Separated Organized Polymerization of an Amphiphilic Monomer and Acrylamide in One-Pot Reaction

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ABSTRACT: It has been first found that separated homopolymerization of an amphiphilic monomer, dodecylglyceryl itaconate (DGI), and acrylamide takes place even in one-pot radical reaction. The DGI molecules form a lamellar liquid crystal of bilayer membranes having the spacing distance of submicrometer and show the iridescent color in the presence of small amount of ionic surfactant. This colored bilayer system can be polymerized together with acrylamide and methylenebis(acrylamide) by photopolymerization to form a hydrogel containing the lamellar structure inside. In this polymerization process, the DGI molecules polymerize alone to form its homopolymer without reacting with any acrylamide and/or methylenebis(acrylamide) molecules. This novel phenomenon has been substantiated by the experimental techniques of SDS−poly(acrylamide) gel electrophoresis, ^{1}H NMR, and IR spectroscopy. This novel homopolymerization of DGI may be resulted from the preferential bond formation between DGI molecules in their organized molecular assembly and is quite interesting with special reference to the chemical reactions in biological systems. The present reaction system may provide a simplest artificial model for the biological reactions in the complicated organized molecular assembling systems.

Introduction

Chemical reactions in organized molecular assemblies are of special interest with reference to biological reactions in living systems. Protein synthesis in the ribosome is a good example of the above chemical reaction systems. Translation of the information on nucleic acid sequence in a m-RNA molecule onto the synthesized protein sequence proceeds in the sophisticated molecular assemblies of the ribosome. Chemical reactions in artificial molecular assemblies must be helpful in understanding the chemical processes in the complicated biological reaction systems.

Dodecylglyceryl itaconate (DGI), an amphiphilic monomer, forms an iridescent lamellar liquid crystalline phase in water in the presence of small amount of ionic surfactant and can be polymerized in this bilayer membrane system.[1−3] Furthermore, this iridescent liquid crystalline structure can be immobilized in hydrogels by means of polymerization of DGI together with gel-forming monomers such as acrylamide and methylenebis(acrylamide).[1−3] It is quite interesting question in this polymerization process whether the DGI molecules are linked with the gel-forming monomers residing in the aqueous phase of the liquid crystal. We have gotten the answer quite accidentally for this question during the experiments of gel electrophoresis using the above hydrogels containing the immobilized bilayer membranes. When the gel was used as the substrate for SDS−poly(acrylamide) gel electrophoresis (SDS−PAGE), the polymeric DGI forming the bilayer membranes was found to be flowed out of the gel. This result strongly suggests that the polymeric DGI molecules are not bound covalently with the polymer gel networks. NMR and IR analysis for the extracted polymer samples from the anode solution of the electrophoresis strongly supported the above assumption, i.e., no covalent bond formation between DGI molecules and gel networks. It is quite interesting to note that the DGI homopolymer can be obtained even in one-pot radical polymerization of binary and/or ternary monomer mixtures.

Experimental Section

Materials. Dodecylglyceryl itaconate (DGI; n-$C_{12}H_{25}OCO$-$CH_2C(=CH_2)COOCH_2CH(OH)-CH_2OH$) was synthesized by essentially the same procedures described in the previous work.[3] The crude product was applied twice to a silica gel column (Wacosil C-200; Wako Pure Chemical Co.) and eluted with a hexane/ethyl acetate mixture (4/6 in weight). The final product (mp = 62−63 °C) was proven to be more than 99% pure by NMR and HPLC analysis. Itaconic anhydride and pyridinium *p*-toluenesulfonate were purchased from Aldrich Chem. Co. 1-Dodecanol and an oil-soluble dye, Yellow AB (1-phenyl-azo-2-naphthylamine), were purchased from Tokyo Chemical Industry Co. Ltd. Monomers and a reaction accelerator for preparation of gel samples were acrylamide (AAm; Wako Pure Chemical Industries Ltd.), *N*,*N*′-methylenebis(acrylamide) (a cross-linker; Wako Pure Chemical Industries Ltd.), and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (an accelerator of polymerization reaction; Wako Pure Chemical Industries Ltd.). All the samples used in this work were guaranteed reagent grade and used without further purification.

Polymerization Procedures. As mentioned previously, DGI monomer molecules form an iridescent lamellar liquid crystalline phase in water in the concentration range of 1−2 wt % of DGI in the presence of small amount of ionic surfactant (0.2−2.0 wt % with respect to DGI).[1−3] In this work, we preferably use sodium dodecyl sulfate (SDS) as an ionic surfactant. This iridescent structure of DGI is maintained even in the aqueous solution of the monomers of acrylamide and *N*,*N*′-methylenebis(acrylamide). The iridescent solution of DGI containing any of the above monomer and cross-linker was photopolymerized by UV light using hydrogen peroxide or Irgacure 2959 as an initiator, and the lamellar structure of the polymeric DGI was immobilized inside the network of

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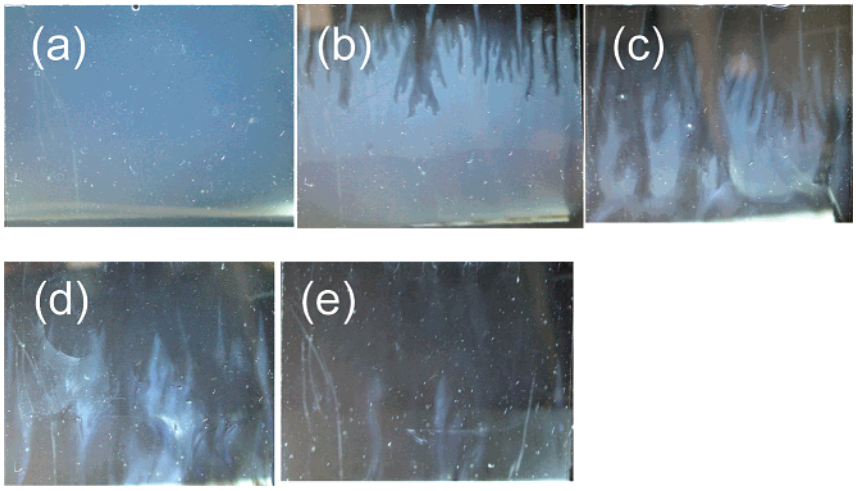

Figure 1. Polariscopic observation of the bilayer-membrane-immobilized hydrogel applied to SDS−polyacrylamide gel electrophoresis at some time intervals: (a) before gel electrophoresis experiment, (b) 1 h after starting gel electrophoresis, (c) 3 h, (d) 5 h, and (e) 7 h. Optical birefringence (the bilayer membrane structure) disappears from the cathode side during the gel electrophoretic experiment due to the solubilization of DGI polymers with SDS micelles.

acrylamide gel.[1−3] The iridescent color shifts to red side during the above polymerization process, since the surface area of the bilayer membranes decreases due to the bond formation between the DGI molecules, thus leading to the increase of the intermembrane distance.[1] Molecular weight of the DGI polymer was estimated by gel permeation chromatography to be about 100 000 when the DGI was polymerized alone (homopolymerization).[1]

To substantiate the separated homopolymerization of DGI in the mixed monomer solutions, we have prepared the DGI polymer samples in the following four kinds of systems.

Sample 1. DGI homopolymer was synthesized as the reference sample. The aqueous iridescent solution of 1.9 wt % DGI containing 0.5 mol % of SDS with respect to DGI was bubbled by argon gas and photopolymerized by UV light (high-pressure mercury lamp, SEN Light Co.; 1000 W) at about 55 °C for 1 h using 0.286 wt % H_2O_2 as an initiator. Polymeric DGI was precipitated out after the polymerization reaction was completed and was filtered. The crude sample was dissolved in chloroform and purified by reprecipitation method in the poor solvent of acetonitrile.

Sample 2. The mixed monomer solution of 0.928 g (∼2.5 mmol) of DGI (containing 0.5 mol % of SDS with respect to DGI) and 0.178 g (∼2.5 mmol) of acrylamide was polymerized in water by the same procedures as above. The precipitated DGI polymer was filtered and then dissolved in chloroform. The chloroform solution of DGI was filtered again to remove the insoluble materials such as SDS and poly(acrylamide). The filtrate was poured into a poor solvent, acetonitrile, to purify the polymeric DGI by reprecipitation. In this procedure, DGI monomer which was soluble in acetonitrile was removed.

Sample 3. DGI was polymerized together with acrylamide and methylenebis(acrylamide) to be immobilized in the poly(acrylamide) gel. The gel sample for SDS−PAGE was synthesized from 1.9 wt % of DGI (containing 0.5 mol % of SDS with respect to DGI), 12.0 wt % acrylamide, 0.08 wt % methylenebis(acrylamide), and 4.5 wt % Tris buffer in a gel electrophoretic cell constructed with two parallel quartz plates (8.0 × 8.4 cm) having a spacing of 1 mm. The polymerization procedures were the same as those for sample 1. The SDS−PAGE experiment was performed as described below using the above gel (sample 3) as the substrate. After the SDS−PAGE was carried out for 7 h, the anodic buffer solution was dried by evaporation as the 1-butanol azeotrope. The dried sample was dissolved into chloroform and filtered to remove the insoluble materials such as buffer components, poly(acrylamide), and SDS. The filtrate was purified by the same reprecipitation method as that for sample 2.

Sample 4. The mixture of DGI and acrylamide in tetrahydrofuran solution was polymerized. In this case, DGI molecules do not form any organized molecular assemblies like bilayer membrane, and two monomers are randomly mixed in the solution. One can expect that two monomers, DGI and acrylamide, are copolymerized. The mixed solution of 10 mL containing 0.928 g of DGI (∼2.5 mmol), 0.178 g of acrylamide (∼2.5 mmol), and 20 mg of AIBN as an initiator was bubbled by argon gas and then polymerized at about 70 °C for 24 h. After the solvent of tetrahydrofuran was evaporated, the crude sample was purified by the same reprecipitation method as mentioned above.

Measurements. SDS−PAGE was carried out at room temperature with a commercial electrophoretic apparatus (BIO CRAFT Type BE-230) utilizing the applied voltage of 280 V. The running buffer solution was 0.3 wt % Tris, 0.146 wt % glycine, and 0.3 wt % SDS for cathode buffer or 0.1 wt % SDS for anode buffer. To visualize the SDS migration in the gel during the SDS−PAGE experiment an oil-soluble dye, Yellow AB, was solubilized into SDS micellar solutions of the cathode buffer. One can see the movement of SDS micelles with yellowish color by the naked eye. NMR measurements were performed with a JEOL ECP-400 NMR apparatus (400 MHz). Each polymer sample of 20 mg (samples 1−4) mentioned above was dissolved in 1 mL of chloroform-d_1, and the solution was employed for NMR measurements. DGI monomer and poly(acrylamide) samples were also measured as the references in chloroform-d_1 and in water, respectively. IR measurements were carried out with an apparatus of Perkin-Elmer type FT-RT spectrometer SPECTROM 2000 utilizing the KBr tablet method.

Experimental Results

SDS−Poly(acrylamide) Gel Electrophoresis. The SDS−PAGE experiment triggered the present research work. We were making a research on the application of the acrylamide gels containing immobilized DGI bilayer membranes to the SDS−PAGE technique and found accidentally an unexpected phenomenon. Figure 1 shows the photographs under crossed polarizers (polariscope) of the gel used for SDS−PAGE at several time intervals. The portion of the gel containing the DGI bilayers looks bright due to the optical anisotropy of the lamellar liquid crystal. One can see that the boundary between bright and dark part of the gel moves from the cathodic to the anodic side as the SDS−PAGE proceeds. The dark part of the gel means that no anisotropic bilayer structure is present there. This result strongly suggests that the polymeric DGI molecules forming bilayer membranes are solubilized into water phase by SDS and flowed out of the gel by the electrophoretic migration.

Figure 2 supports this assumption. In these pictures the SDS micelles are visualized with yellowish color by utilizing the

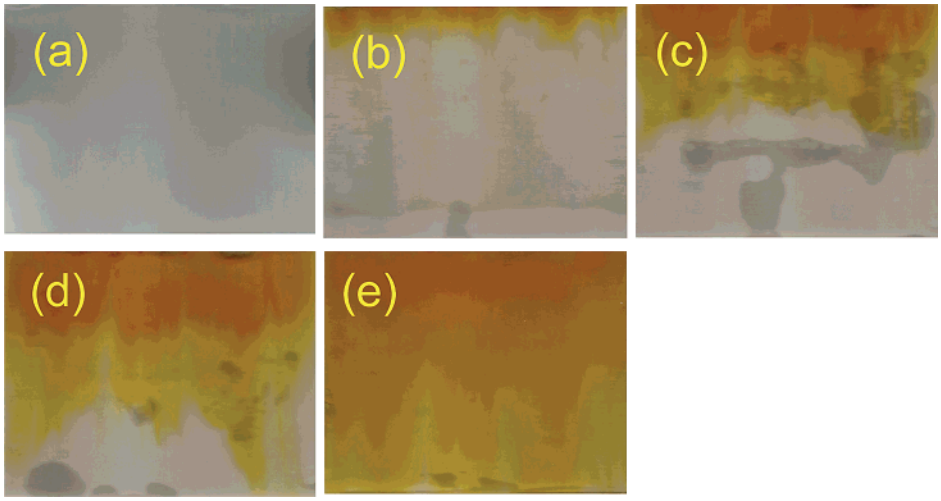

Figure 2. Photographs of the SDS migration into the bilayer-membrane-immobilized gel visualized by solubilized oil-soluble dye Yellow AB: (a) before gel electrophoresis experiment, (b) 1 h after starting gel electrophoresis, (c) 3 h, (d) 5 h, and (e) 7 h. SDS micelles penetrate into the gel from the cathode side due to the electrophoresis.

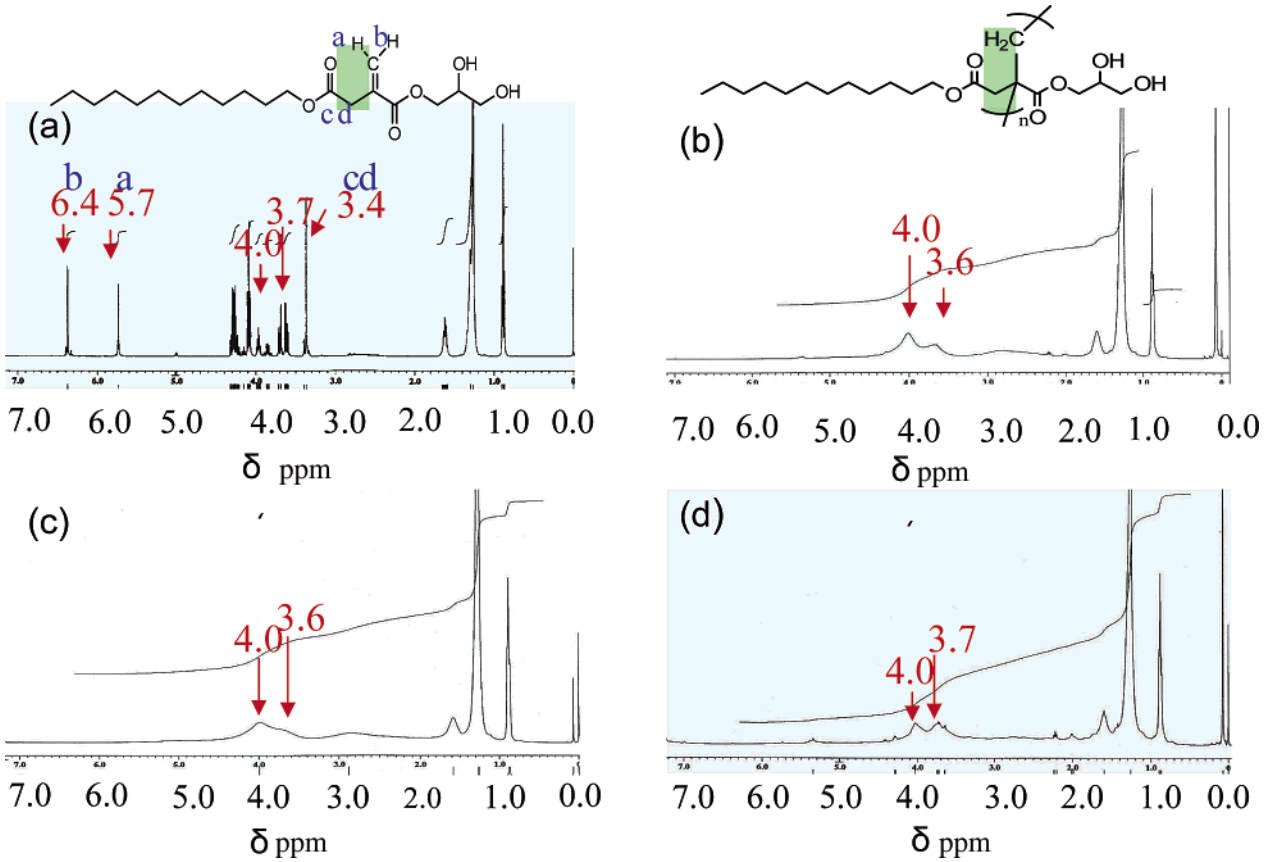

Figure 3. ^{1}H NMR spectra of DGI monomer and the polymer samples prepared by several different procedures: (a) DGI monomer, (b) DGI homopolymer (sample 1), (c) the polymer sample extracted from the reaction mixture of DGI and acrylamide in water (sample 2), and (d) the polymer sample extracted from the anodic buffer (sample 3).

solubilized oil-soluble dye, Yellow AB, in the micelles. The yellowish color (SDS micelles) migrates from the cathodic to the anodic side quite similarly to the movement of the bright and dark boundary in the polariscopic observation mentioned above. These two results of Figures 1 and 2 indicate that SDS micelles solubilize the polymeric DGI and carry it out of the gel to the anodic buffer solution by electrophoretic manner. This can be a strong evidence for no covalent bond formation between polymeric DGI and acrylamide gel networks. If this is not the case, it is impossible for the DGI polymer to be flowed out of the gel.

Identification of DGI Polymers by NMR and IR. To substantiate the above speculation, i.e., DGI forms a homopolymer not linking with poly(acrylamide) gel networks, the DGI polymer samples prepared by the various methods (samples 1−3 mentioned above) were identified by NMR and IR techniques. Figure 3 shows the NMR spectra of DGI monomer (a) and of the sample 1 (b), 2 (c), and 3 (d). One can see clearly that the NMR signals at 5.7 and 6.4 ppm due to the protons attached to the double bond in DGI monomer disappear in the polymer samples. In addition, the sharp signals of glyceryl group in DGI monomer at 3.7 and 4.0 ppm become broad after polymerization.

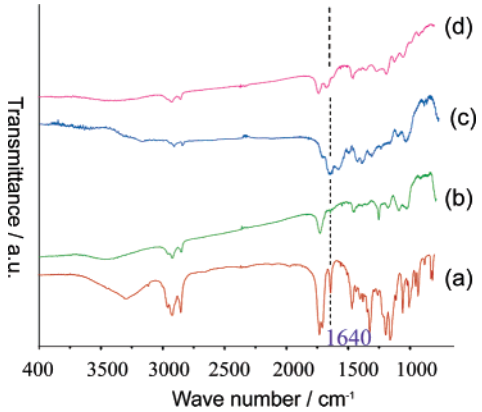

Figure 4. IR spectra of DGI monomer and the polymer samples prepared by several different procedures: (a) DGI monomer, (b) DGI homopolymer (sample 1), (c) the polymer sample extracted from the reaction mixture of DGI and acrylamide in water (sample 2), and (d) the polymer sample extracted from the anodic buffer (sample 3).

Furthermore, the polymer samples extracted from the reaction product of DGI/acrylamide mixture (sample 2) and from the anodic buffer solution (sample 3) show the quite similar spectrum to that of the DGI homopolymer (sample 1). It is then revealed that the DGI molecules form their homopolymers even in one-pot radical polymerization of two (with acrylamide) or three monomer components (with acrylamide and methylenebis-(acrylamide)).

IR spectra for samples 1−3 are shown in Figure 4. The results are basically the same as those of the NMR measurements. The polymer samples extracted from both samples of DGI/acrylamide mixture and the anodic buffer solution are again evidenced to be the DGI homopolymers.

Discussion

Separated Homopolymerization in Mixed Monomer Systems. As mentioned previously, we have first found that homopolymerization of DGI takes place even in the one-pot radical reaction of two and/or three monomer mixtures. Monomer composition in a polymer molecule prepared by copolymerization of a monomer mixture depends, of course, on the copolymerization reactivity ratio, r_1 and r_2, between the monomers.

$$r_1 = k_{11}/k_{12} \quad (1)$$

$$r_2 = k_{22}/k_{21} \quad (2)$$

where k_{ij} is the reaction rate constant of jth monomer with a polymer chain having the radical terminal of ith monomer. According to this copolymerization theory, r_1 (or r_2) has to be infinity and r_2 (or r_1) be 0 to obtain a homopolymer in mixed monomer systems. Unfortunately, we do not have any data of r_1 and r_2 for acrylamide and DGI and make an attempt to see them for similar monomers. The r_1 and r_2 values for dimethyl itaconate (monomer 1) and butyl acrylate (monomer 2) are 0.94 and 0.4, respectively,[4] which means that copolymerization between them should take place in one polymer molecule. Homopolymerization of DGI may also be impossible in the random radical polymerization of the mixed monomer solutions of DGI and acrylamide. We may be able to assume that this novel homopolymerization of DGI is resulted from the preferential bond formation between DGI molecules in their organized molecular assembly of bilayer membranes, as shown schematically in Figure 5.

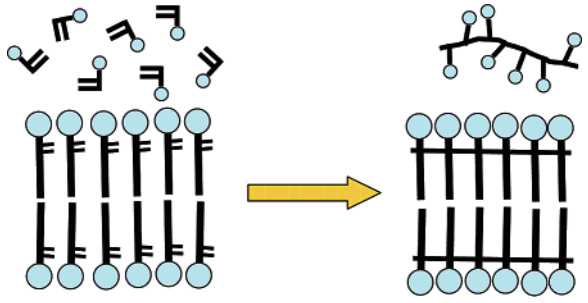

Figure 5. Schematic illustration of polymerization process of DGI and acrylamide in organized molecular assembly of bilayer membranes. A DGI molecule polymerizes preferentially with a neighbored same molecule and forms a homopolymer of DGI.

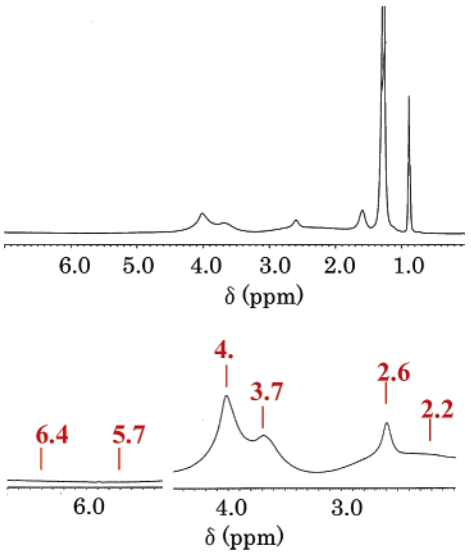

Figure 6. ^{1}H NMR spectrum of the polymer sample extracted from the reaction mixture of DGI and acrylamide in tetrahydrofuran (sample 4).

To substantiate this assumption, we have made one more experiment. Figure 6 shows the NMR spectrum of a polymer sample prepared by radical copolymerization of DGI and acrylamide in tetrahydrofuran (sample 4). One can see an additional peak this time at about 2.6 ppm which can be assigned to acrylamide residue. DGI molecules do not form bilayer membrane structure in organic tetrahydrofuran solvent, and the random copolymerization occurs in this system. We now recognize that the molecular assembly is quite important in chemical reactions.

Although organized polymerization reactions of amphiphilic monomers have been extensively studied so far,[5−21] these works deal with the immobilization of vesicle structures,[5−11] the characteristic polymerization of amphiphilic monomers in solid LB fims,[13−19] and the spontaneous polymerization of a polymerizable surfactant in micellar form,[20,21] etc. Separated organized polymerization to form a homopolymer in one-pot radical reaction has not yet been reported, and the finding in this work is quite new and interesting to note from the viewpoint of polymer chemistry.

A Model for the Chemical Reactions in Biological Systems. In many biological systems chemical reactions take place under the sophisticated molecular assemblies. For instance, the central dogma in biological science, i.e., the protein synthesis according to the information on DNA sequence, proceeds in a sophisticated molecular assembly of ribosome. Assembled multienzyme systems are essentially important in the successive reactions of biosynthesis such as fatty acids synthesis.[22] Our DGI system is

quite interesting also from the viewpoint of the above biological systems. Our system is much simpler compared with the biological ones but may provide an example to demonstrate the importance of molecular assembly for chemical reactions.

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