Artificial metallo-proteins for photocatalytic water splitting: stability and activity in artificial photosynthesis

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SUMMARY

Climate change poses an tough challenge for humanity and, if left unaddressed, its consequences will be dire. Therefore, intensive research towards a means of replacing fossil fuels is ongoing. Dihydrogen is considered a good potential alternative, as its waste product when burned is water, and many sustainable and efficient ways to obtain dihydrogen are being investigated. Photocatalytic water splitting in artificial photosynthesis offers such a means, it can be driven by solar light directly and therefore has great potential to be both efficient and sustainable. However, to put photocatalytic water splitting into practice, the synthetic catalysts and photosensitizers involved in the water oxidation and dihydrogen reduction half-reactions require further optimization. The greatest challenges, especially for catalysts for water oxidation, are their stability and solubility. Artificial metalloproteins offer potential for addressing both challenges, as the protein scaffold can improve aqueous solubility and can provide a direct means of controlling the second coordination sphere of a metal-based catalyst. The protein environment offers a way for further optimization of the activity and to prevent harmful side reactions. In this thesis, different protein scaffolds are explored to support metal-based catalysts and photosensitizers required for the two half-reactions of water splitting. During our analysis, we placed special focus on the stability of our ArMs during water oxidation and dihydrogen evolution catalysis.

In **Chapter 1** the topics of dihydrogen evolution and water oxidation are introduced, firstly by introducing the natural protein systems capable of driving both reactions. For dihydrogen evolution these proteins are the hydrogenases, a multitude of proteins with a variety of cofactors that can support the use and production of H_2. Both reactions are catalysed by varying clusters of iron, nickel, and sulphur. In each case, the clusters are, at least in part, directly coordinated by residues of the protein scaffold, which in this manner directly impacts the reactivity. For driving the more challenging water oxidation reaction nature has only one solution: Photosystem II (PSII). The active core of PSII consists of a manganese cluster and a chlorophyll-containing protein heterodimer, and the challenges that this system faces to prevent photo damage are discussed. This overview is followed by an

introduction to artificial metalloenzymes (ArMs). ArMs combine the advantages of synthetic catalysis, i.e., the variety of (non-)natural reactions that can be catalysed by man-made catalysts and the simplicity of their design, with the strengths of natural metalloenzymes. They allow optimization of the direct environment of the bound catalyst so that reactions can be performed under mild conditions (*e.g.,* neutral pH and room temperature) and so that the selectivity vis-à-vis reactants and products can be improved. Their applications for electron transfer guided catalytic processes are discussed, with a focus on how both coordination spheres around the metal centre can influence its reactivity and stability. Finally, the proteins, photosensitizers, and catalysts employed in this thesis are presented.

Chapter 2 presents a novel screening method for studying interactions between haem proteins and synthetic metal complexes. Semi-native gel electrophoresis is introduced to identify the interaction of metal complexes and haem proteins prepared in the haem-free (*apo*) state. In semi-native gel electrophoresis, a protein in a buffer solution is loaded on a gel containing the denaturing detergent SDS. The mechanism of separation in a semi-native gel is based on the differential stability of the protein scaffold with and without metal complex bound. As the protein moves through the gel matrix, it is exposed to SDS and begins to unfold. The longer the protein can resist unfolding and remains in its compact fold, the lower it ends on the gel. The efficacy of this method was demonstrated in a study of the interaction between the proteins cytochrome b_5 (CB5), haem acquisition system A *Pseudomonas aeruginosa* (HasAp), and myoglobin (Mb), with the cobalt-based water-oxidation catalysts CoSalen and CoPhthalocyanine. Further, the general applicability of this method is shown for the interaction of CB5 with three ruthenium-based complexes and two other cobalt-based water-oxidation catalysts. We found that the size and axial ligands of the metal complex were the primary factors determining the success of ArM formation, and we observed the best successes with the Co-based complexes.

Based on this screening procedure, the combination of CB5 and the water oxidation catalyst complex CoSalen was chosen to design an ArM for photocatalytic water oxidation catalysis, described in **Chapter 3**. Three ArMs were prepared from CB5 with CoSalen, one reacted in a 1:1 (CB5:CoSalen) molar ratio with *apo*CB5, one reacted in a 1:5 molar ratio and one reacted

1:5 with *holo*CB5. *Apo*CB5:CoSalen 1:1 binds a single CoSalen to the binding pocket whereas for *apo*CB5:CoSalen 1:5 and *holo*CB5:CoSalen 1:5 several cobalt complexes bound to the protein scaffold. The *apo*CB5:CoSalen 1:1 complex was found to be catalytically inactive, while *apo*CB5:CoSalen 1:5 and *holo*CB5:CoSalen 1:5 were both catalytically active in photocatalytic water oxidation systems comprising $Ru(bpy)_3^{2+}$ as photosensitizer and $Na_2S_2O_8$ as sacrificial electron acceptor, though with lower activity than CoSalen in solution. CoSalen functions as a precatalyst forming mixed cobalt oxide/hydroxide nanoparticles that are the true active species. In contrast, in the ArMs CoSalen-protein interactions were destabilized during photocatalysis, but nanoparticle formation was not observed. Our data suggests that a weakly interacting, soluble protein-Co species is formed upon irradiation under photocatalytic conditions, and this species acts as the catalytically active species. The CB5 protein scaffold suffered from limited ligand oxidation, crosslinking, and partial unfolding during catalysis, but remained overall surprisingly intact and was capable of strongly improving the solubility of the CoSalen catalyst. The CB5:CoSalen ArMs represent one of the first ArMs capable of catalyzing water oxidation in photocatalytic conditions. The analysis presented here shows the potential as well as the challenges of using a protein scaffold as support of an active metal-based catalyst in the harsh, oxidative conditions of photocatalytic water oxidation.

In **Chapter 4** four fully protein-based systems for photocatalytic dihydrogen evolution were designed and characterized. In two of the systems, myoglobin with its haem substituted for cobalt protoporphyrin (CoPPIX), ArM3, functioned as a dihydrogen evolution catalyst (HEC). The HEC ArM was combined with HasAp with haem substituted for ZnPPIX, ArM1, or for RuPPIX, ArM2, as photosensitizer (PS). In both systems dihydrogen evolution was measured under irradiation in the presence of electron relay methyl viologen (MV^{2+}) and sacrificial donor triethanolamine (TEOA). For the RuPPIX-based PS 385 nm near UV light was used, for the ZnPPIX-based PS 435 nm blue light was employed. A system comprising both ArM1 and ArM3 was capable of driving dihydrogen evolution with a turnover number (*TON*) of 212 per catalyst ArM3, remaining active for 42 h. When ArM1 was replaced by ArM2 as PS, the photocatalytic activity was initially comparable to that of the ArM1-containing system, but after ~13 h a far more active catalyst species was formed showing no sign of loss of activity, leading to a *TON* of 1069 after

42 h. In ArM1 the binding ratio of HasAp:ZnPPIX was 1:1.1. Therefore, when ArM1 was reacted with CoPPIX, ZnPPIX was displaced to form a mixture of HasAp-ZnPPIX and HasAp-CoPPIX that is referred to as ArM5. The binding ratio of HasAp:RuPPIX in ArM2 was 1:2.4. As a result, when ArM2 was reacted with CoPPIX not all RuPPIX was displaced, forming CoPPIX-HasAp-RuPPIX or ArM4. The dihydrogen evolution activity of ArM5 was slightly higher than that of ArM1+ArM3, with a *TON of* 349. The activity of ArM4 exceeded that of ArM2+ArM3 for 17 h, after which the ArM2+ArM3 system overtook ArM4. The *TON* of ArM4 was 492 after 42 h. All types of HasAp-based ArMs showed high photo- and thermostability, though after 42 h of photocatalysis, some protein cleaving was observed. Mb could no longer be seen on gel after photocatalysis in the ArM3 containing systems, while its CoPPIX ligand was still observed. It was hypothesized that when Mb precipitates, the gradually released CoPPIX could bind to ArM2 and form a complex with far superior H_2-evolution properties compared to ArM3, possibly ArM4. In short, two earth-abundant, protoporphyrin IX-based dihydrogen evolution systems were successfully produced that function under blue-light irradiation, though their activity was lower than that of their ruthenium-based analogues. Finally, HasAp showed good potential as a protein scaffold with long lasting stability for supporting both dihydrogen evolution catalysts and photosensitizers.

In **Chapter 5** an analysis is presented that guides the optimal position of a PS with respect to the binding pocket of CB5 for the design of an ArM that combines photosensitizing and catalytic properties. Electron transfer protein CB5 is used as a scaffold to host a $Ru(bpy)_3$-derived photosensitizer, covalently linked *via* a maleimide group to a cysteine residue that was introduced using site directed mutagenesis. This approach leaves the binding pocket free so that in future experiments a catalyst could be coordinated as substitute for ferric haem. Such a system, in which the catalyst and photosensitizer are kept in a well-defined position with respect to each other, can be used to optimize the activity and stability of the protein scaffold and catalyst. Four cysteine mutants were prepared, V66C, S69C, T70C, and L75C, and characterized. Each mutant was found to be well-folded and capable of binding haem, though by a small margin the properties of the L75C and V66C mutants were the most favourable. The efficiency of electron transfer from the photosensitizer to haem was probed via the kinetics of the

build-up of haem in the Fe(II) state. Here the T70C and L75C mutants are found to be the most efficient. A fluorescence comparison led to the conclusion that a cysteine that points away from the protein core reduces back-electron transfer and increases solvent accessibility for interaction with the sacrificial donor. Considering all the above, the L75C mutant is considered the best host for a catalyst and photosensitizer.