Investigating the Key Bridging Ligand in the Active Site of [FeFe] Hydrogenase Enzymes Towards the Development of Artificial Metalloenzymes for Hydrogen Conversion

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1. The Potential of Hydrogen in a Sustainable Future

How do we supply reliable, affordable, and renewable energy to all?

- As renewable energy is an intermittent energy source it requires an energy vector for storage and transportation.1-4
- Hydrogen is the simplest energy vector to manufacture, storing more energy per unit volume and producing zero emissions.5
- Renewables can provide energy to split water into hydrogen and oxygen in an electrolyser, generating chemical energy through strong H-H bonds.6
- Hydrogen can be oxidised to water in a fuel cell to generate electricity.
- There are currently no sustainable catalysts for global hydrogen production/oxidation for use in electrolyser and fuel cells.7,8

2. Hydrogen Enzymes

Nature's hydrogen economy formed billions of years ago with the evolution of hydrogenases.9-20

\[ H_2 = H^+ + H^- = 2H^+ + 2e^- \]

They are advantageous to use in fuel cells and electrolyser as they:
- Exhibit very high catalytic rates under ambient conditions
- Are infinitely renewable and biodegradable
- Display excellent specificity
- Can scavenge fuel and oxidants in very low concentrations
- Are very efficient and fast catalysts when adsorbed on electrode surfaces

However, their industrial applications are limited because:
- They are often unstable under harsh conditions
- Their isolation and purification are arduous techniques, which are difficult to scale up to commercial levels
- Exposure to oxygen causes mainly irreversible deactivation

3. [FeFe] Hydrogenases

The [FeFe] hydrogenase is the fastest-known biological catalyst for hydrogen production.14

This N atom withdraws electron density from the active site, acting as a base and polarising the H-H bond

To investigate this, the -NH group has been replaced by an O atom to form an artificial metalloenzyme with different chemical properties

Native Enzyme  Artificial Metalloenzyme

Further understanding the role of this -NH group and its interactions with H₂ and the enzyme’s protein scaffold is crucial for developing commercial synthetic mimics or bioinspired catalysts.

4. Active-Site Structure: X-Ray Crystallography

Methionine

Proton Pathway

Cysteine

Gas Channel

Vacant coordination site where catalysis occurs

Distorted

Cysteine Partially Migrated

Artificial Metalloenzyme (0.99 Å resolution)

5. Catalytic Activity: Electrochemistry

H₂ oxidation/production depends on whether the applied potential is positive or negative relative to the thermodynamic potential of the H⁺/H₂ couple (E⁰[H⁺/H₂]).

- Exhibits very high catalytic current densities for both H₂ oxidation and production.
- Reversible high-potential inactivation is observed at high positive potentials.

Native Enzyme

- Exhibits smaller current densities and only in the H₂ oxidation direction; no H₂ production observed.
- Irreversible high-potential inactivation is observed at high positive potentials.

Artificial Metalloenzyme

6. Conclusions

- The flipped bridgehead of the artificial metalloenzyme is likely enabled by the smaller size of O and the weakened interactions with the protein scaffold's amino acid residues.
- Highlights the importance that the amino acids have in maintaining the correct bridgehead position geometry for optimal catalysis by interacting with the active site.
- The partial migration of the cysteine residue into the vacant coordination site in the artificial metalloenzyme allows for greater protection of the active site towards O₂ degradation while maintaining catalytic activity.
- This promises a different catalysis is used in a system that utilises a ‘flipped bridgehead’ mechanism for enhanced O₂ stability in artificial metalloenzymes or synthetic catalysts and overcome one of the main issues of the native enzymes that greatly hinders their commercial applicability.

References