Sample Abstract – *Chemistry*

Abstract Title: Mechanistic Exploitation of a Self-Repairing, Blocked Proton Transfer Pathway in an O_2-Tolerant [NiFe]-Hydrogenase

Catalytic long-range proton transfer in [NiFe]-hydrogenases has long been associated with a highly conserved glutamate (E) situated within 4 Å of the active site. We hypothesize that substituting for glutamine (Q) in the O_2-tolerant [NiFe]-hydrogenase-1 from *Escherichia coli* produces a variant (E28Q) with unique properties. These properties have been investigated using protein film electrochemistry, protein film infrared electrochemistry, and X-ray crystallography. At pH 7 and moderate potential, E28Q displays approximately 1% of the activity of the native enzyme, high enough to allow detailed infrared measurements under steady-state conditions. Atomic-level crystal structures reveal partial displacement of the amide side chain by a hydroxide ion, the occupancy of which increases with pH or under oxidizing conditions supporting formation of the superoxidized state of the unusual proximal [4Fe–3S] cluster located nearby. Under these special conditions, the essential exit pathway for at least one of the H^+ ions produced by H_2 oxidation, and assumed to be blocked in the E28Q variant, is partially repaired. During steady-state H_2 oxidation at neutral pH (i.e., when the barrier to H^+ exit via Q28 is almost totally closed), the catalytic cycle is dominated by the reduced states "Nia-R" and "Nia-C", even under highly oxidizing conditions. Hence, E28 is not involved in the initial activation/deprotonation of H_2, but facilitates H^+ exit later in the catalytic cycle to regenerate the initial oxidized active state, assumed to be Nia-SI. Accordingly, the oxidized inactive resting state, "Ni-B", is not produced by E28Q in the presence of H2 at high potential because Nia-SI (the precursor for Ni-B) cannot accumulate. The results have important implications for understanding the catalytic mechanism of [NiFe]-hydrogenases and the control of long-range proton-coupled electron transfer in hydrogenases and other enzymes.

KEY

Abstract contains sufficient background to understand the problem under investigation

Abstract must contain a hypothesis, objective or statement about the problem under investigation

Abstract must contain a brief statement of the experimental methods/methodology used

Essential results must be present in summary form (even if preliminary)

Abstract must contain a conclusion that explains how the work contributes to the hypothesis, objective or statement of problem

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