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EVOLVING LIPID VESICLES IN PREBIOTIC HYDROTHERMAL ENVIRONMENTS

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Abstract. We compared three different kinds of lipid vesicles made of saturated fatty acids, unsaturated fatty acids, and phospholipids for their evolutionary capabilities in a simulated hydrothermal environment. Encapsulation of the glycine monomers enhanced the oligomerization of peptides in all cases. Fatty acid vesicles remained stable at higher temperatures and efficiently utilized heat energy for this synthetic reaction. Phospholipid vesicles were destabilized by higher temperatures, and thus were found to be better suited to enhance synthetic reactions at lower temperatures.

Keywords: decanoic acid, fatty acid, lipid, oleic acid, phospholipid, vesicles

1. Introduction

The participation of lipid vesicles in prebiotic evolution would have required the early presence of lipid molecules such as fatty acids (Deamer and Oro, 1980; Luisi *et al*., 1999). In fact, prebiotic synthesis of fatty acids would have been plausible by means of a Fischer-Tropsch-type reaction using formic or oxalic acids as carbon sources (McCollom *et al*., 1999; Rushdi and Simoneit, 2001). Formic and acetic acids could in turn have been synthesized from carbon dioxide and water in the presence of metal oxides at high temperatures of about 1000 °C on conditions that the products were suddenly cooled down in water at 0 °C (Terada *et al*., 1999). Carbon dioxide contacting heated metal oxides in water would have been commonplace near areas of volcanic activity and in the vicinity of hydrothermal regions on the primitive Earth. These lipids would then have self-assembled into membranous vesicles in the proper aqueous milieu.

Here we compare the functional capabilities of these lipid vesicles made of decanoic acid as a saturated fatty acid (Apel *et al*., 2002), oleic acid as an unsaturated one, and a phospholipid, dipalmitoyl-L-alpha-phosphatidylcholine (DPPC) (Tsukahara *et al*., 2002) to facilitate the oligomerization of small peptides from glycine monomers. Although decanoic acid can be synthesized abiotically, both oleic acid and DPPC are the products of a complex set of enzymatic reactions which would have necessitated the pre-existence of living organisms and so it is doubtful that they existed on the early Earth.

2. Materials and Methods

In this experiment we used a flow reactor constructed to simulate the pressure and temperature conditions of circulating water in a hydrothermal environment, and to examine the role vesicles might have played in the synthesis of oligopeptides from amino acids both on and inside these vesicles (Matsuno, 1997; Imai *et al*., 1999). In the flow reactor, a high-temperature, high-pressure fluid at 180–200 °C at 21 MPa was injected into a low temperature chamber maintained at the same high pressure as the fluid. The fluid circulated in a closed loop from the high temperature chamber to the low temperature chamber which we maintained in the range 0–40 °C depending upon the choice of lipid molecules, and then again back to the high temperature compartment repeatedly. The diameter of the nozzle connecting the high-temperature to the low-temperature chamber was 0.8 mm, and the flow rate was 7.5 mL/min. Samples of the fluid were taken from the low-temperature chamber for measurement at regular time intervals. The total volume of the circulating fluid was 500 mL, and the volume of the high-pressure, high-temperature chamber was 15 mL. The cycle time of the fluid was 62.5 min. However, the cycle time of reactants in the solution in stirring conditions was about 1 min or less because the diffusion of reactants in the large vessel proceeded rapidly (Imai *et al*., 1999). The time required to reach the designated high temperature (starting from room temperature) was about 15 min.

We used three different lipid molecules to make three sets of lipid vesicles. We prepared decanoic acid vesicles in a solution of 50 mM decanoic acid and 100 mM glycine and adjusted the pH to 8.0 by adding NaOH. The pH was then lowered to 7.0 by adding HCl (Apel *et al*., 2002). Oleic acid vesicles were prepared similarly in a solution of 10 mM oleic acid, 100 mM glycine and 1% methanol. The pH was raised to 10.0 in order to dissolve the lipid, and then adjusted to pH 9 with HCl to form the vesicles. DPPC vesicles were made in a solution of 0.5 mM phospholipid and 100 mM glycine at pH 6.25. The preparation of DPPC vesicles followed the standard method detailed by Bangham (Bangham *et al*., 1965). All the preparations and measurements of pH were done at room temperature. Encapsulation or uptake of glycine into or onto the lipid vesicles was in a solution of 100 mM glycine with no added metallic ions. Although divalent ions have previously been shown to enhance oligomerization (Imai *et al*., 1999), our objective was to see how lipid vesicles might function in this process without additional metallic ions. In fact, divalent ions have recently been shown to destabilize fatty acid vesicles in aqueous solution (Monnard *et al*., 2002).

Identification of the oligomeric products was made with an HPLC analysis of the sample fluid (1 mL treated by 1.0% Triton-X100 20 μL). We also examined a solution of 100 mM glycine dissolved in pure water as a control. All samples were analyzed with a Hitachi (L-7420, L-7100, and D-7500) HPLC apparatus with a column Hydrosphere C18 (5 μm by 4.6 mm by 150 mm). The mobile phase consisted of 50 mM KH_2PO_4 and 7.2 mM $C_6H_{13}SO_3Na$, and its pH was maintained

at 2.5 by adjusting the added amount of H_3PO_4. The flow rate of the mobile phase was 0.5 mL/min. The signal was detected by measuring the absorbance at 195 nm.

3. Results

Phase contrasted photomicrographs of decanoic acid vesicles and of oleic acid vesicles are displayed in Figure 1. Decanoic acid vesicles made in the solution of 50 mM decanoic acid and 100 mM glycine were placed in the flow reactor in which the low temperature chamber was maintained at 40 °C set above the melting point of decanoic acid (31.6 °C). The high temperature chamber was set at 200 °C. The time developments of the yields of diglycine and triglycine we obtained are as depicted in Figure 2 along with the yields in the absence of the fatty acids. Oleic acid vesicles made in the solution of 10 mM oleic acid, 100 mM glycine and 1% methanol were put in the flow reactor in which the low temperature chamber was maintained at 25 °C above the melting point of oleic acid 12 °C while the high temperature chamber was set at 200 °C. The time developments of the yields of diglycine and triglycine are displayed in Figure 3 along with the yields in the absence of the fatty acids. The standard deviation of the measurement was 0.05 mM or less for diglycine and 0.002 mM or less for triglycine throughout the entire course of the present work. Each measured value was obtained from three to five independent runs of the similar measurements.

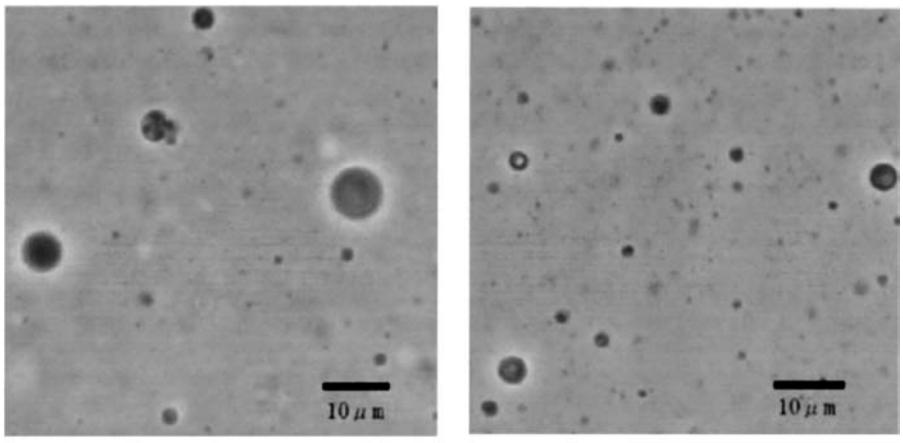

Figure 1. (a) A phase contrasted microscope image of decanoic acid vesicles made in the solution of 50 mM decanoic acid and 100 mM glycine at pH 8.0 by adjusting the amount of NaOH to be added, and then decreased down to 7.0 by adding HCl. (b) A similar image of oleic acid vesicles in the solution of 10 mM oleic acid and 100 mM glycine at pH 10.0 by adjusting the amount of NaOH to be added, and then decreased down to 9.0 by adding HCl. All the preparations were made at room temperature.

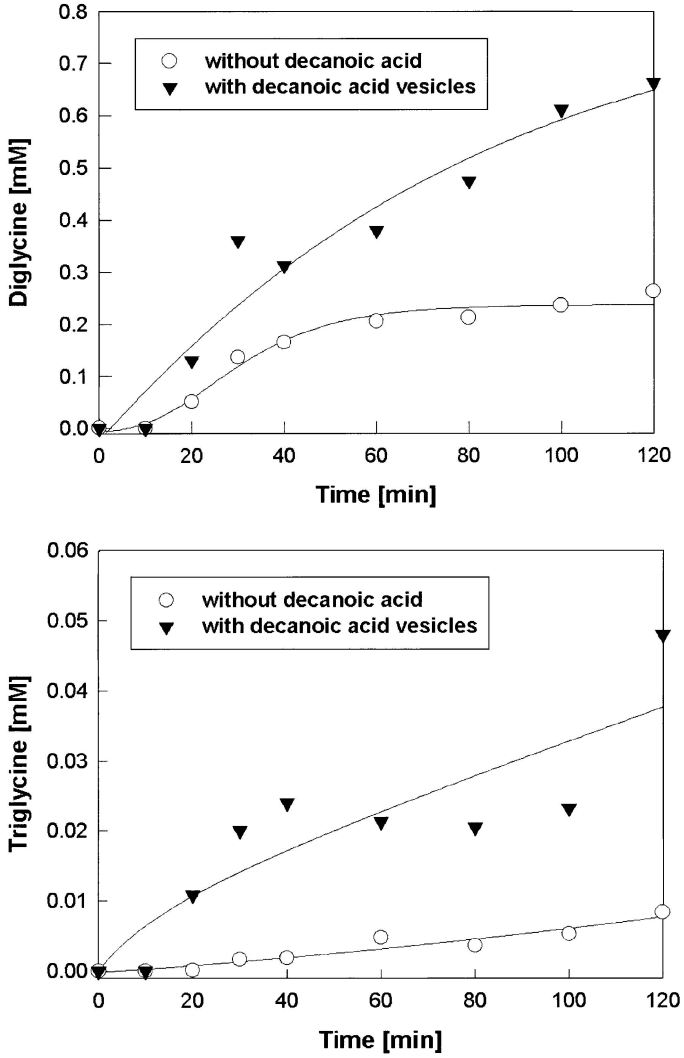

Figure 2. Time developments of the yields of diglycine and triglycine in the presence and absence of decanoic acid vesicles. The solution condition for the absence of decanoic acid vesicles was only 100 mM glycine with neither pH control nor added metallic ions. The time required for reaching the designated high temperture 200 °C as starting from room temperature was 15 min. The low temperature chamber was maintained at 40 °C.

Enhancement of oligomerization of glycine in the presence of lipid vesicles indicates two possibilities. It may be due to catalytic properties inherent in the vesicle surfaces themselves, or due to synthetic reactions proceeding inside the vesicles. In order to distinguish between these two possibilities, we first prepared oleic acid vesicles as with the case presented in Figure 3 and then dissolved the vesicles into micelles by adding surfactant (15 mL of 10% Triton-X 100) to the 500 mL

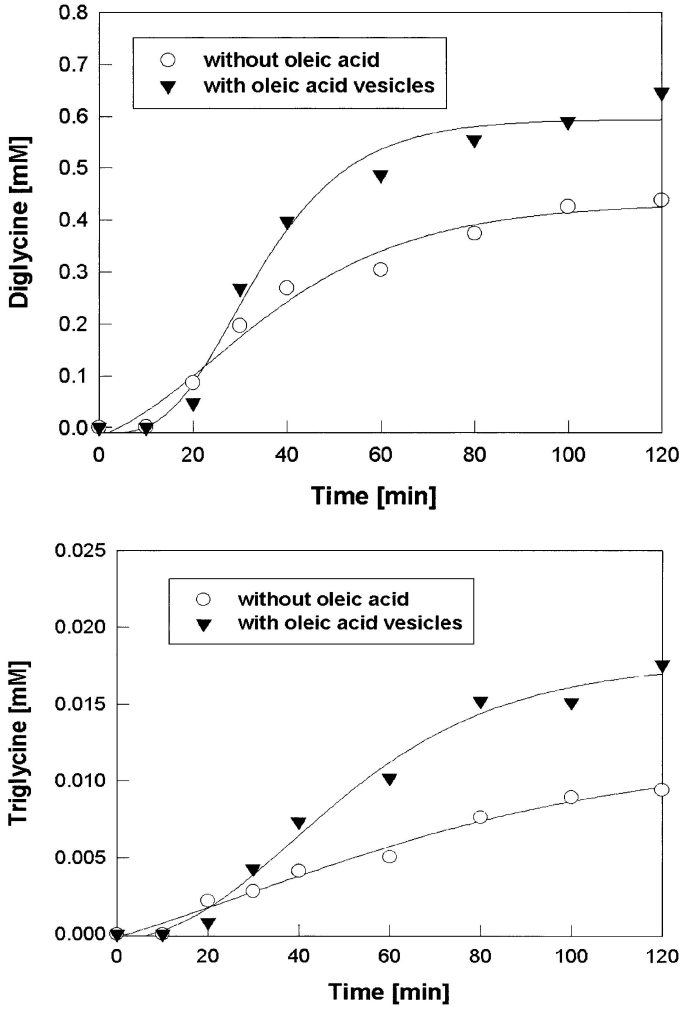

Figure 3. Time developments of the yields of diglycine and triglycine in the presence and absence of oleic acid vesicles. The solution condition for the absence of oleic acid vesicles was only 100 mM glycine with neither pH control nor added metallic ions. The time required for reaching the designated high temperature 200 °C as starting from room temperature was 15 min. The low temperature chamber was maintained at 25 °C.

reaction solution. Time developments of the yields of triglycine are displayed in Figure 4 along with the similar yields in the absence of the surfactant. There was found to be no significant difference in the yields of triglycine between the absence of lipid molecules (c.f., Figure 3) and the absence of lipid vesicles while in the presence of lipid molecules. Since no significant difference in yields was detected, the possibility of lipid molecules serving as catalysts for oligomerization may be dismissed.

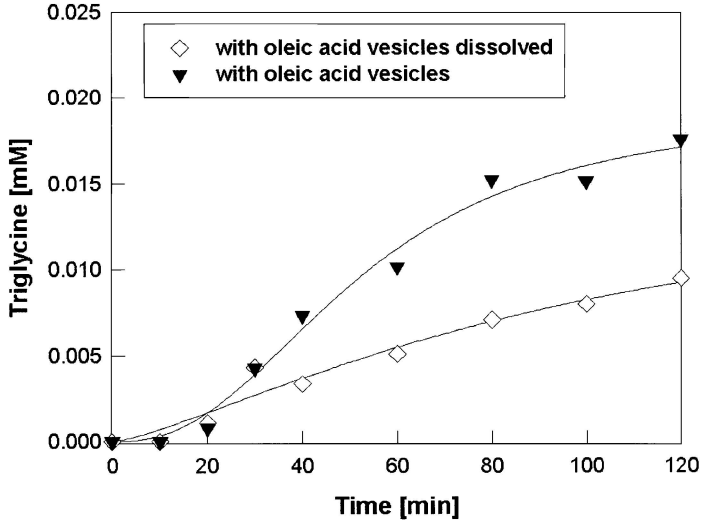

Figure 4. Time development of the yields of triglycine in the reaction solution 500 mL of 10 mM oleic acid, 100 mM glycine, 1% methanol, and 15 mL of 10% Triton-X 100 further added. For comparison, the yields of triglycine from the solution in the absence of surfactant Triton-X 100 are also presented.

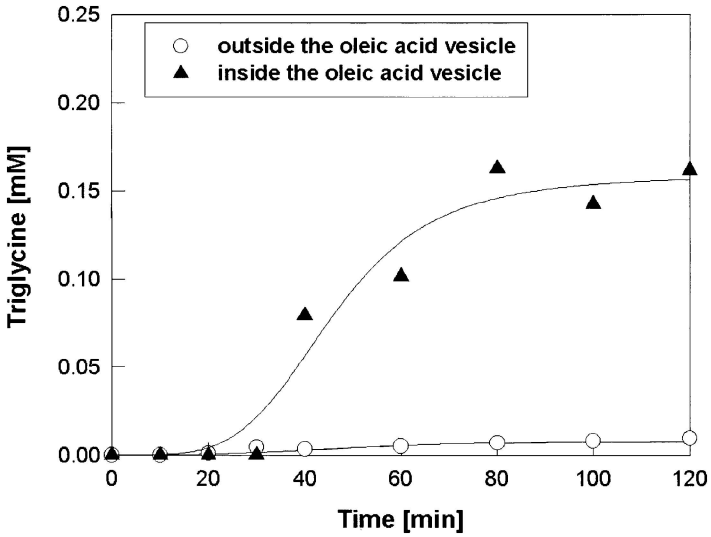

Figure 5. A comparison of the time developments of the yields of triglycine between the inside of the oleic acid vesicles and their outside. The reaction solution was 500 mL of 10 mM oleic acid and 100 mM glycine.

Quantitative estimation of oligomerization of glycine inside the oleic acid vesicles in 10 mM oleic acid solution was demonstrated in Figure 5, along with the result on oligomerization outside the vesicles. The total volume of the vesicles in the solution was independently measured by encapsulating the dye pyranine inside

the oleic acid vesicles when they were formed. The volume measurement was done as separating unencapsulated pyranine from those vesicles containing pyranine by using an exclusion column about 17 cm long and 1.5 cm in diameter (for details, see Apel *et al.* (2002)). The yields of triglycine inside the vesicles were about 20 times more than those outside the vesicles at 120 min after the operation of the flow reactor. The total volume shared by the vesicles was about 5%. In this connection, we also examined the case that the initially prepared 10 mM oleic acid solution contained oleic acid vesicles with no glycine inside the vesicles, while the glycine concentration outside the vesicles was 100 mM. We then observed (data, not shown) that there was no enhancement of the yields of triglycine compared to the case of the similar solution with no oleic acid. This observation again confirmed that enhancement of oligomerization proceeded inside the lipid vesicles.

To further examine the volume effect upon oligomerization inside lipid vesicles, we prepared two different scenarios for phospholipid DPPC vesicles. One was to make DPPC vesicles in the 500 mL solution of 0.5 mM DPPC and 100 mM glycine. The other was to first make DPPC vesicles in the 50 mL solution of 5 mM DPPC and 1 M glycine, and then to dilute the reaction solution to 500 mL by adding water. We ran DPPC vesicles in the flow reactor in which the high temperature chamber was maintained at 180 °C while the low temperature chamber at 0 °C with its pH about 6.5. In fact, an evolutionary significance of phospholipid vesicles is in that they can be formed even at a temperature as low as 0 °C. The

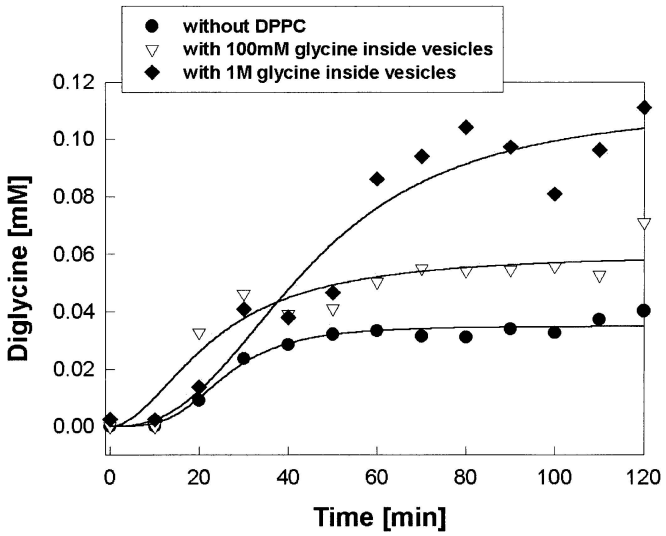

Figure 6. Time developments of the yields of diglycine in the reaction solution in the presence of DPPC vesicles. Although the reaction solution was prepared to be 100 mM glycine, the concentration of glycine inside the DPPC vesicles was set 1 M. For comparison, the results for 100 mM glycine both inside and outside the DPPC vesicles and for only 100 mM glycine with no DPPC are also displayed.

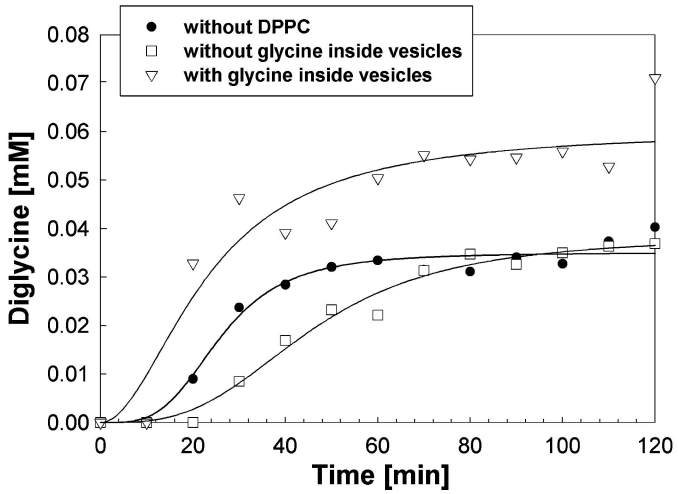

Figure 7. Time developments of the yields of diglycine in the reaction solution of 100 mM glycine in the presence of DPPC vesicles, while the vesicles were prepared in the absence of glycine and then transferred into the 100 m Mglycine solution. The results for 100 mM glycine both inside and outside the DPPC vesicles and for only 100 mM glycine with no DPPC are also displayed.

yields of diglycine with use of DPPC vesicles of 1 M glycine encapsulated in their inside are depicted in Figure 6. The results of the cases for 100 mM glycine inside DPPC vesicles and for only 100 mM glycine with no DPPC are also displayed for comparison. Enhancement of the yields of diglycine with the increase of the concentration of reacting monomers inside the phospholipid vesicles demonstrates that oligomerization of glycine efficiently proceeded inside the vesicles.

The volume effect could also be confirmed by examining the reaction solution of 100 mM glycine into which DPPC vesicles prepared in the absence of glycine were transferred later. The results are displayed in Figure 7. There was no significant difference between the absence of DPPC vesicles present in the solution and the absence of glycine inside DPPC vesicles, as indicating that the lipid vesicles could remain pretty stable in the high temperature region even around 200 °C if the residence time there is limited as in our flow reactor. Such high-temperature stability was also confirmed with both decanoic acid and oleic acid vesicles (data, not shown). This supports the observation that oligomerization can be enhanced in the presence of lipid vesicles if monomers to be oligomerized are sufficiently encapsulated.

4. Discussion

Lipid vesicles made of saturated fatty acids such as decanoic acid could readily have been formed in prebiotic hydrothermal environments on the primitive Earth. Decanoic acid vesicles could be formed and then decomposed to some extent

repeatedly as the reaction fluid including decanoic acid visits each of cold and hot regions in the vicinity of hydrothermal vents in a recycling manner. Encapsulation of monomers to be oligomerized in the lipid vesicles, the latter of which may also be formed at the same time, could certainly have enhanced their oligomerization compared to the cases otherwise. However, decanoic acid vesicles have their own limitations. Decanoic acid vesicles can be formed only above the melting point of the acid about 32 °C, though stable in the solid phase down to 23 °C (Apel, 2003), and in the extremely low concentration of divalent ions or in their total absence (Monnard *et al.*, 2002). Enhancement of oligomerization of monomers encapsulated inside decanoic acid vesicles could have been likely only when the temperature of the cold regions surrounding hydrothermal vents in the ocean was above 32 °C and well before the concentration of metallic ions or ionic strengths in the surrounding seawater became high enough.

Since decanoic acid can easily be synthesized abiotically, for instance, via a Fischer-Tropsch-type reaction, it would be required to assess how robust the process of olygomerization of amino acids could be inside their vesicles. For this purpose, some quantitative aspects of the synthesis of oligoglycine inside the decanoic acid vesicles are displayed in Figures 8 and 9 on the effects of the temperature of the high-temperature region, of the concentration of amino acid glycine and of the concentration of decanoic acid. We thus observed that the enhancement of

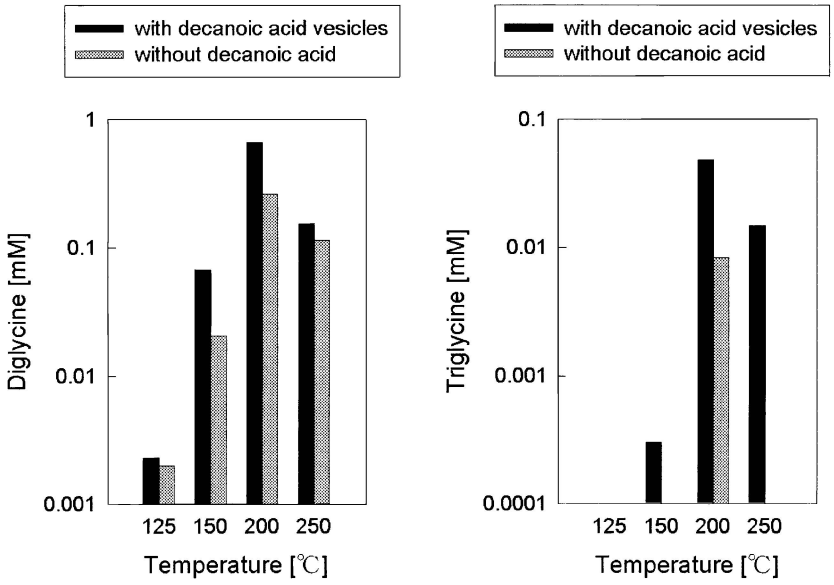

Figure 8. Dependence of the yields of diglycine and triglycine on the temperatures of the high temperature chamber for both cases of presence and absence of decanoic acid vesicles. Comparisons were made at 120 min after the start of the flow-reactor operation, while other conditions were the same as with those in Figure 2.

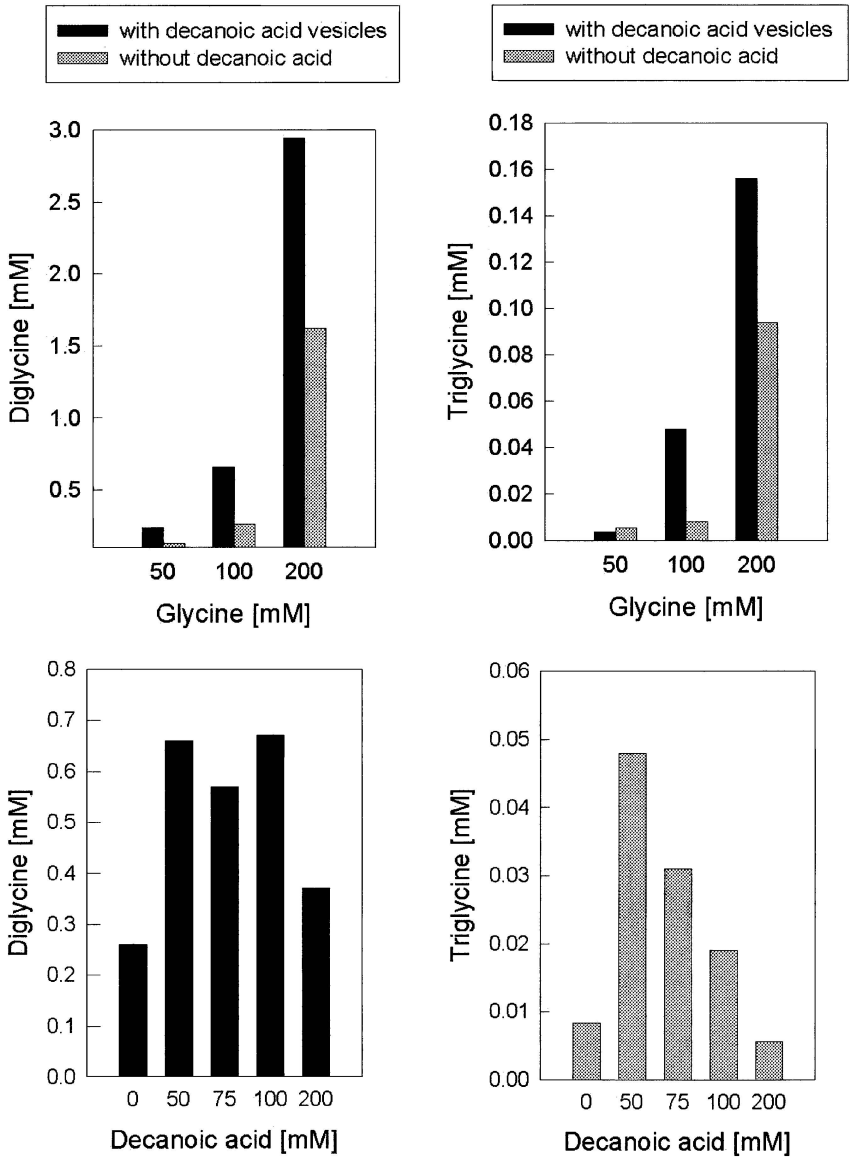

Figure 9. Dependence of the concentrations of glycine and decanoic acid on the yields of triglycine for both cases of the presence and absence of decanoic acid vesicles. Comparisons were made at 120 min after the start of the flow-reactor operation, while other conditions were the same as with those in Figure 2.

oligomerization of glycine could remain pretty robust even if the physical parameters characterizing the hydrothermal environments were varied to some extent.

The evolutionary successors to lipid vesicles of saturated fatty acids might have been those of unsaturated fatty acids such as oleic acid, whose melting point is

about 12 °C that is definitely lower than the melting point of decanoic acid. Oleic acid vesicles could have taken over decanoic acid vesicles as the temperature of the primitive ocean outside of hot hydrothermal vents became less than 32 °C if the synthesis of oleic acid out of its component molecules could become available in the neighborhood environments. When the temperature of the cold regions surrounding hot hydrothermal vents became further below 12 °C, the lipid vesicles that could tolerate such a cold environment must have been those of phospholipid such as DPPC.

From the prebiotic perspective of evolution, however, how the synthesis of unsaturated fatty acids and phospholipids could come into being remains to be seen. Nonetheless, lipid vesicles of saturated fatty acids as with decanoic acid vesicles are readily fabricated even in the current laboratory setup simulating prebiotic conditions. One issue remains to be clarified. How might vesicles of saturated fatty acids have furnished the conditions to encourage the evolutionary emergence and maintenance of unsaturated fatty acids and phospholipids?

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