5. b SUMMARY

The hydrogen evolving and oxygen-sensitive hydrogenase HydA1 from the green alga *Chlamydomonas reinhardtii* reveals high specific activity and is one of the smallest known [FeFe]-hydrogenases. Therefore HydA1 is most useful for structural examination of the unique active site of [FeFe]-hydrogenases, called H-cluster. The amount of HydA1 which can be isolated from one liter of *C. reinhardtii* culture was 1 μg and far too low for biophysical experiments.

Within this work it was for the first time possible to establish a heterologous expression system for catalytically highly active [FeFe]-hydrogenases, using the strict anaerobic bacterium *Clostridium acetobutylicum* as a host. This was shown for two rather similar algal hydrogenases: HydA1 from *C. reinhardtii* and HydA<sub>S,o</sub> from *Scenedesmus obliquus*. Using the StrepTag-technology a highly efficient method for protein purification was implemented. Compared to the native enzymes the heterologously expressed algal hydrogenases also show high specific activity and reveal similar substrate affinity. By adopting the hydA1 sequence from *C. reinhardtii* to the codon usage of the host organism and by introducing an exposed StrepTag sequence, the protein yield was improved from initially 0,1 mg to 1 mg per liter of culture.

In parallel, during this work a homologous expression system for HydA in *C. acetobutylicum* was demonstrated by using the sophisticated method of homologous recombination leading for the first time to the directed genomic integration of a recombinant hydrogenase gene in this organism. This allowed a comparative analysis of HydA and HydA1 by performing enzyme kinetics under identical conditions. Specific activity for HydA proton reduction turned out to be 100fold higher than published before.

Biophysical examinations using pulse-EPR- and Davies-ENDOR-spectroscopy were done with [FeFe]-hydrogenases that had been expressed by *C. acetobutylicum* during this work. Both hydrogenases, HydA1 from *C. reinhardtii* and HydA from *C. acetobutylicum* revealed typical spectra for intact H-clusters. Furthermore, functional enzyme immobilisation at a gold surface was achieved for HydA1 of *C. reinhardtii* within a SEIRAS-cell, allowing infrared spectroscopical examination of this characteristic [FeFe]-hydrogenase within the near future. On the basis of those and similar experiments research on green algal hydrogenases will soon contribute to enzyme structure determination and biotechnological application of [FeFe]-hydrogenases in general.