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Regulation of a cerium(IV)-driven O_2 evolution reaction using composites of liposome and lipophilic ruthenium complexes†

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A composite containing a liposome and a lipophilic ruthenium complex was synthesized to regulate an O_2 evolution reaction using cerium(IV) ammonium nitrate as an oxidizing reagent. We found that the surrounding environment of the reaction centre is an important factor for controlling the O_2 evolution catalytic reaction. We successfully regulated the reaction activity using the linker length of the lipophilic ligand and using the head groups of the phospholipid component.

Sophisticated design of molecular catalysts for medical and industrial applications has been a long-standing goal in the field of catalytic chemistry. While molecular catalysts are designable for high activity and selectivity by adjusting the substituents of their ligands, such designs often require complicated organic synthesis. Furthermore, the low solubility of molecular catalysts interferes with their applications to "green chemistry". One of the promising methods to overcome these limitations involves deposition of molecular catalysts in a confined environment, such as a nanoreactor, constructed from synthetic and biological molecules.[1]

A spherical vesicle, such as a liposome, consisting of a phospholipid bilayer has four types of regions: a hydrophilic inner water phase, a hydrophobic lipid bilayer, and inner and outer surfaces. A hierarchical incorporation of different functional molecules can occur in these vesicles. Such spherical composites that integrate functional molecules are attractive for fabricating catalysts, sensors, drug delivery systems, and nanomaterials.[1,2] In addition, liposomes have advantages as "reaction spaces" because of the ease of controlling their size (nm–μm scale) and their surface charge from the various types of phospholipids that can be employed, and because of their high stability in environmentally friendly aqueous solutions. Hence, catalytic systems based on liposomes, used in organic conversion,[3,4] electron transfer,[5] photocatalytic water oxidation,[6] and hydrogen production,[7,8] have been reported. Previous researchers have succeeded in utilizing lipid membranes as platforms for reactions, whereas regulation of the reactions has been addressed using functional molecules themselves, and not by the environment on the membrane surface.

Here, we report on the preparation of new liposome composites with metal complexes and on a strategy for regulating their catalytic reactions (Fig. 1a). For the functionalization of the liposome surface, we designed new lipophilic metal complexes and succeeded in fixing them on liposomes. In this work, O_2 evolution from water was selected because of its potential application in artificial solar energy conversion and storage.[9–11]

To fix the oxygen-evolving mononuclear ruthenium complex $[Ru(terpy)(bpy)(OH_2)]^{2+}$ (Ru core unit = **cRu**) on the liposome surface,[12,13] two lipophilic Ru complexes having cholesterol derivatives were designed: $[Ru(terpy)(L1)(sol)]^{2+}$ (**1**, L1 = cholesterol 2,2′-bipyridine-5-ylmethylsuccinate) and $[Ru(terpy)(L2)(sol)]^{2+}$ (**2**, L2 = cholesterol 2,2′-bipyridine-5-carboxylate) (Fig. 1b). The high affinity of the cholesterol moiety for phospholipids allowed **1** and **2** to become fixed tightly onto the lipid bilayer.[14] Different linker lengths between bpy and the cholesterol moieties were designed to adjust the position of the Ru cores on the liposome surface and to influence the accessibility of oxidizing reagents. Compounds **1** and **2** were synthesized as described in the ESI.† The UV/Vis spectra of **1** and **2** in acetonitrile were identical to that of the precursor **cRu** (Fig. S1†).[12] The absorption band observed around 460 nm corresponded to the metal-to-ligand charge transfer (MLCT) band. Cyclic voltammograms of **1** and **2** in acetonitrile showed redox couples from Ru(II)/Ru(III) at $E_{1/2}$ = 0.91 and 0.94 V *vs.* Fc/Fc^+, respectively (Fig. S2†). In addition, cyclic voltammograms of **1** and **2** in an aqueous 0.5 M H_2SO_4 solution showed that

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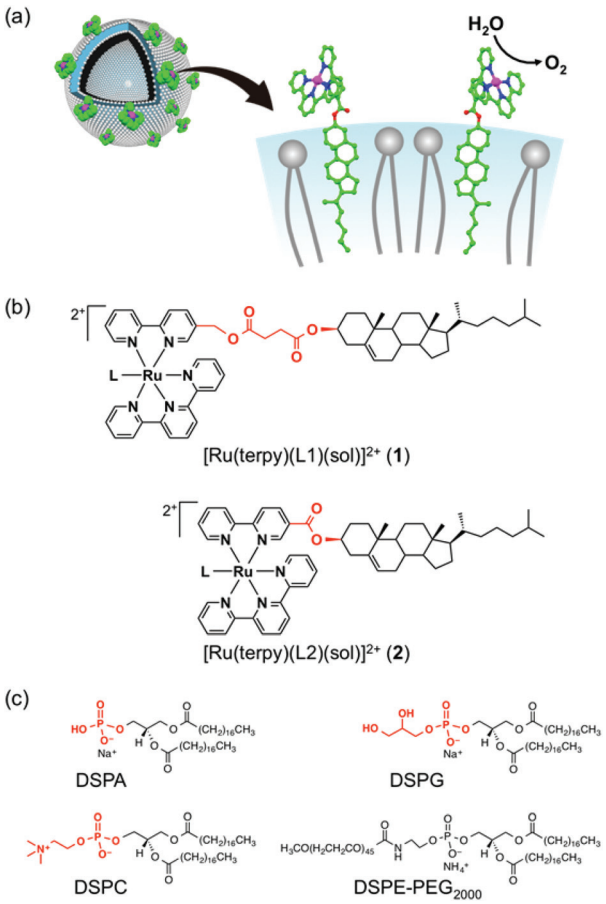

Fig. 1 (a) A schematic representation of the composite of a liposome and a lipophilic Ru complex. The molecular structures of (b) **1** and **2** and (c) the phospholipids used.

electrocatalytic oxygen evolution from water occurred at potentials of *ca.* 1.3 V *vs.* Ag/AgCl (Fig. 2a). These results show that the properties of the prepared lipophilic Ru complexes were the same as that of **cRu**.

We succeeded in crystallizing an analogue of **2**, [Ru(terpy)(L3)(CH$_3$CN)](PF$_6$)$_2$ (L3 = cholesterol 5′-methyl-2,2′-bipyridine-5-carboxylate). The crystal structure shows acetonitrile occupying a reaction site on the Ru core and the relative disposition of the coordinated acetonitrile and the pyridyl ring bearing the cholesterol substituent being in a trans configuration (Fig. 3). This implies that the reaction sites of **1** and **2** were fixed in the lipid bilayers, facing the outer water phase of the liposomes, and oxidants and water molecules could easily access the Ru cores.

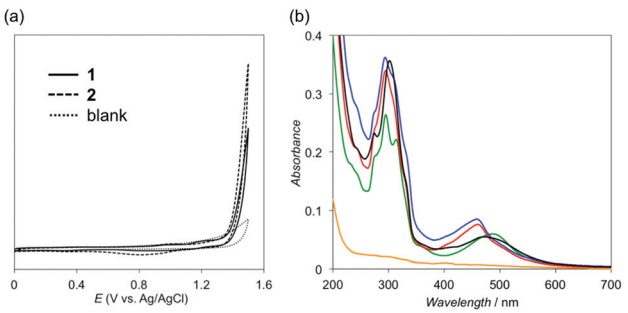

Fig. 2 (a) Cyclic voltammograms of **1**, **2** and a blank solution in aqueous 0.5 M H_2SO_4 under a N_2 atmosphere. (b) UV-visible spectra of **1**·PA (green), **1**·PC (blue), **1**·PG (red), and **2**·PG (black) in H_2O (Ru concentration = 6 μM).

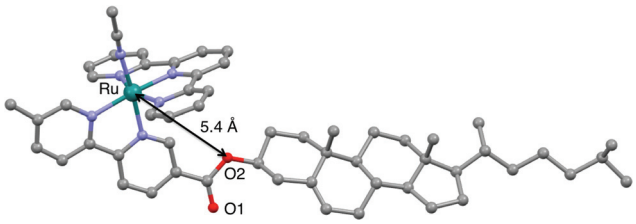

Fig. 3 The crystal structure of [Ru(terpy)(L3)(CH$_3$CN)](PF$_6$)$_2$.

The type of phospholipid used is important in the design of the reaction space surrounding the Ru cores on the liposome surface. Three phospholipids were used in this work: the negatively charged lipids 1,2-distearoyl-*sn*-glycero-3-phosphate (DSPA) and 1,2-distearoyl-*sn*-glycero-3-phospho-(1′-*rac*-glycerol) (DSPG), and the zwitterionic lipid 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC) (Fig. 1c). The size and charge of the phospholipid head group was expected to influence the packing and surface charge of the composites, thereby regulating the accessibility of oxidants to the Ru cores. To stabilize the liposomes under the reaction conditions used (high ionic strength and acidic solutions), phospholipids with a stearoyl group were chosen. Yasuda *et al.* reported that liposomes containing phospholipids having saturated and relatively long fatty acid chains are stable in acidic solutions.[15] In addition, 3.6 mol% of 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[methoxy(polyethylene glycol)-2000] (DSPE-PEG$_{2000}$) was added to each composite to reduce any aggregation and fusion of the liposomes.[16]

Liposome composites were prepared using a lipid thin film hydration method with a 9 mol% chloroform solution of **1** or **2** followed by sonication (Table 1). Formation of the composite was confirmed directly using confocal laser scanning microscopy (CLSM), in which a giant unilamellar vesicle was used for visualization (Fig. S3†), and the contents of **1** and **2** were determined using inductively coupled plasma atomic emission spectroscopy. The UV-Vis spectra of the samples are shown in Fig. 2b. **1**·PC and **1**·PG exhibited an MLCT absorption maximum at 457 nm, which is almost the same as that of **1** in acetonitrile. However, the MLCT absorption maximum in **1**·PA and that in **2**·PG were red-shifted to 495 nm and 472 nm when compared with that of **1** (at 456 nm) and **2** (at 465 nm) in acetonitrile, respectively. The Ru cores of **1**·PA and **2**·PG could be influenced by the phosphate groups of DSPA and by those of DSPG, because the MLCT band of cRu in 50 mM Na_2HPO_4 showed a shift similar to those of **1**·PA and **2**·PG (Fig. S4†).

Table 1 $Z_{average}$ diameter and zeta potential of the liposome composites

Composite	Lipophilic Ru complex[a]	Phospholipid[b]	$Z_{ave.}$ diameter (nm)	Zeta potential (mV)	Turnover number after 12 h
1·PA	**1**	DSPA	65.1	−56.5	133
1·PC	**1**	DSPC	108.1	30.5	118
1·PG	**1**	DSPG	41.6	−49.9	145
2·PG	**2**	DSPG	56.7	−50.8	53
PA	—	DSPA	74.4	−78.5	—
PC	—	DSPC	63.3	−36.3	—
PG	—	DSPG	50.7	−70.1	N.D.

[a] The concentration of the lipophilic Ru complexes was 9 mol%. Liposomes without lipophilic Ru complexes contained 9 mol% of cholesterol instead of **1** and **2**. [b] All composites contained 3.6 mol% DSPE-PEG_{2000}.

The average particle size of the composites was measured using dynamic light scattering (Table 1). The $Z_{ave.}$ diameters of **1**·PA, **1**·PG, and **2**·PG were in the range 40–65 nm, and were almost the same as that of the liposomes without lipophilic Ru complexes, except for **1**·PC. The average particle size of **1**·PC (108 nm) was about twice as large as that of PC. Because larger liposomes commonly have a smaller degree of curvature and have a more closely packed lipid bilayer,[17] the Ru complex moieties of **1** in **1**·PC could interact electrostatically with DSPC head groups. Furthermore, the fixation of the lipophilic Ru complexes on the liposome surface was confirmed from the zeta potential. The zeta potentials of all the liposome composites were more positive than those of the non-complexed liposomes (Table 1). This observation is attributed to the positive charge of the Ru core of **1** and of **2**. The zeta potentials of the composites depended on the type of phospholipid head group. **1**·PC containing zwitterionic DSPC was determined to have a positive surface charge (30.5 mV), although other composites had a negative surface charge. This result suggests the liposome surface as the location of the Ru cores, and that the charges surrounding the Ru cores were controlled by the types of phospholipid head groups.

The evolution of O_2 was examined at 20 °C under an Ar atmosphere in the presence of 195 mM $(NH_4)_2[Ce(NO_3)_6]$ as an oxidant and 0.08 mM of the liposome composites (Fig. 4). The stability of the composites under the reaction conditions was confirmed using CLSM. The reaction between **1**·PG and 40 equiv. of $(NH_4)_2[Ce(NO_3)_6]$ was monitored spectrophotometrically (Fig. S5†). The spectrum of the mixture after 12 h showed the bleaching of the MLCT band, corresponding to the oxidation of the Ru^{II} species by Ce^{4+}.[12] After addition of an excess of ascorbic acid to the reaction mixture, the MLCT band of **1** was recovered.[12] In addition, the reaction mixture evolved a negligible amount of carbon dioxide during the reaction, which was confirmed by gas chromatography. These results provided good corroboration for the robustness of **1**·PG during the O_2 evolution reaction. **1**·PG exhibited the highest catalytic activity, which was of the same order as that of **cRu**.[12] The total amount of O_2 evolved by **1**·PG was 23.2 μmol, which is 2.7 times greater than that evolved by **2**·PG. **1**·PG and **2**·PG have comparable surface environments regarding $Z_{ave.}$ diameter and zeta potential; the difference in catalytic reactivity can thus be explained by the location of the Ru cores on the liposome surface, which was adjusted by the linker lengths of L1 and L2. The distance between the Ru and the ester oxygen atom O2 in the crystal structure of $[Ru(terpy)(L3)(CH_3CN)](PF_6)_2$ (analogue of **2**) is *ca.* 5.4 Å, which is less than the thickness of the PG head group (*ca.* 9 Å) (Fig. 3).[18] Thus, the Ru core of **2**·PG would be embedded in the head group region, resulting in a reduced accessibility of Ce^{4+} ions. In addition, the O_2 evolution behaviour of these composites changed depending on the types of phospholipids. The activities of **1**·PG and **1**·PA, whose surfaces are net negatively charged, were significantly higher than that of **1**·PC, whose surface is net positively charged. In the case of **1**·PA, the O_2 evolution began without an induction period. To help understand the initial process of the reaction, the reaction between the composites and 40 equiv. of $(NH_4)_2[Ce(NO_3)_6]$ was monitored by UV-visible spectroscopy (Fig. S6†). Soon after mixing **1**·PA with Ce^{4+}, the spectrum showed rapid bleaching of the MLCT band. In contrast, the MLCT bands of **1**·PC and **1**·PG gradually decayed. This means that the relatively small and negatively charged head group of PA is effective in promoting the accessibility of Ce^{4+} ions to the Ru core. This result suggests that (1) the location of the Ru cores

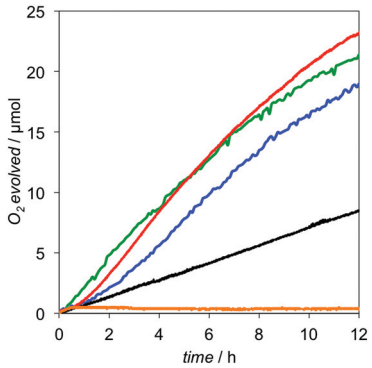

Fig. 4 The evolution of oxygen from an aqueous 195 mM $(NH_4)_2[Ce(NO_3)_6]$ solution in the presence of **1**·PA (green), **1**·PC (blue), **1**·PG (red), **2**·PG (black), and PG (orange).

is on the liposome surface, and (2) the charge of the phospholipid head group is an important consideration in the development of catalytic systems based on liposome space.

Conclusions

We fabricated O_2 evolution catalysts using liposomes and lipophilic ruthenium complexes. We found that the surrounding environment of the ruthenium reaction centre is an important factor controlling the O_2 evolution reaction. In particular, the position of the Ru cores on the liposome surface influences the accessibility of oxidizing reagents, and the reaction activity can be regulated by the linker length of the lipophilic ligand and by the phospholipid head group. This is a new method for regulating molecular catalyst systems. Compared with the reactivity of the photocatalytic water oxidation system using Ru complexes with vesicles reported by Hansen *et al.*,[6] our system showed lower catalytic reactivity than our system. Because the types of catalysts and reactions are completely different in these two systems, it is not easy to determine the reason for the different reactivity. One possible reason is that the lipophilic complexes were installed on the outer and inner surfaces in the current method. Because oxidants could not pass through the lipid bilayer, the complexes fixed on the inner surface do not participate in the reaction. The catalytic activity of the composites would be improved by establishing a method that selectively fixes the metal complexes on the outer surface.

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