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Nucleobase Mediated, Photocatalytic Vesicle Formation from an Ester Precursor

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It is well-known that amphiphiles will self-assemble into nanostructures under the right conditions. Notably, many fatty acids spontaneously form vesicles in water when the solution pH is adjusted close to the pK_a of the acid. This ability and their simple synthesis by Fischer−Tropsch reactions[1] under prebiotically plausible conditions have led to the suggestion that fatty acids preceded phospholipids as a primitive cellular (or cellular-like) membrane.[2−4]

Our interest is in the production of amphiphiles from precursor molecules and their subsequent self-assembly into vesicles because the dynamics of such a system may give insight into the types of chemical processes and couplings that could occur under prebiotic conditions. We have designed a chemical system in which fatty acid amphiphiles are generated from precursor molecules by visible light photolysis.

We report the use of photoinduced electron transfer to drive reductive cleavage of an ester to produce bilayer-forming molecules; specifically, visible photolysis in a mixture of a decanoic acid ester precursor, hydrogen donor molecules, and a ruthenium-based photocatalyst that employs a linked nucleobase (8-oxo-guanine) as an electron donor generates decanoic acid (Figure 1).[5,6] The overall transformation of the ester precursor to yield vesicles represents the use of an external energy source to convert nonstructure forming molecules into amphiphiles that spontaneously assemble into vesicles. The core of our chemical reaction system uses an 8-oxo-G-Ru photocatalyst, **2a**, a derivative of [tris(2,2′-bipyridine)-Ru(II)]$^{2+}$.

To understand the thermodynamics of this central catalytic reaction, one must consider the photoredox properties of [Ru(II)(bpy)$_3$]$^{2+}$, which are summarized in Figure 2 in the context of the chemistry employed in our system.[7] It is well-known that visible photoexcitation of [Ru(II)(bpy)$_3$]$^{2+}$ generates a metal-to-ligand charge transfer (MLCT) state that is both a better oxidant and reductant than the ground state.[7] This excited state has been used by many researchers to initiate electron transfer reactions.[8] Our system is designed to proceed via a reductive quenching pathway from the excited state (the left side of the diagram in Figure 2) and proceeds only if the donor possesses an oxidation potential less negative than ~ -1.0 V.

Guanine, the most easily oxidized conventional nucleobase, is not a sufficiently strong electron donor to provide an electron to the Ru MLCT excited state.[9] However, 8-oxo-G satisfies this requirement.[10] We attached (see Figure 1, compound **2a**) the 8-oxo-G to one of the bipyridine ligands via an electronically insulating alkane bridge to avoid significant changes in the redox potential from that of the parent [Ru(II)(bpy)$_3$]$^{2+}$ complex. An electron must be provided by the 8-oxo-G moiety (Figure 2, Reaction 1) generating the reactive element [Ru(II)(bpy^0)$_2$(bpy^{-1})]$^{1+}$ before the cleavage reaction can proceed. Once generated, the Ru^{1+} complex can then transfer charge to the picolinium ester providing carboxylic acid in the presence of a hydrogen source (Figure 2, Reaction 2).

Photolysis of a reaction mixture using visible light with all the components present gives substantial conversion to products within 24 h. The initial reaction mixture is a solution without visible structures (Figure 3A). As the photolysis proceeds, the solution becomes opalescent suggesting that large amphiphile structures are forming. Examination of these solutions via epifluorescence microscopy reveals that, at low conversions (<20 to ∼30%), oil-in-water emulsion compartments begin to form. By the time ∼44% conversion is achieved (Figure 3B), they are well established. When the critical vesicular concentration (CVC) is surpassed at ∼45−50% conversion, significant numbers of bilayer vesicular structures are observed along with oil-in-water emulsion compartments (Figure 3C). We observe that the CVC for the mixture of precursor ester and decanoic acid (DA) is considerably less than that of pure DA but have not observed the formation of vesicles with the precursor by itself under any conditions. At ≳65% conversions (Figure 3D) nearly all the structures that are observed are bilayer vesicles or tubules. These epifluorescence micrographs clearly show the evolution of the initially structureless system to one containing large numbers of membrane bilayer vesicles, demonstrating the spontaneous formation of organized structures from the precursor molecules. When the same experiment was performed using the guanine analogue of the photocatalyst (Figure 3E, 3F), no structures are observed with the exception of some of the fatty acid precursor that "phase-separated" from solution.

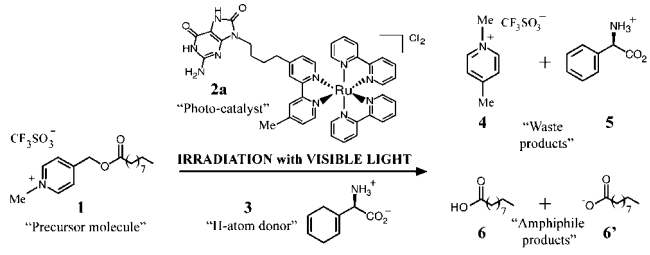

Figure 1. A summary of the nucleobase-mediated photochemical production of decanoic acid from its picolyl ester precursor.[5,6] The precursor **1** is dispersed in an aqueous buffered solution containing the electron donor linked photocatalyst **2a** and the dihydrophenylglycine hydrogen donor **3**. Upon irradiation, fatty acids [**6** protonated and **6′** deprotonated] form and self-assemble into membranous structures once the critical vesicle concentration of the precursor/fatty acid mixture is reached. Two "waste" compounds, *N*-methyl picolinium **4** and phenylglycine **5**, are also produced.

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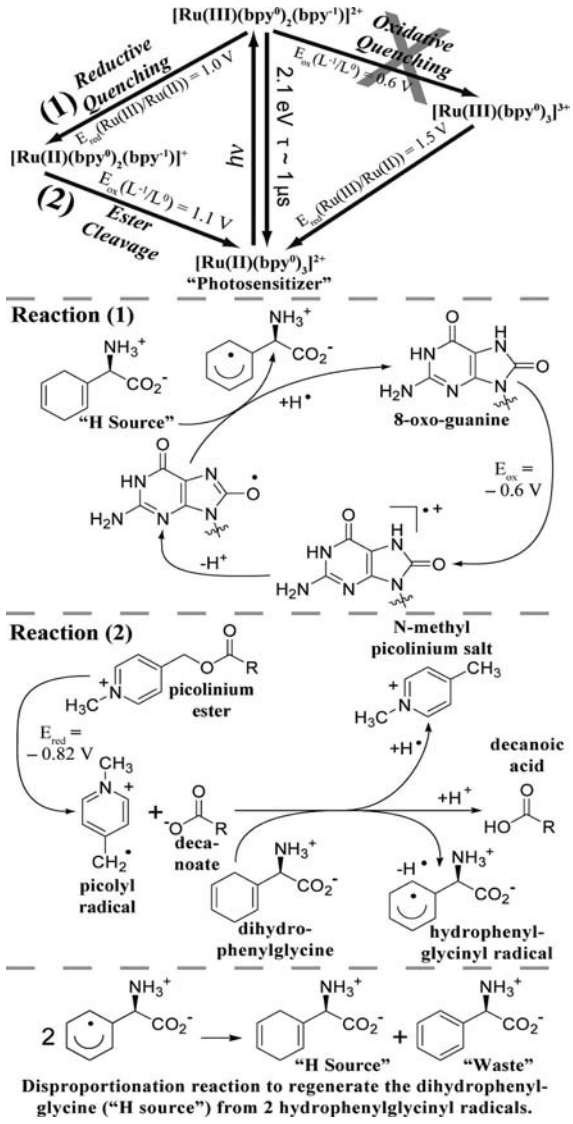

Figure 2. A summary of the reductive and oxidative pathways for the $[Ru(II)(bpy)_3]^{2+}$ complex showing the redox potentials and reactions of the photocatalyzed ester cleavage reaction. Only the reductive quenching pathway is thermodynamically feasible. All electrode potentials are relative to NHE from Vlcek et al.[5−7,10]

When the reaction was monitored using ^{1}H NMR spectroscopy (Figure 4), the clean conversion of a 15 mM concentration of precursor ester to the final products of the reaction was seen when using only a 1 mM concentration of the 8-oxo-G-Ru catalyst. Also, the NMR signals at ∼0.85, 1.25, and 1.60 ppm arising from the H atoms on the alkyl chains of the decanoate groups that are produced show significant broadening at ∼40% conversion, which becomes more pronounced as the reaction continues. At high conversions, these NMR signals are nearly broadened into the baseline as is expected if the alkyl chains of the amphiphile products have restricted motion due to their incorporation into organized structures.[11] These structures are destroyed when the reaction mixture is diluted with water (D_2O) such that the concentration of decanoic acid falls below its CVC (trace f, Figure 4).

We also performed the photocatalytic reaction using a 405-nm laser and monitored the reaction with FTIR spectroscopy. This system is more amenable to quantitative analysis of the changing concentrations of the reactants than the NMR experiments and has allowed us to collect reproducible kinetic data for the reaction. For the FTIR experiment, a short-path length cell (<30 μm) was used

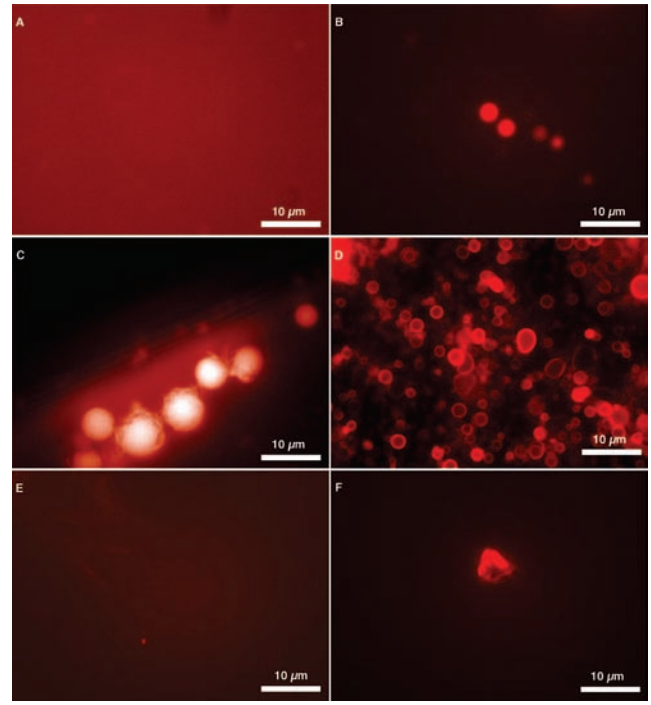

Figure 3. Epifluorescence micrographs of aggregate formation mediated by the photolysis reaction. All reaction samples are stained with 2.5 μM Nile Red, which strongly associates with hydrophobic molecular regions and permits the visualization of bilayer structures (D). The epifluorescence micrographs were taken under UV-illumination and observed through a 550−560 nm filter. (A−D) The 8-oxo-G linked photocatalyst: (A) at t = 0 h with 0% conversion; (B) after 6-h irradiation with ∼44% conversion, oil−water emulsion structures likely coated by fatty acid are freely moving in the medium; (C) after 8-h irradiation with ∼47% conversion, both emulsion and membranous structures are observed. Note the apparent shedding of material from the emulsion structures; (D) after ∼65% conversion at 24 h. Vesicles and other membranous structures are already readily apparent at ∼50% conversion (not shown). (E and F) The guanine analogue of the photocatalyst: (E) at t = 0 h with 0% conversion; (F) at t = 24 h. The bright spot visible is some of the fatty acid precursor that has "phase-separated" from solution.

because the resulting limited attenuation of the excitation beam yields an essentially flat excitation profile. This change results in an increased reaction rate relative to that of the NMR experiments, giving 50% conversion within 3 h. The results from two experiments using this short-path length cell with the decanoic acid precursor are shown in Figure 5, and the results from several other experiments are found in the Supporting Information (SI).

Several conclusions can be drawn from the kinetic data. Most importantly, when **2a** is used, the reaction proceeds smoothly, cleanly, and catalytically. When we attempted to use the analogous guanine linked complex (G-Ru) as the photosensitizer, only a small amount of decanoic acid was produced. The generation of *N*-methyl picolinium carbinol (5−7% conversion) as indicated by NMR results when the guanine complex was used as a photosensitizer suggests that the slow background hydrolysis of the ester becomes the predominant reaction in the guanine case. These results demonstrate that **2a** is essential for the reaction to occur and that the G-Ru species shows no catalytic rate enhancement, essentially duplicating the results obtained from the Ru sensitizer with no nucleobase in the system. Furthermore, the reaction is clearly dependent upon the presence of the Ru complex and a specific nucleobase (8-oxo-G).

The initial quantum yield for the reaction was determined to be 0.44% in the laser photolysis experiments of Figure 5 (full details in SI). Given that the reaction slows with time, this quantum yield

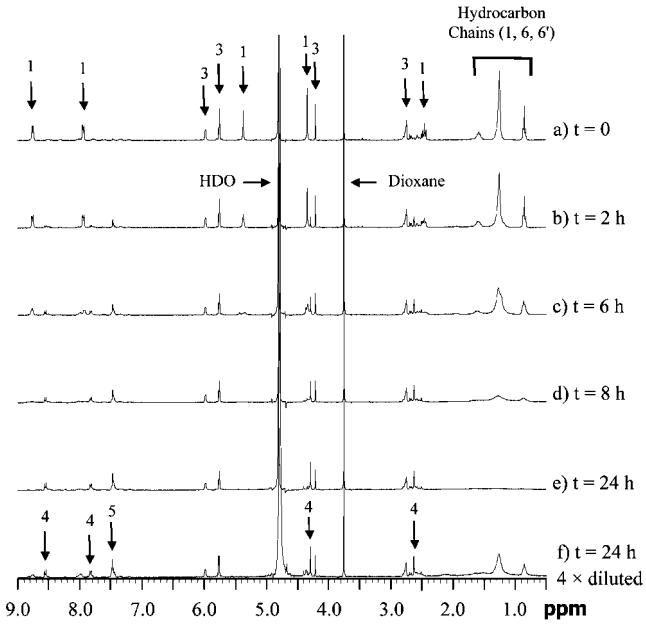

Figure 4. Photolysis of a 15.0 mM solution of ester precursor, with 1.0 mM of the nucleobase−catalyst complex, and 15.75 mM dihydrophenylglycine in D_2O (100 mM phosphate buffer at pD = 7.0) when illuminated with a 150 W tungsten spotlight, followed by ^1H NMR for different durations of exposure to light. The samples were photolyzed in a water bath maintained at 20 °C. All traces are normalized to the same dioxane peak integral value. For different times, the conversions are (a) 0%, (b) ∼22%, (c) ∼44%, (d) ∼47%, and (e) ∼65%. The bottom trace (f) shows the ^1H NMR of the same material as in (e), but after dilution and with the signal strength scaled to regain the initial dioxane calibration signal strength. The dilution destroys the vesicular structures allowing the alkyl signals from amphiphile products to return to the spectrum. Compound numbers refer to Figure 1.

is an upper limit. Other experiments using HPLC analysis of the product mixtures show that >90% of the Ru catalyst remains after 24 h of reaction. We have not explicitly measured the luminescence quantum yield of the catalyst or the guanine derivative but have observed that the luminescence lifetimes of these complexes are the same as that of the $[Ru(II)(bpy)_2(4,4'Me_2bpy)]^{2+}$ model complex at room temperature in solution. This suggests that quenching by the oxo-G moiety is inefficient and is consistent with the observed low quantum yield.

In summary, we have devised and demonstrated a chemical process in which an 8-oxo-G molecule directly mediates the photochemical conversion of an ester precursor into a fatty acid product that self-assembles into a vesicle. This is in contrast to earlier work in which a simple direct photolysis of a precursor resulted in vesicles.[12] The overall production of fatty acid molecules leading to the spontaneous formation of vesicles presented here is driven by an external energy source rather than by supplying activated components that spontaneously react to yield vesicle building blocks. The net result is a chemical reaction system that is controllable and creates structures capable of growth.

In order for this chemical system to exhibit more of the necessary functions of a self-replicating network, several further steps will clearly be necessary.[13] All the species must be colocated into/onto the container, the 8-oxo-G molecule must be incorporated into an oligomer or polymer with a base sequence that has the potential for templated replication[14] and kinetic regulation of the photolysis

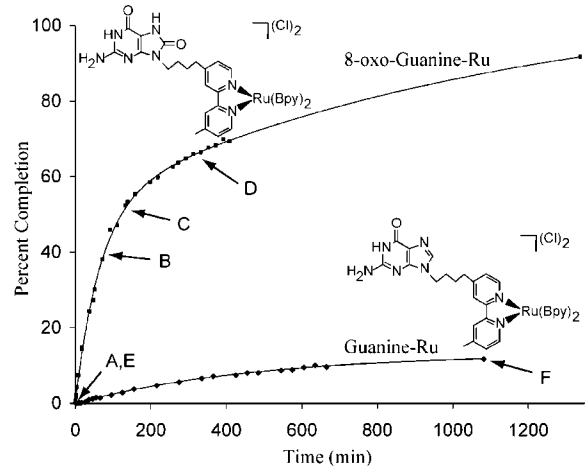

Figure 5. Kinetics of the reaction of decanoate precursor in the presence of 8-oxo-G-Ru catalyst (■) or G-Ru complex (◆). Conversion is calculated using the loss of the absorbance due to the ester carbonyl stretching vibration at 1740 cm^{-1}, an area free of interfering absorbance changes. There is no reaction beyond background with the guanine Ru complex. Labels A−F correspond to the same conversion levels shown in the images of Figure 3 but occur at different times due to changes in the experimental setup (light intensities, wavelengths, and pathlengths).

chemistry, and the system must continue to function under these conditions. Our ongoing research efforts are striving to address these issues.

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Supporting Information Available: A description of the synthetic procedures and physical data for the new compounds including NMR spectra is provided along with descriptions of the FTIR analyses used to monitor reaction progress. This material is available free of charge via the Internet at http://pubs.acs.org.

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