rightarrow RCOO^-(RCOOH)_n + nH_2O_{aq} \quad (1)$$

In the gas phase reaction 1 is exothermic for all measured n (1–6), and with $n = 6$ the reaction is exothermic by 13 kcal/mol. This stabilization of the $RCOO^-$ anion would decrease the pK_a for acid dissociation by 9.5 pK units. In the vesicles, the stabilizing effect will be decreased by solvation and unoptimized hydrogen bonds although the latter can be overcome by solvent bridged geometries.

A similar effect can also explain the stabilization by alcohols. The assemblies $RCOO^-(ROH)_n$ are also more stable than the hydrated analogues $RCOO^-(H_2O)_n$, although the stabilizing effects are smaller than with the neutral RCOOH molecules. However, in pure acids the vesicles disappear at

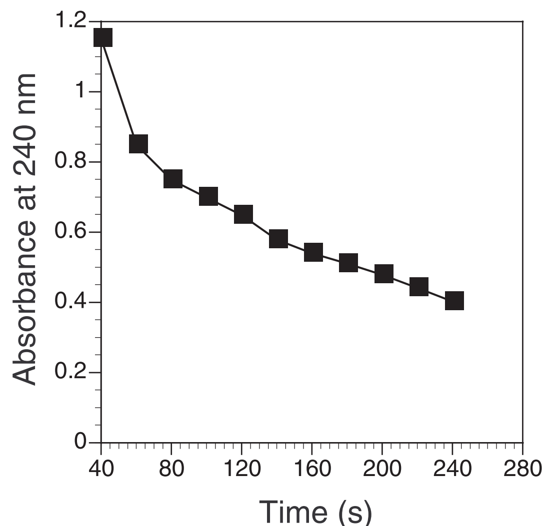


Fig. 8. Activity of encapsulated catalase. The decrease of hydrogen peroxide absorbance at 240 nm was monitored, and the encapsulated catalase was clearly able to perform its catalytic function of degrading the peroxide to water and oxygen. In the control experiment, in which the catalase was added after vesicles had formed, the addition of protease eliminated all catalytic activity.

high pH values when neutral acid molecules are not available to stabilize the aggregates, while the neutral ROH molecules remain available to stabilize the aggregates. This accounts for the observation in Fig. 4 showing stable vesicles up to high pH values. The stabilization of $RCOO^-$ by ROH molecules also explains why less acid is required to form vesicles, in that less RCOOH is necessary when the assemblies are stabilized by ROH molecules.

The data suggest that the vesicles contain stabilized $RCOO^-(RCOOH)_n$ or, with alcohols, $RCOO^-(ROH)_n$ aggregates. The thermochemistry of cluster models shows that carboxylic acids form aggregates with solvent displacement much more readily than other functional molecules because of the combination of several factors: (1) the ready ionization of the carboxylic groups, (2) their relatively weak bonds to water molecules, and (3) their strong bonds to neutral carboxylic acids and alcohol molecules. Of course, the system is more complex and includes hydrophobic bonds between the hydrocarbon chains and other solvation effects. However, the formation of these stabilized hydrogen bonded aggregates at the hydrophilic head groups of the bilayers explain the main features of vesicle formation by carboxylic acids in the pK_a range, and the stabilizing effects of alcohol molecules. The relative contributions of the hydrophilic vs. hydrogen bonding effects could be examined using shorter chain alcohols, where only the hydrogen bonding effects will contribute.

4.2. Significance of vesicle formation in prebiotic evolution

The minimal requirements for membrane formation are also important for evolutionary processes leading to the origin of cellular life on the early Earth [8,9]. Bilayer vesicles were observed to form from organics in the Murchison carbonaceous chondrite [10,11], presumably from carboxylic acids which are relatively abundant in the meteorite. It seems plausible that early membranes could form from such simple components and provide bilayer barrier functions for the earliest forms of cellular life. In this regard, it is significant that both acids and alcohols can be synthesized by Fischer–Tropsch reactions under simulated geothermal conditions [12], and are present in meteorites and in irradiated interstellar ices [13]. These components were imported to the early Earth by interstellar dust particles and meteorites [14], and they can be extracted under aqueous planetary conditions [15]. The present results show that under aqueous conditions in the parent asteroids or on the early Earth, such compounds would be able to form membranes and encapsulate biopolymers, thereby contributing to biogenic processes. It is of interest whether other simple molecules such as the polycyclic aromatic compounds that are abundant in prebiotic environments, can also stabilize the carboxylic acid membranes. These studies are now in progress.

Acknowledgements

The authors thank Sam Ridino for technical help. This research was supported by NASA grants NAG5-4665 and SC-00-35. Partial funding by the Marsden Fund grant LIU901 for one of the authors (M.N.M.) is also acknowledged.

References

[1] J.M. Gebicki, M. Hicks, Nature 243 (1973) 232–234.
[2] W.R. Hargreaves, D.W. Deamer, Biochemistry 17 (1978) 3759–3768.
[3] P.A. Bachman, P.L. Luisi, J. Lang, Nature 357 (1992) 57–59.
[4] P. Walde, A. Goto, P.-A. Monnard, M. Wessicken, P.L. Luisi, J. Am. Chem. Soc. 116 (1994) 7541–7547.
[5] R. Shew, D.W. Deamer, Biochim. Biophys. Acta 816 (1985) 1–8.
[6] B.E. Cohen, A.D. Bangham, Nature 236 (1972) 173–174.
[7] M. Meot-Ner (Mautner), D.E. Elmore, S. Scheiner, J. Am. Chem. Soc. 121 (1999) 7625–7635.
[8] D.W. Deamer, J. Oró, BioSystems 12 (1980) 167–175.
[9] D. Segré, D.W. Deamer, D. Lancet, Orig. Life Evol. Biosphere 31 (2001) 119–145.
[10] D.W. Deamer, Nature 317 (1985) 792–794.
[11] D.W. Deamer, R.M. Pashley, Orig. Life Evol. Biosphere 19 (1989) 21–33.
[12] T.M. McCollom, G. Ritter, B.R.T. Simoneit, Orig. Life Evol. Biosphere 29 (1999) 153–166.
[13] J.P. Dworkin, D.W. Deamer, S.A. Sandford, L.J. Allamandola, Proc. Natl. Acad. Sci. USA 98 (2001) 815–819.
[14] C. Chyba, C. Sagan, Nature 355 (1992) 125.
[15] M. Mautner, R. Leonard, D.W. Deamer, Planet. Space Sci. 43 (1995) 139–147.