Exploring novel viologens as redox mediators for electrochemically-driven enzymatic reactions

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Introduction

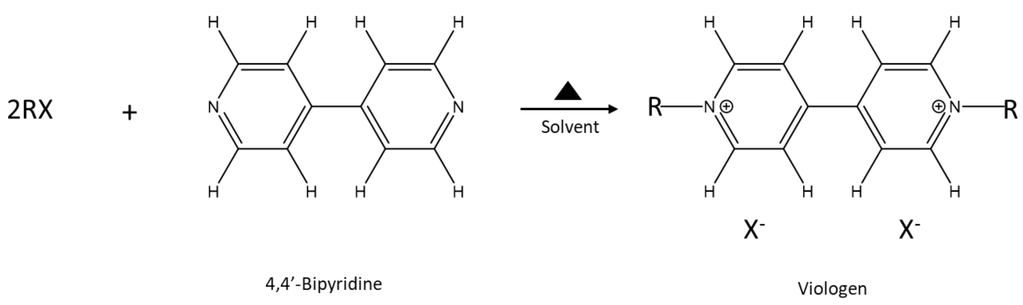
Enzymes are great candidates for the sustainable production of chemicals,[1] environmental remediation,[2] green energy and fuel production,[3] *etc*. However, the co-factors used by these enzymes (such as NADPH) to drive these processes are very costly to industrially produce, unstable and typically consumed during the reaction process.

Viologens, 1,1′-disubstituted-4,4′-bipyridinium salts, are organic redox species that can be used in place of NADPH as mediators for redox enzymes. Much is unknown of the Viologen radical ($V^{+\bullet}$) as a mediator and the fully reduced viologen (V^0) has never been successfully demonstrated to be an NADPH replacement with FAD-dependent enzymes like GR. In this study, using the reduction of oxidized glutathione by glutathione reductase as a model system, a rationally designed library of viologens covering a range of polarities and functional groups were explored as electron transfer mediators for (bio)-electrocatalysis. Through a series of electrochemical investigations, the reduction potential was found to be the primary determining factor for electron transfer between the viologen radical and enzyme.

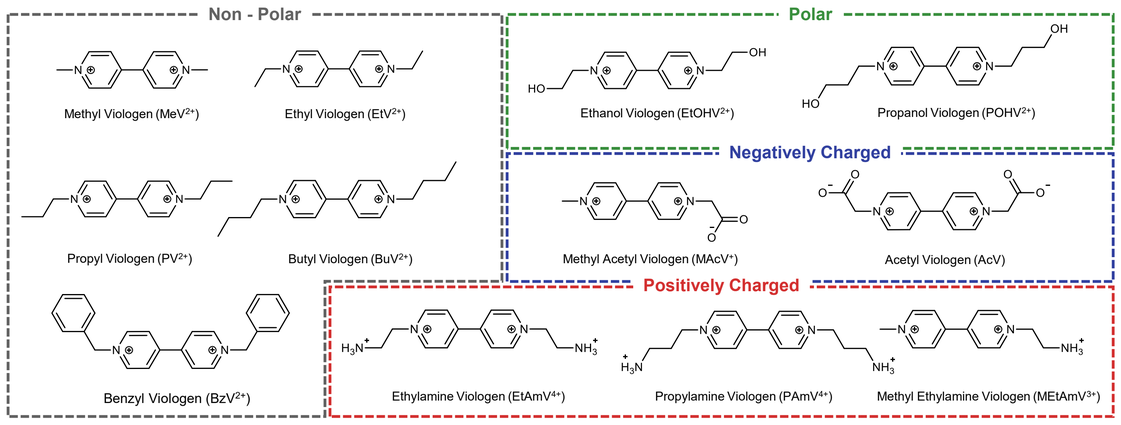
Through enhancing the solubility of viologen such that the fully reduced state (V^0) remained soluble, we demonstrate a much-widened window of usable viologen potentials. In doing so, we describe for the first time a highly efficient electron transfer to a flavoenzyme promoting the catalytic reaction in the absence of co-factors. As such, our study provides a platform for broadening the scope for using viologens as mediating agents for electrochemically-driven enzymatic processes.

Methodology

Synthesis of Viologens

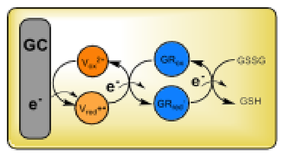


R – Alkyl, Acetate, Alkylamine
X – Halide
▲ – 90 – 120 °C
Solvent – Acetonitrile (ACN), Dimethylformamide (DMF)



CYCLIC VOLTAMMETRY (CV)

Standard components:
- Tris buffer/KCl (electrolyte)
- 10 mM GSSG (Green)
- 1 mM viologen (blue)



1. $V^{2+} + e^- \rightarrow V^{+\bullet}$
2. $V^{+\bullet} + GR \rightarrow V^{2+} + GR^-$
3. $GR^- + GSSG \rightarrow GR + GSH$

Experiments performed with and without
- 0.1 mM gluthathione reductase enzyme (red)

Current Enhancement Factor (CEF) = Max. Reduction Current with GR / Max. Reduction current without GR

Where CEF>1 indicates a catalyzed reaction

Conclusion

- Through the rational design and creation of a diverse library of viologens, we have demonstrated that reduction potential is the defining characteristic for mediating bioelectrocatalytic reactions with high efficiencies by specifically, using the reduction of GSSG by GR as a model reaction.
- Additionally, the kinetics of the reaction could be finely tuned through the chemical properties of the modified viologens, and particularly how they interact with the surface topology of the enzyme.
- Critically, by modifying viologen so that it remains soluble in the fully reduced form, we were able to perform highly efficient bioelectrocatalysis using the second redox potential of viologen; the first time fully reduced viologens have been reported to mediate electron transfer to a flavoenzyme.
- This work demonstrates the potential of viologen mediators for replacing NADPH (and associated recycling) in promoting biocatalytic redox reactions. The simplicity of this reaction system in circumventing the requirement for expensive co-factors will be of great benefit to those working on industrially relevant transformations with a wide range of redox enzymes.
- Investigating the mechanism of the electron transfer process at the second reduction potential, and deciphering whether it is independent of the structural binding of the viologen at the active site of flavoenzyme, will be the focus of future work.

Bibliography

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2. D. A. Koomson *et al., Renew. Energy*, 2022, **189**, 1851–1853 1375-1382.
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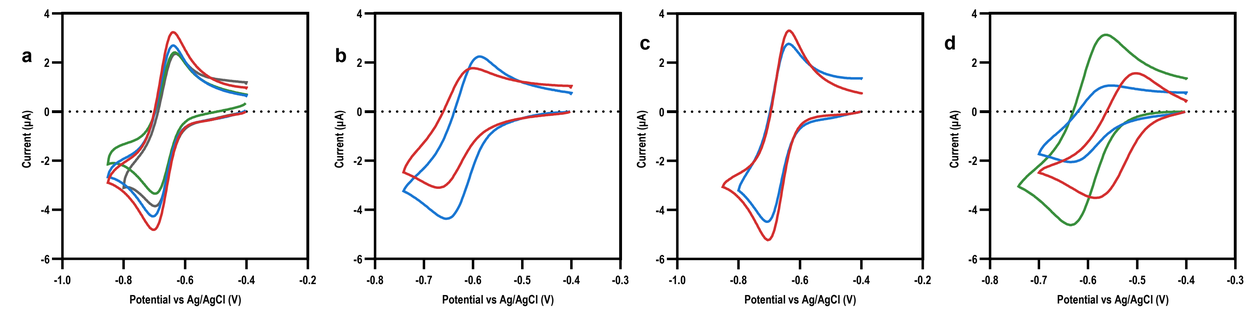
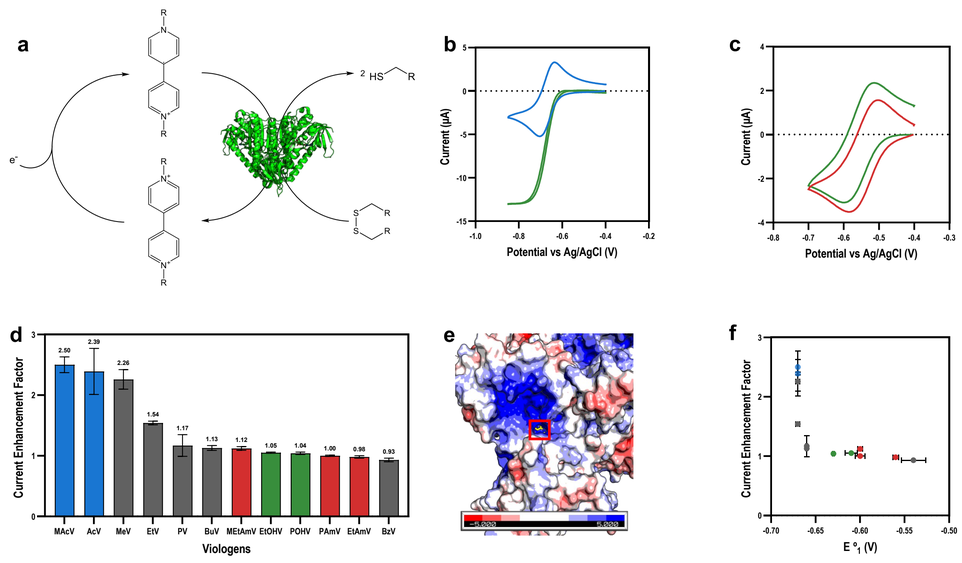
Results & Discussion

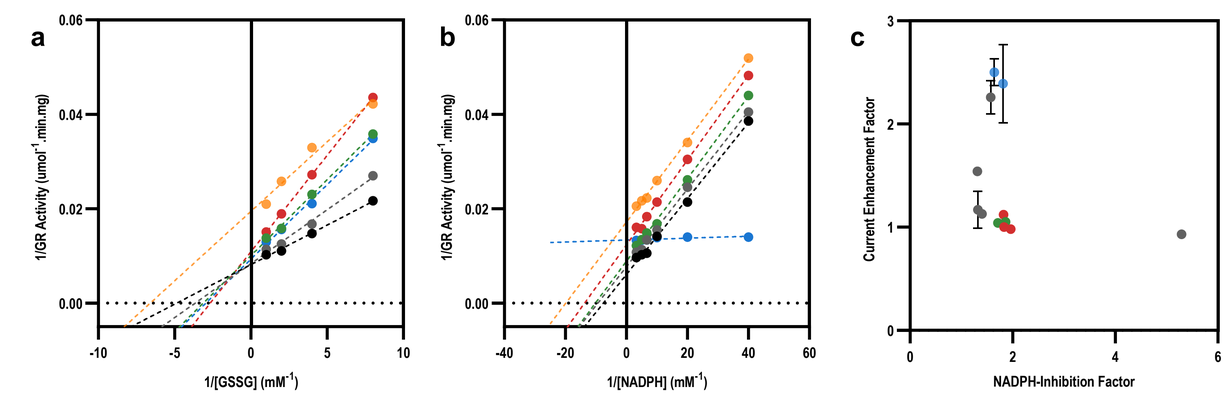


❖ **Electrochemistry of viologen library at first reduction potential.**

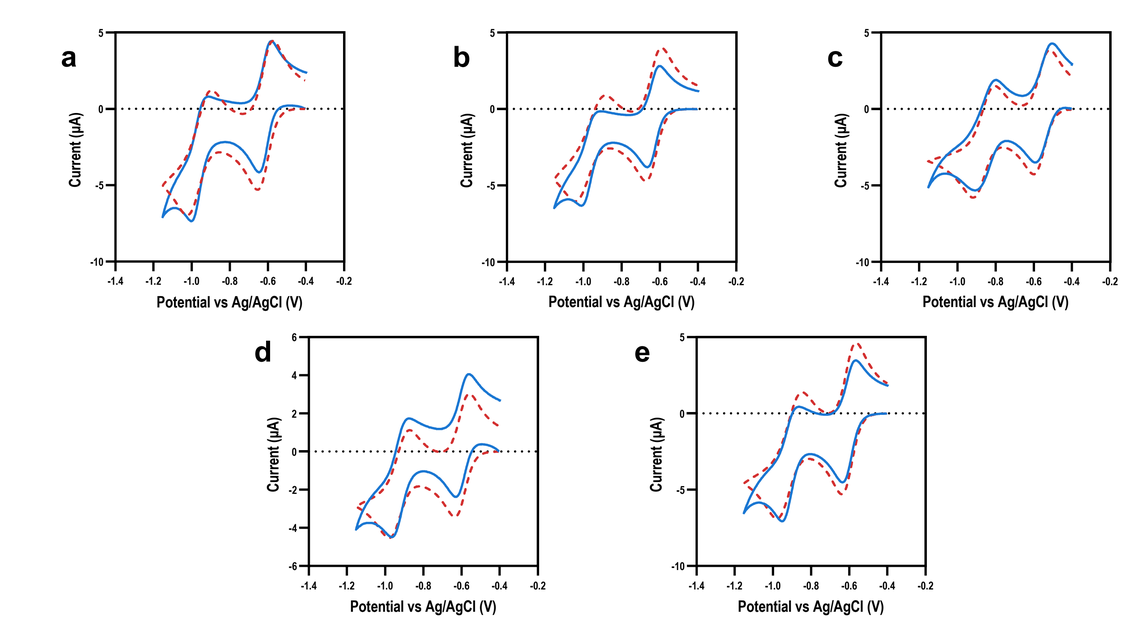
❖ Cyclic voltammograms showing first redox peaks for: (a) non-polar viologens (MeV – red, EtV – blue, PV – Green, BuV – grey); (b) polar viologens (EtOHV – red, and POHV – blue); (c) negatively charged viologens (AcV – blue, and MAcV – red); and (d) positively charged viologens (EtAmV – red, PamV – blue, and MetAmV – green).



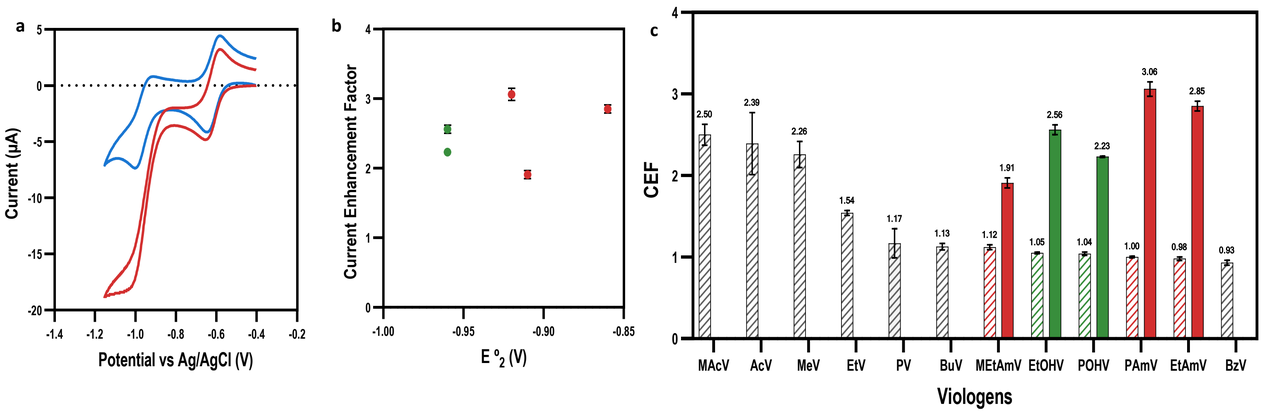
❖ **Mediating bioelectrocatalysis at the first reduction potential.** (a) Schematic illustration of bio-electroreduction of GSSG in the presence of GR with viologen as the electron transfer mediator. (b) Cyclic voltammograms showing example of bio-electrocatalytic reduction of GSSG by GR with MAcV as the electron transfer mediator (green) with control reaction for reference (blue). (c) Cyclic voltammograms showing example of no bio-electrocatalytic reduction of GSSG by GR was observed with EtAmV as the electron transfer mediator (green) with control reaction for reference (red). (d) Current enhancement factor (at E^o_1) for the viologen library. (e) Surface representation of GR (PDB: 2HQM[26]) showing positive charges surrounding active site (FAD highlighted in the red box). (f) Plot of current enhancement factor against E^o_1.



❖ **a – Lineweaver-Burk plot showing inhibition of GSSG at GR active site in presence of viologens:** uninhibited control (black), MeV (grey), BzV (orange), EtOHV (green), EtAmV (red), and AcV (blue)**; b – Lineweaver-Burk plot showing inhibition of NADPH at GR active site in presence of viologens:** uninhibited control (black), MeV (grey), BzV (orange), EtOHV (green), EtAmV (red), and AcV (blue); **c – Current enhancement factor versus NADPH-inhibition factor of the viologens.**



❖ **Second redox cyclic voltammograms (solid blue line) of viologens with their corresponding simulated fits (dotted red line):** a – EtOHV; b – POHV; c – EtAmV; d – PamV; e – MetAmV.



❖ **Mediating bioelectrocatalysis at the second reduction potential.** (a) Cyclic voltammogram showing example of bioelectrocatalysis at the second reduction potential, with EtOHV with GSSG in the presence of (red) or absence of (blue) GR. (b) Plot of current enhancement factor against E^o_2. (c) Current enhancement factors for the viologen library at E^o_1 (dashed) and E^o_2 (solid).