Investigation of Fe-Fe hydrogenase via electrochemistry and scanning probe microscopy: Towards single molecule catalysis

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The *Clostridium acetobutylicum* [FeFe]-hydrogenase HydA has been investigated as a hydrogen production catalyst through the use of electrochemical and scanning probe microscopy techniques. Hydrogenases utilize either a [Ni-Fe] or a [Fe-Fe] complex at their active site, which unlike fuel cell catalysts, contain no precious metals. Precious metal catalysts such as platinum and palladium likely are not sufficiently abundant to be useful for terawatt scale energy transduction. In addition to not containing precious metals, hydrogenases are only reversibly poisoned by carbon monoxide where irreversible inactivation of platinum is observed.

When adsorbed to a pyrolytic graphite edge (PGE) electrode, the *Clostridium acetobutylicum* [FeFe]-hydrogenase (*Ca*HydA) shows a characteristic hydrogen production signal at roughly -0.55 V vs. Ag/AgCl; similar to the signal observed for hydrogen production on a platinum electrode. While the exact orientation of single *Ca*HydA molecules on the PGE has not been extensively characterized, it is suspected that one orientation would be favorable for the highest efficiency during catalytic turnover due to the location of 4Fe-4S clusters that act as an electron transport chain. To test this, *Ca*HydA will be attached to self assembled monolayers (SAM's) on gold surfaces and characterized by scanning tunneling microscopy (STM). The residues on the surface of the protein will interact with the various terminal groups introduced on the SAM attaching it to the surface, while not disturbing the structure of the active protein. Work has begun on this aspect of the project and several STM images have been obtained. This method of attachment could also orient the molecule, allowing for a more uniform orientation of molecules on the macroscopic electrode surface. Once attached to the surface, via the SAM, STM will be used to characterize the *Ca*HydA and distinguish single enzyme molecules. Once a single molecule is identified, the tip of the STM can be held steady while the bias voltage between the tip and the surface is changed. Negative of the H^+/H_2 couple, a current for the catalytic turnover of a single enzyme molecule may be detected, and related to the ensemble behavior seen in cyclic voltammograms on PGE electrodes.

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