The Activation Process of *D.desulfuricans* ATCC 27774 [NiFe] Hydrogenase

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In recent years, we have determined the 3-D structure of [NiFe] hydrogenase from the sulphate- and nitrate-respiring bacteria *D.desulfuricans* ATCC 27774. The active site of this enzyme, which catalyses the reversible reaction $H_2 \leftrightarrow 2H^+ + e^-$ is constituted of a Ni Fe heteronuclear diatomic metal core bonded to the protein chain by four cysteine residues, two of which bridge the metal atoms. The Fe atom is further coordinated by two CO and one CN ligands.

In the simplest description, three states are usually considered for the active site: unready, ready and active. These states have been characterised by EPR spectroscopy for several hydrogenases from different organisms.

Our crystallographic studies allowed us to obtain structural details of the active site in each one of the three states. A key result that emerged from this study was evidence for the coupling between Cys 536, Glu 24 (a highly conserved residue in [NiFe] hydrogenases from *Desulfovibrio* and related organisms) in proton transport off the active site. Cys 536 may even be implicated in the activation of the H-H bond prior to its heterolytic cleavage.

These results have in turn led to a proposed mechanism for the activation process of this enzyme, supported by DFT calculations.

Keywords: hydrogenase, active-site structure, activity and mechanism of enzymes