

Mineral Surface Directed Membrane Assembly

Martin M. Hanczyc · Sheref S. Mansy · Jack W. Szostak

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Abstract The transition from non-living to living matter may have resulted from the self-organizing properties of organic molecules and their interactions with a chemically rich inorganic environment. We have shown that a solution containing RNA, fatty acids and clay produces structures that contain a potentially catalytic surface (clay) and a potential informational biopolymer (RNA) encapsulated within a membrane. This highlights the ability of mineral surfaces to bring together and organize key components of primordial life. We have extended our analysis of mineral-mediated vesicle catalysis to include other natural minerals and synthetic surfaces of varying shape, size, and charge density. Our results show that while RNA polymerization on minerals may be restricted to the surface environment provided by montmorillonite, vesicle formation is enhanced in the presence of disparate types of surfaces. A model is presented in which new sheets of amphiphiles form just proximal to a surface. Similar interactions between amphiphiles and minerals on early Earth may have resulted in the encapsulation of a diverse array of mineral particulates with catalytic properties.

Keywords amphiphile · fatty acid · micelle · mineral · montmorillonite · pyrite · RNA · vesicle

M. M. Hanczyc · S. S. Mansy · J. W. Szostak
Howard Hughes Medical Institute and Department of Molecular Biology,
Massachusetts General Hospital, Boston, MA, USA

J. W. Szostak (✉)
Department of Molecular Biology, and Center for Computational and Integrative Biology 7215,
Simches Research Center, Massachusetts General Hospital, 185 Cambridge Street,
Boston, MA 02114, USA
e-mail: szostak@molbio.mgh.harvard.edu

Present Address:
M. M. Hanczyc
ProtoLife Srl and the European Center for Living Technology, Venice, Italy

Introduction

Cellular life must have started with a much simpler organization than that found in even the simplest extant organisms. Such primitive life would have had to rely upon the self-organizing properties of its components and the input of energy and material from the environment to execute very basic cellular processes. We reported previously that physico-chemical forces acting on simple membranes can drive a cell-like growth and division cycle (Hanczyc and Fujikawa et al., 2003). In addition we showed that the environment can play a critical role in organizing proto-biological materials in a way that could have led to the first cell. Specifically, we demonstrated that montmorillonite clay assists in the assembly of bilayer membrane vesicles from fatty acids. This ability of clay to influence the formation of supramolecular structures led to the hypothesis that clay could have been involved not only in the polymerization of nucleic acid polymers (Ferris and Ertem, 1993; Ferris and Hill et al., 1996; Ertem, 2004), but also in the formation of the first prebiotic compartments containing clay (a catalytic surface) and RNA (a potential genetic polymer).

Simple, single-chain fatty acids have long been known to self-assemble into supramolecular structures such as micelles and vesicles (Gebicki and Hicks, 1973; Gebicki and Hicks, 1976). Fatty acids in a bilayer membrane are in rapid exchange with the aqueous environment (Walde and Wick et al., 1994). Such amphiphiles can also interact with solid surfaces. The interaction of amphiphiles with solid surfaces often involves adsorption due to chemical or physico-chemical forces through covalent bonds, hydrogen bonds, ion exchange, van der Waals forces, and hydrophobic effects (Giles, 1982). Previously we have shown that fatty acids can adsorb to mineral surfaces and that certain mineral surfaces can promote vesicle formation (Hanczyc and Fujikawa et al., 2003).

In this paper we explore the mechanism of mineral-mediated vesicle formation and provide a larger picture of the parameters necessary to see this effect. In particular we test a diverse set of mineral surfaces to determine how variability in particle size, shape, composition, charge and surface area affects vesicle assembly. We also test the possibility that soluble material leached from the mineral particles accounts for the vesicle assembly effect, and examine possible effects of mineral particles on the phase transition from micellar to vesicular structures. Finally we investigate whether the site of vesicle assembly is on or proximal to the mineral surface and propose a model for mineral-mediated vesicle assembly.

Materials and Methods

Solution preparation

Since the rate of vesicle formation from micelles may be affected by the presence of fine particles, all solutions were filtered through 0.02-micron syringe filters (Whatman, Florham Park, NJ) then purged with argon gas.

Micelle preparation

Neat oil of myristoleic acid, palmitoleic acid, or oleic acid (Nu-Chek Prep, Inc.) was added to dilute NaOH and mixed by vortex. This resulted in a solution of 80 mM amphiphile in micelle form at $\text{pH} > 10$. This solution was agitated (Barnstead Labquake shaker) under argon overnight before use.

Mineral preparation

Aluminum silicate (angular particles of diameter 0.2–6.0 μm), alumina (angular particles of diameter 0.6–6.0 μm), glass microspheres (1–40 μm), and calcium carbonate (elongated spheres of diameter 0.1–8.0 μm) were obtained from Duke Scientific (Palo Alto, CA); montmorillonite K10 and white quartz (>230 mesh) from Fluka (Switzerland); lead oxide (1–2 μm particles), talc powder (<10 μm particles), diamond powder (synthetic monocrystalline particles of 1 μm diameter) from Aldrich (Milwaukee, WI); expanded vermiculite D4 and expanded perlite 000 from Whittemore Co. (Lawrence, MA); sodium silicate solution, polytetrafluoroethylene (Teflon particles of 0.22 μm) and, gold powder (spheres of 1.3–3 μm diameter) from Sigma–Aldrich (St. Louis, MO); homo-ionic sodium-montmorillonite (gift from R. Liu), kaolin monohydrate (Acros; New Jersey), Zeeospheres W-210 and G-200 (synthetic hollow silica–alumina ceramic spheres of 1–40 μm in diameter; 3 M Corporation, St. Paul, MN), hydroxyapatite (Bio-Rad; Hercules, CA), anthracite (sample from the mines of Duryea, PA), hydrotalcite Syntal HAS 696 (Sud-Chemie; Germany), hydrotalcite Hybot MA1 (Bystricko; Czech Republic), blackrock desert (sample from Nevada), tungsten powder M20 (mean diameter 1 μm) (Sylvania; Towanda, PA), pyrite from Logrono, Spain (John Betts, New York).

When a particulate material was supplied in a form too large to form a dispersion, the sample was broken into small particles with a mortar and pestle. To isolate the fines of a mineral preparation, the sample (2–5 g) was added to 40 ml water in a 50 ml tube, mixed by vortex and allowed to settle for 10 min. The portion of the mineral preparation that remained dispersed in water was removed to a new tube then centrifuged. The supernatant was discarded and 40 ml of 100% ethanol was added and mixed by vortex to disperse the pellet. The sample in ethanol was then centrifuged to produce a pellet and the supernatant was discarded. The pellet was lyophilized to dryness overnight. Stocks of mineral fines were made at 0.1 g/ml in water.

Silica spheres were produced as described (Stöber and Fink et al., 1968) but with altered amounts of reagents to produce sub-micron diameter spheres. Recipes were as follows: 6 nm spheres: 200 ml ethanol, 16 ml TEOS, 4 ml NH_4OH ; 12 nm spheres: 200 ml ethanol, 16 ml TEOS, 6 ml NH_4OH ; 50 ml ethanol, 4 ml TEOS, 2 ml NH_4OH ; 75 nm: 50 ml ethanol, 4 ml TEOS, 3 ml NH_4OH ; 170 nm: 50 ml ethanol, 6 ml TEOS, 5 ml NH_4OH . The ethanol and TEOS were mixed together first in a round bottom flask. The NH_4OH was then added to the mixture. The mixture was stirred at 300 rpm. The solutions became turbid over the course of minutes to hours as the reaction proceeded and the spheres formed. After an overnight synthesis the spheres (1 ml) were purified by dialysis into water (1 l) over night using Spectra Por CE dialysis membranes, MWCO 500. The water was changed after 3 h and again after an additional 14 h. Reaction yield was determined post-dialysis by lyophilizing and weighing a portion of spheres. Mean diameter was determined by DLS.

Vesicle formation reaction

Reaction conditions were standardized in order to compare the effects of various surfaces and minerals. Reactions contained either no mineral or mineral at 0.02, 0.1, and 0.5 mg/ml in 0.2 M sodium bicine pH 8.5 (Sigma–Aldrich, St. Louis, MO). Turbidity readings were taken at 400 nm and 25°C using a Cary 1E UV–Visible spectrometer. Eighty millimolars of micelles were added to the buffered reaction mixture to a final concentration of 10 mM and mixed briefly with a pipette. The change in turbidity was monitored over time and slopes were calculated for the steepest part of the turbidity curve, usually 0.1 to 4 min past micelle addition. Reactions took place in disposable 1.5 ml semimicro UV plastic cuvettes (Plastibrand). The pH was monitored with a Corning 430 pH meter and pH paper.

Testing of soluble fraction

A dispersion of 0.5 mg/ml montmorillonite in sodium bicine was prepared as above. After tumbling the dispersion overnight, the sample was centrifuged in a Beckman–Coulter Allegra 6 at 2,000 rcf (relative centrifugal force) for 10 min. The supernatant was removed from the pellet and filtered through a Whatman 0.02 μm syringe filter to remove any small clay particles. The pellet was resuspended in buffer. Both the supernatant and pellet fractions were tested for vesicle formation by the addition of 10 mM myristoleate micelles as above.

The divalent ion chelators EDTA (ethylenediaminetetraacetic acid) and EGTA (ethylene glycol bis(2-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid) (Sigma–Aldrich, St. Louis, MO) were used to sequester soluble divalent cations that might influence the reaction. Ten millimolars of myristoleate micelles were added to a buffer containing 0.2 M sodium bicine pH 8.5 and 1 mM or 10 mM EDTA and EGTA. In addition, half of the samples contained 0.1 mg/ml montmorillonite. Change in absorbance was monitored at 400 nm and 25°C as above.

Fluorescence confocal microscopy

Microscopy was performed on a Leica Confocal TCS SP microscope with Melles Griot HeNe, ArKr, and Ar lasers and Leica software. To confirm vesicle formation during the mineral dispersion tests, 4.5 μl of the sample was placed on a slide, and 0.5 μl of 100 μM Rhodamine 6 G was added to the sample and mixed prior to microscopic examination.

Estimation of critical aggregate concentration

Fatty acid micelles were added to a buffered solution (final concentration of 0.2 M sodium bicine, pH 8.5) at varying concentrations with or without 0.1 mg/ml montmorillonite present. After 1 h the reactions were stirred and the absorbance taken at 400 nm at 25°C. Data was plotted as absorbance vs. concentration of fatty acid. Single samples were prepared in order to determine the general trend over a range of fatty acids concentrations: 1–20 mM for myristoleic acid (14:1) and 0.1–10 mM for palmitoleic acid (16:1). From this data the concentration at which the turbidity starts to increase with an increase in amphiphile concentration was estimated. Three different concentrations near this point were analyzed in triplicate. The results were plotted as absorbance at 400 nm versus concentration of fatty acid. The cac was estimated from the x -intercept.

Light scattering

Dynamic light scattering (DLS) data was collected on a temperature controlled PD2000/ Batch instrument (Precision Detectors, Inc. Franklin, MA) at 25°C. When the reaction products included both vesicles and dispersed minerals, the size distribution of the vesicles was determined after centrifugation of the samples at 1,610 g rcf for 10 min to remove minerals from the dispersion.

Estimation of fatty acid concentrations

Fatty acid concentrations were estimated using the fluorometric free fatty acid indicator ADIFAB (AcryloDated Intestinal Fatty Acid Binding protein; Molecular Probes, Eugene, OR) according to manufacturer's instructions.

Surface coating experiment

Fifty millimolars of myristoleate with 0.2 mol% NBD-PE (*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine, triethylammonium salt; Molecular Probes, Eugene, OR) or Rh-DHPE (Lissamine rhodamine B 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine, triethylammonium salt; Molecular Probes, Eugene, OR) was prepared by adding dye-PE 5 mM stock in chloroform to 1 ml methanol containing myristoleic acid. The solution was mixed by vortexing in a round bottom flask. The methanol and chloroform was evaporated using a Büchi 461 rotovap with vacuum at 40 mbar for 5 min. Dilute alkaline water was added to the remaining oil in flask, which was solubilized by vortexing. The resulting micellar solutions were stored in glass vials under argon and protected from light.

Glass microspheres (0.5 μ l; 1–40 μ m in diameter in 0.1 g/ml dispersion) were combined with 10 μ l of 50 mM myristoleate micelles with 0.2 mol% Rh-DHPE. The volume of the sample was increased by adding 500 μ l water resulting in a final concentration of 1 mM myristoleate (below the cac). The samples were agitated (Barnstead Labquake shaker) at room temperature over night under argon. The samples were centrifuged at 1,610 g rcf for 2 min in an Eppendorf centrifuge 5415D and the supernatants removed. The pellets were dispersed by the addition of 25 μ l of water. Coating of the glass microspheres was monitored by confocal microscopy.

Of the coated spheres, 2.5 μ l were mixed with 1.5 μ l 50 mM myristoleate micelles with 0.2 mol% NBD-PE and 1 μ l 1 M sodium bicine pH 8.5. The final concentration of myristoleate was 15 mM, which is above the cac, and at a pH where vesicles will form. When buffer alone was added to amphiphile-coated spheres, no vesicles were produced. One hour after addition of micelles to the coated spheres, the samples were analyzed by confocal microscopy.

The surface area of the glass microspheres was calculated using an average particle diameter of 5 μ m (estimated from microscopy), a density value of 2 g/cm³, and the weight of spheres added to a sample (typically 0.25 mg). The surface area of the added amphiphile was calculated using a value of 0.35 nm² for the area of the fatty acid head group.

For controls, separate batches of 50 mM myristoleate micelles with either 0.2 mol% Rh-DHPE or NBD-PE were mixed together at dye ratios of 50:50 and 90:10, respectively. Five hundred microliters of water was added to the mixed micelles resulting in a final myristoleate concentration of 1 mM as described above. Glass microspheres (0.25 mg) were then added, and the samples tumbled overnight under argon. Spheres were separated from solution by centrifugation at 16.1 rcf for 2 min. Supernatant was removed and 25 μ l water added to pellet. The control spheres coated with myristoleate and mixed dyes were visualized by confocal microscopy.

Teflon particles coated with fluorinated phospholipids

Thin lipid films were made by rotary evaporation of chloroform solutions of 1-Palmitoyl-2-Oleoyl-*sn*-Glycero-3-Phosphocholine (POPC) or 1-palmitoyl-2-(16-fluoropalmitoyl)-*sn*-glycero-3-phosphocholine (F-DPPC). Rehydration with a 6% (w/v) Teflon particle dispersion yielded a mixture containing 8 mg/ml lipid. The dispersion was incubated at room temperature for 20 min followed by centrifugation (National Labnet Co. mini centrifuge C-1200) for 4 min. The supernatant was discarded and the pellet was resuspended in 450 μ l water. This pelleting/resuspension procedure was repeated six times. After the final resuspension, the particles were allowed to settle for 20 min. The portion of the solution

containing dispersed particles was removed and used for vesicle formation experiments. For myristoleic acid an identical procedure was used except that 4 mg of neat oil was added to 0.5 ml of a 6% (w/v) Teflon particle solution.

Teflon particles were tested by dispersing 8 $\mu\text{g/ml}$ particles in a solution containing 0.2 M sodium bicine, pH 8.5. Reactions were initiated by the addition of myristoleate micelles (10 mM fatty acid) and monitored as described under Vesicle Formation Reaction.

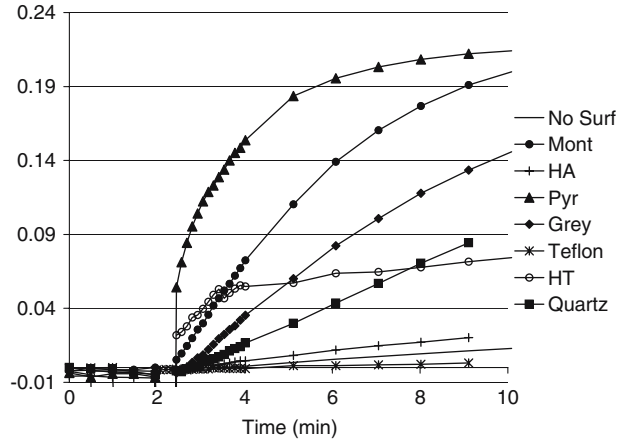
Results and Discussion

Vesicle assembly in the presence of different surfaces

Some mineral surfaces assist in vesicle assembly (Hanczyc and Fujikawa et al., 2003) but the mechanism and the parameters underlying this effect have not been determined. We devised a simple test to search for mineral-directed vesicle formation by monitoring the phase transition from myristoleate (C14:1) micelles to lamellar vesicles as the pH was lowered. By adding dilute myristoleate at pH 10 to a solution buffered with 0.2 M sodium bicine at pH 8.5, vesicles formed from micelles over the course of minutes to hours as monitored by absorbance at 400 nm. Previously we found that trace amounts of montmorillonite clay dispersed in the solution increased the rate of this phase transition by a factor of up to 100 (Hanczyc and Fujikawa et al., 2003).

Can surfaces other than montmorillonite affect the micelle to lamellar vesicle phase transition and if so, in what ways? To address this issue 24 different types of surfaces were tested separately in vesicle assembly assays. Since these surfaces varied in composition, surface charge, shape, and available surface area, the results serve only as a qualitative exploration of the magnitude of the catalytic effect of a wide range of materials. The results of the vesicle assembly assays for a representative set of surfaces are shown in Figure 1. In order to standardize this assay for all surface types, samples were prepared in cuvettes with 0.2 M sodium bicine pH 8.5, and either 0, 0.02, 0.1, or 0.5 mg/ml mineral dispersion. Vesicle formation was monitored by turbidity measurements at 400 nm. The resulting vesicle assembly curves (Figure 1) illustrate how certain particles, namely montmorillonite, pyrite, quartz, hydrotalcite, and Gray Zeeospheres™ (a synthetic ceramic), accelerate vesicle formation. Other surfaces, such as hydroxyapatite and Teflon particles, do not affect the rate of the transition and are comparable to the no surface control. Each surface was tested in this way for rate enhancement, and relative rate enhancement was calculated for each sample by taking the ratio of the greatest initial slope of the reaction over the slope for the no surface control. Accordingly, a value of 1 indicates that there was no rate enhancement. These results, shown in Figure 2, indicate that the majority of surfaces tested accelerated the rate of turbidity change in the vesicle formation assay. These surfaces include natural and synthetic aluminosilicates, natural and synthetic glasses, metals, metal oxides, carbonaceous minerals and also sample of desert sand. To be certain that an increase in turbidity signified the formation of vesicles, an aliquot from each assay was analyzed by microscopy for the presence of vesicles. It was found that vesicles were formed from micelles in all cases even when no rate enhancement was observed with only one exception. When micelles were added to a dispersion of calcium carbonate particles, only lipid aggregates formed (data not shown). This aggregation is likely due to the presence of soluble calcium ions that are known to interact with fatty acids and form insoluble calcium carboxylates (Deamer and Dworkin et al., 2002).

Figure 1 Turbidity change upon micelle addition. Myristoleate micelles were added to buffered solutions after the 2-min mark. The initial absorbance of all solutions was recorded and then the micelles were added. The data is shown as change in absorbance over time. The buffered solutions contained either no mineral surface (*No Surf*; *solid line*) or a fine dispersion of montmorillonite (*Mont*; *solid circle*), Hydroxyapatite (*HA*; *cross*), pyrite (*Pyr*; *solid triangle*), gray zeospheres (*gray*; *solid diamond*), Teflon (*asterisk*), hydrotalcite (*HT*; *open circle*), or quartz (*solid square*).



Given the diversity of materials investigated and apparent variation of characteristics such as composition, surface charge and shape, no single one of these parameters appears to be required for the promotion of vesicle assembly. However, some of these characteristics may modulate the magnitude of the catalytic effect. From the assays, the notable materials that do not promote vesicle formation are hydroxyapatite and Teflon particles. In both cases vesicles form at rates comparable to assays in which no mineral surface is added. For Teflon, it is likely that the lack of adsorption of fatty acid to the surface of the particle is the factor responsible (see below). The reason that hydroxyapatite, a calcium phosphate, does not affect the rate of vesicle formation is most likely the formation of an insoluble and inactive fatty acid precipitate that coats the particle surface.

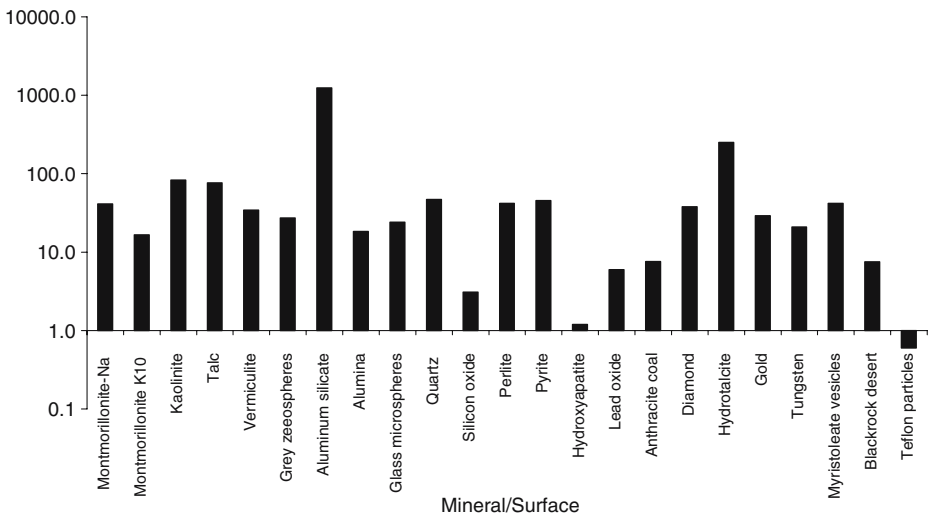


Figure 2 Effects of different minerals and surfaces on vesicle assembly. All minerals and surfaces were tested for their ability to catalyze vesicle formation under standardized assay conditions. The relative rate enhancement is calculated as the largest initial slope of change in turbidity over time divided by the slope of the no surface control. A value of 1 represents no effect of the added surface on vesicle assembly.

The process of surface-enhanced vesicle formation is more general than clay-mediated RNA polymerization. RNA polymerization requires very specific conditions, including very specific ways of processing the clay catalyst (Ertem, 2004). The conditions on the early earth that would favor RNA polymerization on clay would probably have been quite rare. In contrast, vesicle assembly in the presence of clay could occur under more general conditions that favor the lamellar phase and therefore perhaps be more common.

Does a soluble agent assist vesicle assembly?

We tested whether the presence of soluble cations released from the mineral montmorillonite affected the rate of vesicle assembly. It is known that divalent cations affect the organization of fatty acids even at sub-millimolar concentrations (Deamer and Dworkin et al., 2002). We added the divalent ion chelators, EDTA and EGTA, at 1 and 10 mM to a standard vesicle formation reaction with and without a 0.1 mg/ml montmorillonite dispersion (Figure 3a). There is no apparent difference in the rate of formation of vesicles when chelators are added indicating that soluble metal ions, such as magnesium and calcium from montmorillonite, are not influencing vesicle formation.

We also performed vesicle assembly tests using the soluble fraction of a montmorillonite dispersion alone. A dispersion containing 0.5 mg/ml montmorillonite was prepared and the particles were separated from the supernatant by centrifugation. Very fine particles were

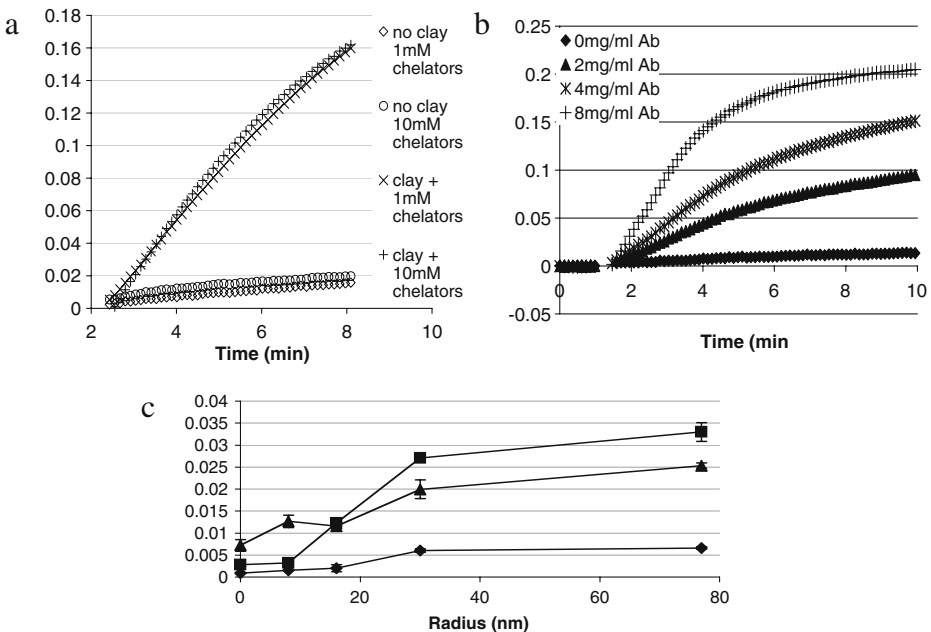


Figure 3 Effect of chelators and silica spheres on the rate of turbidity change in vesicle assembly reactions. (a) Micelles were added to buffered solutions containing either 1 or 10 mM EDTA and EGTA with and without a dispersion of montmorillonite clay. (b) The change in turbidity was monitored upon the addition of micelles to a buffered solution containing 0, 2, 4, or 8 mg/ml silica spheres of diameter 6 nm. (c) The initial slope of the change in turbidity over time was monitored as fatty acid micelles were added to dispersions of silica spheres of different radii. In each solution the amount of surface area provided by the spheres was kept constant at $3.7 \times 10^{16} \text{ nm}^2$. *Diamonds* represent turbidity values for oleate micelles, *squares* palmitoleate, *triangles* myristoleate.

removed by passing the supernatant through 0.02 μm syringe filters. Both pellet and filtered supernatant fractions were tested in the standard vesicle formation test. We observed that only the solid mineral fraction was able to catalyze vesicle formation, whereas the filtered supernatant yielded data equivalent to that of control reactions without minerals present.

Fatty acid supramolecular structures are very sensitive to pH. The pH values of the dispersions and assays were monitored for any alteration in pH caused by the addition of minerals. No changes in pH were found (data not shown), as expected due to the high buffer concentration employed (0.2 M sodium bicine, pH 8.5).

Clay and the critical aggregate concentration

The cac is the critical concentration of amphiphile needed for lamellar vesicle structures to form. Once the concentration of an amphiphile rises above the cac, vesicles will form spontaneously given a favorable environment. Moreover, the rate of vesicle assembly increases as the amphiphile concentration rises above the cac. We therefore sought to determine if minerals increase the rate of vesicle formation by lowering the effective cac of the amphiphile. We measured turbidity over a broad range of amphiphile concentrations: 1–20 mM for myristoleic acid (14:1) and 0.1–10 mM for palmitoleic acid (16:1), at a wavelength of 400 nm and a temperature of 25°C. From this data the approximate point at which the turbidity starts to increase with an increase in amphiphile concentration was determined. Three replicate samples were then prepared at each of three concentrations above the approximate cac with or without montmorillonite clay at 0.1 mg/ml (Figure 4). The cac was estimated by determining the value of the x -intercept. For myristoleate with and without clay the estimated cac values were 3.66 and 3.68 mM, respectively. For palmitoleate with and without clay the values were 0.24 and 0.26 mM, respectively.

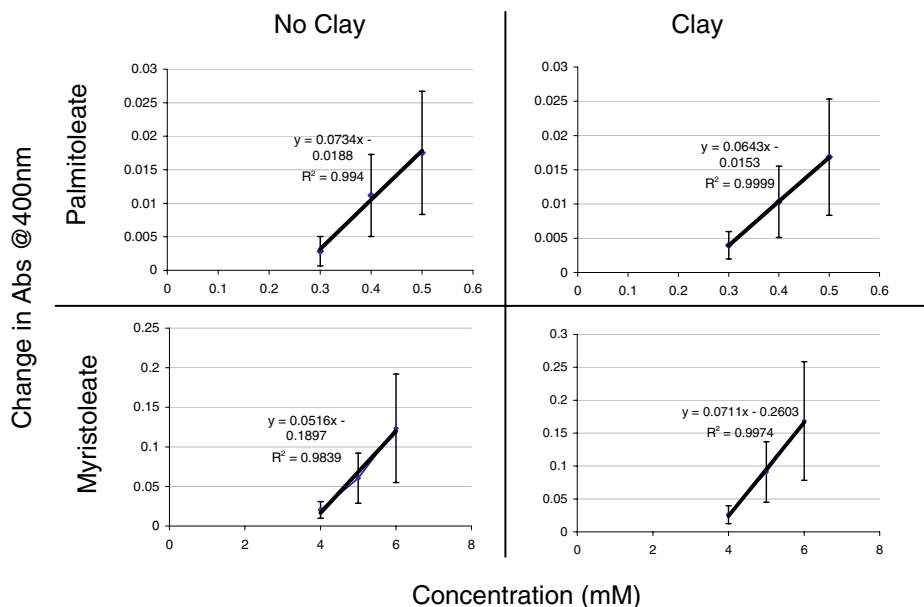


Figure 4 The effect of montmorillonite clay on the cac. Samples in triplicate containing varying amounts of the fatty acids myristoleate and palmitoleate were prepared and turbidity values recorded with and without montmorillonite clay.

Therefore, the addition of the clay dispersion to the vesicle formation reaction does not appear to shift the critical concentration at which amphiphiles assemble into stable lamellar vesicles.

We conclude that the enhancement in the rate of vesicle formation in the presence of many different minerals is unlikely to be due to the introduction of a soluble agent to the solution, a change in the pH of the solution, or a change in the cac of the fatty acids. The mineral surface itself is the most likely cause of the observed rate enhancements.

Highly curved silica surfaces

To focus on the surface parameters necessary to promote vesicle formation, we explored whether there is a lower limit for particle size below which vesicle formation does not occur. Fatty acids assembling on a small spherical surface may be limited by the significant membrane curvature energy when the principle radius of curvature is less than 20 nm. This would result in less effective surface catalysis when the radius of curvature is very small. We synthesized a series of silica spheres with different diameters by the Stöber method (Stöber and Fink et al., 1968). After synthesis each population of spheres was characterized by dynamic light scattering (DLS) to determine the average sphere diameter and to confirm monodispersity. Each population was tested in a vesicle formation reaction, and all showed rate enhancement. Even the smallest spheres of 6 nm diameter promote vesicle formation (Figure 3b). The diameters of these smallest particles are only about five-fold larger than the spherical micelles added to the reaction. Therefore it appears that even very small particles can serve as nucleation sites for amphiphile organization and vesicle formation. Since these spheres are probably smaller than the smallest possible vesicles (Cornell et al., 1982), nucleation most likely involves the formation of small patches of membrane that can continue to grow at their edges independently of the silica spheres.

How does the rate enhancement vary with surface curvature? Each population of silica spheres with a defined diameter was tested in a vesicle formation assay. Varying amounts of spheres were added so that the total surface area was held constant at $3.7 \times 10^{16} \text{ nm}^2$.

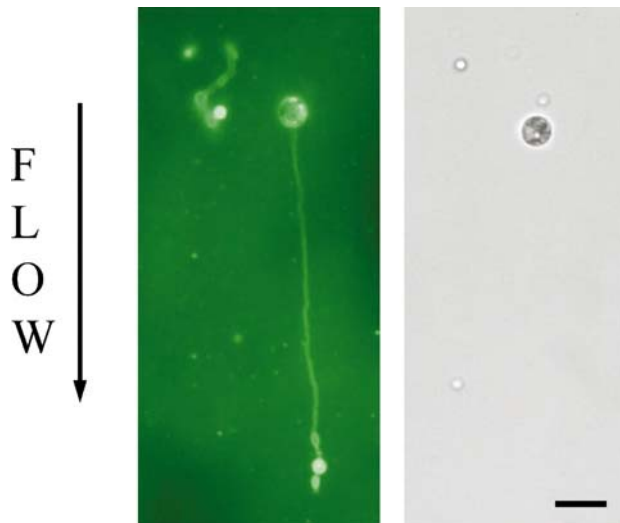
The initial rate of vesicle formation increases with increasing silica sphere diameter (Figure 3c). This trend holds true for myristoleic, palmitoleic and oleic acids (C14:1, C16:1, and C18:1, respectively). Because increased turbidity can result from the formation of either more or larger vesicles, this experiment cannot distinguish between these possibilities. Nevertheless, the total available surface area is clearly not the only determinant of vesicle formation, and surface curvature also plays a role.

Does vesicle formation occur on or proximal to the mineral surface?

As previously reported, when fatty acid micelles are added to a mineral surface three main effects occur: the initial rate of vesicle formation is accelerated, the mineral surface becomes coated with amphiphile, and some of the mineral particles become encapsulated within the resultant vesicles (Hanczyc and Fujikawa et al., 2003). To visualize surface-assisted vesicle formation in real time, we added NBD-PE labeled fatty acid micelles to a buffered solution of synthetic ceramic microspheres placed on a microscope slide. We were able to see the formation of vesicles streaming off a microsphere just after micelle addition (Figure 5) indicating that vesicle assembly occurs in close proximity to the mineral surface.

One hypothesis for how surfaces assist in vesicle assembly is that the amphiphiles are brought together by adsorption on the mineral surface, and that vesicles are produced directly from that adsorbed layer of amphiphiles. To test this hypothesis, 0.25 mg of glass

Figure 5 Fluorescence and bright-field micrographs of a synthetic ceramic microsphere (*gray zeosphere*) soon after NBD-PE labeled fatty acid micelles were added to a dispersion of spheres. *On the right* the fluorescence micrograph shows that the zeosphere is labeled with the NBD-PE as well as the membranous structures that have incorporated the NBD-PE. *In bright field on the left*, only the large zeosphere is visible. There is a flow across the slide in the direction indicated by the *arrow*. Size bar, 5 μm .



microspheres with diameters in the 1–40 μm size range were first coated by the addition of excess fatty acid/Rh-DHPE mixed micelles to a final amphiphile concentration of 1 mM. The estimated surface area of the glass microspheres in the 0.25 mg sample is $1.5 \times 10^{14} \text{ nm}^2$ and the estimated head group surface area of total added amphiphile is $1.1 \times 10^{17} \text{ nm}^2$. The microspheres were then separated from the unadsorbed micelles by centrifugation. The coating on the glass microspheres consists of both fatty acid and Rh-DHPE as confirmed by quantitative ADIFAB analysis of the remaining unadsorbed fatty acid and fluorescence microscopy and spectrophotometry of the DHPE-conjugated rhodamine dye in both the adsorbed and unadsorbed fractions, respectively. Excess fatty acid/NBD-PE mixed micelles were then added to the coated microspheres resulting in a 1,000-fold excess of added surface area compared to the surface area of the precoated spheres. The glass microspheres promote vesicle formation both alone (Figure 2) and when precoated with amphiphile (data not shown). If vesicles assemble directly on the mineral surface, we would expect the red Rh-DHPE label to be diluted as it forms vesicles and is replaced by the material labeled with green NBD-PE. As shown in Figure 6, the microspheres were coated with adsorbed Rh-DHPE (shown in red, Figure 6a, b) and the added micelles containing NBD-PE formed vesicles (shown in green, Figure 6c, d). Notably, the label on the precoated spheres does not become mixed with the added micellar NBD-PE label. Control micrographs of microspheres coated with known mixtures of fatty acid/Rh-DHPE/NBD-PE micelles (Figure 6g, h) demonstrate that even a 10% mixing of labels would be detectable in this assay. Figure 7 shows that the coated glass microspheres and resultant vesicles are in close proximity, yet the green label introduced with the subsequent micellar amphiphiles does not mix with the precoated surface. We conclude that although the amphiphiles coat the particles during vesicle formation, the resultant vesicles do not form in direct contact with the mineral surface.

Activation of Teflon particles by coating with fluorinated lipids

An intriguing possibility is that the amphiphile coating a surface can be the actual catalyst. Support for this possibility stems from the fact that Teflon, a hydrophobic and lipophobic

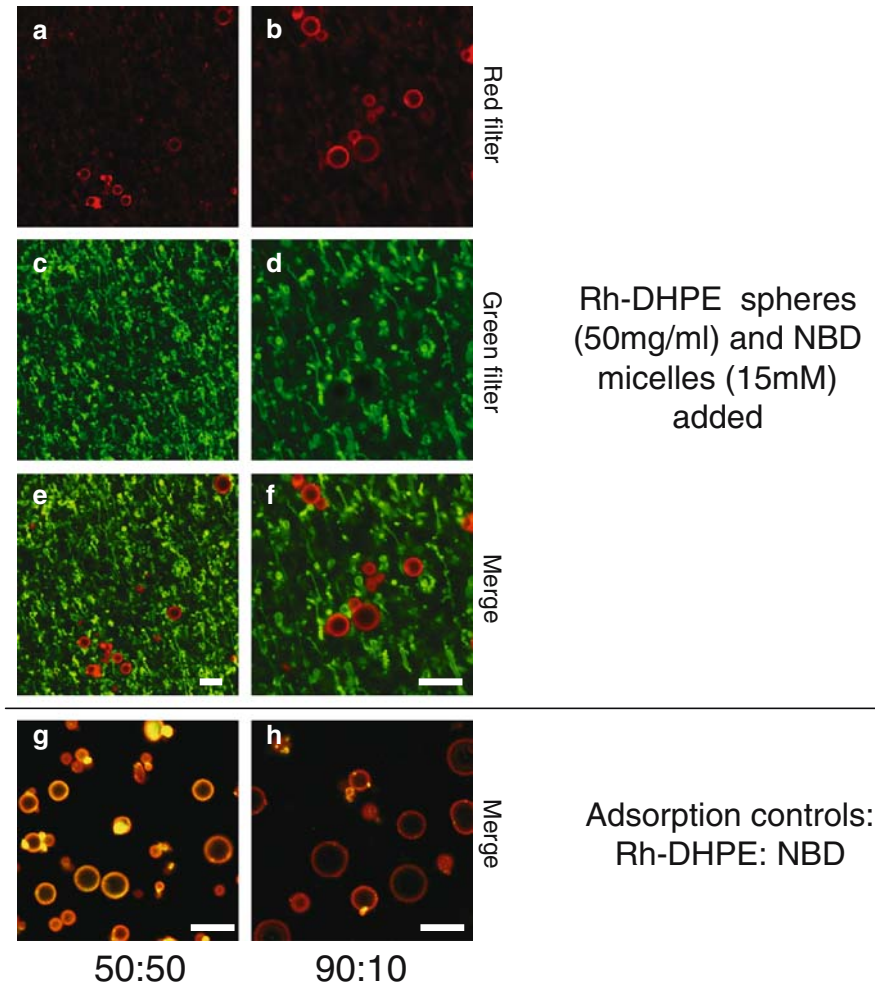


Figure 6 Confocal micrographs of coated glass microspheres and vesicles. Glass microspheres were coated by adsorption of a mixture of myristoleate and Rh-DHPE. Coated spheres were put into a buffered solution and then excess myristoleate micelles with NBD-PE were added. (a, c, e) and (b, d, f) show the results of two independent experiments. The micrographs in the *top section* show the partitioning of dye label on the microspheres (a, b) and the vesicles (c, d) using *red and green filters*, respectively, and then as merged images (e, f). As controls, microspheres were coated with myristoleate micelles and a mix of Rh-DHPE: NBD-PE in the ratios of 50:50 (g) and 90:10 (h). Bar: 5 μ m.

material that does not significantly interact with fatty acids or phospholipids, does not promote vesicle formation (Figures 1 and 2). Also, the rate of vesicle formation is reduced when quartz cuvettes used in the vesicle assembly assay are coated with Trichloro(1H,1H,2H,2H-perfluorooctyl)silane (data not shown). To test whether an amphiphile coating is necessary for the catalysis of vesicle formation, we sought to convert Teflon from an inactive to an active surface by using an amphiphile coating. We used a fluorinated amphiphile, F-DPPC (1-Palmitoyl-2-(16-Fluoropalmitoyl)-sn-Glycero-3-Phosphocholine), capable of interacting with other fluorinated species to coat the Teflon particles. Then fatty acid micelles were added and monitored for vesicle formation. We observed rapid vesicle formation in the presence of coated Teflon

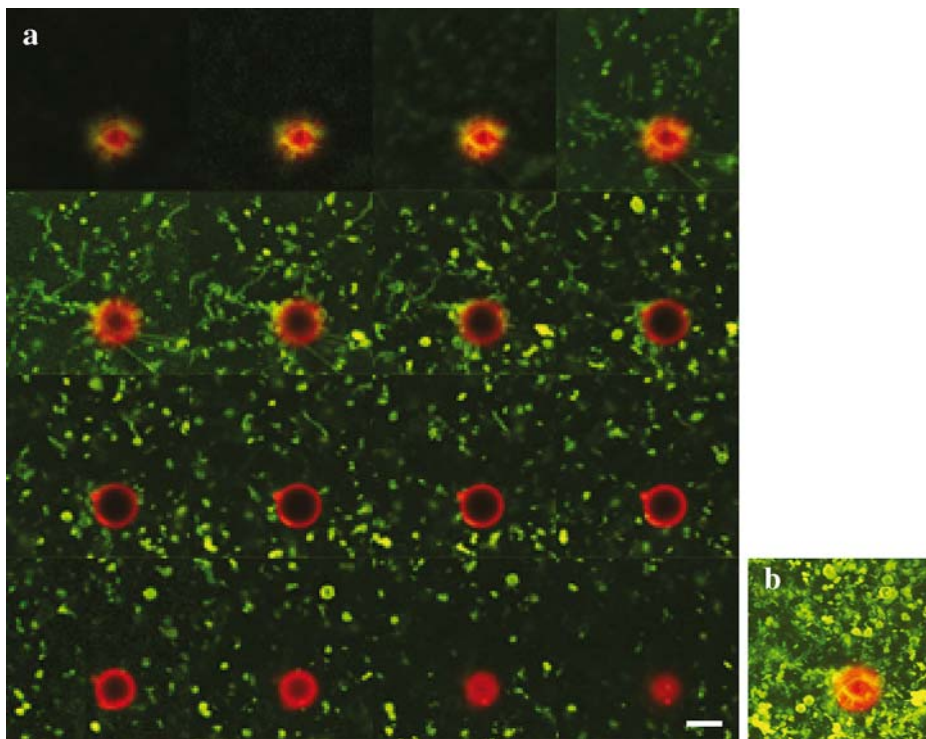


Figure 7 Confocal micrograph of a glass microsphere coated with fatty acid/Rh-DHPE with an excess of fatty acid (*NBD-PE*) added. (a) 16 images from *z*-axis slices, (b) compiled image from (a). Size bar, 5 μm .

particles thereby demonstrating the conversion of Teflon from a surface incapable of promoting vesicle assembly to one that catalyzes vesicle formation simply by coating the surface with F-DPPC (Figure 8).

Discussion of the model

When fatty acid micelles are added to a buffered solution containing a dispersion of particles, vesicles are produced at an accelerated rate, the particles become coated with amphiphile and particles are often encapsulated in the vesicles. Given these close associations between particle and amphiphile, we initially favored a model in which fatty acids adsorb directly to a surface and continue to grow as lamellar sheets. These sheets then fold onto themselves to produce vesicles. This model predicts a reaction limited by the available surface area of the particle as shown in Figure 3b and (Hanczyc and Fujikawa et al., 2003). In addition, growing lamellar sheets detach (when large enough) from the particle surface and form vesicles, freeing the particle surface to accept more fatty acids. By first coating glass microspheres with fatty acids and then adding a vast excess of fatty acid micelles, we determined that the excess fatty acids do not mix with the adsorbed layer of fatty acid on the particle surface, and the resultant vesicles do not form from continual turnover of a layer of fatty acid that is in direct contact with the particle surface (Figure 6). These microscopic observations argue strongly against the original model, with the caveat that the fluorescent microscopy tracks a fluorescent dye-labeled phospholipid and not the actual fatty acids.

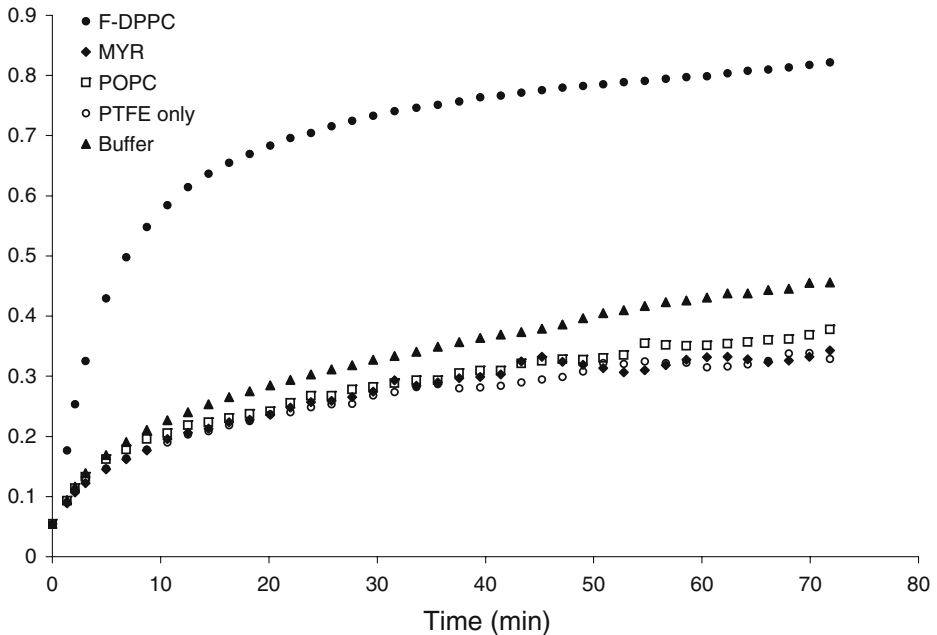


Figure 8 Coated Teflon mediated vesicle assembly. Teflon particles were incubated with myristoleic acid (*MYR*, diamonds), POPC (*squares*), F-DPPC (*filled circles*), or without incubation (*PTFE only*, open circles) followed by repeated washing steps to remove unbound amphiphiles. Subsequently, myristoleic acid (10 mM) micelles were added to a solution containing the coated mineral and turbidity changes were measured at 400 nm. The reaction was also monitored in the absence of added particulates (*Buffer*, triangles).

Since we are able to see that vesicles form proximal to the particles suspended in solution (Figures 5 and 7), we now propose a model where the fatty acids assemble into sheets close to the particle surface. In certain cases it may be a coating of amphiphile on the particle surface that serves to assist in vesicle formation. For instance in the case of Teflon particles, it was first necessary to pre-coat the surface with amphiphiles in order to see catalysis of vesicle formation (Figure 8). It is unknown whether all surfaces tested need to be coated with fatty acid or whether some can catalyze vesicle formation directly without an adsorbed layer of lipid. The amphiphile may be organized as a supported bilayer or monolayer and the precise organization may be dictated by the characteristics of the surface. Fluorescence Recovery After Photobleaching (FRAP) and X-ray diffraction experiments may reveal the arrangement of amphiphiles adsorbed to the surface.

It has been hypothesized that fatty acid micelles added to preformed fatty acid vesicles form a layer surrounding the existing vesicle and this may form the basis for how one layer of amphiphile may influence and organize subsequent layers (Chen and Szostak, 2004). These sheets when large enough can close on themselves to form vesicles. Previous studies have reported on the autocatalysis of the transition of fatty acid micelles to vesicles by the presence of preformed vesicles (Walde and Wick et al., 1994). This effect results in an enhanced rate of vesicle formation comparable to the results presented here for various surfaces (see myristoleate vesicles in Figure 2). Perhaps the surface of the particles becomes coated in a way that mimics the lamellar structure of fatty acid vesicles. If so, then there would be a shared mechanism between surface-mediated vesicle formation and vesicle autocatalysis.

A surface may influence the rate of vesicle formation by providing an environment that produces a high local concentration of lipid. The electrostatic attraction of micelles to a region close to the surface would increase the local concentration and favor nucleation of larger aggregates. The growing aggregates could then be released, where they could continue to grow and eventually form vesicles. Under typical reaction conditions where the concentration of amphiphile added is above the *cac*, vesicles would form readily and remain stable in the surrounding buffer. However, when the vesicle formation assay was preformed with dilute amphiphile below the *cac*, no vesicle formation was observed (data not shown). Even if the area in close proximity to a surface favors the accumulation of amphiphile and the subsequent formation of vesicles, the surrounding solution must be able to support and stabilize the formed vesicles.

Many of the tested surfaces have interesting properties that may play a role not only in vesicle formation but also in RNA polymerization (Ferris and Ertem, 1992; Ferris and Ertem, 1993; Ferris and Hill et al., 1996; Ertem, 2004). For example, the natural aluminosilicate, montmorillonite, is a smectitic mineral and may provide internal surfaces where amphiphiles can adsorb. This property of the mineral provides an even greater potential surface area for vesicle assembly. Experiments so far have been unable to distinguish whether the initial amphiphile aggregation occurs between the platelets of these minerals or only on the external surface of the particles. However, other aluminosilicate minerals, both natural and synthetic, that are not smectitic also promote vesicle formation, showing that inter-layer aggregation is not necessary. Another mineral that has been hypothesized to be involved in early biogenetic processes is pyrite (FeS_2). The formation of this mineral from hydrogen sulfide and ferrous ions has been postulated to be a prebiotic driving force for carbon fixation (Wächtershäuser, 1988). Pyrite particles also catalyze membrane assembly, and could presumably become encapsulated within vesicles, raising the interesting possibility of mineral catalyzed metabolic transformations within vesicles.

Mineral-assisted vesicle assembly appears to be a remarkably general phenomenon. We expect that a closer examination of mineral-assisted vesicle assembly will reveal specific aspects of mineral composition and structure that affect the mechanism by which membrane assembly is catalyzed. Such studies may clarify the processes that lead to the encapsulation of mineral particles within vesicles, and the assembly of compound vesicles containing both small vesicles and mineral particles. In light of the known catalytic effects of various mineral surfaces, a better understanding of mineral-membrane interactions will be helpful in assessing the potential roles of composite mineral-vesicle particles in the origin of life.

Conclusions

The interactions of simple, single-chain amphiphiles with many different surfaces results in the organization of membranes and the formation of vesicles. This effect could have played a key role in the organization and formation of the first cell-like structures on the early earth. Since mineral particles have been implicated in very early chemistries and polymerization reactions (Bernal, 1951; Wächtershäuser, 1988; Ferris and Hill et al., 1996; Sowerby and Cohn et al., 2001; Sowerby and Petersen et al., 2002; Monnard, 2005), it is intriguing that minerals might have also been involved in the formation of yet another essential component of life—the cellular membrane. Mineral-mediated vesicle formation occurs with many disparate types of minerals and is therefore a more general property than clay-catalyzed RNA polymerization.

The formation of vesicles in the presence of minerals occurs proximal to the mineral surface and is not due to the release of some soluble factor or change in the micelle to vesicle transition concentration. In addition, the rate of vesicle formation is influenced not only by the available surface area of the mineral, but also by the curvature of the surface. Mineral surfaces that are intrinsically negatively charged may directly stimulate membrane formation, while in other cases an adsorbed layer of amphiphiles may coat the mineral particle and serve as the catalyst for subsequent vesicle formation. If such mechanisms operated on the prebiotic Earth, diverse types of mineral particulates with a variety of catalytic properties may have been efficiently encapsulated within membrane vesicles.

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