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Catalytic photoinduced electron transport across a lipid bilayer mediated by a membrane-soluble electron relay

B. Limburg, E. Bouwman, S. Bonnet*

Unidirectional photocatalytic electron transfer from a hydrophilic electron donor encapsulated in the interior of a liposome, to a hydrophilic electron acceptor on the other side of the membrane, has been achieved using the simple membrane-soluble electron relay 1-methoxy-N-methylphenazinium (MMP⁺). The total amount of photoproduct (>140 nmol) exceeds the number of moles of MMP⁺ present (125 nmol), thus showing that the transport of electrons is catalytic.

To accomplish efficient artificial photosynthesis, two complimentary redox reactions must be fuelled by visible light irradiation. In both photoreactions the generation of a long-lived charge-separated species is required, and the greatest challenge in photocatalysis is to overcome charge recombination. In order to do so, the reduced electron acceptor and the oxidized electron donor must be physically separated. In green plants for example such separation is realized by large protein molecules embedded in the thylakoid membrane of chloroplasts. The dissymmetry of biological membranes allows for unidirectional electron transfer to occur from the lumen to the stroma. However, such photocatalytically active protein assemblies are highly complex and fragile, and artificial systems based on simpler, more robust molecules are required for building artificial devices capable of solar fuel production.

Among the numerous tools offered by supramolecular photocatalysis liposomes holds great promise. They offer a simple way to organize molecular components in space and mimic biological photosynthesis. By segregating electron donors and acceptors on each side of a lipid membrane, it should be possible to create long-lived charge-separated states that, in combination with two different catalysts, would allow for producing solar fuels. Electron transfer across the liposomal membrane to electron acceptors such as quinones, Co(bpy)₃³⁺ or viologens has been investigated intensively in the past. However, especially with viologens some ambiguity about the true nature of the charge separation was later revealed. It was shown that in those systems electron transport occurred due to diffusion of the reduced electron acceptor through the membrane, after which it remained occluded in the interior of the liposome. Thus, because the electron-transporting molecule never returned to its original location, it was unable to turn over for producing a true catalytic reaction. Up to now, there are only few systems known in the literature that catalytically transport electrons across membranes. However, all of them contain a complicated molecular structure to realize the charge separation across the membrane.

Herein, we report the truly catalytic unidirectional photoinduced electron transfer from the interior of a liposome to its exterior, based largely on commercially available compounds. This study is inspired by the widely used "WST-1" cell-counting assay, in which a water-soluble electron acceptor, the 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium anion (WST1⁻, Scheme 1), is reduced to the corresponding formazan dye (Fz1²⁻, Scheme 1) by addition of two electrons and one proton. Usually the electrons and protons are generated inside living cells in the form of nicotinamide adenine dinucleotide (NADH), which is too hydrophilic to cross the cell membrane. The WST-1 assay requires the membrane-soluble relay 1-methoxy-N-methylphenazinium cation (MMP⁺, Scheme 1) to transport the biological reducing equivalents from inside the cell to the WST¹⁻ acceptor situated on the other side of the membrane. By analogy, we envisioned that biomimetic liposomes containing a water-soluble electron donor in their interior (ethylenediaminetetraacetic acid, H₄EDTA) and a photosensitizer in the membrane would be able to unidirectionally transport the photoelectrons to the WST¹⁻ molecules located outside the liposomes, provided that MMP⁺ is added to the system. We describe below photocatalytic
Dye-functionalized liposomes were prepared in which the amphiphilic photosensitizer 1-[4-(iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium anion (WST1), the membrane-soluble electron relay 1-methoxy-N-methylphenazinium cation (MMP), their reduction products Fe3+ and MMPH, respectively, and the lipophilic zinc porphyrin photosensitizer [1]Cl.

A typical evolution of the UV-vis spectrum of the irradiated 1a. Clearly, a band ascribed to the Fz1+ oxidation potential of ~ +0.8 V was observed due to Fe3+ and its oxidation potential to Fe4+ in the Soret band of [ZnTMPyP]Cl at 449 nm. Instead the Soret band of the porphyrin bleached to around half of 141 nmol after 1 hour of irradiation than the number of moles of MMP present (125 nmol) in the system (Figure 1b), indicating that MMP functioned as a true catalyst in this system.

When the electron relay MMP was omitted from LA, formation of Fe3+ was not observed. Instead the Soret band of the porphyrin bleached to around half of its initial intensity (Figure S1a). It was thus clear that system LA2 requires MMP to perform charge separation, and that the electrons cannot be transferred directly from HEDTA to WST1 because they are positioned in different aqueous phases separated by an impermeable lipid membrane. Furthermore, in LA2 only half the porphyrin molecules were bleached, probably those on the inside of the membrane that were photoreduced by HEDTA. In system LA, triton X-100 was added to LA prior to the reaction, resulting in disruption of the liposomes and release of HEDTA.

### Table 1. Bulk concentrations and initial quantum yields for the different photocatalytic liposomes described in this report.

<table>
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<tr>
<th>System</th>
<th>MMP</th>
<th>Triton X-100</th>
<th>O2</th>
<th>EDTA</th>
<th>ZnSO4</th>
<th>ψ0 (%)</th>
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<td>-</td>
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<td>-</td>
<td>0.84</td>
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For the preparation of lipidosome samples LA and LB, see ESI. Bulk concentrations before extrusion and/or size exclusion: DPPC (1.04 mM), NaDSPE-PEG2K (10.4 µM), [1]Cl (16.7 µM). Final concentration of [1]Cl = 2.1 µM, as determined by UV-vis. MMP added as methylsulfate salt. For these samples, Na2EDTA was encapsulated inside the liposomes. To obtain the same osmolality inside and outside the liposomes, all solutions were prepared using isotonic NH4OAc (379 mOsm, pH = 7). These samples contained twice more liposomes per unit volume than LA (DPPC: 2.08 mM, NaDSPE-PEG2K: 20.8 µM, [1]Cl: 33 µM).

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into the bulk solution. In such conditions neither formation of Fz1\(^{2+}\), nor bleaching of the porphyrin was observed upon light irradiation. Since the bulk concentration of HEDTA\(^{3-}\) was 0.11 mM (see ESI), i.e. ~50 times lower than the bulk concentration of Zn\(^{2+}\), the released HEDTA\(^{3-}\) was fully bound to the Zn\(^{2+}\) ions thereby losing its ability to donate electrons in system LA\(_2\).

In order to investigate in which way physical separation of the electron donor and acceptor by the lipid membrane influences the rate of the photocatalytic reaction, the photocatalytic system LB\(_2\) was developed, which contained both the electron donor and acceptor on the outside of the lipidosome membrane (Figure 2, bottom). The liposomes used for these systems, called liposome sample LB, were prepared by standard extrusion methods from a lipid film of DPPC, 1 mol\% NaDSPE-PEG2K and 1.6 mol\% \([\text{1}]\)Cl\(_4\) hydrated in aqueous NH\(_4\)OAc (pH = 7.0). To this liposomal solution were added an isotonic HEDTA\(^{3-}\) buffer at pH=8.0, WST1\(^{-}\), and the electron relay MMP\(^{2+}\), to form system LB\(_2\) (see Table 1). System LB\(_2\) was deaerated and irradiated as described above, which also led to the formation of Fz1\(^{2+}\). A much lower initial photoelectron transfer quantum yield \(\varphi_0 = 0.90(9)\%\) was found for this system compared to LA\(_1\), indicating that the physical separation of the electron donor and acceptor in LA\(_1\) leads to a more stable separation of charges (Figure 1b). In system LB\(_2\), the photoreduced electron acceptor Fz1\(^{2+}\) is in the same aqueous phase as the photosensitizer, so that it may also serve as an electron donor and quench the porphyrin excited state, or react with porphyrin cation radicals. Such reactions diminish the overall quantum yield of the photoreaction. When MMP\(^{2+}\) is left out from this mixture (system LB\(_2\)), the reaction is only slightly inhibited (\(\varphi_0 = 0.72\%\)) compared to LB\(_2\) (Figure 1b), confirming that electron transfer from HEDTA\(^{3-}\) to WST1\(^{-}\) in LB\(_2\) occurs within a single aqueous phase where no membrane needs being crossed. The addition of triton X-100 to system LB\(_2\) (i.e., system LB\(_3\)) leads to an increase in the efficiency of the photoreaction, confirming that in LA\(_3\) triton X-100 quenches the photoreaction solely by disrupting the liposomes.

Many photocatalytic systems are not active in the presence of dioxygen, which would pose a problem in a photocatalytic fuel production system based on water oxidation. Thus, the sensitivity of systems LA\(_1\) and LB\(_1\) to O\(_2\) was investigated. When system LB\(_1\) was not deaerated (system LB\(_3\)) the presence of O\(_2\) did not significantly influence the rate of the photoreaction, nor did it cause any induction period common to this type of systems due to the initial competition between WST1\(^{-}\) and O\(_2\) to accept the first photoelectrons.\(^{4,9}\) In contrast, when system LB\(_1\) was not deaerated (system LB\(_3\)) complete bleaching of the Soret band of the porphyrin was observed and no Fz1\(^{2+}\) was produced (Figure 1b). The striking difference between systems LB\(_2\) and LB\(_3\) shows that one of the catalytic intermediates in LA\(_1\) and LB\(_1\) is unstable in the presence of O\(_2\). We hypothesized that such an intermediate would be MMPH, as it plays a key role in the photocatalytic mechanism of system LA\(_1\). In a control experiment, system LA\(_1\) without WST1\(^{-}\) was irradiated in the absence of O\(_2\); in this reaction MMPH was produced, which could be detected by UV-vis spectroscopy (Figure S2, left). After bubbling air through the solution the reaction was fully reversed: all MMPH was oxidized back to MMP\(^{2+}\) (Figure S2, right). Thus,
photocatalytic electron transfer through the membrane such as that observed in system L\textsubscript{A}\textsubscript{4} is sensitive to dioxygen because the reduced electron relay is rapidly oxidized by air. Based on the observations described above the mechanism depicted in Figure 2 (middle) is proposed for system L\textsubscript{A}\textsubscript{4}. First MMP\textsuperscript{2+}, introduced in the bulk aqueous phase, diffuses through the membrane into the liposomes (a),\textsuperscript{3} where it is photoreduced with HEDTA\textsuperscript{3–} (b). This reaction presumably occurs via reductive quenching of [1]\textsuperscript{4+} by HEDTA\textsuperscript{3–} (high local concentration, 125 mM), followed by reduction of MMP\textsuperscript{2+} by [1]\textsuperscript{3+}. Oxidative quenching would be thermodynamically feasible (the redox potential of the MMP\textsuperscript{2+}/MMPH couple is +0.06V vs. NHE)\textsuperscript{40}, however kinetically unlikely due to the low concentration of MMP\textsuperscript{2+} (42 µM). MMPH produced inside the liposomes can diffuse through the membrane in absence of O\textsubscript{2}, thus transporting the electrons and protons out of the liposome (d). Reduction of WST\textsuperscript{1} by MMPH outside of the liposomes forms Fz1\textsuperscript{2–} (e) and regenerates MMP\textsuperscript{2+}, as reported for the WST-1 cell counting assay. MMP\textsuperscript{2+} can then diffuse through the membrane again and turnover further. Since the amount of Fz1\textsuperscript{2–} produced (e), monitored by UV-vis, exceeds the amount of MMP\textsuperscript{2+} present at the start of the reaction (Figure 1a), MMP\textsuperscript{2+} must be concomitantly regenerated in the bulk aqueous phase, i.e., MMP\textsuperscript{2+} must be catalytic.

In summary, unlike previous studies in which electron transfer across lipid bilayers occurred concomitantly with the stoichiometric transport and occlusion of the electron acceptor into the membrane, directional electron transfer across a DPPC bilayer is unambiguously demonstrated here by combining a membrane-soluble electron relay, hydrophilic electron donors and acceptors situated inside and outside liposomes, respectively, and a membrane-bound photosensitizer. In this system the MMP\textsuperscript{2+} electron relay truly acts catalytically in absence of oxygen, but under air it is oxidized and cannot fulfill its electron-transporting role. Under inert atmosphere, the actual transport of electrons across the membrane does not limit the reaction rate, as the initial quantum efficiency of the reaction through the membrane (LA\textsubscript{4}) is three times higher than when the donor and acceptor are both positioned on the same side of the membrane (LB\textsubscript{4}). Keeping the photoreduced electron acceptor physically separated from the electron donor allows for preventing product inhibition, i.e., quenching of the porphyrin excited states by Fz1\textsuperscript{2–}, which would otherwise diminish the quantum efficiency of the photo-reduction. Finding a membrane-soluble electron relay that is stable in the presence of dioxygen will be required for the future development of supramolecular photocatalytic systems in which water oxidation and proton reduction catalysts are combined.

Notes and references