SUMMARY

The catalytic cofactor of [FeFe]-hydrogenases (H-cluster) is embedded in a highly conserved hydrophobic pocket of the protein. The H-cluster consists of a [4Fe-4S]-cubane (4FeH), coordinated by four cysteines, and a [2Fe-2S]-cluster (2FeH). The Fe-ions of the 2FeH-cluster (Fe_p, Fe_d) carry biologically unusual ligands and Fe_d constitutes the site for catalytic turnover. The 2FeH-subcluster exhibits only one covalent bond to the peptide, wherefore several noncovalent interactions of highly conserved residues inside the H-cluster vicinity are probably essential for structural stability and catalytic function.

The structure function relationships of these residues were analysed via site-directed mutagenesis (SDM) in the enzymes of Clostridium pasteurianum (Cpi) and Chlamydomonas reinhardtii (CrHydA1). The mutations of CpiK_{358} (CrHydA1K_{228}) and CpiM_{497} (CrHydA1M_{415}) confirmed an essential contribution of these two residues to stability or insertion of the 2FeH-site, since a specific loss of the corresponding FTIR and EPR signals was observed. Mutation of two other residues in contrast resulted in variants with a structurally intact H-cluster but a strongly altered catalytic activity. For CpiC_{299S} (CrHydA1C_{169S}) no activity was detectable, while EPR and FTIR spectroscopy revealed a redox state of the H-cluster not observed in CrHydA1 or Cpi before. This state closely resembled the inactive H_{trans} state of DdH from Desulfovibrio desulfuricans and was thought to be characteristic for the reductive activation of this aerobically purified enzyme. Mutation of CpiM_{353} (CrHydA1M_{223}) in contrast resulted in a decrease of catalytic activity of about 80% compared to wild type. The interaction of this residue was therefore concluded to be not essential for catalysis, nevertheless seems to constitute a “fine tuning element” for the level of catalytic activity. The information of the catalytic impact of single amino acids of the H-cluster vicinity obtained by SDM might allow further optimisation of artificial H-cluster resembling catalysts.

In the second project described in the present work SDM was applied to an [FeFe]-hydrogenase for the generation of solar hydrogen producing nanoconstructs. These constructs consist of photosystem I (PSI) from Synechococcus spec PCC 7002 and [FeFe]-hydrogenase HydA from Clostridium acetobutyllicum, electrochemically linked by a dithiol wire. The initial system produced light driven hydrogen by a rate of 30.3 µmol H_2 mg chl^{-1} h^{-1}, which was successfully optimised by almost two orders of magnitude to a rate of 2832 µmol H_2 mg chl^{-1} h^{-1}. Thus nanowiring enables the establishment of an efficient electron transfer connection between two different proteins or protein complexes, where electron transmission does not occur naturally. The achieved electron transfer rates in the optimised system are even comparable to the electron throughput of PSI during oxygenic photosynthesis. The dithiol wired PSI-hydrogenase nanoconstructs outcompete all other PSI based
systems for light driven hydrogen production in catalytic efficiency reported so far and exhibit a functional stability over a period of 100 days under anoxic conditions. Furthermore, this coupling principle solely depends on the modification of the binding motif of surface exposed [4Fe-4S]-cubanes. As these cubanes are widely distributed among metalloenzymes, the system should as well be suitable for the electrochemical coupling of other redoxproteins.